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SUMMARY

SOIL SCIENCES

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MISCELLANEOUS

SOIL
SCIENCES

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DETERMINATION OF THE EFFECT OF SILICON, MYCORRHIZA AND PHOSPHORUS BACTERIA APPLICATION ON INCREASING PHOSPHORUS UTILIZATION EFFICIENCY AND STEM RESISTANCE IN SUNFLOWER (*Helianthus annuus*)

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Abstract

Today, the intensive use of chemical fertilizers causes various environmental problems. Inoculation of microbial preparations as an alternative to chemical fertilizers to plants can be an effective method for sustainable production efforts. Silicon application shows potential to increase nutrient uptake by roots and nutrient availability in the rhizosphere through complex mechanisms, can increase P availability in the soil. In this study, the effects of silicon application and inoculation of phosphorus solubilizing bacteria and mycorrhiza on sunflower plant growth and phosphorus utilization efficiency were investigated. Oil sunflower seed, Glomus etunicatum, mycorrhiza complex and with phosphorus bacteria were inoculated. Si application was also made at doses of 0-30-60 kg/da. After the sowing of the experimental plants, mycorrhiza and P bacteria were inoculated. The experiment was terminated when 50% of the flowers bloomed. According to the results, average plant height, flower wet and dry weights showed significant differences with Si treatments, Si with mycorrhiza and P bacteria treatments. 30 Si kg/ha dose had more positive effects on plant growth.

Key words*: silicon, phosphorus, mycorrhiza, phosphorus bacteria.*

INTRODUCTION

Silicon, which is not classified as an absolutely essential element for plants, has actually been observed to have beneficial effects in various plant species and environmental conditions. It has been reported that Si has the potential to increase the uptake of nutrients in the rhizosphere and root zone through complex mechanisms (Pavlovic et al., 2021). Si has been described by various researchers as an “agriculturally essential element” due to its ability to increase plant resistance to plant diseases and pests and stressful conditions such as drought, metal toxicity, salinity and sodium (Detmann et al., 2012; Klotzbücher et al., 2018; Liang et al., 2015). Some plant species are minimally affected by Si fertilization compared to other plants (Coskun et al., 2019).

Silicon has been reported to play an important role in P nutrition as well as in the uptake of other plant nutrients (Schaller et al., 2021; Pavlovic et al., 2021). Also, Si has been shown to have a mitigating effect in wheat (Kostic et al., 2017; Neu et al., 2017), tomato (Zhang et al., 2019), paddy (Pati et al., 2016; Hu et al., 2018), maize (Owino-Gerroh and Gascho, 2005), potato (Soltani et al., 2017; Soratto et al., 2019) plants under conditions of limited availability of phosphorus. Si has been reported to be effective in alleviating P deficiency by increasing root uptake and utilization of phosphorus within plant tissues (Neu et al., 2017; Zhang et al., 2019).

Although the use of phosphorus fertilizers is recommended to eliminate P deficiency in soil, there are factors that reduce the efficiency of phosphorus use in acidic and calcareous soils. In recent years, it has been reported that arbuscular mycorrhizal fungi (AMF), phosphate solubilizing bacteria (PSB) and Si application can be effective and economical solutions to increase the availability and efficiency of phosphorus in soil (Etesami et al., 2021). In this study, the effects of inoculation of sunflower plants with different doses of Si, a phosphorus solubilizing bacterium and two different mycorrhizae on plant growth parameters were investigated.

MATERIALS AND METHODS

The experiment was conducted as a pot experiment in greenhouse conditions in the spring of 2024. Before the experiment was established, leek seeds were inoculated (500 spore/pot) with the existing *Glomus etunicatum* mycorrhiza species obtained from previous projects and mycorrhiza isolated from natural plant roots from the mining area under greenhouse conditions. When the inoculated seeds develop as plants and reach the height of 30-40 cm, the underground part of the plant was taken along with the soil, mixed thoroughly and stored at +4°C until use. Thus, the propagation of these mycorrhizae from leek roots was carried out.

The experimental soil was collected from a depth of 0-30 cm, sieved through a 4 mm sieve, and filled into the pots by weighing (4 kg of soil pot^{-1}) as an oven-dried basis. The experiment was conducted in a factorial design with three replications and three factors:

Mycorrhiza: *Glomus etunicatum and natural mix mycorrhiza inoculation* (500 spores/pot to a depth of 10 cm from the soil surface) and without inoculation (M0).

Phosphorus bacteria were applied from a commercial preparation (*Bacillus pumilus*) with inoculation (B+) and without inoculation (B-).

Silicon application: $Si(OH)_4$ containing 26% water soluble silicon from a commercial product (Agrisilica) was used. Application doses were 0 (Si0), 30 (Si30) and 60 (Si60) kg Si/da.

Sunflower seeds (MAY M96CL02 oil sunflower seed) were sown 8 seeds in each pot and diluted to 5 plants after emergence.

The analysis results of the soil used in the experiment are presented in Table 1.

Table 1. Initial physicochemical properties of selected soil used in pots

Parameters	Results	Commentary	Literature of analysis method
pH (1:2.5 soil:water)	8.8	Strong alkaline	(Richards, 1954)
EC (1:2.5 soil:water) ($\mu S\ cm^{-1}$)	428	Lightly Salt	(Richards, 1954)
$CaCO_3$ (%)	38.23	High	(Bayraklı, 1986)
Organic matter (%)	1.45	Little	(Walkley and Black, 1934)
Texture class	Loam		(Gee and Bauder, 1986)
Ca ($mg\ kg^{-1}$)	3415	Sufficient	(Thomas, 1982)
Mg ($mg\ kg^{-1}$)	368	Sufficient	(Thomas, 1982)
K ($mg\ kg^{-1}$)	210	Sufficient	(Carson, 1980)
Na ($mg\ kg^{-1}$)	114		(Thomas, 1982)
P ($mg\ kg^{-1}$)	34.7	More	(Olsen and Sommers, 1982)
Fe ($mg\ kg^{-1}$)	3.86	Medium	(Lindsay and Norvell, 1978)
Zn ($mg\ kg^{-1}$)	3.75	Sufficient	(Lindsay and Norvell, 1978)
Mn ($mg\ kg^{-1}$)	1.03	Deficiency	(Lindsay and Norvell, 1978)
Cu ($mg\ kg^{-1}$)	0.72	Sufficient	(Lindsay and Norvell, 1978)
B ($mg\ kg^{-1}$)	2.65	More	

After sowing, the experimental plants were irrigated according to the field capacity of the soil, and the plants were waited until the formation of the flower plate to see the inoculation and effectiveness of mycorrhiza and P bacteria. The experiment was finished when 50% of the flowers opened after four months of development. N-P-K fertilizer was applied to the plants once during the growing period. During harvesting, the plants were cut above the soil, the above-ground parts and root parts of the plants were taken separately and brought to the laboratory. Wet weights of above-ground stems and flowers and dry weights after drying at 65°C were taken on a precision balance. Mycorrhizal inoculation status of plant roots was determined in "Nikon ECLIPSE E 100" (Koske and Gemma, 1989; Giovenetti and Mosse, 1980). Chlorophyll was measured twice and plant height was measured three times at one month intervals throughout the experiment.

Statistical analysis of the values obtained as a result of the research, it was carried out with the Minitab 18 Statistical Software package program. All data (Si, Mycorrhiza, and P bacterium interactions) were subjected to the Tukey's multiple comparison test to determine the statistical significance of the treatment effects.

RESULTS AND DISCUSSIONS

The results showed that silicon application had a significant effect on plant height, flower weight and mycorrhizal hyphae formation. Similarly, Si*Mycor., Si*P bac. and Si*Mycor.*P bac. interactions also showed significant effects on plant height, plant top and flower weights and mycorrhizal hyphae formation (Table 2). Leaf chlorophyll contents varied between 21.62-32.66 on average (Figure 1), plant height values varied between 36.67-48.80 cm, 49.87-63.33 cm, 77.87-89.73 cm in three different periods and the highest values were in Si60-Mmix-PBac+ treatment (Figure 2). In ornamental sunflower, silicon treatment resulted in the formation of thick and straight stems, increased flower and stem diameters and height, and improved sunflower quality compared to untreated controls (Kamenidou and Cavins, 2008).

Table 2. Analysis of the variances for the measured traits

Applications	Leaf Chlorophyll		Plant height measurements			Plant weights		Flower weights		Mycorrhiza		
	1	2	1	2	3	wet weight	dry weight	wet weight	dry weight	Arbüscul (%)	Pouch (%)	Hyphae (%)
Si doses	ns	ns	**	**	ns	ns	ns	**	**	ns	ns	*
Mycorrhiza	ns	ns	ns	ns	ns	**	**	**	**	**	**	**
P bac.	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Si*Myc	ns	ns	*	*	ns	**	**	**	**	ns	ns	ns
Si*P bac.	ns	ns	**	**	*	**	**	**	**	ns	ns	ns
Myc*P bac.	ns	ns	ns	ns	ns	ns	*	**	*	ns	ns	*
Si* Myc*P bac.	ns	ns	*	*	ns	**	**	**	*	ns	*	**

ns, nonsignificant; *, significant at P≤0.05**, significant at P≤0.01.

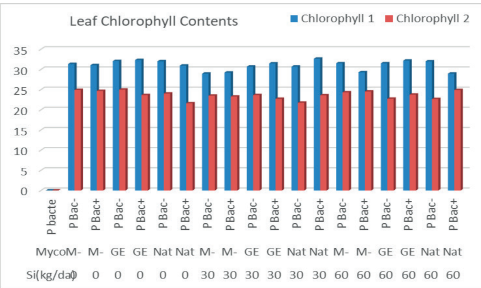

Figure 1. The effects of Si (Si0; Si30; Si60 kg/da), Mycorrhiza (with, *G.etunicatum*; *Natural mix*; without, M-) and P Bacteria (with, P Bac+; without, P Bac-) on the leaf chlorophyll contents

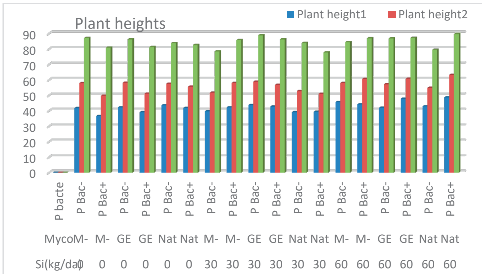

Figure 2. The effects of Si, Mycorrhiza and P Bacteria on the plant heights (cm)

Wet and dry weight of the above-ground part of the plant increased significantly with mycorrhiza application; this increase was especially higher in Si30-Mycorrhiza treatments. Similar situation was also observed in mycorrhiza and P bacteria co-application. In Si30-*G. etunicatum*-P Bac+ treatments, the wet weight of the above-ground part was 144.62 g and the dry weight was 32.71 g, while the wet weight was 113.81 g and the dry weight was 27.74 g in the control treatment (Figure 3).

When the effect of soaking the seed in different concentrations of nano-silicon solutions on the germination characteristics of sunflower was investigated in the pre-sowing revitalization processes, it was observed that germination time was significantly reduced and root length, average daily germination, seedling vigor index and final germination percentage were better (Janmohammadi and Sabaghnia, 2015).
In another study, it was observed that wheat root length and root relative yield increased with 100 mg/kg silicon application (Mali and Aery, 2008).

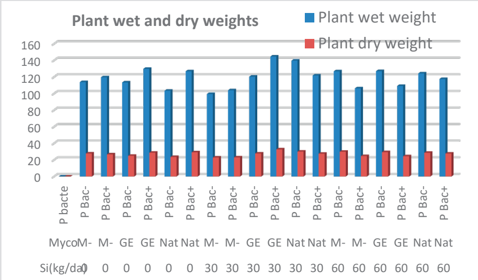

Figure 3. The effects of Si, Mycorrhiza and P Bacteria on the plant wet and dry weights (g)

While flower wet and dry weight did not change with phosphorus bacteria application, it increased significantly with mycorrhiza application. This increase was especially in Si30-Natural mix mycorrhiza treatment. The highest flower wet and dry weight values were again in Si30-*G. etunicatum* and natural mix mycorrhiza treatments (Figure 4).

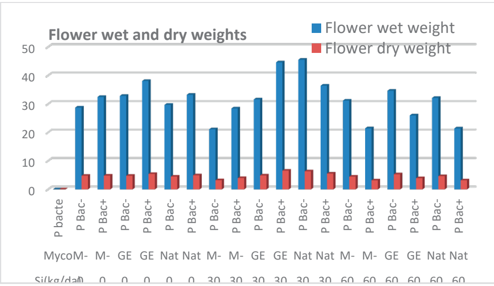

Figure 4. The effects of Si, Mycorrhiza and P Bacteria on the flower wet and dry weights (g)

It has been reported that the interaction between Si and other nutrients such as N, P, K in the soil-plant system has a positive effect on paddy yield, especially through increasing Si availability in the soil and increasing nutrient transfer from the stem to the grain (Huang et al., 2024).

In our study, it was observed that the effect of silicon in combination with mycorrhiza inoculation increased both plant aboveground parts and flower weight.

Arbuscule, hyphae and pouch (%) values indicating mycorrhizal inoculation in plant roots showed significant increases with mycorrhizal inoculations, while phosphorus bacteria treatment did not cause significant differences in these values.

The highest inoculation to the plant root was especially in the Natural mycorrhiza mix treatment compared to the control and *G. etunicatum*.

In the triple interactions, these values did not change with silicon doses.

In this case, it can be said that mycorrhizae isolated from the natural environment are more effective than *G. etunicatum* and have a higher positive effect on plant growth (Figure 5, Table 3).

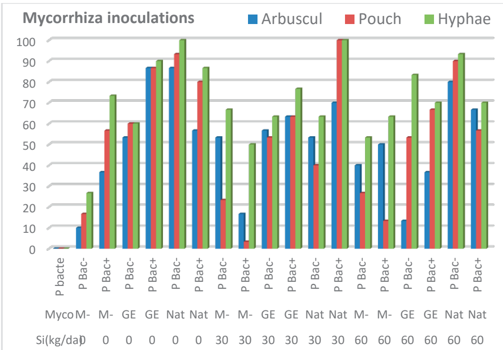

Figure 5. The effects of Si, Mycorrhiza and P Bacteria on the mycorrhiza inoculations (%)

Table 3. The effects of Si (Si0; Si30; Si60 kg/da) and Mycorrhiza (with, *G. etunicatum*; Natural mix; without, M-) on the plant values

Si (kg/da)	Myco.	Leaf Chlo. 1	Leaf Chlo. 2	Plant height 1(cm)	Plant height 2(cm)	Plant height 3(cm)	Plant wet weight (g)	Plant dry weight (g)	Flower wet weight (g)	Flower dry weight (g)	Arbü. (%)	Pouch (%)	Hyph. (%)
0	M-	31,2	24,8	39,3	53,9	84,0	116,7	27,2	30,6	4,8	23	37	50
0	G. etun.	32,2	24,3	40,8	54,7	83,8	121,6	26,9	35,4	5,1	70	73	75
0	Nat.	31,5	22,8	42,8	56,7	83,3	115,2	26,4	31,5	4,7	72	87	93
30	M-	29,1	23,4	41,1	54,9	82,2	101,7	23,0	24,8	3,6	35	13	58
30	G. etun.	31,1	23,2	43,3	57,9	87,6	132,5	30,2	38,1	5,7	60	58	70
30	Nat.	31,7	22,7	39,3	52,0	80,9	130,7	28,7	41,0	6,0	62	70	82
60	M-	30,4	24,4	45,0	59,4	85,8	116,6	27,2	26,3	3,8	45	20	58
60	G. etun.	31,9	23,3	45,0	59,0	87,1	118,1	26,9	30,3	4,6	25	60	77
60	Nat.	30,5	23,8	45,9	59,2	84,6	120,9	28,1	26,8	3,9	73	73	82

CONCLUSIONS

Overall, this study showed that silicon in combination with mycorrhiza had a significant effect on all measured traits; this increase was especially in the Si30-Natural mix mycorrhiza treatment.
This study showed that silicon and natural mycorrhiza inoculation together have significant and positive effects on sunflower plant growth parameters; further studies should be carried out by examining plant nutrient uptake in this regard. In previous studies, it has already been stated that silicon is an agronomically necessary element due to its positive effects on plant resistance mechanism; however, it is understood that new studies should be carried out on the mechanism of action of this element and its effect on different plants should be investigated.

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ASSESSMENT OF HYDROCARBON CONTAMINATION USING GEOPHYSICAL METHODS AND ITS IMPACT ON SOIL QUALITY IN AGRICULTURAL CONTEXT

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Abstract

This paper presents an integrated investigation of hydrocarbon-contaminated soils near the Petromidia refinery using advanced geophysical and hydrogeological methods, with implications for soil sciences in agriculture. The main goal was to identify and monitor contamination to assess its impact on soil quality and support sustainable land management strategies. Methods included electrical resistivity measurements (Vertical Electrical Soundings - VES), ground-penetrating radar (GPR) with 100 and 500 MHz antennas, and hydrogeological drilling for soil and groundwater sampling. Geophysical data were integrated with hydrogeological results to develop a hydrogeophysical model. The study revealed contaminants as thin hydrocarbon films within sandy layers and localized accumulations along NW-SE fault zones. Contamination is influenced by active infiltration, precipitation, and continuous pollutant influx. The resulting hydrogeophysical model accurately mapped the spatial distribution and migration pathways of pollutants, emphasizing soil vulnerability in the area. These findings are critical for assessing impacts on soil fertility and the agricultural potential of adjacent lands. This integrated approach provides a solid basis for remediation strategies and sustainable soil resource management.

Key words: *hydrocarbon contamination, geophysical methods, VES, GPR, hydrogeophysical model, soil quality.*

INTRODUCTION

Soil and groundwater contamination with hydrocarbons is a major environmental issue, significantly impacting agricultural land quality and water resources. This paper analyzes the extent and characteristics of hydrocarbon pollution in the petroleum discharge area of Vadu commune, Constanța County, where two pits measuring 50 × 100 m have been used for petroleum waste disposal. Over time, these deposits have led to extensive soil and groundwater contamination, reducing land fertility, excluding it from agricultural use, and posing risks to local ecosystems and human health.

Given the petroleum pollutant leaks into the subsurface and considering that once formed, the pollution plume moves randomly at a depth between 1 and 2 meters, it is necessary to use electromagnetic investigation methods, specifically the vertical electrical sounding (VES) method with a Schlumberger array, in parallel with ground-penetrating radar (GPR) investigations using single-frequency antennas (100 MHz; 500 MHz). The two methods validate each other, ultimately resulting in a more accurate stratigraphic succession and an exact determination of the water table level. These methods enabled the identification of hydrocarbon accumulation zones and the analysis of their interaction with the local geological structure, fault systems, and groundwater flow.

In addition to geophysical investigations, photogrammetry was used to develop a digital terrain model (DTM), providing a detailed representation of microrelief variations and potential pollution-induced alterations. Soil and groundwater samples were collected and analyzed for total petroleum hydrocarbons (TPH), pH, organic carbon content, and heavy metal concentrations, further validating geophysical findings.

This integrated hydrogeophysical approach not only precisely delineated the affected area but also improved the understanding of contaminant infiltration, retention, and dispersion processes in the subsurface environment. The obtained results are crucial for developing efficient remediation strategies, supporting the recovery of affected land, and facilitating its reintegration into sustainable agricultural use (Anghel, 2024).

Furthermore, the study contributes to optimizing hydrocarbon pollution monitoring methodologies and enhancing predictive capabilities regarding contaminant evolution in similar environments, thereby offering valuable insights for environmental protection and land management policies.

MATERIALS AND METHODS

Study Area

This area, located a short distance from the Mamaia resort, is notable for its sandy soils alternating with clay, presenting a strong flow of groundwater but also of contaminating substances. The spillage of petroleum products through pipelines in the area bordering the locality of VADU was the main motivation in choosing the investigation area. Thus, geophysical mapping allowed for the determination of the thickness and extension of the petroleum products' settling area (Alimohammadi & Butt, 2020).

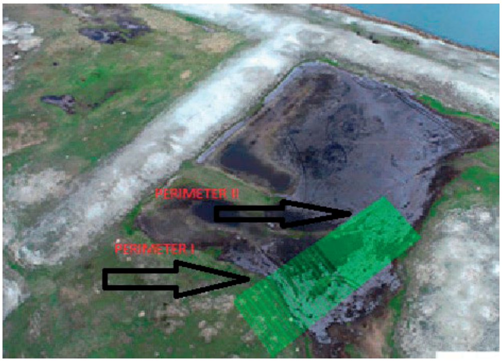

Figure 1. Network of profiles related to perimeters I and II investigated with two antennas (100MHz and 500MHZ)

Data Acquisition

Methodology of using the GPR - NOGGIN system

First of all, it can be said that there is a perfect similarity between GPR and seismic refraction. Thus, the radar transmitter located on the antenna emits a short-duration pulse characterized by high frequency, which can vary in the range of 10 MHz - 10 GHz. The propagation of the electromagnetic wave through the ground occurs at a speed that depends on the characteristics of the terrestrial electric field.

Propagating in the investigated environment, the electromagnetic wave can encounter an object or surface characterized by different electrical properties, so that part of its energy is reflected to the surface, and the remaining energy continues to penetrate deeper into the investigated environment (Figure 1).

The GPR reception system captures the reflected wave, which is subsequently recorded by the NOGGIN 500 system (Figure 2). The travel time of the pulse from the surface to the reflective interface and back is analyzed based on these recordings. Water content is an important element in determining the electrical properties of geological structures. The volume of water from the pores of rocks can influence the electrical properties of the investigated terrain.

The type of rock, the material, or the water that fills the existing mini-fractures played an essential role in determining the accuracy of the GPR (Ground penetrating radar) system.

These variations in electrical properties cause partial reflection of the transmitted signal.

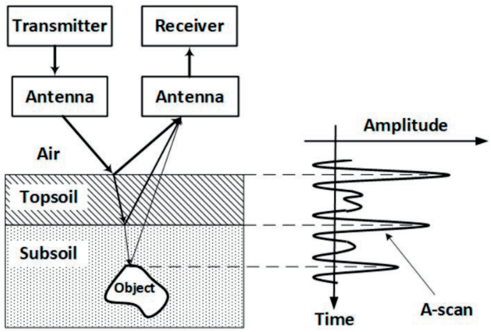

Figure 2. Synthetic scheme regarding the propagation of electromagnetic waves in the subsoil - shape and amplitude intensity (after Ukaegbu et al., 2019)

The propagation of high-frequency radio waves in the soil is based on two essential factors: velocity, which quantifies how fast the wave propagates through the soil, and attenuation, which gives us an image of the weakening of the signal as it propagates through the investigated medium. The conductivity of the material and the dielectric properties can characterize the speed and attenuation of electromagnetic waves in the investigated medium. The dielectric constant (relative permittivity) is a term used to describe the response of the material at high frequencies (10-1000 MHz) and can be characterized by the relative permittivity, in this case, two frequencies,s 100 MHz and 500 MHz. At very high frequencies (10-1000 MHz), in the case of limestones where the conductivity does not exceed 10 mS/m, the propagation speed of electromagnetic waves is not dependent on frequency. To determine the extension of the pollution plume in the subsoil, its position, and its depth, we must take into account the propagation speed of the electromagnetic wave, which is dependent on the dielectric constant.

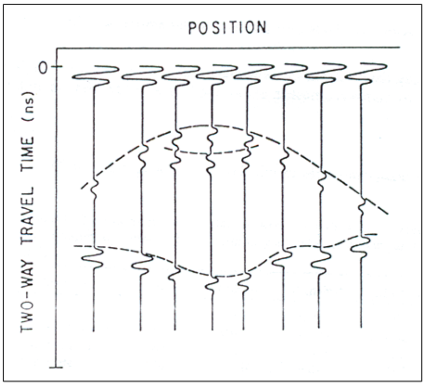

Figure 3. Generation of a radargram within the data acquisition process

The type of material can influence the speed of the electromagnetic wave and the degree of attenuation, according to Table 1.

Table 1. Dependence of Radar Wave Velocity and Attenuation on Material Type

Material	ε_r Relative Permittivity	σ (mS/m) Conductivity	V (m/ns) Velocity	α (Db/m) Attenuation
Air	1	0	0.30	0
Distilled water	80	0.01	0.033	2×10^{-3}
Freshwater	80	0.5	0.033	0.01
Seawater	80	3×10^{4}	0.01	10^{3}
Dry sand	3-5	0.01	0.15	0.01
Saturated sand	20-30	0.1-1.0	0.06	0.03-0.3
Limestone	4-8	0.5-2	0.12	0.4-1
Clay	5-40	2-1000	0.06	1-300
Granite	4-6	0.01-1	0.13	0.01-1
Dry salt	5-6	0.01-1	0.13	0.01-1
Ice	3-4	0.01	0.16	0.01

Conductivity (mS/m) – determines how much energy is absorbed by the material. High-conductivity materials, such as clay (2-1000 mS/m) or seawater (30,000 mS/m), rapidly attenuate the radar signal, limiting the penetration depth (Figure 3).

Signal attenuation (dB/m) – represents the loss of energy as the radar wave propagates through the material. For example, air and dry sand have very low attenuation (~0 dB/m and 0.01 dB/m), allowing deep penetration. In contrast, seawater (1,000 dB/m) or highly saturated clay (>300 dB/m) absorb the radar signal almost completely, significantly reducing the depth of investigation.

Materials with low permittivity and low conductivity (e.g., dry sand, ice, air, dry rock) allow deep radar signal penetration, making them ideal for geological and archaeological investigations.

Materials with high permittivity and/or high conductivity (e.g., water, wet clay, saturated sand) reduce radar signal penetration, limiting the depth of investigation and making it difficult to detect subsurface structures.

Water content and soil moisture are critical factors, as even small amounts can drastically alter the soil's electrical properties, affecting the depth and clarity of GPR surveys.

This interpretation helps in understanding how GPR can be effectively used depending on the soil and material characteristics. The GPR method measures the signal travel time, which must be determined with nanosecond precision. Resolution refers to the system's ability to distinguish between two closely spaced signals. There is always a trade-off between investigation depth and GPR resolution. Studies have shown that a 100 MHz frequency offers an optimal balance between penetration depth, resolution, and device portability. When a deeper investigation is required, a lower resolution is acceptable, whereas higher resolution demands a reduced penetration depth.

Geological surveys rely on detailed stratigraphic information to determine groundwater flow direction, especially in areas with high concentrations of dissolved salts. GPR plays a key role in defining subsurface geology and assessing the parameters that influence fluid movement through rocks.

In most cases, GPR is operated in reflection profiling or tomography mode (Figure 2), where the antennas are moved along the survey line. Determining the depth of buried bodies, their shape, size, and inclination using the speed of propagation of electromagnetic waves is a process that can also be applied in the case of seismic refraction where the elastic wave is generated mechanically. However, it is important to note that GPR equipment remains costly, and data interpretation can be challenging due to numerous interferences, particularly from power transmission networks. Additionally, the presence of clay layers, which act as natural barriers, can render the method ineffective for deep investigations.

Despite these limitations, GPR remains the geophysical method with the highest resolution capability. Georadar investigations were carried out on two designated areas using 100 MHz and 500 MHz antennas. Each survey area measured 30 meters in length and 20 meters in width. The survey grid for each perimeter consisted of both longitudinal and transverse profiles.

For the first perimeter, measurements were conducted along longitudinal profiles spaced at 0.5 meters and transverse profiles spaced at 2 meters (Figure 4). In this case, a 100 MHz antenna was used.

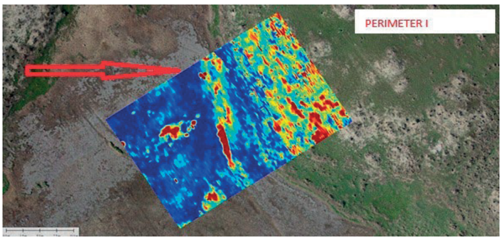

Figure 4. Ground-Penetrating Radar (GPR) Depth Section (0.5 m) - Perimeter I

In Perimeter I, investigated with the 100 MHz antenna (Figure 4), measurements followed a grid of transverse and longitudinal profiles, maintaining a spacing of 0.5 meters between longitudinal profiles and 2 meters between transverse profiles.

As shown in Figure 5, the placement of the survey area on the orthophotomap highlights that 30% of the area is located in an unpolluted zone, while 70% falls within the petroleum discharge zone.

The positioning of the perimeter was carefully selected to ensure that data acquisition captured information from both polluted and unpolluted areas. This approach enables a comparative analysis during data processing, allowing radargrams to reveal the effects of petroleum contamination more clearly. The data was positioned in real-time using the GPS integrated into the Noggin system. The preliminary processing and interpretation were carried out in the field immediately after the data acquisition phase was completed.

As part of the data processing and interpretation process, GPR sections were generated at depths of 0.5 m, 1 m, and 1.5 m. For a depth of 0.5 m, the GPR section highlights high-intensity reflections in the unpolluted area (red-yellow color) and low-intensity reflections, characteristic of the area heavily polluted with hydrocarbons (blue color). As shown in Figure 5, the section corresponding to a depth of 0.5 m highlights high-intensity reflections in the non-polluted area (red-yellow color) and low-intensity reflections, characteristic of the heavily hydrocarbon-polluted area (blue color).

Thus, in this case, a clear delineation of the heavily hydrocarbon-polluted area can be observed both at the surface and in depth.

For Perimeter II (Figure 1), investigated using the 500 MHz antenna (Figure 5), measurements were conducted along both transverse and longitudinal profiles, with a fixed spacing of 0.5 meters between them.

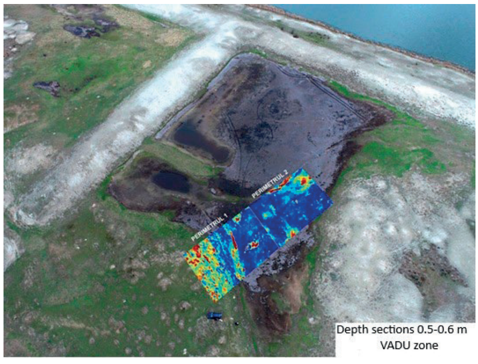

Figure. 5 GPR Depth Section (0.5 m) - Perimeter I, and (0.6 m) - Perimeter II

As shown in Figure 5, the placement of the perimeter on the orthophotomap highlights that it is located entirely (100%) within the petroleum spill zone.
The perimeter was deliberately positioned to ensure that only data from the polluted area was recorded during the acquisition process. This approach allows for a comparative analysis of the radargrams during data processing, emphasizing the impact of petroleum pollutants when investigated using the two types of antennas (Bachu & Bennion, 2009).
During the data processing and interpretation phase, it was observed that the hydrocarbon spill area, encompassed within the two perimeters, can generate low-intensity reflections on the depth sections, both when using the 100 MHz antenna and the 500 MHz antenna for data acquisition.
The analysis of the radargrams presented in Figure 6 shows that the petroleum-contaminated area produces very low-intensity reflections on the radargrams, compared to the distinct hyperbolas observed in the area with vegetated soil.

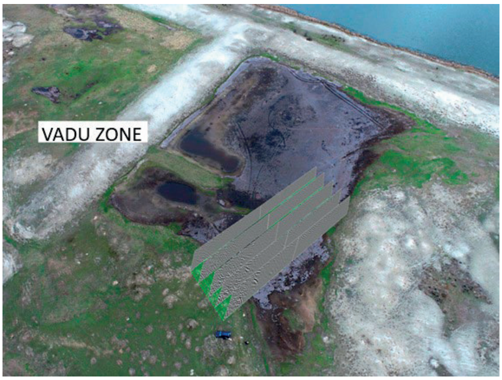

Figure 6. Positioning of the radargrams on the two perimeters was carried out with a fixed spacing of 5 meters between them

This experiment, which compares data obtained using two types of antennas under conditions of intense pollution, represents a first-of-its-kind study and highlights the crucial role that the ground-penetrating radar (GPR) method can play in investigating and identifying heavily polluted areas in depth.
Considering the thickness of the pollutant layer, approximately 1.5 meters, and the continuity of the low-reflection zone up to this depth, we can conclude that the GPR method is highly effective even when the pollutant is not present at the surface, allowing for its detection in depth in the form of a contamination plume.

Figure 7. GPR data acquisition using the 100 MHz and 500 MHz antennas

***Electrical Resistivity Measurements* (*VES*)**
The electrometric investigation (Figure 7) of the perimeter was conducted along a network of parallel profiles (Cassiani et al., 2004). Based on this analysis, it was deemed appropriate to generate apparent resistivity sections to highlight the presence of a quasi-homogeneous resistive horizon, associated with a gravelly sand layer located beneath the petroleum-contaminated horizon.
Below this resistive horizon lies a low-resistivity domain, corresponding to the aquifer level, which was also confirmed through piezometric boreholes in the area.
In the studied area affected by petroleum pollutant discharge, two shallow boreholes were drilled, and sediment samples were collected every 20 cm using a manual corer.

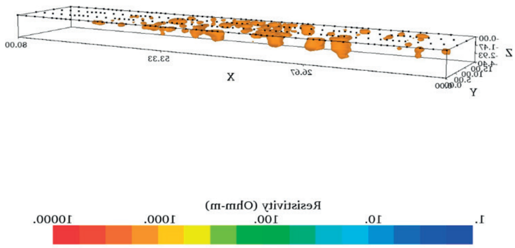

Figure 8. 3D representation of the inverted apparent resistivity section (Rez2Inv), highlighting the high-resistivity zones corresponding to the polluted areas (Loke, 2018)

The collected soil samples were analyzed using a DR 2000 analyzer (Domenico & Schwartz, 1998). In the first borehole, hydrocarbon concentrations of 1 ppm were identified down to a depth of 40 cm, while in the second borehole, similar concentrations were detected down to a depth of 1.4 m (Figure 1). These analyses contributed to the calibration of both the electrometric method and the ground-penetrating radar (GPR) method (Covaliu et al., 2024).

Figure 9. Field data acquisition using the Vertical Electrical Sounding (VES) method (McNeill, 1990)

RESULTS AND DISCUSSIONS

The investigations conducted in the hydrocarbon-contaminated area near the Petromidia refinery (Vadu zone) have highlighted the presence of pollution in both soil and subsoil, impacting land quality and its potential agricultural use. The parallel use of geophysical and hydrogeological methods led to the delineation of the polluted areas and the determination of the degree of contamination. Using the vertical electrical survey method for the determination as well as georadar profiling, significant differences (contrasts) were highlighted between the contaminated and uncontaminated perimeters. The apparent resistivity sections identified a quasi-homogeneous gravel sand horizon located below the hydrocarbon-polluted layer, which was highlighted by the apparent resistivity sections. The sudden decrease in resistivity below this layer marked the aquifer level, which was also confirmed by piezometric drilling. The pronounced hyperbolas present on the georadar sections are characteristic of areas with unaltered soil and are in contrast to the low-intensity reflections characteristic of hydrocarbon-contaminated soils. Thus, it was possible to clearly delineate the pollution plumes and their migration paths into the subsoil. The local geological structure, precipitation and active infiltration influenced the contaminant migration pathways, which were outlined by the resulting hydrogeophysical model. The sustainability of agricultural crops (Reynolds, 2011) depends largely on the degree of soil contamination. The importance of geophysical methods for detecting and monitoring soil pollution is vital for sustainable agriculture. The protection of agricultural resources and the environment depends on the data collected that provide a solid basis for remediation strategies and sustainable agricultural land management.

CONCLUSIONS

The high level of soil and subsoil contamination, with direct implications for the use of agricultural land, was highlighted by the investigations carried out in the area of oil product spillage from the Petromidia refinery in the Vadu region. Hydrogeological, GPR and electrometric investigations identified the presence of hydrocarbons infiltrated into the soil, with significant accumulations reaching depths of up to 1.4 m (Iorga et. al, 2024). These petroleum products have dispersed unevenly within the sand and gravel layer, forming an extended pollution plume. Hydrocarbon pollution significantly alters the soil's physical and chemical properties, reducing its fertility and its ability to sustain agricultural crops. The presence of hydrocarbons disrupts soil structure, hinders water and nutrient absorption, and negatively affects beneficial microorganisms essential for plant growth.

Given the high concentrations of pollutants and the persistent nature of hydrocarbons in the soil, the investigated land is not suitable for agricultural use without remediation measures. Risks to human health and local ecosystems may arise when pollutants could enter the food chain as a result of the cultivation of contaminated agricultural land (Sultan et al., 2014).

To restore the land and reintegrate it into agricultural use, urgent remediation actions are required, such as bioremediation, soil washing, or excavation of the contaminated layer.

The assessment of the evolution of contamination and the effectiveness of remediation techniques depends largely on long-term monitoring strategies (Oprea et al., 2024).

This study has demonstrated that geophysical methods are effective tools for identifying and mapping soil contamination, providing essential data for decision-making in land management. Integrating these methods into remediation programs can help mitigate the impact of contaminants on the environment and agriculture.

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STUDY ON THE ANALYSIS OF THE MAIN AGROPRODUCTIVE PROPERTIES OF THE EUTRIC PSAMOSOL IN DOLJ

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Abstract

The study analyzes the main agroproductive properties of eutric psamosols in Dolj County, identified on large areas between Craiova and Dăbuleni, in areas with dunes subject to wind deflation. They contain a very high percentage of sandy material, being very dry and permeable. These soils benefit from a deficient moisture regime, are much poorer in humus, clay and nutrients, which is why they have a very low natural fertility, being generally used as poor quality pastures. Based on the results carried out in the field and in the laboratory, it is recommended that in order to be cultivated, these soils require radical improvement works, including: irrigation, organic and mineral fertilization with large quantities of fertilizers, combating wind deflation and cultivating with plants specific to sandy areas. The application of improvement measures has increased the bonitization scores, which demonstrates that eutric psamosols can also achieve medium fertility.

Key words: *agroproductive properties, eutric psamosol, amelioration measures, bonitation grades, fertility.*

INTRODUCTION

The achievement of quantitative and qualitative productions, which ensure a decent standard of living for the population, is closely related to the in-depth study of soil as the main means of production in agriculture.

Of the diversity of soils that we encounter in our country, psamosols (sands and sandy soils) have the weakest productive capacity, are permanently subject to the process of wind deflation and have a very low capacity to retain water in the soil. The content of nutrients is very low and the natural potential is very low.

Starting with 1955, complex research was carried out to learn about the types of soils and their agro-productive potential, in the sandy area of Dolj County, starting with the sands of Tâmbureşti. Research on these lands was intensified starting with 1959 when the Central Station for Plant Culture on Sands was established in Bechet.

Pedological research on sandy lands is a necessity for establishing methods for their rational use, taking into account the fact that their properties change through agricultural use, which makes it necessary to be constantly up to date with the situation regarding the properties and productive capacity of these lands.

According to Pop (1977), the surface of sands and sandy soils on the Danube terraces is approximately 120,000 ha.

These sands come from materials brought by the Danube from the mountainous area of Oltenia and which, under the action of the wind, were carried and deposited in the northeast in the form of dunes. Near the river, the dunes have an orientation parallel to the direction of the water flow, and further inland they acquire an orientation perpendicular to the flow, due to the action of the Austrul wind, which blows from northwest to southeast.

In the Desa-Ciuperceni area we have mobile dunes that can reach heights of up to 15 m, and in the Băileşti area we have old consolidated dunes with heights between 5 and 10 m. The length of the dunes in southern Oltenia can reach up to 3-4 km, with a width of 50 m to 800 m in the interdunes.

The two sand areas of Oltenia unite in the area where the Danube and Jiu terraces connect, between the localities of Dobreşti and Sadova.

MATERIALS AND METHODS

The research method aims at the following more important objectives (Călina et al., 2023; Călina et al., 2000): knowledge of the natural conditions for the formation of psamosols in

Dolj County. This aspect required an in-depth study from the geomorphological, lithological, hydrographic, hydrological, climate and vegetation points of view of the studied area; identification and study of eutric psamosols in Dolj County. Documentation was done in the field by executing and studying soil profiles in points covering the entire studied area and documentation at the specialized institution OSPA Dolj; determination of the main physical, hydrophysical and chemical properties of eutric psamosols in Dolj County.
To achieve the established objective, field research was carried out, during which several soil profiles were morphologically analyzed and samples were collected from the most representative ones, to be analyzed in the laboratory; establishing the suitability of psamosols for different crops and methods of use.
The soil profiles were deepened to approximately 200 cm. Soil samples were collected from each horizon and transported to the specialized laboratory, where mechanical, hydrological and physico-chemical analyses were performed as (Canarache, 1990, Florea et al., 1987, Călina et al., 2000):
- the color of the horizons is determined using the Munsell Soil Color Charts, for wet and dry soil samples;
- the granulometric composition was determined by treating the soil with sodium pyrophosphate solution and separating the granulometric fractions by the wet sieving method (coarse sand), the siphoning method (fine sand) and the pipetting method, using the Kubiena mechanical pipette (dust and clay);
- the texture classes were established using the texture triangle;
- the density (D, g/cm^3), was determined by the pycnometer method, using the pump to remove air from the soil samples;
- the apparent density (Da, g/cm^3) was determined by the metal cylinder method, with a volume of approximately 100 cm3 for soil samples collected in natural settings;
- total porosity (Pt %), was calculated with the relationship: Pt % = (1- Da / D) 100;
- the maximum hygroscopicity coefficient (CH %) was determined according to the Mitscherlich method, using a 12% sulfuric acid solution;
- the wilting coefficient (CO%) was determined by calculation based on the maximum hygroscopicity coefficient, using the relationship: CO % = CH % 1.5;
- the moisture equivalent (EU) was determined by centrifuging the wet soil sample at a centrifugal force of 1000 times the gravitational acceleration;
- the usable water capacity (CU %) was determined by calculation based on the moisture equivalent and the wilting coefficient, using the relationship: CU % = EU % - CO %;
- humus (%) was calculated by the Walklei-Black method, treating soil samples with concentrated sulfuric acid and potassium dichromate;
- total nitrogen (Nt %) was determined using the Kyeldhal method;
- mobile phosphorus (P mg/100 g soil) was determined by the Egner-Riehm-Domingo method, using a photocolorimeter;
- mobile potassium (K mg/100 g soil) was determined by the Egner-Riehm-Domingo method, using a flame photocolorimeter;
- soil reaction (pH value) was determined by the potentiometric method, in 1/2.5 aqueous solution;
- calcium carbonate (Ca CO3 %) was determined using a Scheibler calcimeter in percentages.

RESULTS AND DISCUSSIONS

Distribution

According to Mihalache (2006, 2008) psamosols (solified sands) occupy an area of about 370,000 ha, which represents 1.6% of the country's surface. They are found in the Oltenia Plain on the left side of the Danube and Jiu in the area of Călmăţui, Ialomiţa and Buzău and the Tecuci Plain (Hanul Conachi). Psamosols are widespread in the steppe and forest-steppe area, therefore in areas with low precipitation, with high intensity winds, usually near rivers, lakes and the sea, on low relief units (plains, meadows). Characteristic for sands is the relief of dunes and interdunes, subject to wind deflation.
Cotet (1957) considers that both the sands on the left bank of the Jiu and those located along the Danube have a common origin, the primary material being brought by waters from the

Southern Carpathians. The sandy soils here represent alluvial deposits of the old Jiu riverbeds, as well as subsequent deposits of material that were brought by the Jiu and the Danube, transported and shaped by the wind.

Tufescu (1966) attributes the origin of the sands from the central part of Oltenia to deposits of Levantine age of lacustrine origin in which the wind played the role of shaping and transporting. The fluvial origin of the sands in this area is only attributed to the southern extremity of the area, located near the Danube.

The same fluvial origin of the sands and sandy soils on the left bank of the Jiu is also considered by Chiriţă and Bălănică (1958) in this case the sandy material being brought by the Jiu from the mountainous and hilly area, deposited in the plain and then blown away by the wind and deposited in the form of dunes. The relief of this area is particularly rough presenting many elevations as a result of the transport and deposition of sand by the wind in dune and interdune formations.

According to Maxim and Gaţă (1971) the sandy soils on the Jiu terraces present some physical and chemical properties different from those on the Danube terraces. The sandy soils on the Jiu terraces have a more uniform texture, but coarser than those on the Danube terraces which are formed from much more varied material. The chemical properties are very similar, except for humus which is richer in the sands on the Danube terraces.

Agropedological characterization of eutric psamosol

The formation and evolution of sands and sandy soils in our country occurred over time under the influence of pedogenetic factors specific to each sandy area. In general, sands in Romania have a fluvial and aeolian origin.

Flowing waters eroded, transported and deposited sandy material, after which it was transported by winds at different distances from the watercourse and deposited in the form of dunes and interdunes.

The sands and sandy soils on the left bank of the Jiu River were formed under the strong influence of this river, which eroded the material from the highlands (mountains, hills) and deposited it in the lowlands (plains), after which it was blown away by winds, especially on the left bank of the watercourse.

The natural factors that influenced the formation of psamosols were: relief, climatic conditions, parent material, vegetation, hydrography, hydrology.

The alternations of diurnal and seasonal temperatures result in the fragmentation of the superficial layer into smaller fragments, which can be removed, thus exposing new portions of rocks to the effect of disaggregation and volume increase by freezing. Both the penetration of water into the cracks of the rock, as well as the considerable pressures exerted by frost, by the growth of plant roots in these cracks, contribute intensely to the crumbling of hard rocks.

The water from precipitation together with the air that comes into direct contact with the surface of the rock fragments, causes the triggering of a new process of a chemical nature, namely alteration. Through chemical alteration, the soil radically changes its composition, so that the primary minerals of the rock are transformed into new minerals, into secondary minerals. Precipitation, being an important agent of alteration, determines the dissolution, hydration and hydrolysis of minerals (Coteț, 1973)

The water from precipitation penetrates the soil, determining the entrainment and deposition of salts and colloidal substances at different depths, playing an important role in the formation of the soil profile, respectively of the horizons.

The indirect action of climate as a factor in soil formation refers to the role of vegetation in the formation and evolution of the soil. The development of vegetation is closely linked to the climate.

The climate in the southern area of Oltenia is temperate-continental with average temperatures ranging between 10.5-11.5^{0}C, higher values being recorded in the summer months (22-23^{0}C) and lower in the winter months, below 0^0 C.

The monthly temperature variation has an upward trend from January (-2.6^{0}C in Craiova and -2.9^{0}C in Bechet) to July, when it reaches a maximum (23.0^{0}C in Craiova and 23.1^{0}C in Bechet), then gradually decreases. The multiannual average air temperature is 10.9^{0}C in Craiova and 11.3^{0}C in Bechet. The temperature difference between the middle of the warmest month (July 23.0^{0}C) and the month with the lowest temperature (January - 2.6^{0}C) is 25.6^{0}C.

In terms of precipitation, the multiannual average is 545.4 mm at the Craiova meteorological station and 519.0 mm at the Bechet meteorological station. The rainiest month was June (66.4 mm) at Craiova, and the month with the least precipitation was February (33.4 mm) at Bechet, resulting in a difference of 33.0 mm.

In the case of these soils, a rather big problem is also given by winds, especially in spring when the land is not covered by vegetation, this leading to the scattering of sandy material from the dunes and the formation of real sandstorms. Analysing the frequency and average wind speed at the Craiova and Bechet meteorological stations, it is observed that the highest speed is the winds that blow from the west in winter (4.8 m/s), and the highest frequency is the winds that blow from the west in summer (30.5 m/s). At Bechet, westerly and northeasterly winds predominate, and at Craiova the intensity and frequency of the winds is higher than at the other station.

In conclusion, if the climatic elements of the researched area are analyzed, it is observed that the microclimate aspect of the plain area is preserved with a regime of high temperatures and low precipitation combined with winds that blow frequently throughout the year, especially in spring, summer and autumn, this requiring the taking of special measures in order to ensure optimal conditions for plant development.

Regarding vegetation, it is known that the biological factor represented by plant and animal organisms has a determining role in the solidification process. Higher plant organisms, plants with chlorophyll associated with lower plant organisms, bacteria and fungi without chlorophyll, together form specific biocenoses that play a much more important role in the solidification process than animal organisms. Grassy and woody vegetation influences the solidification and humification processes through the quantity, quality and method of transformation of organic residues that remain in the soil. On sandy soils, vegetation has a weaker development compared to other soils. Natural grassy vegetation is represented by various species, such as: *Digitaria sanguinalis*, *Bromus tectorum*, *Cynodon dactylon*, *Trifolium arvense*, *Agropyrum repens*, *Vicia vilosa*, *Tragus racemosus*, *Trifolium campestre* and in agricultural crops there are weeds such as: *Delphinium consolida, Convolvulus arvensis, Cirsium arvensis, Portulaca oleracea, Setaria viridis, Sorghum halepense, Lepidium draba, Chenopodium album, Tribulus terrestris, Sambucus ebulus, Xanthium strumarium, Anthemis ruthenica, Euphorbia virgata, Ranunculus arvensis, Solanum nigrum*. Natural forests appear sporadically in this sandy area and are made up of acacias (*Robinia pseudacacia*) such as those from Tâmbureşti, Mârşani, Malu Mare, Daneţi, or pedunculate oak (*Quarcus peduncullata*) and shrubs such as *Rosa canina, Crataegus monogyna, Morus alba, Pirus piraster, Ligustrun vulgare* (Șorop et al., 1990; Răduțoiu et al., 2018).

Morphological characterization of eutric psamosol

Eutric psamosols are found on large areas between Craiova and Dăbuleni, in areas with dunes subject to wind deflation. From the studies carried out, these soils are generally found on the dune crests and on the slopes and due to the fact that their composition includes a very high percentage of sandy material, these soils are very dry and permeable. These soils are very poor and are generally used as poor quality pastures, because they have a very low fertility with a low content of organic material.

For the study of this type of soil, a soil profile executed in the southern part of the Tâmbureşti locality is presented (Figure 1). The soil has an Ao-AC-C type profile.

Figure1. Soil profile on a eutric psamosol

- **Ao horizon**: 0-41 cm depth, dark brown (10 YR 4/3) when wet and grayish brown (10 YR 5/3) when dry; unstructured or mono-granular; porous; loose; frequent filiform roots; frequent sand grains with diameter over 1 mm; weak colloid films on the surface of the sand grains; gradual transition.

- **AC horizon**: 41-80 cm depth; dark yellowish brown (10 YR 4/4) when wet, yellowish brown (10 YR 5/6) when dry; sandy-loamy texture, unstructured, porous; medium compact, to loose fibrous roots; rare synclitic material; weak colloid films on the surface of the sand grains; gradual transition.

- **Horizon C**: under 80 cm depth; yellowish color (10 YR 5/6) when wet and whitish yellowish color (10 YR 7/6) when dry; sandy texture; unstructured; porous; loose; frequent large diameter quartz sand grains; rare small diameter skeletal material.

Physical-mechanical properties of eutric psamosols

Interpreting the results of laboratory analyses (Table 1) it is found that the soil has a very high coarse sand content of 73.0% in the C horizon and decreases to 70.5% in the Ao horizon.

Table 1. Physical-mechanical properties of eutric psamosol

Horizon	Depth -cm-	Granulometric composition %				Textural Class	BD	D	TP
		Coarse sand 2-0.2 mm	Fine sand 0.2-0.02 mm	Dust 0.02-0.002 Mm	Clay < 0.002 mm		g/cm³		
Ao	0-41	70.5	17.2	6.6	5.7	NL	1.46	2.62	44
AC	41-80	71.6	17.9	5.1	5.4	NL	1.49	2.63	44
C	over 80	73.0	18.2	3.6	5.2	N	1.51	2.64	43

The fine sand content varies along the profile from 17.2% in the Ao horizon to 18.2% in the C horizon. The dust content decreases from 6.6% at the surface to 3.6% in depth. The clay content also varies from the surface where 5.7% was recorded to 5.2% in the C horizon. The granulometric composition determines the soil texture in the first two horizons to be sandy-loamy, and in the last horizon sandy. The bulk density presents values ranging between 1.46 g/cm^3 at the surface and 1.51 g/cm^3 in depth. The density increases from 2.62 in the Ao horizon to 2.64 in the C horizon. This determines that the total porosity of the soil is between 44-43%.

Hydrophysical properties of eutric psamosols

The hydrophysical indices show low values that correlate very well with the clay content (Table 2).

Table 2. Hydrophysical properties of eutric psamosols

Horizon	Depth (cm)	HC (%)	WC (%)	ME (%)	SC (%)
Ao	0-41	0.7	1.7	4.1	2.4
AC	41-80	0.6	1.6	3.8	2.2
C	over 80	0,5	1.5	3.5	2.0

The hydroscopicity coefficient (HC) has values ranging between 0.7% in the Ao horizon and 0.5% in the C horizon.
The wilting coefficient (HC %) has lower values compared to mollic psamosols, ranging from 1.7% at the surface to 1.5% in depth.
The moisture equivalent (ME) also has lower values, compared to the first soil, so that its value decreases from 4.1% in the first horizon to 3.5% in depth.
The water storage capacity (SC) is low, the capacity value for useful water being between 2.4% in the Ao horizon and 2% in the C horizon.

Chemical properties of eutric psamosols
This soil is less well supplied with organic matter, the humus content being between 0.52% at the surface and 0.25% in depth (Table 3).

Table 3. The main chemical properties of eutric psamosols

Horizon	Depth (cm)	Humus (%)	Nt (%)	P_2O_5	K_2O	pH	SH	SB	T	V (%)
				me/100 g. soil		(H_2O)	me/100 g. soil			
Ao	0-41	0.52	0.044	3.5	5.4	6.1	1.1	3.9	5.0	78
AC	41-80	0.37	0.029	3.1	5.1	6.6	0.7	3.5	4.2	83
C	over 80	0.25	0.020	2.7	4.2	6.9	0.4	3.2	3.6	88

The total nitrogen content (Nt %) is also low and ranges from 0.044% in the Ao horizon to 0.020% in the C horizon.
The mobile phosphorus content P_2O_5 ranges between 3.5 and 2.7 me/100 g. soil.
The potassium content (K_2O) decreases from 5.4 me/100 g. soil. and 4.2 me/100 g. soil. The soil reaction is weakly acidic.
The pH is between 6.1 and 6.9, the sum of exchangeable bases varies across the profile from 3.9 in the first horizon to 3.2 me/100 g. soil in the C horizon.
The total cation exchange capacity has values ranging from 5.8 me/100 g. soil Ao and 3.6 me/100 g soil in the C horizon.
The base saturation degree (V %) is between 78 and 88%.
In conclusion, eutric psamosols benefit from a deficient moisture regime, are poor in humus, clay and nutrients, which is why they have a very low natural fertility.
In order to be cultivated, these soils require radical improvement works, including: irrigation, organic and mineral fertilization with large quantities of fertilizers, combating wind deflation and cultivation with plants specific to sandy areas.

Soil evaluating in natural conditions
The growth and fruiting of agricultural plants depends on the entire complex of ecological factors, which can provide conditions from minimum to optimal for plant development.
The quantitative expression of these conditions for each natural factor and for each soil property is achieved using indicators or evaluation coefficients, which can have values from 0 to 1.
At the value of "1" of the evaluation coefficient, the conditions provided for a plant by the natural factor or soil property taken into account are optimal and the highest production can be obtained. At the value of "0" of the evaluation coefficient, the development conditions are minimal and the respective plant is not recommended to be cultivated on the soil and in the respective area, because the production obtained is economically inefficient.
The value of the evaluation coefficients was established for different natural factors and for different soil properties, taking into account and processing data obtained in research and production, establishing average values for each natural factor and soil property.
The agricultural plants for which the values of the evaluation coefficients have been established are: pastures; hayfields; apple; pear; plum; cherry; apricot; peach; vines, wine varieties; vines, table varieties; wheat; barley; corn; sunflower; potato; sugar beet; soybeans; peas-beans; flax; hemp; alfalfa, clover; vegetables.
Depending on the values of the evaluation coefficients obtained by each plant, the evaluation score is calculated. Under natural conditions, evaluation scores can have between 0 and 100 points.

When the evaluation score is maximum (100), the plant finds optimal development conditions in the area and on the respective soil, obtaining high and economically efficient productions. The lower the value of the score, the lower the production, the lower the income obtained, bringing a lower profit.
Depending on the values of the evaluation scores obtained by the plants, the favourability classes can be established. Fertility class I has 90-100 points and class X has a score between 1-10.
Eutric psamosols can be found on dune plateaus and their slopes. Due to the high content of sand (coarse and fine) which occupies an average of 90% and the low content of fine material (dust and clay), around 10%, these psamosols do not retain water and nutrients.
Having a deficient humidity regime, the plants find harsh conditions for development, so that most evaluation scores are below 30 points, and the favourability classes are VII, VIII and IX, i.e. weak and very weak.
The lowest evaluation scores were obtained by hemp with 13 points, clover 14 points and hay grass 17 points.
The best grades were obtained by apricot with 51 points and peach with 46 points. It is known from agricultural practice that these two plant species are well suited to the conditions provided by sandy areas. Most crops fall into the VIIIth fertility class (14 crops), and 5 crops fall into the IXth fertility class. So, the two classes include 19 crops out of the total of 24, which highlights the very low natural fertility of this soil.

Soil evaluating in ameliorative conditions

Psamosols are generally considered to have a low natural fertility potential, because they have coarse textures, do not retain water, are poor in nutrients and are subject to the process of wind deflation.
In order to successfully and economically efficiently cultivate them, they must be characterized and classified from a technological point of view, applying specific improvement works.
By "technological characterization of agricultural lands" is meant a specialized work, through which agricultural lands are defined and classified parametrically, in terms of their physical, mechanical, chemical, biological, morphometric, morphological, climatic, etc. properties, properties that imprint different behaviours of soils in the production process, requiring a complex of agricultural technologies and improvement measures to increase their productive capacity (Teaci, 1980).
The technological characterization activity is carried out based on data obtained from the assessment of agricultural lands, taking into account a whole series of characteristics and correlations between pedogenetic factors and soil properties, such as: suitability for irrigation, prevention and control of excess moisture; flood ability; prevention and control of salinization, erosion; mechanization technologies; specifics of soil works; organic and mineral fertilization; prevention and control of pollution; reaction correction, etc.
Within each technological category of improvement works, classes and subclasses of lands have been separated, which group the lands according to the intensity of the restriction or the need to perform a certain improvement intervention.
All improvement works contribute to the improvement of natural factors and soil properties, positively influencing the growth and fruiting of plants.
For each improvement work, some "potentiation" coefficients have been established by which the evaluation coefficients are multiplied.
The potentiation coefficients are included in the annex tables that can be found in the "Methodology of Elaboration of Pedological Studies", produced and published by I.C.P.A Bucharest in 1987.
With each potency coefficient granted for a certain improvement work, the evaluation coefficient obtained by the plant under normal cultivation conditions is multiplied. Through potentiation, the value of the evaluation grades increases and therefore the productive potential of the improved lands is higher.
All improvement works through potentiation can raise the evaluation score to a maximum of 100 points. The only exception to this rule is irrigation.

This work positively influences several pedogenetic factors (humidity, temperature, groundwater, texture, microclimate), therefore it can lead to an increase in the evaluation score to over 100 points. In this case, 5 more classes from XI to XV are added to the 10 fertility classes.

On eutric psamosols, following the multiplication of the evaluation coefficients by the potentiation coefficients corresponding to the improvement works performed, the evaluation scores increased by somewhat higher percentages.

The more obvious increase is explained by the fact that eutric psamosols being less productive, the effect of the treatments is more obvious.

On eutric psamosols, after improvement, the best results were obtained for apricot with 83 points; peach with 79 points and vegetables with 72 points. Weaker results were recorded for hemp with 26 points; for clover with 30 points and for hayfields with 36 points.

For all other crops, after enhancement, the scores were between 40 and 60 points, which demonstrates that after improvement even eutric psamosols can acquire medium fertility.

This conclusion is also highlighted by the usual score for the arable land use form which is 56 points, which means the 5th class of favorability, i.e. medium fertility.

CONCLUSIONS

The formation and evolution of sands and sandy soils in our country has occurred over time under the influence of pedogenetic factors specific to each sandy area. In general, sands in Romania have a fluvial and eolian origin.

Eutric psamosols are found on large areas between Craiova and Dăbuleni, in areas with dunes subject to eolian deflation. They occupy the plateaus and slopes of sand dunes, have a deficient water regime because they do not retain water and therefore present a very low natural fertility. They have an A0-AC-C type profile.

Being rich in sand (over 90%) and poor in dust and clay, they do not retain water and nutrients. They have a coarse texture, are very poor in humus and colloidal complex, which is why they have a low cation retention and exchange capacity.

In order to be cultivated, these soils require radical improvement works, including: irrigation, organic and mineral fertilization with large amounts of fertilizers, combating wind deflation and cultivating with plants specific to sandy areas.

Due to the deficient humidity regime, plants find harsh conditions for development, so most of the evaluation scores are below 30 points, and the favourability classes are VII, VIII and IX, i.e. weak and very weak. The lowest evaluation scores were obtained by hemp with 13 points, clover with 14 points and meadow grass with 17 points, and the highest ones by apricot with 51 points and peach with 46 points.

On eutric psamosols, following the multiplication of the evaluation coefficients by the potentiation coefficients corresponding to the improvement works carried out, the evaluation scores increased by somewhat higher percentages. The more obvious increase is explained by the fact that eutric psamosols are less productive, the effect of the treatments is more obvious. After improvement, the best results were obtained for apricot with 83 points; peach with 79 points and vegetables with 72 points. Weaker results were recorded for hemp with 26 points; clover with 30 points and hayfields with 36 points.

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SOIL QUALITY IN PADDY FIELDS OF COASTAL KARNATAKA, INDIA

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Abstract

Coastal soils are typically nutrient-dense, acidic and extremely saline. Synthetic N:P:K supplementation and biofertilizers are essential for paddy cultivation. Global warming causes the ozone depletion, creating a harsh agro-environment for crop plants and beneficial soil organisms. Physico-chemical analysis was carried out for the twenty-five soil samples collected from the paddy fields of Coastal Karnataka in the present study. The results of the soil experiment indicated that TMR$_3$ (S$_2$) was more acidic (4.06), and the pH was essentially supported by the extremely low amount of organic carbon material in the HK (S$_2$) region (0.63%). With the exception of sulphur (S), which is less available and negatively correlated with other soil properties, it also exhibits a positive correlation with micronutrients (Ca, Mg, Zn, Cu, Fe, Mn and B). The highest content of macronutrients is nitrogen 502.00 kg/ac in the GSUK region, phosphate 27.30 kg/ac in the SK (S$_1$), and potassium 402.66 kg/ac in the HUK region. The results indicate that the more acidic-saline soil is harmful to rice crops, and less beneficial to agriculture.

***Key words**: paddy soil, algal bloom, cyanobacteria, global warming, synthetic and biofertilizers.*

INTRODUCTION

Soil is a region found directly on the surface of the Earth that serves as a natural growing medium for plants. They differ from their parent materials in a number of physico-chemical and bio-morphological ways. The Earth's surface contains loose mineral and organic matter that reflects the effects of genetic and environmental factors, such as climate, temperature, and water variations, as well as macro and microorganisms that function throughout time on parent material and are regulated by solution. The quantity of different sized inorganic particles in soil is a significant determinant of its texture. Understanding soil texture is necessary, which produces awareness of soil behaviour, including sensation, colour, sound and cohesion. Sand, silt and clay particle proportions determine the physical properties of soil, including its texture. Given that many plants require soil that drains properly, soil drainage is an important factor to consider. Plants that have poorly drained soil may experience stunting or even death as a result of inadequate oxygen reaching their roots. The soil system is composed of clay particles and the pores that link them. Soils with a structure that promotes plant development contain stable aggregates. Clays are very chemically active and retain nutrients on their surface better than other inorganic components. As soon as these nutrients are released into soil water, plants can start using them. Like nutrients, water also clings to the soil's top, although it is difficult for plants to consume. Iron, silicon, and aluminium make up the majority of the chemical components of soil's inorganic minerals, but they don't do much to satisfy the nutritional needs of plants. The organic matter component of soil comes from biodegradable components. Organic matter in the soil contributes to stable soil aggregation by binding soil particles together. Organic elements are regularly added by soil-dwelling plants through their roots and excrement. This organic waste is broken down by microbes, which releases nutrients for other plants to thrive on. Certain proteins, starches and sugars can all be easily broken down by microorganisms. The presence of significant organic content and less easily degradable materials like lignin and tannin gives the soil its black colour. An essential subgroup of semi-wetland, paddy soils are characterized by water saturation, which controls soil development as well as the kinds of plant and animal populations. The pedogenic characteristics and shape of paddy soil differ from those of dry land because it floods for several months of the year (Kawaguchi & Kyuma, 1977). Iron and manganese may form in paddy soil as a result of the repeated puddling and drying (redox cycle) of dissolved iron and manganese that build up in the B horizon (Kurniati et al., 2016). Field crops may face challenges due to the pedogenic characteristics and shape of paddy soil, even if a crop rotation plan is in place prior to planting. Top-layered paddy soil is less conducive to farming. Plow pan layers will negatively impact root development and crop nutrient availability. Paddy soil morphology varied generally according to the intensity of rice farming (i.e., the length of time the soil was immersed) during a year. The distinct features of paddy soil, which are made up of minerals, water, air, and soil organic materials (SOMs), include the soil's texture, structure, and porosity. The numerous minerals, organic matter, and nutrients that are present, determine the soil's chemical composition. The pH of the soil regulates the amount of organic matter, soil organism activity, and mineral concentration. The coastal parts of Karnataka are classified according to their soil geography as red, black, lateritic, alluvial-colluvial, forest, and coastal soils (laterite or coastal alluvium). Most coastal soils are clay and loamy in texture, with small amounts of silt and sand, saline to very saline, low to well-drained conditions, low to high organic matter content, and deficiencies or toxicity in certain minerals (Velayutham et al., 1999; Shamsudheen & Dasog, 2005; Paul & Rashid, 2017). They are also less productive because of their low organic content (Patil & Anil Kumar, 2014), which is usually a primary indicator of the poor physical quality of coastal soils. A negligible quantity of nitrogen, ranging from 1 to 1.5% is also present (SRDI, 2001). Similarly, most coastal soil shortages in copper and zinc are associated with low phosphorus concentrations (Karim et al., 1990). High salinity negatively affects agriculture claim (Mahajan et al., 2015), because the inherent functional characteristics of soil fertility are changed by both natural and man-made influences. Moreover, a range of abiotic stresses affect coastal soils. The coastal agricultural area is mostly marshy, moist, saline, alkaline and acid sulphate. The majority of coastal soils have an acidic pH with the top soil surface being primarily acidic and the lower areas being slightly alkaline to neutral. This ecosystem's low land and water productivity is therefore mostly caused by unfavourable weather, deteriorating soil health and poor water quality. Thus, it is necessary to create appropriate agro-strategies for higher agricultural yields. By using natural resources and a population of people and livestock, coastal Karnataka's economy depends on tourism and agriculture to give both rural and urban dwellers a steady level of living and food security. There is also a vast variety of plants from tropical rainforest and mangrove ecosystems. Thus, the present work aims to give detailed note on soil properties of paddy field in Coastal Karnataka which supports the agriculture.

This study aims to analyse the impact of saline environments and soil properties on the water logged paddy fields ecosystem in Coastal Karnataka. By investigating the region's unique soil characteristics and their influence on paddy cultivation, the research seeks to provide insights into sustainable agricultural practices and soil management strategies tailored to saline environments.

MATERIALS AND METHODS

Study Area

A remarkable natural ecosystem, the Coastal Karnataka, which includes portions of the Sahyadri Mountains and Western Ghats, lies between the lithospheric and hydrospheric sections of the Arabian Sea's Western Peninsula, and is home to Konkan region. The state of Karnataka covers roughly 320 km^2 of the Arabian Sea. The distribution of the region stretches to the plains of Kerala to the south, at latitudes of 15° 19' 2.1972" N and longitude of 75° 42' 50.0040" E. Over 1000 km^2 on the southwest coast of India, from Karwar, the capital of Uttara Kannada, to Mangalore, the principal city of Dakshina Kannada, lies Coastal Karnataka also known as the Kanara and Karavali region and it is a historically significant area.

Method of soil sampling

A total of 1000 grams of soil samples were taken from each sampling site using (particular spot utilizing) the Z and V-shaped sampling methods. The soil samples were dug up to a depth of 30 cm using a digger, and the samples were subsequently sealed in the sterile zip bags. From the Coastal Karnataka taluks, a total of 25 paddy field soil samples were collected during the period of February 2023 to May 2024 as shown in Table 1. The soil samples were subsequently air-dried under shaded laboratory conditions to maintain their physical and chemical properties for analysis.

Macro and micro nutrient evaluation

The colour, texture, type, moisture, density, pH, electrical conductivity, organic carbon, nitrogen, phosphate, potassium, magnesium, sulphur, manganese, iron, copper, and zinc of soil in agricultural ecosystems were estimated by the standard laboratory methods for physico-chemical examination listed in Table 2.

Table 1. Soil samples collected from the paddy fields of Coastal Karnataka

Collection site code	Village or place	Taluk	District	Geographical coordinates (D˚M′ S″)	
				Latitude (N)	Longitude (E)
HK (S_2)	Halga	Karwar	Uttara Kannada	14˚52′ 24.9″	74˚13′ 17.5″
KK (S_1)	Kinnar	Karwar		14˚52′ 00.2″	74˚12′ 23.8″
SK (S_1)	Sawantwada	Karwar		14˚53′ 14.6″	74˚09′ 48.6″
$AKUHR_1$ (S_1)	Hosur	Ankola		14˚35′ 44.1″	74˚22′ 58.0″
$AKSR_2$ (S_2)	Shiroor	Ankola		14˚36′ 03.1″	74˚21′ 51.4″
GKR_2 (S_1)	Gokarna	Kumta		14˚32′ 52.7″	74˚20′ 05.3″
$GKTR_3$ (S_2)	Torke (Gokarna)	Kumta		14˚33′ 27.3″	74˚21′ 02.7″
DJUK	Durgi	Joida		15˚20′ 15.8″	74˚32′ 07.1″
BSUK	Bisalkoppa	Sirsi		14˚32′ 37.1″	74˚49′ 32.7″
GSUK	Ghattikai	Siddapur		14˚31′ 02.4″	74˚50′ 32.8″
YUK	Yallapur	Yallapur		14˚59′ 08.7″	74˚43′ 12.6″
HUK	Halyal	Halyal		15˚19′ 53.1″	74˚44′ 38.7″
KGMUK	Kop Gotgudi	Mundagod		14˚45′ 40.0″	75˚01′ 28.1″
DUK	Dandeli	Dandeli		15˚14′ 39.3″	74˚37′ 37.4″
UDR_3	Doddanagudde	Udupi	Udupi	13˚21′ 46.2″	74˚49′ 42.9″
UDR_4 (S_2)	Devinagar	Udupi		13˚20′ 52.7″	74˚49′ 01.0″
$BKUS_1$	Brahmavar	Brahmavar		13˚25′ 52.3″	74˚45′ 39.1″
BKU	Kundapura	Kundapura		13˚37′ 07.5″	74˚41′ 48.6″
KKU	Karkala	Udupi		13˚13′ 55.3″	74˚53′ 34.0″
TMR_3 (S_2)	Thiruvali	Mangaluru	Dakshina Kannada	12˚55′ 12.1″	74˚55′ 00.2″
TUB	Thannirapantha	Beltangady		12˚52′ 59.3″	75˚14′ 46.0″
BSDK	Bellare	Sulya		12˚39′ 43.3″	75˚22′ 21.6″
KPDK	Kemminje	Puttur		12˚44′ 56.4″	75˚13′ 36.1″
KKDK	Kadaba	Kadaba		12˚44′ 49.6″	75˚27′ 59.6″
KBDK	Kula	Bantwala		12˚46′ 40.3″	75˚08′ 35.1″

Table 2. Physico-chemical properties of soil and standard analytical methods

Depth (cm)	Parameters	Analytical method	References
0 to 30	Texture	Hydrometric	Das et al. (2020)
	Moisture (%)	Gravimetric	Das et al. (2020)
	Density (g/cm^3)	Core	Jabro et al. (2020)
	pH (1:2.5)	Potentiometry	Jackson (1973)
	EC (ds/m)	Conductometry	Jackson (1973)
	OC (%)	Wet oxidation	Walkley & Black (1934)
	N (kg/ac)	Alkaline permanganate	Subbiah & Asija (1956)
	P (kg/ac)	Olsen's P	Olsen et al. (1954)
	K (kg/ac)	Flame photometry	Richards (1954)
	Ca (meq/100g)	Versenate titration	Jackson (1973)
	Mg (meq/100g)		
	S (ppm)	Turbidometry	Black (1965)
	Zn (ppm)	DTPA extraction AAS	Lindsay & Norvell (1978)
	Cu (ppm)		
	Fe (ppm)		
	Mn (ppm)		
	B (ppm)	Hot water soluble extraction	Devi & Sumathy (2017)

Statistical analysis

The IBM SPSS statistics, version 20 software and XLSTAT applications were used to analyse the soil properties using principal component analysis, Pearson correlations, and cluster analysis for qualitative cluster characteristics of locations based on geography.

RESULTS AND DISCUSSIONS

The results show significant variations in the physicochemical properties of coastal paddy field soils.

The coastal soil is laterite to medium black in colour, with a loamy texture that also contains sand and silt. BKU had the highest moisture content in this analysis, measuring 48.00±0.57, whereas HUK, GSUK, AKSR$_2$ (S$_2$), and KK (S$_1$) had intermediate moisture contents, measuring 25.00±0.57 to 26.00±0.57. Lastly, very low moisture content was found in GKR$_2$ (S$_1$) that is 18.00±0.57. The density in TMR$_3$ (S$_2$) is high at 49.99±48.50, whereas GKTR$_3$ (S$_2$) displays a medium range of 6.18±4.61 and TUB had a significantly lower value of 1.28±0.0, as shown in Table 3.

Table 3. Physical properties of collected soil samples from the different paddy fields of Coastal Karnataka

Site (code)	Moisture	Density	Colour	Type	Texture
HK (S$_2$)	23.50 ± 0.28^{i,j,k}	1.54 ± 0.08^{b}	Laterite yellow	Laterite	Sandy loam
KK (S$_1$)	25.00 ± 0.57^{h,i}	1.72 ± 0.15^{b}	Laterite yellow	Laterite	Sandy loam
SK (S$_1$)	22.00 ± 0.57^{k}	1.45 ± 0.0057^{b}	Laterite yellow	Laterite	Sandy loam
AKUHR$_1$ (S$_1$)	23.00 ± 0.57^{j,k}	1.52 ± 0.057^{b}	Laterite yellow	Laterite	Sandy loam
AKSR$_2$ (S$_2$)	26.00 ± 0.57^{h}	1.61 ± 0.057^{b}	Laterite yellow	Laterite	Sandy loam
GKR$_2$ (S$_1$)	**18.00 ± 0.57^{l}**	1.44 ± 0.011^{b}	Laterite yellow	Laterite	Sandy loam
GKTR$_3$ (S$_2$)	22.00 ± 0.57^{k}	6.18 ± 4.61^{b}	Laterite yellow	Laterite	Sandy loam
UDR$_3$	23.00 ± 0.57^{j,k}	1.52 ± 0.0088^{b}	Laterite yellow	Laterite	Sandy loam
UDR$_4$ (S$_2$)	23.00 ± 0.57^{j,k}	1.54 ± 0.0057^{b}	Laterite yellow	Laterite	Sandy loam
TMR$_3$ (S$_2$)	24.00 ± 0.57^{i,j}	**49.99 ± 48.50^{a}**	Laterite yellow	Laterite	Sandy loam
DJUK	30.00 ± 0.57^{f}	1.45 ± 0.011^{b}	Laterite yellow	Laterite gravel	Sandy loam
BKUS	41.00 ± 0.57^{b}	1.48 ± 0.0057^{b}	Laterite yellow	Laterite gravel	Sandy loam
KBDK	35.00 ± 0.57^{d}	1.42 ± 0.0082^{b}	Laterite yellow	Laterite gravel	Sandy loam
BSUK	38.00 ± 0.57^{c}	1.43 ± 0.0057^{b}	Laterite yellow	Laterite	Silt loam
GSUK	26.00 ± 0.57^{h}	1.42 ± 0.0088^{b}	Laterite yellow	Laterite	Silt loam
YUK	41.00 ± 0.57^{b}	1.35 ± 0.0057^{b}	Laterite yellow	Laterite	Silt loam
KGMUK	42.00 ± 0.57^{b}	1.36 ± 0.0057^{b}	Laterite yellow	Laterite	Silt loam
DUK	36.00 ± 0.57^{d}	1.40 ± 0.057^{b}	Laterite yellow	Laterite	Silt loam
KKU	28.00 ± 0.57^{g}	1.37 ± 0.0088^{b}	Laterite yellow	Laterite	Silt loam
TUB	28.00 ± 0.57^{g}	**1.28 ± 0.0057^{b}**	Laterite yellow	Laterite	Silt loam
BSDK	41.00 ± 0.57^{b}	1.36 ± 0.0057^{b}	Laterite yellow	Laterite	Silt loam
KPDK	38.00 ± 0.57^{c}	1.34 ± 0.0057^{b}	Laterite yellow	Laterite	Silt loam
KKDK	32.33 ± 0.88^{e}	1.37 ± 0.0057^{b}	Laterite yellow	Laterite	Silt loam
HUK	26.00 ± 0.57^{h}	1.37 ± 0.0088^{b}	Laterite yellow	Medium laterite	Silt loam
BKU	**48.00 ± 0.57^{a}**	1.38 ± 0.0057^{b}	Medium black	Medium black	Silt loam

The mean of the replicates ± standard error (SE) is represented for each value. A significant difference (Duncan's multiple range test, $P < 0.01$) was demonstrated by a one-way ANOVA with distinct letters in a column representing probability values.

According to recent research findings, soil characteristics have an impact on plant crops, the environment, and beneficial soil organisms. But the best soil texture material preserves pore space, which implies that air and water may support life. Paddy field soil contains complex chemical properties that are more crucial for plant development and the beneficial biological life of the soil shown in Table 4. The pH of the soil varied significantly across the samples. KGMUK had the highest pH at 6.44±0.01, while HUK and HK (S$_2$) exhibited medium pH levels of 5.33±0.14 to 5.34±0.08, and finally lowest pH was observed in TMR$_3$ (S$_2$) at 4.06±0.01. Both beneficial microbial populations and plants are influenced by pH (Zhang et al., 2017; Li et al., 2020). Although it naturally varies based on the surroundings, the acidity level is increased by the use of specific fertilizers (Song et al., 2022). However, due to the acidifying nature of coastal soils, a high algal biomass content leads to breakdown and CO_2 enters the soil through coastal waters; an extensive fertilizer application, on the other hand, produces the biological content. In the interaction of soil health with ecosystems and the environment, pH plays a critical role in ensuring the development of high-quality agricultural products and food security. But according to Gentili et al. (2018), pH controls both major and minor nutrient absorption. Rice, groundnuts, cucumbers, tuber crops, and other agro-crops along the coastline are under stress due to the low to moderate pH range (4.06±0.01 to 6.44±0.01), which is almost acidic.

Table 4. Chemical evaluation of soil samples collected from paddy fields of Coastal Karnataka

Sample I D	**Chemicals Parameters**		
	pH (1:2.5)	**EC (ds/m)**	**OC (%)**
HK (S_2)	$5.34 \pm 0.08819^{g,h}$	1.41 ± 0.057^{b}	$\mathbf{0.63 \pm 0.057^{j}}$
KK (S_1)	$5.44 \pm 0.13868^{f,g}$	$\mathbf{3.61 \pm 0.11^{a}}$	0.71 ± 0.086^{j}
SK (S_1)	4.25 ± 0.00577^{m}	$0.05 \pm 0.0057^{i,j}$	$1.08 \pm 0.0057^{d,e}$
AKUHR$_1$ (S_1)	$4.16 \pm 0.00577^{m,n}$	$0.07 \pm 0.0057^{h,j}$	$1.14 \pm 0.0033^{c,d}$
AKSR$_2$ (S_2)	5.88 ± 0.03512^{b}	0.94 ± 0.020^{c}	$0.94 \pm 0.0082^{f,g,h,i}$
GKR$_2$ (S_1)	4.44 ± 0.02028^{l}	0.04 ± 0.0057^{j}	$1.07 \pm 0.011^{d,e,f}$
GKTR$_3$ (S_2)	$4.53 \pm 0.01155^{k,l}$	$\mathbf{0.04 \pm 0.0057^{j}}$	$0.97 \pm 0.011^{e,f,g,h}$
UDR$_3$	4.23 ± 0.00577^{m}	$0.06 \pm 0.0057^{h,i,j}$	$0.95 \pm 0.0082^{e,f,g,h,i}$
UDR$_4$ (S_2)	4.56 ± 0.00882^{k}	$0.06 \pm 0.0057^{h,i,j}$	$0.92 \pm 0.0082^{g,h,i}$
TMR$_3$ (S_2)	$\mathbf{4.06 \pm 0.01202^{n}}$	$0.07 \pm 0.0082^{h,i,j}$	$0.96 \pm 0.0082^{e,f,g,h}$
DJUK	4.95 ± 0.01764^{j}	$0.34 \pm 0.014^{d,e}$	$\mathbf{1.70 \pm 0.11^{a}}$
TUB	$5.23 \pm 0.00577^{h,i}$	$0.22 \pm 0.0057^{f,g}$	$0.96 \pm 0.0082^{e,f,g,h}$
BSUK	5.64 ± 0.01155^{d}	0.43 ± 0.088^{d}	$1.25 \pm 0.014^{b,c}$
BSDK	$5.84 \pm 0.01453^{b,c}$	$0.15 \pm 0.0082^{g,h,i,j}$	$1.26 \pm 0.0082^{b,c}$
KPDK	$5.47 \pm 0.00882^{e,f}$	$0.30 \pm 0.057^{e,f}$	1.36 ± 0.0057^{b}
BKUS	5.76 ± 0.00577^{c}	$0.18 \pm 0.0057^{g,h}$	1.30 ± 0.057^{b}
GSUK	$5.25 \pm 0.00882^{h,i}$	$0.35 \pm 0.0082^{d,e}$	$0.96 \pm 0.0082^{e,f,g,h}$
KKDK	5.15 ± 0.00882^{i}	$0.24 \pm 0.012^{e,f,g}$	$1.03 \pm 0.0082^{d,e,f,g}$
YUK	4.95 ± 0.00577^{j}	$0.26 \pm 0.0057^{e,f,g}$	$0.95 \pm 0.011^{e,f,g,h,i}$
KBDK	5.20 ± 0.05774^{i}	$0.16 \pm 0.0082^{g,h,i}$	1.34 ± 0.0082^{b}
HUK	$5.33 \pm 0.01453^{g,h}$	$0.30 \pm 0.057^{e,f}$	$1.26 \pm 0.088^{b,c}$
KGMUK	$\mathbf{6.44 \pm 0.01453^{a}}$	$0.17 \pm 0.0082^{g,h}$	$1.14 \pm 0.012^{c,d}$
DUK	$5.55 \pm 0.01202^{d,e}$	$0.32 \pm 0.0057^{e,f}$	1.35 ± 0.0082^{b}
BKU	$5.82 \pm 0.00577^{b,c}$	$0.18 \pm 0.0057^{g,h}$	0.84 ± 0.011^{i}
KKU	$5.25 \pm 0.00882^{h,i}$	$0.17 \pm 0.0082^{g,h}$	$0.87 \pm 0.011^{h,i}$

The mean of the replicates ± standard error (SE) is represented for each value. A significant difference (Duncan's multiple range test, $P < 0.01$) was demonstrated by a one-way ANOVA with distinct letters in a column representing probability values.

The electrical conductivity is then examined and found to be higher in KK (S_1) at 3.61±0.11, and medium in HUK, KPDK, and DJUK at 0.30±0.05 to 0.34±0.01. Finally, in TMR$_3$ (S_2), UDR$_4$ (S_2) and UDR$_3$, it is extremely low at 0.04±0.00. The combined effect of pH and electrical conductivity (EC) on pH variations brought on by both natural and man-made farming procedures (fertilizer application) indicates that salinity increases electrical conductivity. Although higher nitrogen concentrations caused plant stress, which affects rice production grade, they did not increase the pH range with EC. According to Huang et al. (2017), nitrogen fertilization frequently increases the pH and electrical conductivity of soil. Most plants usually relate to the range of 0.8 to 2.5 mS/m, although salt-sensitive plants require 1 to 2.6 mS/m. In this case, coastal agriculture is generally supported by the total EC (0.04±0.0 to 3.61±0.11). Additionally, the proportion of soil organic carbon is very low in HK (S_2) (0.63±0.05), medium in GKR$_2$ (S_1) and KKDK (1.03±0.0082 to 1.07±0.011), and high in DJUK (1.70±0.11).

Naturally, coastal soils with lower fertility levels support less organic matter; exposure to organic carbon has a positive correlation with these soil microbial activities (Nisha et al., 2018), and pH has a positive correlation with the inter-root bacterial population in maize (Wang et al., 2018). Jin et al. (2020) claim that treating the soil with rice straw successfully preserved soil organic carbon.

However, with the help of soil microorganisms the plant waste progressively dissolves down after harvesting, raising the amount of organic carbon elements. The results showed that the amount of organic matter in coastal paddy soils ranged from 0.63±0.05 to 1.70±0.11, which is low to moderate. The composition of organic matter in soil is more popular for microbial diversity than the composition of sand, despite the fact that organic matter both directly and indirectly serves as a primary source of food and energy for the microbial community (Rashid et al., 2016; Naylor et al., 2022).

Biological processes that enrich soil organic matter with carbonates are driven by the photosynthetic contribution of primary producers and photosynthetic microorganisms such as cyanobacteria (Singh et al., 2016). According to Plante & McGill (2002), soil contains cyanobacterial discharges. The development of stable macro-aggregates and a reduction in the soil's C:N ratio brought on by the breakdown of organic waste are important aspects of remediation. Prior to the discovery of macro and micronutrients in this parametric investigation, soil matter was more than just a physico-chemical property, as indicated in Table 5. Here, nitrogen (N), phosphate (P), and potassium (K) are macronutrients; calcium (Ca), magnesium (Mg), sulphur (S), zinc (Zn), copper (Cu), iron (Fe), manganese (Mn) and boron (B) are micronutrients. Furthermore, during this experiment a significant nitrogen concentration (502.00±0.57) was found in GSUK. In DUK, DJUK and YUK a modest range of nitrogen levels (351.00±0.57 to 337.00±0.57) was found. Lastly, UDR_3 showed a relatively low nitrogen concentration (221.00±0.57). More importantly, plants use inorganic conditions like ammonium nitrate to absorb nitrogen-limited organic resources. Ammonia volatilization and the use of chemical fertilizers are two natural processes that increase the pH of soil. The nitrogen needs of rice crops were satisfied by chemical fertilizers (Lin et al., 2013). The primary element controlling rice crop is nitrogen; fertilizers can damage rice crops and increase global temperatures, which further increases the likelihood of global warming as a result of CO_2 produced from paddy fields (Park et al., 2023). Growth of paddy fields, therefore elevated levels of nitrogen-based fertilizers might negatively affect rice crops and increase global temperatures, which would increase the likelihood of global warming as a result of CO_2 emissions from paddy fields (Park et al., 2023). Coastal paddy soil has moderate to high levels of accessible soil nitrogen, ranging from 221.00±0.57 to 502.00±0.57, according to this analysis. Manure is being substituted by microbial-based biofertilizers (Tirol et al., 1982), which is a very efficient source of nitrogen and increases the production of rice (Roger & Kulasooriva, 1980). Therefore, using biofertilizers like cyanobacteria, *Azotobacter*, *Azospirillum*, *Rhizobium*, and so on can improve agricultural land both economically and environmentally. In sandy soils, nitrate from core soil seeps around the root zone, and in flooded soils, nitrogen fixation by bacteria converts gaseous nitrogen into a form that plants can use. However, nitrate from core soil is not immediately available to plants. Additionally, by raising the soil's N and P content and promoting development through the secreting of phytohormones, nitrogen-fixing bacteria have a major effect on plant growth (Hameeda et al., 2008). Phosphate content is another important macronutrient that is moderately available in KKU, BKU, DUK, HUK, BSDK, BSUK and HK (S_2) at 14.26±0.08 to 16.80±0.05, while it is high in SK (S_1) at 27.30±0.65. However, the phosphate concentration in TMR_3 (S_2) and UDR_4 (S_2) is extremely low at 8.00±0.57. Phosphate is critical for rice production and influences crop productivity. Its excessive use, along with the presence of heavy metals like Pb, Hg, and As in rice, increases the risk of food insecurity (Cao et al., 2009; Dang et al., 2016). The overuse of phosphate fertilizers affects paddy crops and can reduce yields. The easy and natural availability of nitrate and the rise in phosphate content have a virtuous effect on the domination of soil microbes (Zancan et al., 2006; Yandigeri et al., 2011). These parameters are also effective indicators of anthropogenic compression to the agro-environment (Zutshi et al., 1984), and field soil benefits from this as well (Roger & Kulasooriya, 1980).

Table 5. Micro and macro nutrient concentration of soil samples collected from paddy fields of Coastal Karnataka

Sample I D	Macronutrients			Micronutrients							
	N (kg/ac)	P (kg/ac)	K (kg/ac)	Ca (meq/100 g)	Mg (meq/100 g)	S (ppm)	Zn (ppm)	Cu (ppm)	Fe (ppm)	Mn (ppm)	B (ppm)
HK (S_2)	324.00 ± 0.57^{k}	16.30 ± 0.057^{e}	92.23 ± 0.62^{i,j}	5.43 ± 0.29^{i}	2.76 ± 0.18^{j,k}	14.50 ± 0.30^{c}	0.62 ± 0.12^{e,f}	0.41 ± 0.057^{g,h}	11.50 ± 0.31^{b}	9.17 ± 0.54^{h}	0.27 ± 0.03^{i,j}
KK (S_1)	265.16 ± 0.60^{q}	10.70 ± 0.057^{k}	116.00 ± 0.57^{h,i}	10.20 ± 0.05^{a}	5.40 ± 0.057^{g}	10.55 ± 0.0088^{g}	**0.22 ± 0.0057^{h}**	0.56 ± 0.0057^{e,f,g,h}	14.56 ± 0.0057^{b}	11.22 ± 0.0057^{f}	0.62 ± 0.0057^{f}
SK (S_1)	229.16 ± 0.60^{r}	**27.30 ± 0.65^{a}**	56.18 ± 0.60^{k,l}	2.20 ± 0.05^{k}	2.00 ± 0.57^{l,m}	11.70 ± 0.24^{f}	**0.22 ± 0.0057^{h}**	0.50 ± 0.057^{f,g,h}	17.16 ± 0.60^{b}	9.23 ± 0.033^{h}	0.45 ± 0.0088^{h}
AKUHR$_1$ (S_1)	364.16 ± 0.60^{h}	11.70 ± 0.11^{j}	51.43 ± 0.18^{k,l,m}	5.56 ± 0.17^{i}	2.50 ± 0.057^{k,l}	10.51 ± 0.057^{g}	0.42 ± 0.0088^{g}	0.33 ± 0.011^{h}	19.55 ± 0.020^{b}	11.24 ± 0.020^{f}	0.26 ± 0.011^{j}
AKSR$_2$ (S_2)	283.80 ± 0.15^{p}	12.60 ± 0.11^{h,i}	227.53 ± 0.23^{d,e}	6.46 ± 0.17^{h}	3.23 ± 0.088^{i,j}	11.73 ± 0.12^{f}	0.25 ± 0.0088^{h}	0.42 ± 0.0088^{g,h}	19.63 ± 0.011^{b}	12.54 ± 0.011^{d,e}	0.32 ± 0.0088^{i}
GKR$_2$ (S_1)	400.50 ± 0.11^{d}	11.70 ± 0.11^{j}	35.00 ± 0.57^{l,m}	2.40 ± 0.11^{k}	1.33 ± 0.088^{n}	11.53 ± 0.14^{f}	0.32 ± 0.0088^{g,h}	0.55 ± 0.0057^{e,f,g,h}	**392.37 ± 380.81^{a}**	8.93 ± 0.014^{h}	0.52 ± 0.0088^{g}
GKTR$_3$ (S_2)	265.70 ± 0.11^{q}	18.60 ± 0.15^{d}	**22.26 ± 0.0088^{m}**	**1.70 ± 0.11^{l}**	0.66 ± 0.088^{o}	16.26 ± 0.088^{b}	0.53 ± 0.017^{f}	0.33 ± 0.014^{h}	17.50 ± 0.11^{b}	9.25 ± 0.014^{h}	0.25 ± 0.0088^{j}
UDR$_3$	**221.00 ± 0.57^{s}**	13.53 ± 0.088^{g}	81.33 ± 0.088^{j,k}	2.70 ± 0.057^{j}	1.20 ± 0.057^{n}	9.53 ± 0.011^{h}	0.43 ± 0.011^{g}	**0.26 ± 0.011^{h}**	**4.55 ± 0.012^{b}**	**3.12 ± 0.0088^{k}**	0.45 ± 0.014^{h}
UDR$_4$ (S_2)	368.00 ± 0.57^{g}	**8.00 ± 0.57^{l}**	57.22 ± 0.012^{k,l}	2.33 ± 0.088^{k}	1.56 ± 0.088^{m,n}	11.54 ± 0.0088^{f}	0.28 ± 0.0057^{h}	0.72 ± 0.0088^{d,e,f,g}	14.53 ± 0.011^{b}	13.00 ± 0.57^{c,d}	0.45 ± 0.0057^{h}
TMR$_3$ (S_2)	368.00 ± 0.57^{g}	**8.00 ± 0.57^{l}**	45.34 ± 0.012^{l,m}	8.64 ± 0.011^{e}	**0.60 ± 0.057^{o}**	10.52 ± 0.0088^{g}	0.32 ± 0.0057^{g,h}	0.96 ± 0.0057^{c,d}	17.40 ± 0.057^{b}	9.020 ± 0.0057^{h}	**0.23 ± 0.088^{j}**
DJUK	351.00 ± 0.57^{i}	10.30 ± 0.11^{k}	126.00 ± 0.57^{g,h}	7.05 ± 0.0088^{g}	5.73 ± 0.088^{f,g}	8.18 ± 0.017^{j,k}	0.75 ± 0.014^{d}	0.73 ± 0.011^{d,e,f,g}	7.56 ± 0.12^{b}	10.66 ± 0.088^{g}	**1.36 ± 0.0057^{a}**
TUB	391.00 ± 0.57^{e}	20.20 ± 0.057^{c}	395.00 ± 0.57^{a}	8.64 ± 0.011^{e}	4.80 ± 0.057^{h}	7.63 ± 0.088^{l}	**1.20 ± 0.057^{a}**	1.05 ± 0.0057^{b,c,d}	6.00 ± 0.57^{b}	5.70 ± 0.11^{j}	0.75 ± 0.011^{e}
BSUK	403.33 ± 0.88^{c}	15.40 ± 0.11^{f}	301.00 ± 0.57^{c}	6.57 ± 0.011^{h}	3.66 ± 0.088^{i}	12.93 ± 0.012^{d}	0.94 ± 0.0088^{b}	0.86 ± 0.0088^{c,d,e,f}	5.95 ± 0.011^{b}	14.63 ± 0.14^{b}	0.84 ± 0.011^{d}
BSDK	431.00 ± 0.57^{b}	15.40 ± 0.11^{f}	402.00 ± 0.57^{a}	8.95 ± 0.008^{d}	4.73 ± 0.088^{h}	16.46 ± 0.12^{b}	0.66 ± 0.088^{d,e}	**2.00 ± 0.57^{a}**	7.70 ± 0.11^{b}	7.70 ± 0.11^{i}	1.02 ± 0.0057^{b}
KPDK	297.00 ± 0.57^{o}	10.30 ± 0.11^{k}	241.33 ± 0.88^{d}	9.60 ± 0.057^{b,c}	6.20 ± 0.057^{e,f}	9.03 ± 0.011^{i}	0.95 ± 0.011^{b}	0.85 ± 0.011^{c,d,e,f}	12.00 ± 0.57^{b}	**15.83 ± 0.011^{a}**	0.85 ± 0.011^{d}
BKUS	302.00 ± 0.57^{m,n}	13.00 ± 0.57^{g,h}	283.33 ± 1.20^{c}	9.50 ± 0.11^{b,c}	9.76 ± 0.088^{b}	**20.36 ± 0.088^{a}**	0.87 ± 0.0057^{b,c}	1.06 ± 0.0088^{b,c,d}	12.40 ± 0.11^{b}	10.36 ± 0.088^{g}	1.03 ± 0.011^{b}
GSUK	**502.00 ± 0.57^{a}**	19.66 ± 0.088^{c}	342.00 ± 0.57^{b}	9.32 ± 0.0088^{c}	8.60 ± 0.057^{c}	**6.94 ± 0.0088^{m}**	0.94 ± 0.0088^{b}	1.02 ± 0.0057^{b,c,d}	12.20 ± 0.057^{b}	10.36 ± 0.088^{g}	0.95 ± 0.0057^{c}
KKDK	315.00 ± 0.57^{l}	10.20 ± 0.057^{k}	217.00 ± 0.57^{d,e}	8.60 ± 0.057^{e}	5.60 ± 0.057^{g}	7.95 ± 0.014^{k}	0.75 ± 0.012^{d}	0.77 ± 0.011^{d,e,f,g}	12.36 ± 0.14^{b}	10.36 ± 0.088^{g}	0.84 ± 0.0088^{d}
YUK	351.00 ± 0.57^{i}	10.20 ± 0.057^{k}	151.00 ± 0.57^{g}	9.63 ± 0.0057^{b}	6.36 ± 0.088^{e}	8.11 ± 0.095^{j,k}	0.95 ± 0.011^{b}	0.84 ± 0.014^{c,d,e,f}	12.00 ± 0.57^{b}	10.50 ± 0.11^{g}	1.03 ± 0.0088^{b}
KBDK	303.66 ± 0.88^{m}	21.20 ± 0.057^{b}	130.40 ± 0.11^{g,h}	7.82 ± 0.0088^{f}	8.70 ± 0.057^{c}	8.36 ± 0.088^{j}	0.77 ± 0.0088^{c,d}	1.20 ± 0.057^{b,c}	10.43 ± 0.011^{b}	13.23 ± 0.066^{c}	1.05 ± 0.0088^{b}
HUK	297.66 ± 0.88^{o}	14.26 ± 0.088^{g}	**402.66 ± 0.88^{a}**	6.94 ± 0.014^{g}	8.00 ± 0.57^{d}	8.30 ± 0.11^{j}	0.75 ± 0.014^{d}	1.03 ± 0.0057^{b,c,d}	11.53 ± 0.014^{b}	10.42 ± 0.0088^{g}	0.96 ± 0.0057^{c}
KGMUK	301.00 ± 0.57^{n}	12.23 ± 0.088^{i,j}	202.00 ± 0.57^{e,f}	**10.46 ± 0.0088^{a}**	**22.50 ± 0.057^{a}**	10.40 ± 0.057^{g}	0.77 ± 0.011^{c,d}	1.05 ± 0.011^{b,c,d}	12.23 ± 0.088^{b}	12.26 ± 0.12^{e}	0.94 ± 0.0057^{c}
DUK	337.00 ± 0.57^{j}	16.30 ± 0.11^{e}	64.50 ± 48.25^{j,k,l}	8.56 ± 0.0088^{e}	4.50 ± 0.11^{h}	9.83 ± 0.0011^{h}	0.70 ± 0.057^{d,e}	0.93 ± 0.012^{c,d,e}	10.73 ± 0.0088^{b}	10.23 ± 0.088^{g}	1.03 ± 0.0088^{b}
BKU	302.33 ± 0.88^{m,n}	14.70 ± 0.11^{f,g}	293.33 ± 1.45^{c}	9.63 ± 0.011^{b}	5.70 ± 0.057^{f,g}	8.36 ± 0.14^{j}	0.87 ± 0.0088^{b,c}	1.40 ± 0.057^{b}	12.26 ± 0.088^{b}	10.73 ± 0.011^{f,g}	1.03 ± 0.011^{b}
KKU	381.00 ± 0.57^{f}	16.80 ± 0.057^{e}	180.40 ± 0.11^{f}	7.66 ± 0.01^{f}	8.66 ± 0.088^{c}	12.23 ± 0.088^{e}	0.96 ± 0.0088^{b}	1.02 ± 0.0057^{b,c,d}	18.30 ± 0.057^{b}	10.60 ± 0.057^{g}	0.83 ± 0.0088^{d}

The mean of the replicates ± standard error (SE) is represented for each value. A significant difference (Duncan's multiple range test, $P < 0.01$) was demonstrated by a one-way ANOVA with distinct letters in a column representing probability values.

Since cyanobacteria are thought to be significant P sinks, they are constantly causing P-transformations. The quantity of phosphate available to rice plants is increased by the absorption of phosphate during cell growth and its excretion in soluble organic form during decomposition, which results in mineral orthophosphates and phosphate containing compounds (Manal et al., 1999). In addition, soil microbial interactions can use heterocyclic chemicals, produce organic acids, or mobilize different insoluble forms of inorganic phosphate. When cyanobacteria are employed as biofertilizers, Nisha et al. (2014) reported phosphomonoesterase activity facilitates the binding of organic phosphorus in soil. Concurrently, the medium low and high phosphate levels in paddy field soil were found to vary from 8.00±0.57 to 27.30±0.65. As a result of that, potassium availability, the last significant macronutrient, is higher in HUK at 402.66±0.88 and varies from 130.40±0.11 to 202.00±0.57 in KKU, KGMUK, KBDK and YUK at medium doses decreasing to 22.26±0.00 in $GKTR_3$ (S_2). For healthy plant growth and the presence of beneficial soil organisms, potassium (K^+) is one of the most sought-after macronutrients in soil. However, it has a direct effect on the growth, productivity, and dispersion of living organisms in soil media that contain carbonates and organic carbon (El-Gamal et al., 2016) and is intimately linked to cationic salts like Na^+, Ca^{+2} and Mg^{+2} (Shi & Wang, 2005). The potassium values in the paddy field soil parametric research ranged from 22.26±0.0088 to 402.66±0.88. Rashid et al. (2016) state that N:P:K based fertilizers are frequently used to maintain potassium levels in rice crop paddy fields. When excessive N fertilizer is applied, the soil's N content increases but the P and K concentrations remain unchanged (Clark, 1982; Stiling & Moon, 2005; Sarwar, 2012; Rashid et al., 2016). Excessive potassium fertilizer application reduces plant soluble proteins, nitrogen concentration, and carbohydrate content (Salim, 2002b). Similarly, since cyanobacteria initially developed cyanobacterial biofilms on the soil's surface, high pH and potassium rich topsoil have been used as biomarkers of cyanobacteria in agricultural settings (Alghanmi & Jawad, 2019). Now, focusing on micronutrients, calcium is mostly available in KGMUK (10.46±0.0088), moderately available in DUK, KBDK, KKDK, TUB, and TMR_3 (S_2) (7.82±0.0088 to 8.64±0.011), and finally available in $GKTR_3$ (S_2) at a lesser concentration of 1.70±0.11. Calcium (Ca) is by far the most prevalent cationic micronutrient in soil. According to Zutshi et al. (1980), cationic calcium is abundant in areas such as the Himalayas and coastal regions that include exposed limestone-rich rocks. White & Bradley (2003) claim that hydration bonding increases the pH range of soil; as a result, calcium affects plant physiological processes and rice crop growth (Gentili et al., 2018).

According to this analysis, the calcium concentration of the soil in the coastal paddy field varied from 1.70±0.11 to 10.46±0.0088. Macro and micronutrients including N, P, Mg, OC, Na, K and calcium build up as reserve traces after microbial biomass breaks down, leading to soil enrichment (Yanni & Abd-El-Rahman, 1993; Mandal et al., 1999; Kaushik, 2014). The microbial community is altered by more calcium and carbonate, which also alters the soil's chemistry. The combination of calcium and nitrogen changes the active reaction of photosynthetic bacteria, according to study by Piccioni & Mauzerall (1978). In the end, this impact on photosynthesis causes an excess of oxygen to accumulate in the atmosphere. Additionally, to stabilize carbonic calcium ions in a soluble form and reduce the amount of sodium ions in the soil, cyanobacteria and other soil microbes generate oxalic, organic, succinic, and lactic acids (Bhatnagar & Roychoudhury, 1992). Applying soil calcium topically can reduce nickel toxicity in soil (Aziz et al., 2014). The current available magnesium content is very low in TMR_3 (S_2) at 0.60±0.057, moderate (4.73±0.088 to 9.76±0.088) in BSDK, DJUK, BKU, KKDK, BKUS, TUB, and KK (S_1), and high in KGMUK at 22.50±0.05. Magnesium, an essential structural component of chlorophyll pigments, facilitates photosynthetic processes in autotrophs such as plants, algae and cyanobacteria. According to this investigation, the soil's total magnesium availability varied between 0.60±0.05 and 22.50±0.05. Magnesium is one of the most important cationic salts that supports the growth and spread of plants as well as the health of the soil microbial community. Magnesium levels in the soil promote leaf girth and stability. However, insufficient supply causes rice leaves to lose their chlorophyll, which decreases the rate of photosynthesis and resulting in stunted and dry shoots (Shaul, 2002; Shabala & Hariadi, 2005). A sufficient amount of magnesium promotes photosynthesis and other physiological functions in plants (Ding et al., 2006). Sulphur concentrations in $AKUHR_1$ (S_1), SK (S_1), KK (S_1), $AKSR_2$ (S_2), GKR_2 (S_1), UDR_4 (S_2), TMR_3 (S_2), and KGMUK are in the medium range (10.40±0.05 to 11.73±0.12), while GSUK has the lowest concentration (6.94±0.0088). BKUS has the highest concentration (20.36±0.08). Sulphur content in this study was low to moderate, ranging from 6.94±0.00 to 20.36±0.08. Positive interactions with other soil factors were not seen. The amount of sulphur in the soil has a major effect on plant growth and affects the girth and elongation rate of rice grains and straw (Ram et al., 2014). According to Tripathi et al. (1992), a formulation of soil organic matter with a high sulphur content was found to be sufficient in enhancing the potential for soil organism and plant physiological activities. Furthermore, the zinc concentration was the lowest (0.22±0.0057) in SK (S_1) and KK (S_1), moderate in DJUK, BSDK, KKDK, and DUK (0.66±0.088 to 0.75±0.014), and much higher in TUB (1.20±0.05). Zinc levels in soil decrease when nitrogen is added (Salim, 2002a). As per Westerman (1990) and Horneck et al. (2011), the available zinc concentration in the soil ranged from medium to high (0.22±0.00 to 1.20±0.05) in this analysis. Low yield circumstances, especially in onion crops, are caused by inadequate zinc concentration. In contrast, the use of biofertilizers neutralizes the zinc concentration (Khokhar, 2019) and increases crop yields (Dake et al., 2011; Manna & Maity, 2015; Babaleshwar et al., 2017). Copper concentrations currently range from moderate (0.72±0.00 to 0.93±0.01) in UDR_4 (S_2), DJUK, KPDK, KKDK, YUK, and DUK, to very low (0.26±0.01) in UDR_3 and exceeding 2.00±0.57 in BSDK. Plants primarily use copper (Cu) conductivity in soil to move cationic elements; in extremely saline soil conditions, copper and electrical conductivity are positively correlated. Copper concentrations in the studied coastal paddy soils ranged from 0.26±0.01 to 2.00±0.57, which are regarded as partially low and high, according to the data. A rice plant that has taken up the right amount of copper from the soil is considered healthy. However, Cu toxicity can sometimes negatively affect rice plant growth, including root and shoot development, as well as grain quality when elevated copper levels are present (Thounaojam et al., 2012; Htwe et al., 2020).

At 392.37±380.81, GKR_2 (S_1) has the highest available iron concentration, whilst UDR_3 has the lowest at 4.55±0.01. Additionally, India's forest and coastal soils are "Congo red" due to the high concentration of iron ores in them. An iron level of 4.55±0.012 to 392.37±380.81, as determined by soil analysis, is sufficient for plants and agricultural produce. Furthermore, soil microorganism biomass rises with soil iron (Fe) levels (Alghanmi & Jawad, 2019). In general, N:P:K in combination with Cu, Zn, and Fe increases plant crop yield via influencing stem strength and girth (Fouda, 2016). Samaranayake et al. (2012) state that low iron weakens stems and roots, while high iron is harmful to stem and root cells. In this experiment, manganese availability is highest in KPDK (15.83±0.01), moderate in $AKSR_2$ (S_2) (12.54±0.011), and lowest in UDR_3 (3.12 ± 0.0088). The amount of accessible manganese (Mn) in the soil increased from 3.12±0.008 to 15.83±0.01 in the analysis. Appearances of iron and zinc reduce the amount of Mn accessible in soils (Chaudhry & Wallace, 1976; Alam, 1982). Although manganese is essential for plant growth, an increasing amount of it may be harmful to plants (Rajput et al., 2021). Similarly, plants slow down photosynthesis when exposed to high Mn concentrations, especially in rice fields (Lidon & Teixeira, 2000a; 2000b).

Accordingly, the accessible boron concentration in TUB and KK (S_1) is generally available (0.62±0.00 to 0.75±0.01), with DJUK having the highest concentration (1.36±0.0057) and TMR_3 (S_2) having the lowest value (0.23±0.08). In summary, the micronutrient boron (B) concentrations in the soil samples of medium low to high in rice fields ranged from

0.23±0.088 to 1.36±0.0057. With an emphasis on the onion crop, Khokhar (2019) researched on boron concentrations to increase plant yield coinciding with other works (Smriti et al., 2002; Dake et al., 2011; Manna & Maity, 2015; Babaleshwar et al., 2017). This leads to a lack of mineral boron because the soils of the majority of countries have low levels of organic saturation and granular structure (Takkar et al., 1989; Razzaq & Rafiq, 1996; Borkakati & Takkar, 2000).

Principal component analysis, one of several statistical methods employed in this study, yielded six rotated components, PC1, PC2, PC3, PC4, PC5, and PC6, listed in Table 6. Thus, according to this parametric statistic, PC1 has the largest percentage of variance and cumulative percentage of all six components; in contrast to PC6, which has the highest eigenvalue, PC1's eigenvalue is lower than the other components'. With a variance percentage of 5.752, a cumulative percentage of 35.951, moisture content of 0.8, pH of 0.817, potassium as a macronutrient of 0.828, and micronutrients of calcium (0.866), magnesium (0.648), zinc (0.773), copper (0.812) and boron (0.756), PC1 is ideally balanced. Comparing these parameters to other parameters, they are the highest-grade parameters in this analysis. With an organic carbon content of 0.816 and a density of 0.087, the PC2 component is significantly higher than the other components.

Table 6. Principal component analysis of soil properties

Parameters	**Rotated component matrix**					
	PC1	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**
% of variance	**5.752**	1.953	1.502	1.241	1.178	1.102
Cumulative %	**35.952**	12.209	9.391	7.756	7.361	6.886
Eigen values	35.952	48.161	57.551	65.307	72.668	**79.554**
Moisture	**.811**	.222	.245	.007	-.194	.109
Density	-.242	**.087**	.049	-.886	-.009	.023
pH	**.817**	-.205	.258	.256	-.089	.218
EC (ds/m)	-.001	-.825	**.281**	.092	-.150	-.010
OC %	.180	**.816**	.297	.000	-.038	-.031
N (kg/ac)	.339	.072	-.084	-.238	**.782**	-.112
P (kg/ac)	.073	.117	-.719	**.405**	-.167	.082
K (kg/ac)	**.828**	-.049	-.213	.012	.142	-.018
Ca (ppm)	**.866**	-.191	.245	.199	-.072	-.183
Mg (ppm)	**.648**	.060	.277	.252	-.145	-.059
S (ppm)	-.047	-.015	-.042	-.007	-.013	**.962**
Zn (ppm)	**.773**	.227	-.178	.107	.072	-.247
Cu (ppm)	**.812**	.229	-.076	-.237	.195	.135
Fe (ppm)	-.398	.072	.144	.270	**.743**	.105
Mn (ppm)	.199	.080	**.721**	.146	-.071	.011
B (ppm)	**.756**	.366	.169	.230	.032	-.231

The cumulative percentage is 12.209, the variance percentage of 1.953, and the Eigen value as 48.161.

The PC3 data set shows that manganese (0.721) and electrical conductivity (0.281) are the most readily obtainable elements.

The eigen value is 57.551, the cumulative percentage as 9.391, and the variance percentage represents 1.502. With an eigen value of 65.307, a cumulative percentage of 7.756, and a percentage of variance of 1.241, PC4 has a higher phosphate content (0.405) than the other six components. The PC5 component's Eigen value has 72.668, its cumulative percentage is 7.361, and its variance percentage as 1.178. The remaining six components have modest levels, with the two highest being 0.782 nitrogen and 0.743 iron.

Finally, among the six components' soil parameters, PC6 has the highest content, explaining 1.102 percent of variance, 6.886 cumulative percentage, and 79.554 eigen value interaction with sulphur (0.962).

Figure 1 displays the results of principal component analysis, which shows both positive and negative correlation between the parameters in a biplot spanning four quadrants.

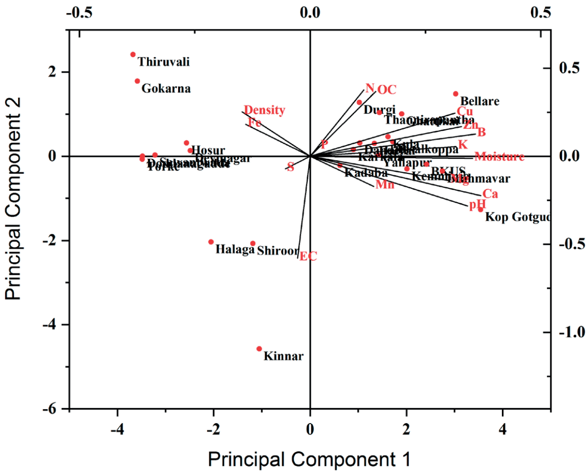

Figure 1. Principal component biplot analysis of 25 paddy field locations: correlations among physicochemical properties (moisture, density, pH, EC, OC, N, P, K, Cu, Fe, Mn, Mg, S, Zn, Ca and B)

It should be noted that the density is positively correlated with nitrogen (N), sulphur (S), and iron (Fe), but negatively correlated with organic carbon (OC), copper (Cu), zinc (Zn), boron (B), potassium (K), moisture, phosphate (P), magnesium (Mg), manganese (Mn), electrical conductivity (EC), and pH in two components. In contrast to nitrogen, organic carbon, copper, zinc, boron, phosphate, potassium, magnesium, manganese, calcium, pH, and EC, only density and sulphur (S) show a positive link with iron.

Sulphur is positively correlated with density, iron, and EC, but negatively correlated with N, OC, Cu, Zn, B, K, P, moisture, Mg, Mn, Ca and pH. Nitrogen, organic carbon, copper, zinc, boron, phosphate, potassium, magnesium, manganese, calcium, moisture and pH are all positively connected, according to this association.

On the other hand, there is a negative correlation between density, iron, sulphur, and EC. Magnesium, Mn, Ca and pH, on the other hand, correlate favourably with N, OC, Cu, Zn, B, P, K, moisture and EC and negatively with S, Fe, and density. However, EC and pH are positively correlated, as are all macro and micro variables except density, Fe and S.

A higher pH concentration is invariably the result of a higher moisture content, as indicated by the basic expression of the Pearson correlation on the physico-chemical characteristics of soil in Table 7.

Organic carbon and current electrical conductivity are negatively correlated. Potassium and moisture are positively correlated. There is also a positive correlation between calcium and moisture, pH, and potassium. There is a positive correlation between magnesium and moisture, pH, and calcium. Zinc also has a positive relationship with moisture, pH, calcium, magnesium, and potassium. Copper is positively correlated with moisture, pH, nitrogen, potassium, calcium, magnesium, and zinc. Finally, boron is positively correlated with organic carbon, pH, calcium, magnesium, zinc, copper, and moisture. Therefore, whereas negativity should either be constant or result from holdings that are equal to or less than one, positive always influences growing parameters.

Figure 2 illustrates the experimental cluster analysis of soil characteristics under four groups in a graph, with sites assigned based on virtually identical qualitative features with geography. The seven locations that comprise cluster one are Shiroor, Kop Gotgudi, Kemminje, Kadaba, Halyal, Brahmavar, and BKUS. The only location that comprises the second cluster is Gokarna. The third cluster has thirteen locations, including Yallapur, Torke, Thiruvail, Sawantwada, Kula, Kinnar, Karkala, Hosur, Halaga, Durgi, Doddanagudde, Devinagar, and Dandeli. Finally, the fourth cluster consists of four locations: Bellare, Bisalkoppa, Ghattikai, and Thannirapantha.

Table 7. Pearson correlations among physical and chemical properties of soil

Soil properties	Moisture	Density	pH	EC	OC	N	P	K	Ca	Mg	S	Zn	Cu	Fe	Mn	B
Moisture	1															
Density	-.188	1														
pH	.708**	-.386	1													
EC	-.142	-.099	.244	1												
OC	.297	.044	.063	-.420*	1											
N	.049	.085	.069	-.205	.093	1										
P	-.089	-.274	.004	-.146	-.070	-.059	1									
K	.499*	-.257	.641**	-.051	.121	.345	.135	1								
Ca	.734**	-.388	.794**	.283	.112	.172	-.064	.628**	1							
Mg	.549**	-.247	.679**	-.014	.182	.027	-.001	.391	.676**	1						
S	.026	.009	.117	.017	-.039	-.086	.058	-.067	-.201	-.108	1					
Zn	.611**	-.266	.482*	-.276	.219	.368	.170	.648**	.649**	.471*	-.195	1				
Cu	.686**	.036	.536**	-.218	.291	.438*	.085	.666**	.548**	.412*	.025	.515**	1			
Fe	-.335	-.031	-.250	-.099	-.030	.206	-.124	-.267	-.323	-.194	.048	-.276	-.171	1		
Mn	.324	-.120	.370	.121	.254	.064	-.222	.044	.302	.294	-.050	.103	.072	-.102	1	
B	.712**	-.359	.555**	-.124	.544**	.234	.022	.535**	.725**	.570**	-.247	.679**	.656**	-.166	.206	1

*At the 0.05 level (2-tailed), the correlation is significant.
**At the 0.01 level (2-tailed), the correlation is significant.

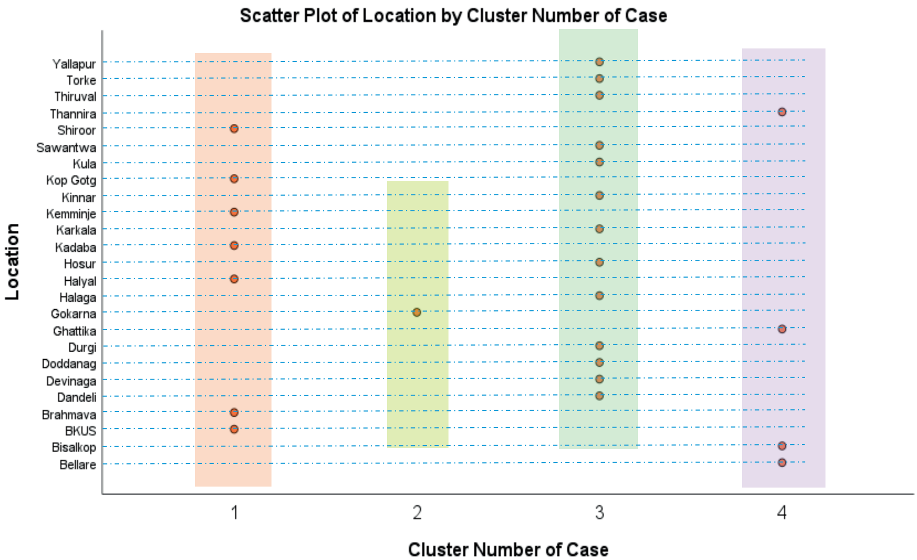

Figure 2. Using the k-means clustering method, a cluster diagram was created, and cluster number case scores were used to distribute locations

CONCLUSIONS

Coastal Karnataka's soil type advantageous because of a sufficient scope of agro-climatic influence, which boosts coastal crop yields. A tolerable pH and a supportive EC with micro and macronutrients are essential for rice growing, which is the agricultural technique that makes Coastal Karnataka popular for its heritage and culture. This is the case for Coastal Karnataka, where large-scale distributed algal biomass in marine habitats supports pH and OC parameters in extremely saline soil environments linked to electrical conductivity, and where synthetic fertilization has allowed soils to become acidic. Furthermore, the high nitrogen concentration is a crucial macronutrient for rice crops. For this subject, comfortable concentrations of nitrogen availability may be supplemented by N fertilization (inorganic form); rising global temperatures generate global warming processes, which in turn cause ozone depletion. The nutrients needed by the plant are supplied by N:P:K fertilization in this parametric analysis. However, excessive application of organic and manure fertilizers in combination with synthetic fertilizers is toxic to plants; as a result, agricultural crops that contain beneficial soil microorganisms are stressed and by these physiological and mechanical activities in the agricultural environment. Due to synthetic fertilization or biofertilization (microorganisms), soil phosphate and potassium in this examination also seem to be in good condition. This is because cyanobacteria are naturally occurring P-sinkers in agricultural soils. Lastly, all other micronutrients were available in good concentrations and had positive correlations with pH, EC, OC, and macronutrients, with the exception of sulphur, which had a negative correlation and was present in delicate levels. The interactions between beneficial soil microbes and plants in the soil profile, and soil and agricultural economics are the only focus of this study.

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DYNAMICS OF SOIL MOISTURE UNDER THE MAIN FIELD CROPS (WHEAT, MAIZE, SUNFLOWER, PASTURE) ON A CLAY-LOAM SOIL IN THE SOUTH-WEST OF ROMANIA

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Abstract

The water consumption of plants is influenced by species, variety, vegetation periods, the degree of root development and intensified by the type of soil and the type of practical agricultural work. This paper presents the evolution soil moisture in 2022 year, depending on the technology applied to wheat, corn, sunflower and pasture crops. Analysing the evolution of soil moisture, under the 4 crops, during the agricultural year, it was possible to observe differences in soil moisture between the applied technologies and, of course, also between crops. For the analysed area, respectively on a clay loam soil, the utilization of the water from the precipitation is better achieved in the conditions of the preparation of the germinal bed by plowing.

Key words*: soil moisture, tillage, precipitation.*

INTRODUCTION

Soil moisture (SM) is a parameter that in agricultural technology should be very well known and tracked.

Globally there has been since 2009, the International Soil Moisture Network (ISMN) which was initiated to serve as a centralized facility of soil moisture (SM) data available worldwide (Dorigo et al., 2021). The ISMN gathers SM measurements collected by a multitude of organizations, harmonizes them across international scientific units, and stores them in a database. Users can freely retrieve data from this database through an online web portal (https://ismn.earth/en/) (Dorigo et al., 2021).

At national level, there is the Romanian Soil Moisture Network (RSMN), managed by the Romanian National Meteorological Administration, consisting of 19 stations homogeneously distributed throughout Romania, for 13 stations the data are also available in the ISMN database. The network aims to create a framework for the assessment of current and future soil surface moisture products (0-0.05m) obtained through satellite monitoring (Ortenzi et al., 2024). However, there is limited coverage due to the high variability of SM, it is difficult to obtain estimates of soil moisture over large areas, as well as missing data for certain demarcated areas.

SM directly or indirectly influences a series of actions/processes on soil and plants (Ortenzi et al., 2024; Wang et al., 2019), such as: erosion (wind, water, harvesting, landslides), soil biodiversity (Babaeian et al., 2019), soil compaction, soil salinization, soil contamination, soil nutrients, soil pH (Calistru et al., 2024) carbon content (Trugman et al., 2018), desertification, soil degradation, drought (Gu et al., 2019), flooding, evapotranspiration, crop yields (Babaeian et al., 2019), Plant health, applied agrotechnics (Partal & Oltenacu, 2022), production costs (Maleknia et al., 2023), etc.

The movement of water in the soil determines how nutrients reach the disposition of the roots both in a tilled soil and in a soil covered by natural grasslands (Bălan et al., 2024 a, b). The movement of water from the surface of the soil, of excess water (free water), when the soil moisture is above the value of the field capacity has a negative influence, in addition to the phenomenon of erosion and leaching and there are constant changes in terms of morphological and physical properties, hydro-physical, chemical and biological factors, compared to soils not affected by this phenomenon (Bălan et al., 2024b; Popescu et al., 2024).

Due to the importance and extensive use of SM information, Numerous measurement and monitoring capabilities have been developed in recent years (electromagnetic sensors, tensiometer, reflectometry, cosmic ray neutron, gamma ray, neutron probe, remote sensing,) From one-point measurement to global determinations (Babaeian et al., 2019). The number of SM networks continues to grow, but most of these networks have evolved without international standardization and thus present challenges for validating the correct estimation of SM (Caldwell et al., 2022)

In general, gravimetric measurements and electromagnetic (EM) sensor arrays are considered to be the most reliable means for the direct and accurate determination of SM moisture in the soil profile (Babaeian et al., 2019).

Soil moisture, or soil water content (SWC), can be determined using electromagnetic sensors buried in the ground, which infer SWC from an electromagnetic response. This signal can vary considerably depending on the texture and mineralogy of the soil, the salinity of the soil or the electrical conductivity and temperature of the soil; Each of these can have different impacts depending on the sensor technology, in addition, poor ground contact and sensor degradation can affect the quality of these readings over time (Caldwell et al., 2022).

The soil retains water in a mixture, called the soil solution, through the action of surface tension that attracts water molecules to soil particles.

The moisture content of the soil is influenced by several factors: the hydraulic properties of the soil, the types of soil texture, the slope, surface infiltration and runoff, and mainly the evolution of the climate (Maleknia et al., 2023).

A main feature of the soil that influences the water regime is water permeability, in soils with good water permeability, water infiltrates and can be kept by the soil at a great depth (loam soils), while soils with low water permeability (clay soils) the soil is soaked with water, puddles appear on the surface, creating anaerobic conditions (Grumeza, 2005).

In recent years, the evolution of the climate is very varied, so that the summer months become drier, rainfall is uneven, and extreme weather causes large production losses. At the Romanian level, Ontel et al. (2021) determined through a series of indices (soil moisture anomaly, soil water index, standardized precipitation index, land surface temperature anomaly, normalized difference vegetation index anomaly) the following years as dry: 2007, 2011-2012 excessively dry, and the years 2009, 2019 and 2020 as dry years. Data demonstrate a recurrence period of 3 events at 10 years.

For the period 2000-2013, throughout the country, and thus in the studied area, S-V Romanian Plain, there were five years of extreme drought and three years of excessive rains (Constantin et al., 2015).

Most studies and research on ensuring the water needs for field crops show that the most important role is played by rainfall. Thus, the researches carried out by Popescu (2001) in the period 1996-1998, by Pandrea (2012) in 2008-2010 with irrigated wheat, corn and sunflower crops showed that rainwater is the major source of water supply from soil and plants, ranging from 50% to over 90%. It is clear that in order to use rainwater effectively, appropriate technologies must be applied that lead to better soil water retention, plant water supply and minimal evaporation.

MATERIALS AND METHODS

The research and determinations carried out in this work aim to follow the dynamics of soil moisture. The determinations were made in 2022 on 6 variants for the following field crops: wheat, corn, sunflower and natural pasture.

The working method consisted of determining the soil water content (SWC) by the gravimetric method. Soil samples were taken from a depth of 20 cm during the vegetation period (Table 2). Gravimetric water content is measured by weighing a soil sample, drying the sample to remove the water, then weighing the dried soil.

As outlined above, this method is the safest for determining soil moisture (SM).

The soil belongs to the Chernisols class, the vermic Chernozem type, characterized by:

- loam-clay texture (34-36% clay);
- humus content is small 2.5-3%;
- soil pH 6.2-6.7;

- total porosity 50-52% at start 0-40 cm;
- wilting coefficient (WC) in layer 0-20 is 11.4-12.9%, and in layer 0-80 it is 12.8-13.5% (100-135 mm);
- field capacity (FC) falls in the middle class with a value of 23.1-24.5%, in the 0-20 cm layer, and in the 0-80 cm layer it is 23.1-23.4% (230-260 mm);
- total water capacity is 29.3%;
- bulk density is 1.26-1.31 (g/cm^3).

For the interpretation of the resulting humidity data, field capacity (FC), permanent wilting (WC) and minimum ceiling at 1/2 (MC) were also determined in advance. SWC data has also been converted to mm.

The working methods presented were made for the following variants (crops and agricultural technologies):

a.1) Wheat (Wht.1): – The preparation of the seedbed was carried out with a disc harrow (10-15 cm), the cultivated area was 49 ha, the previous crop was sunflower. The preparation of the seedbed consisted of 3 passes with the disc harrow, one immediately after harvesting (August 22, 2021), and the others during the autumn before sowing. Before the last processing with the disc, the basic fertilization was carried out. Sowing was carried out on October 10-12, 2021 at a depth of 4-5 cm. The harvest was done at a humidity of 13% of the grains, between July 12-15, 2022 obtaining an average yield of 6280 kg/ha.

a.2) Wheat (Wht.2): – ploughed at a depth of 22-25 cm plus two shredding and leveling works of the soil, the cultivated area was 55 ha. The predecessor plant was rapeseed. Between June 21-34, 2021, the entire surface was ploughed at a depth of 22-25, and immediately afterwards a disc harrow pass was made. On October 7, 2021, basic fertilization was carried out, followed by soil tillage with a combiner, and sowing was done between October 10-12, 2021. The harvest was done at a humidity of 14% of the grains, between July 12-15, 2022 with a yield of 6740 kg/ha.

b.1) Maize (Mz.1): – plowing at 30 cm depth plus seedbed cultivators, the cultivated area was 41 ha. The plowing was done at a depth of 30 cm between November 18-22, 2021. The previous harvest was sunflower. Spring seedbed preparation consisted of two passes with seedbed growers from April 3 to 5, 2022. The signing started between April 13-15, 2022 with a density of 60000 plants/ha, at 70 cm between the rows and at 7-8 cm depth. There was no weed infestation because herbicide was carried out both pre-emergent and in vegetation. On May 12, a mechanical sweep was made. The harvest was done between September 15-16, 2022, obtaining a yield of 4410 kg/ha corn grains with 15% moisture.

b.2) Maize (Mz.2): – plowing at 20-25 cm plus disc harrow, the cultivated area was 44 ha. The previous harvest was wheat, and immediately after its harvest, a ploughing was made at 20-25 cm between July 18-22, 2021. The preparation of the seedbed consisted of a disc harrow processing on 1-2 March 2022 and then a processing on 4-5 April 2022. Sowing was carried out between April 13-15, 2022 with a density of 60000 plants/ha, at 70 cm between the rows and 7-8 cm deep. There was no weed infestation because herbicide was carried out both pre-emergent and in vegetation. No more soil tillage was carried out during the vegetation period. The harvest was carried out mechanically between September 15-16, 2022 with a yield of 4850 kg/ha at 15.5% humidity.

c) Sunflower (Sfl.): – plowing at 24-27 cm plus disk harrow, the cropped surface was of 45 ha. The previous crop was wheat. The plowing was performed at 24-27 cm between 18-22 July 2021. The seedbed preparation was performed by disk harrow tillage at 1-5 march 2022 and then, at 28 march there was made tillage by disk harrow. Sowing was performed at 4-6 April 2022 with a density of 58,000 plants/ha, at 70 cm between rows and 4-5 cm depth. There was no weed infestation because herbicide was carried out both pre-emergent and in vegetation. No more soil tillage was carried out during the vegetation period. The harvesting started at 5 September 2022, at 9% moisture of sunflower kernels with an yield of 2740 kg/ha.

d) Natural pasture (Np) – farmed by grazing with animals, cattle and sheep.

In order to analyze soil moisture data, it is necessary to know the climatic data, especially rainfall and temperature. In this case, the climatic data of the 2021-2022 agricultural year from the ARDS-Caracal weather station, Olt County, were used.

In addition, the rainfall recorded in the agricultural year 2021-2022 was corrected with that recorded in the field.
With the help of these data, an improved Walter-Lieth climate diagram (Walter et al., 1960) was made. This chart provides a generalized representation of temperature and precipitation values for the time of year. The temperature and precipitation scales are fixed in the chart in a ratio of 1:2 and 1:3, making it easy to compare different periods.

RESULTS AND DISCUSSIONS

The land where the determinations were made is located in the Oltenia Plain, in the western part of the Romanian Plain and belongs to a farm in the south of Olt County, in the southwestern region of Romania.
The dominant relief is flat, on certain areas with low slopes of 2-5%, the altitude is 58 m, the groundwater is found at 3-5 m, and from a hydrological point of view the land belongs to the hydrographic basin of the Olt River, with influences from the Danube itself. The land is located on the first terrace of the Danube River, on the left side, about 20 km away.
The hydrological regime of the soil depends on a number of external factors, climate, relief, groundwater intake. Among these factors, the climate has a special role. Climate acts on SM both positively through precipitation and negatively through evaporation and transpiration, along with the other climatic elements: light, heat/temperature, solar radiation, wind.
From the meteorological data of the southern area of Olt County (Table 1) it can be seen that the average annual temperature is 10.61ºC. The lowest temperature is recorded in January (-3.0ºC), and the highest is recorded in July (22.7ºC) and August (21.9ºC). From the determination of the multiannual average values, it can be seen that the extreme values recorded both negative and positive values.
The average annual temperature was 12.43ºC (Table 1), so there was a pronounced warming compared to the multiannual average of 1.99ºC. Except for October, March and April, all other months of the 2021-2022 agricultural year had temperatures above the multiannual averages, even the winter months. It can be seen that this winter the average temperatures did not reach negative values.

Table 1. The average temperatures and rainfall during 2021-2022 agricultural year

Months													
	X	XI	XII	I	II	III	IV	V	VI	VII	VIII	IX	Annual average
Temperature (ºC)													
2021–2022	10,18	7,32	2,57	1,96	4,13	4,48	11,11	18,17	23,03	25,42	24,8	18	12,60
Multiannual	11,3	4,9	-0,5	-3,0	-0,6	4,8	11,2	16,6	20,5	22,7	21,9	17,6	10,61
Deviation	-1,12	2,42	3,07	4,96	4,73	-0,32	-0,09	1,57	2,53	2,72	2,90	0,40	1,99
Rainfall (mm)													
2021 - 2022	101,4	28,0	60,8	19,2	4,8	13,2	77,8	44,6	14,2	30	50,2	55,4	499,60
Multiannual	40.4	40.3	39.4	33.3	30.4	34.9	43.6	64.9	67.0	52.9	50.7	39.6	537,4
Deviation	61,00	-12,30	21,40	-14,10	-25,60	-21,70	34,20	-20,30	-52,80	-22,90	-0,50	15,80	-37,80

The rainfall data (Table 1) for the agricultural year 2021-2022 are spatio-temporally fluctuating and unevenly distributed, thus values between 4.8 mm (February 2022) and 101.4 mm (October 2021) were recorded. During the vegetation period (2022), rainfall ranged from 14.2 mm in June to 77.8 mm in April, 44.6 mm in May, 30 mm in July, 50.2 mm in August and 55.4 mm in September. All summer months record values below the multi-annual average, with a significant deficit for certain months (June).
The total amount of precipitation in the agricultural year 2021-2022 is lower than the sum of the multiannual average (499.60 mm compared to 537.4 mm-My.a), there is a deficit of 37.8 mm (Table 1, Figure 1).
The distribution of precipitation during the vegetation period was much smaller compared to the multiannual average (Table 1).

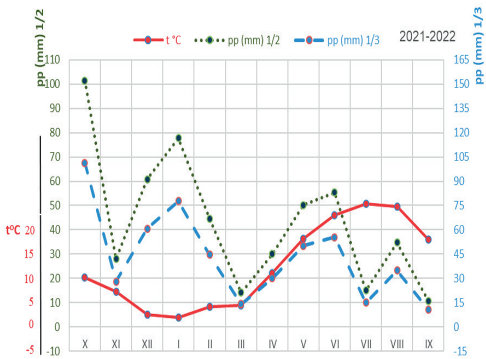

Figure 1. Climate diagram for 2021-2022 agricultural year

The climate data recorded in this case are also confirmed by other researchers, who note the same trend of increasing temperature and uneven distribution of precipitation.

In a long-term study of the evolution of the weather in Romania (temperature over 122 years, and precipitation over 146 years) it was shown that the average temperature over the entire agricultural year, but also during the vegetation period, increased from 10.3°C in 1897-1898 and reaching 12.7°C, and precipitation tends to decrease values, especially during the vegetation period (Șumuleac L. et al., 2020).

In case of an increase in average temperatures by 2ºC, the water requirement for corn will be 61% above the current requirement, and in the case of a temperature increase of 5ºC, the water requirement will be 74% above the current requirement, in an irrigated system (Nițu A. et al., 2023).

By translating these climatic data into Walter-Lieth charts (Figure 1), it is possible to identify periods of the year with excess moisture or moisture deficit. Thus, as can be seen, it can be seen that the more significant rainfall in the autumn of 2021 ensured a high level of humidity, and the summer period of 2022 is dry.

In most previous years, plants suffered from a lack of water in the soil, but 2021 was a rainy year, with rainfall reaching 711.6 mm compared to the multiannual average of 537.4 mm (Cioboata M. et al., 2024).

At first glance, this rainfall could be sufficient for crops, especially wheat, in terms of ensuring the initial water supply.

In the studied area, for the depth of 20 cm, the field capacity (FC) is 23.4% (59.9 mm), the wilting coefficient (WC) is 12.9% (33.02 mm), and the minimum ceiling (MC) calculated at 1/2 of the active humidity range is 18.15% (46.46 mm).

The evolution of soil moisture (SM) is dependent on the amount of precipitation. Soil samples were taken for the 4 crops analyzed from April 12, 2022 to September 15.

Figure 2 shows the SM dynamics for the 2 technological variants of the wheat crop Wht.1 (DH), Wht.2 (Pl,dh). In April, the SM level for both variants is above the MC (minimum ceiling), in May when wheat has the highest water consumption, the SM drops below MC, but does not reach the WC. The rainfall recorded at the end of May determined the recovery of MS, right at the beginning of June, before harvesting, when the wheat was towards maturity. The SM was higher in the Wht.2 (Pl,dh) variant, on all three months of wheat vegetation, but continued after harvesting. On the analyzed soil, the preparation of the seedbed by ploughing plus disc harrow favored the storage of water in the soil.

Figure 2. Soil moisture dynamics in winter wheat crop (mm) and after harvest

In the maize crop, for both variants, Mz.1 (Pl30) and Mz.2 (Pl25) SM decreases from sowing to harvest, the same trend for sunflower (Figure 3).

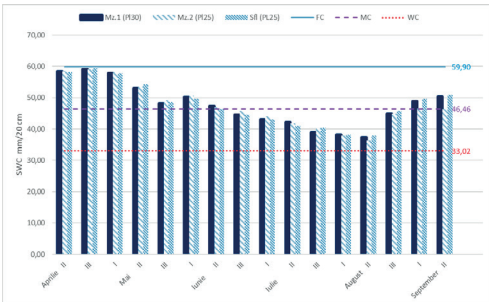

Figure 3. Soil moisture dynamics in maize and sunflower crops (mm)

In April, when both crops were established, rainfall was also recorded, and the SM at a depth of 20 cm was very close to the FC, 58-59 mm. The SM in May for both crops has values above the MC, starting with the last decade of June, the SM value decreases below the MC, and in mid-August the SM approaches the WC value. Both cultures Mz.1 (Pl30), Mz.2 (Pl25) and Sfl. (Pl25) have an SM of 36-37 mm, and the WC is 33.02 mm. The rainfall recorded in the second half of August determines the recovery of SM.

As for the dynamics of soil moisture (SM) on pasture, it can be seen that it depends on both precipitation and air temperatures.

The highest SM values were recorded in April, May and the first half of June, when they are above the MC (Figure 4). The next period is characterized by a very low SM level on grasslands, until the last determination SM was below MC. This is also due to high air temperatures.

Figure 4. Dynamics of soil moisture with pasture (mm)

The analysis of SM dynamics (Table 2 and Figure 5) for the four researched crops and 6 variants for determining soil moisture, during 2022 presents the following aspects:

- at the beginning of the vegetation period, when the first determination was made, on 12.04.2022, it can be seen that the soil water reserve is high for all 6 soil samples, being sufficient and normal for this period;
- at the first determination of SM, the highest values are for corn and sunflower soils (22.7-22.8%), wheat soils have 20.8-21.4%;
- at the next determination, on 22.04.2022, there are significant differences between crops. Wheat has the lowest soil moisture (19.4% and 19.8%) compared to the other crops: corn - 23.1%, sunflower 23.2%, meadow 21.7%;
- the SM determinations in May are between 17.2-22.6%, the wheat variants have the lowest values, and for Wht.1 (DH) and Wht.2 (Pl,dh) the SM of the second decade and the third decade of May is below the MC (in wheat the wilting phenomenon is observed). In the third decade of May, all variants have SM very close to MC (18.8-19.5%);
- the low SM values in May are due to the lack of precipitation, between May 1 and May 26 only 8.4 mm was recorded, on May 27-28 there were 35.2 mm of precipitation, which influenced the subsequent SM values;
- in June there are only 14.2 mm of precipitation, which led to a decrease in SM for all variants. In the first and second decade of June, the SM is between 18.0-19.8%, being very close to the MC (18.15%), in the last decade the SM for all variants is below the MC, the values being between 16.2-17.9%;
- the SM values in July are definitely the most influenced by climatic factors. The total rainfall this month was 30 mm, with a maximum of 13.6 mm on July 26. SM, the average air temperature recorded the highest values (25.42^{0}C). In this month, the SM for all variants is below the value of the minimum ceiling (MC) of 18.15%, the SM is between 13.9-17.2%. Np has the lowest SM values, close to permanent wilting (12.9%);
- in August the SM remains below the MC, values between 13.9-17.9% are determined, except for the harvested wheat soils, where the SM is 18.2-18.4% in the third decade of August, a value influenced by 21.2 mm rainfall on August 22 and probably by the plant residues on the soil surface;
- the latest determinations of the SM indicate slight increases close to the MC, even above, values influenced by the rainfall at the end of August and in the first decade of September (38.8 mm). In the soils of Np, the SM values remain below the MC.

From the analysis of the "crop plant" factor, it can be seen that there are differences between them. SM varies between crops depending on the vegetation stage. This being a certain thing. According to previous research, water consumption (ET) is different. Thus, for the research area, Nistor A. et al. (2017) determined a water consumption, for the crops followed in this work, in the soil layer 0-75 cm, as follows (m^3/ha/day): for wheat 29 April, 41 May, for corn 18 in April, 26 in May, 39 in June, 59 in July, 42 in August, 24 in September, and for sunflowers 16 in April, 35 in May, 56 in June, 58 in July and 26 in August.

Table 2. Evolution of soil moisture (SM) during the vegetation period, sampling data, researched variants

	April		May			June			Juli			August			September	
Decad (sampling of day)	II (12)	III (22)	I (3)	II (13)	III (23)	I (2)	II (12)	III (23)	I (4)	II (14)	III (25)	I (5)	II (16)	III (26)	I (5)	II (14)
Wht.1(DH)	20.8	19.4	18.8	17.9	17.2	18.8	18.0	16.2	16.1	15.3	15.3	15.1	15.1	18.2	20.1	19.7
Wht.2 (Pl,dh)	21.4	19.8	19.2	18.1	17.6	19.0	18.8	17.1	16.6	16.1	15.7	15.6	15.1	18.4	20.3	19.9
Mz.1 (Pl30)	22.8	23.1	22.6	20.7	18.8	19.6	18.5	17.4	16.8	16.5	15.2	14.9	14.6	17.5	19.1	19.7
Mz.2 (Pl25)	22.8	23.1	22.7	20.9	19.2	19.8	18.4	17.9	17.2	16.3	15.7	14.8	14.2	17.5	18.2	18.1
Sfl (PL25)	22.7	23.2	22.6	21.2	19.0	19.4	18.1	17.4	16.8	16.0	15.8	14.9	14.8	17.9	19.4	19.9
Np	21.6	21.7	20.5	19.9	19.5	19.8	18.9	17.2	13.9	15.2	14.8	14.8	13.9	15.3	15.9	16.8
FC = 23.40% = 599.04 m^3/ha = 59.9 mm, WC = 12.90% = 330.24 m^3/ha = 33.02 mm, MC = 18.15% = 464.64 m^3/ha = 46.46 mm																
Wht.1 (DH) – wheat/ disc harrow; Wht.2 (Pl,dh) – wheat/ploughing + disc harrow; Mz.1 (Pl30) – maize/ploughing at 30 cm; Mz.2 (Pl25) – maize/ploughing at 25 cm; Sfl (PL25) – sunflower/ploughing at 25 cm; Np – natural grassland.																

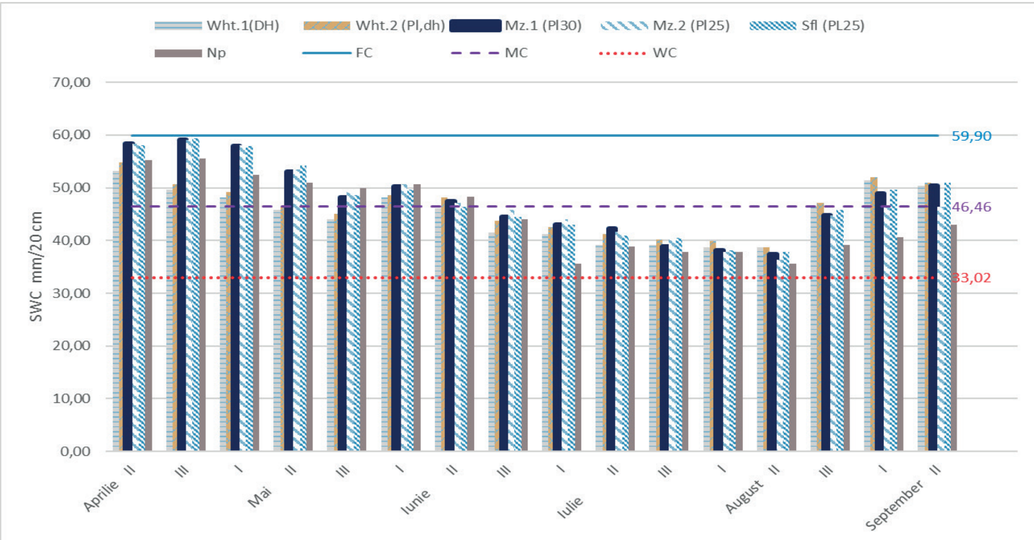

Figure 5. Centralized soil moisture dynamics

Also from the point of view of crops, it is worth analysing the fact that in June after the wheat harvest, the SM was higher in the plots (variants) covered by vegetation, respectively corn and sunflower, compared to plots on which there was wheat and Np.

From the analysis of the influence of cultivation technology on the evolution of soil moisture (SM) it is found (Table 2):

- for the wheat variants Wht.1 (DH), Wht.2 (Pl,dh) it can be observed that the variant Wht.2 (Pl,dh) where the seedbed preparation was carried out by ploughing with the pulse plough with disc harrow passes, SM has higher values compared to Wht.1 (DH) where the seedbed preparation was carried out only by disc harrow passages;
- at the first determinations of SM, at the beginning of vegetation, the lowest values were for the Wht.1(DH) variant (20.8%) and for Np (uncultivated land) (21.6%);
- the determinations after the wheat harvest indicate higher values also for plot Wht.2 (Pl,dh), compared to plot Wht.1 (Pl,DH);

- the tillage of the Mz.1 (Pl30), Mz.2 (Pl25) maize variants differs in that at Mz.1 (Pl30) the surface soil is better shredded, due to the use of seedbed cultivators, compared to the other variant where the disc harrow was used;
- for the Mz.1 (Pl30), Mz.2 (Pl25) maize variants, the SM values are very close, slightly higher at Mz.2 (Pl25);
- the sunflower variant Sfl (PL25) where the tillage was ploughed plus the disc harrow records SM values close to the corn varieties, with small differences during the vegetation period;
- the natural grassland variant (Np) registers the lowest SM values;
- in the Np variant, the lowest value (13.9%) of SM close to the WC value (12.9%) was determined;
- from the comparisons of SM between Np and the other variants where the soil was tilled, the conclusion can be drawn that the tillage of the soil on the surface can prevent evaporation.

CONCLUSIONS

From the aspects presented during the work, several conclusions and interpretations can be drawn, as well as perspectives for carrying out other research.

The most relevant conclusions are summarized.

The year 2022 in which the research was carried out was a dry year, recording rainfall below the multiannual average (-37.8 mm), with extremely hot summer months and very low rainfall in quantity. The average annual temperature was 12.6ºC, 1.99ºC above the multiannual average.

The level of precipitation directly influences the evolution of soil moisture, both during vegetation and before the vegetation season.

The accumulation of precipitation in autumn and winter can influence the soil moisture (SM) in the vegetation period. This explains the fact that the variants where the soil was ploughed, the storage of water in the soil was favoured, and the result was by determining higher values of SM as well as higher productions.

The vegetation development of crops causes a higher water consumption, but they also have a role in mitigating the evaporation of water from the soil surface, through shading, soil cover.

Soil tillage most of the time causes the loss of water from the soil, but there are situations when a good infiltration of water into the soil is subsequently found, Or situations when the soil is shredded very finely and the infiltration slows down.

It should be noted that the physical properties of the soil, the agricultural equipment used, the time of execution of agricultural works influence the infiltration and maintenance of water in the soil in the short and long term.

From soil moisture data, as well as yield results, we can conclude that the best option was soil preparation by ploughing.

Determination of SM at the surface does not provide clear information on the water consumption of crops

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RESEARCHES ON WATER AVAILABILITY IN FUNCTION OF SOIL TILLAGES

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Abstract

We have researched the water lose in conventional, no-till with and without mulch layer in vegetation vessels and we have found that the upper layer in no-till without mulch layer system conducted to lose of much more water which results in poorer plant development. We have considered that the mulch layer prevents the water lose and improve the better root growing and plant developing. The conventional tillage as well as the minimum tillage helps creating an upper layer with larger pores which slow the water loses, too. Better results of minimal tillage can also be explained by better water keeping due to the loosened upper 5-7 cm of soil. Our conclusion is the need to manage the residue layer in order to reduce water loss which results in better root growth, plant development and better yield than conventional system in high evaporative climates.

Key words*: no-till, mulch, water.*

INTRODUCTION

Like other areas of human activity, agriculture is evolving. If for thousands of years people have cultivated the land by using plowing as a basic work, in recent decades a new method has emerged, namely that of sowing directly into the not tilled soil. It seems, at first glance, inconceivable; against the customs of our ancestors, but the results prove that, through this technique, the soil maintains its fertility and the productions are even higher. In addition, expenses are much reduced, a farmer can work a larger area and earn better.

It is true that research in this field was forced by the oil crises of the 1970s, but this has proven beneficial. The first were American researchers from the universities of Kentucky, Indiana and Ohio who still have the oldest experiences mounted on the topic of no-till.

The soil has only been vigorously tilled by plowing very recently, and plants have been able to grow in the artificially tilled soil since they first appeared on Earth.

These conditions, however, presupposed the existence of a layer of plant debris left over from previous years, in various stages of physical breakdown and microbiological decomposition.

By trying to crop the land without tillage but also without the layer of plant debris, we will create the conditions for the formation of a compacted superficial layer resulting from self-compaction – the basic tillage and preparation of the germination bed applied for decades have led to the fragmentation of soil aggregates and their more compact placement – and the action of rain (Figure 3).

This layer, having lower capillarity than the one in depth, extracts water from the deeper layers, from the active root zone, and loses it through evaporation; in addition, as it dries, its cohesion increases and the root can no longer grow normally in this layer.

No longer able to explore a large volume of soil and no longer having sufficient water, the access of oxygen being, also, hindered by the reduced spaces through which air exchange takes place, the plants growing in these conditions have given extremely low yields.

In order to grow plants without doing soil tillage, which is considered by many researchers abroad to be unnecessary and harmful, the mulch layer on the soil surface is essential. Its role, first and foremost, is to maintain water in the soil and, hence, the advantages but also the disadvantages that arose:

- decrease in soil cohesion and facilitation of root growth;
- improvement of plant water supply;
- increase in the availability of mineral elements;

- formation of a stable hydrological structure;
- slowing down the rate of decomposition of plant resources and the gradual release of nutrients;
- reduction of soil temperature in the summer and reduction of amplitudes between day and night;
- increase in the number of living things in the soil;
- radical decrease in erosion by water or wind;
- reduction of humus losses and increase in soil fertility.

The disadvantages consist of:
- decrease in soil temperature at sowing;
- acidification of the superficial soil layer due to leaching and the predominance of fungi in the decomposition of organic matter;
- increase in the proportion of large, airy spaces within the soil porosity, with effects on the leaching of substances from the soil solution;
- decrease in the soil's water capacity by reducing the capillary spaces that retain useful water;
- technological difficulties in creating this layer;
- the need to change agricultural machinery.

The most important advantages of this system are:
- lower labor requirements;
- higher financial benefits;
- increased soil fertility and reduced erosion (https://istro.org/; http://www.rolf-derpsch.com/en/no-till/; https://conservationagriculture.org/app/uploads/2019/02/STEPS-TO-NO-TILL-ADOPTION-R.-DERPSH.pdf; https://www.no-tillfarmer.com/articles/12148-worlds-longest-continuing-no-till-plots-at-ohio-state-hit-60-years; https://en.wikipedia.org/wiki/No-till_farming; https://pt.wikipedia.org/wiki/Plantio_direto; https://journals.usamvcluj.ro/index.php/promediu/article/view/11870; Triplett G. B. et al., 2007).

The negative effects of intensive and repeated soil tillage on humus content, erosion, water infiltration, flora and fauna, and nutrient loss lead to physical, chemical, and biological degradation of the soil, which in turn leads to lower yields over time and reduced soil productivity.

The introduction of no till requires, first of all, good documentation of experimental results and farmers' experience in different pedoclimatic conditions, the availability of good and cheap herbicides, suitable agricultural machinery, the use of rational rotations that also include green manures or cover crops to create the mulch layer, and a change in mentality.

This new technology is not a fashion but will be imposed by economic and ecological needs. Romania, which has a temperate climate, similar to that of the USA, Argentina, Chile, Paraguay, etc. must follow their example and start research that will lead to the adoption of this new agricultural system (Table 1).

If the first attempts of our researchers failed due to the lack of suitable agricultural machinery and herbicides, now is the time to revitalize this research cause there are very good and cheap herbicides and agricultural machinery, for a start, these can be imported. We need, in addition to the fundamental research in this field, a direct link with farmers. Through their direct involvement, by organizing demonstration plots directly on their farms, information can be disseminated more easily and directly.

Table 1. The surfaces cropped on no till technology around the world till 2018-2919

World's continents	Surface, thousand hectares	Amount of continent cropland
South America	83,001	68.7
North America	65,882	33.6
Australia and New Zealand	23,309	74.0
Asia	17,482	3.6
Russia and Ukraine	6,920	4.5
Europe	5,584	5.2
Africa	445	1.1
World no-tilled surface	205,399	14.7

Source: Kassam, A. et al.,2022.

In Romania, the area cultivated with no-till technology was 583 thousand hectares in 2019, but it is growing very strongly thanks to the new generation of farmers who are becoming more informed and adapting much better.

MATERIALS AND METHODS

Two experiments were located at the Economic Sector of the Botanical Garden of the University of Craiova. The first experiment was conducted in the field and included 5 treatments in 3 replications. The treatments tested were:

- V1: control variant, plowing in autumn and spring bed preparation by harrowing;
- V2: no-till without mulch layer;
- V3: no-till with pea mulch layer
- V4: no-till with triticale mulch layer mixed with peas;
- V5: no-till with triticale mulch layer

The surface of an experimental plot is 40 m^2, i.e. 6 rows at 70 cm on a length of 8 m. The soil tillage was carried out manually, with a spade, in the case of basic soil tillage, simulating plowing and the preparation of the germinal bed, in the spring, with a hoe, simulating disc tillage. The mulch layer was obtained by cultivating peas (V3), a mixture of peas and triticale (V4) and triticale (V5). These special crops for creating the mulch layer (cover crops) were sown in early spring, in March, when it was possible to go out into the field. At the end of April, the mulch crops were herbicided with Roundup and, after a week, chopped to create a layer of plant residues on the soil surface (Figure 1).

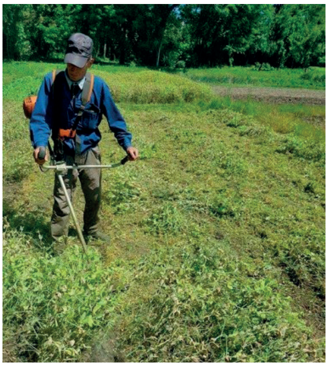

Figure 1. Cutting pea plants for mulch (original)

Weed control was achieved through 2 treatments with Mistral, based on nicosulfuron, because there was a massive infestation with Bermuda grass weed (*Cynodon dactylon*) (Figure 2). During the vegetation period, determinations of the current soil moisture were made by the direct method, by drying in an oven, on two dates: June 10 and July 15. Determinations of the bulk soil density were also made, also by the direct method, after taking the soil in Nekrasov cylinders, on two depths: 0-10 and 10-20 cm. The height of the corn plants was also measured, before harvesting.

Figure 2. Treatment with a layer of pea mulch after applying the Mistral herbicide (original)

In order to determine the amount of water lost by comparing the two systems, we organized an experiment in vegetation pots using the three types of texture: sandy, loamy and clayey. Three vegetation pots were filled with soil from each type of texture. For each type of texture, three types of soil cultivation were organized:
1. scratched at 5-7 cm with a knife;
2. no-till without a layer of mulch;
3. no-till with a layer of mulch.

The vegetation pots were prepared in October and were left outside all winter and spring, to acquire a natural settlement.

The determination of the mass of the vegetation pots began in the spring, in March, and continued until July 15. The weightings were made, as far as possible, daily. At the beginning of May, corn was sown in each vegetation pot and one plant was maintained in each cylinder. In addition to determining the water loss through evapotranspiration, which was done by weighing the vegetation pots, the height of the plants, the dimensions of the ears and roots were also determined. Also, the textural and chemical properties of the three soil types were determined.

RESULTS AND DISCUSSIONS

Results on field experiment

Results obtained in the first year of experimentation:

The results highlight that the main effect of tillage is to keep water in the soil, available to the roots. In the absence of tillage, it forms a superficial compacted layer of 3-5 cm that extracts water from depth and loses it through evaporation. Direct sowing in no-till land requires the presence of a mulch layer to prevent evaporation of water from the soil (Figure 3).

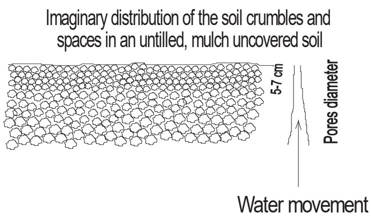

Figure 3. Water movement in a no-till not covered soil (original)

The sowing was done on May 5th and the plants sprouted after 5 days due to the rain (Figure 4).

Figure 4. Seedbed preparation and sowing in the mulch layer variant from the previous year, alongside the tilled variant (original)

Soil moisture was very strongly influenced by tillage or the presence of mulch. Two determinations of soil moisture and bulk density were made, on June 15 and July 10. These results are presented below (Table 2).

Table 2. Soil moisture and bulk density, depending on soil tillage and the presence of the mulch layer and its nature and thickness (original)

Treatment	Depth (cm)	15 June		10 July	
		U, %	Bd, g/cm^3 at 5-15 cm	U, %	Bd, g/cm^3 la 5-15 cm
V1	0-10	13.9	1.30	12.9	1.35
	10-20	14.3		13.5	
V2	0-10	13.5	1.45	12.5	1.44
	10-20	14.5		12.8	
V3	0-10	14.3	1.27	13.5	1.35
	10-20	14.0		13.8	
V4	0-10	14.5	1.33	12.8	1.30
	10-20	14.7		13.0	
V5	0-10	14.8	1.30	13.4	1.35
	10-20	14.9		13.9	

On October 15, the plots were harvested. Due to the fact that the Botanical Garden is a public place, about half of the ears disappeared. For this reason, we made an assessment of the production on each plot, by counting the plants and calculating the production by multiplying the number of plants by the average mass of an ear in each experimental plot. Plant height was also determined, also at harvest. These data were statistically interpreted by analysis of variance. The results are presented in Table 3.

Table 3. Corn yield and plant height depending on soil tillage and mulch layer, at the Botanical Garden of the University of Craiova (original)

Treatment	Height, cm	Yield, kg/ha	%	Diff. kg/ha	Sign.
V1	230	7,973	100	Ctrl,	-
V2	120	3,512	44	-4,461	000
V3	210	6,410	80	-1,563	0
V4	170	4,421	55	-3,553	000
V5	120	2,798	35	-5,175	000

DL 5%=1,156kg/ha; DL 1%=1,623 kg/ha; DL 0.1%= 2,291kg/ha

From these results we can conclude that tillage gave the best yields and the tallest corn plants (Table 3).
The no-till variant with peas as a mulch layer was the best of the three types of mulch due to its thickness and nitrogen content. This variant was close in terms of production and plant height to the control variant, it shows. This proves the possibility of applying no-till technology, provided that the mulch layer is created as thick as possible, with high coverage and rich in nitrogen.
The variants with a mixture of triticale and peas or only triticale, used to create the mulch layer, gave poor results due to the insufficiency of plant material and its poorer nitrogen content.
From this data we can easily see that the best way to keep water in the soil for use by plant roots is to keep it covered with a thick layer of mulch that has a C/N ratio close to 25 to 1. Tilling the soil causes water to be retained in the soil by forming wider spaces in the tilled layer, wider than the ones below. This slows down the water's access to the soil surface, where it is lost through evaporation. This is why tilled soil produces good yields. Untilled soil without a mulch layer forms a superficial layer a few cm more compact than in depth. This leads to the migration of water from the soil to the surface because narrower spaces

attract water more strongly than wider spaces. Once it reaches the surface, the water evaporates very quickly, within a few hours, causing the soil to dry out and harden, which results in plant failure (Figure 5).

Figure 5. No-till variant without mulch layer.
It is observed that the plants have hardly grown at all (original)

Figure 6. The conventional treatment.
The plants developed normally (original)

Figure 7. Pea covercrop treatment (original)

Results obtained in the second year of experimentation.

The sowing was done on April 30. During that period there was a lot of rain and the corn plants grew very well in the first 2 weeks. This year we applied the herbicide Adengo, based on isoxaflutole 225 g/l + thiencarbazone-methyl 90 g/l + cyprosulfamide (safener) 150 g/l. The application was done in early post-emergence. We were surprised that this herbicide did not controlled the species Digitaria sanguinalis which grew unhindered, especially in the plots with and without mulch.

Humidity determinations were made on June 1 and July 10.

Table 4. Soil moisture at June 1 and July 10, depending on the base tillage and the presence of the mulch layer (original)

Treatment	Depth (cm)	Soil moisture, %	
		1 june	10 july
V1 - plow	0-10	12.5	14.4
	10-20	12.9	14.8
V2 – no-till no mulch	0-10	11.7	13.2
	10-20	12.0	13.5
V3 – no-till pea mulch	0-10	13.4	15.4
	10-20	14.2	16.3

From these data we can see that the no-till variant with pea mulch retained the best water in the soil and the no till variant without a mulch layer had the lowest actual soil moisture at both dates (Table 4).

On September 14, the experimental plots were harvested and the yield was weighed. Plant height measurements at harvest were also made. The results are presented in the Table 5.

Table 5. Corn plant height and ear production per hectare depending on basic soil tillage and the presence of mulch layer (original)

Treatment	Plant height, cm	Yield, kg/ha	%	Diff. kg/ha	Sign.
V1 - plow	210	6,845	100	Mt.	-
V2 - no-till no mulch	120	540	7.8	- 6,305	000
V3 - no-till pea mulch	220	7,250	105	405	*

DL 5% = 225 kg/ha; DL 1% = 480 kg/ha; DL 0.1% = 680 kg/ha

From these results we can conclude that the no-till variant with pea mulch yielded better results than the tilled variant this year. The no-till variant without a layer of mulch yielded almost no harvest (Table 5, Figures 6 and 7).

Similar results have been obtained by (https://growingformarket.com/articles/cover-cropping-notill-systems; https://ohioline.osu.edu/factsheet/SAG-11; http://dasnr54.dasnr.okstate.edu:8080/notill2015/publications/no-till-handbook/Chapter%2012.pdf; https://rodaleinstitute.org/science/articles/choosing-the-best-cover-crops-for-your-organic-no-till-vegetable-system/; https://www.gardeningknowhow.com/edible/grains/cover-crops/no-till-cover-crops.htm; Jacobs A.A. et al., 2022)

Results on pot experiment

From the results of the weightings of the clay soil pots, we can see that during the spring no significant differences were obtained between the variants, probably due to the reduced evaporative potential of the atmosphere in these conditions. However, between the three cylinders of the clay texture, differences can be observed regarding the speed and amount of evaporated water. Thus, the tilled pot and, especially the one covered with mulch recorded lower values of the amount of evaporated water compared to the uncovered and no tilled pot. This can be observed almost every day of weighing. Another aspect of soil moisture is that it is much more fluctuating in the no tilled and uncovered pot than in the tilled or mulched ones.

The following period, from early June to early July, is characterized by very large amounts of water lost through evapotranspiration due to the increase in atmospheric temperature, the decrease in pressure and, consequently, the increase in evaporative potential by increasing the amount of water vapor at which the dew point is reached.

During this period, the greatest differences between vegetation pots are observed. Thus, while the tilled and no tilled-uncovered pots lost water very quickly (almost 1 kg per week), the soil covered with mulch, even no tilled, lost only 1.458 kg of water throughout the month (Table 6).

Table 6. Dynamics of the mass of vegetation pots in an experiment carried out at the Botanical Garden of the University of Craiova (original)

D	CM	CT	C	LM	LT	L	SM	ST	S
22	27	27	27	22	22	22	27	27	27
29	28	28	28	23	23	22	29	28	28
6	28	28	28	23	23	22	28	28	28
11	28	28	28	22	23	22	28	28	28
25	28	28	29	23	23	23	29	29	29
13	28	28	28	23	23	23	29	29	29
15	27	28	28	23	23	22	28	28	28
17	27	28	28	23	23	22	28	28	28
21	26	27	27	22	22	21	27	27	27
23	25	26	26	21	22	21	27	27	27
25	25	26	26	21	21	21	26	26	26
1	26	27	28	21	22	21	28	28	27
5	28	29	30	23	24	23	29	30	30

Legend: D - days within march to july; CM - clay mulch; CT - clay tillage; C - clay, no-mulch, no-till; LM - loam mulch; LT - loam tillage; L - no-mulch, no-till; SM - sand mulch; ST - sand tillage; S - no-mulch, no-till.

The soil temperature at a depth of 5 cm, on May 20, at 11^{00} clock was as follows: CM (clay mulch) = 17°C; CT (clay tilled) = 19°C; C (uncovered clay) = 19°C; LM (loam mulch) = 16°C; LT (loam tillage) = 21°C; L (uncovered loam) = 18°C; SM (sandy mulch) = 18°C; ST (sandy tillage) = 22.5°C; S (uncovered sandy soil) = 22°C.

The mulch layer greatly reduces the evaporation of water from the soil in all soil textural types, but especially in clayey soil. In the spring, when water evaporates more slowly, the method of tillage or soil maintenance did not determine obvious differences between the treatments.

Tilled soil loses less water than no tilled and uncovered soil due to the larger spaces created on the soil surface by tillage, spaces that are larger than those in the depths.

No-tilled and uncovered soil forms a superficial layer of 5-7 cm of soil with lower capillarity than that in the depths. This causes water to migrate from the depths to this superficial layer, which quickly loses it through evaporation. The rapid loss of water through evaporation from no-till bare and soil causes soil hardening, less water available to the plant, a very poor overall development of the plant and, ultimately, a very poor or no yield (Table 7).

Table 7. Air-dried corn cob mass, root length and volume as a function of soil texture and tillage method (original)

Tillage and mulch	Specification	Soil texture		
		Clay	Loam	Sandy
No-till mulch	Cob mass (g)	Missing data	11.149	13.558
	Root lenght (cm)	32.0	27.0	32.0
	Root volume (cm^3)	6	7	6
Tillage	Cob mass (g)	16.500	7.647	10.481
	Root lenght (cm)	32.5	25.0	32.0
	Root volume (cm^3)	6	4	6
No-till bare	Cob mass (g)	12.097	8.482	8.950 (no grains)
	Root lenght (cm)	26.0	24.0	27.0
	Root volume (cm^3)	4	3	4

The roots of corn plants developed differently depending on both the textural type of soil and the way the soil was tilled and maintained. Thus, in no-till soil covered with mulch, fine roots are observed located closer to the soil surface compared to tilled soil. Also, no till soil without mulch causes the formation of a less developed root due to soil hardening caused by rapid water loss through evaporation.

CONCLUSIONS

Conclusions regarding the field experience

Conclusions after the first year of experimentation:

The conventionally tilled variant gave the highest production in the first year of experimentation, of 7,973 kg/ha.

The lowest productions were obtained by the variant with triticale mulch (2,798 kg/ha) and the variant without mulch layer (3,512 kg/ha), very significantly negative compared to the classic variant.

The variant with pea mulch gave, in the first year of experimentation, a production close to the classic tilled variant (6,410 compared to 7,973) significantly negative.

The variant with pea mulch mixed with triticale gave an intermediate production between the classical tilled one and the one with pea mulch.

From these results we can conclude that tillage gave the best productions and the tallest corn plants.

The no-till variant with peas as a mulch layer was the best of the three types of mulch due to its thickness and nitrogen content. This variant was close in terms of production and plant height to the control variant, it shows. This proves the possibility of applying the no-till technology, provided that the mulch layer is created as thick as possible, with high coverage and rich in nitrogen. The variants with a mixture of triticale and peas or only triticale, used to create the mulch layer, gave poor results due to the insufficiency of plant material and its poorer nitrogen content.

Conclusions after the second year of experimentation.

From the results obtained in the second year of experimentation, we can conclude that the no-till variant with pea mulch gave better results than the tilled variant. The no-till bare treatment almost did not yield a harvest.

Conclusions regarding the experience in vegetation pots.

The mulch layer greatly reduces the evaporation of water from the soil in all textural types of soil, but especially in clayey soil.

In the spring, when water evaporates more slowly, the method of tillage or soil maintenance did not determine obvious differences between the variants.

Tilled soil loses less water than untilled and uncovered soil due to the larger spaces that are created on the soil surface by tillage, spaces that are larger than those in the depth.

No-till bare soil forms a superficial layer of 5-7 cm of soil with lower capillarity than that in the depth. This causes water to migrate from deep into this surface layer, which quickly loses it through evaporation.

The rapid loss of water through evaporation from uncultivated and uncovered soil causes soil compaction, less water available to the plant, very poor overall plant development, and ultimately very poor production.

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ALGAL BIOFERTILIZERS FROM THE GENUS *Nostoc*: A SUSTAINABLE ALTERNATIVE FOR THE CULTIVATION OF *Echinaceae purpurea* L. IN THE CONDITIONS OF THE REPUBLIC OF MOLDOVA

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Abstract

The present paper presents the experimental results established upon combined application of algal biofertilizers from the genus Nostoc, the species Nostoc gelatinosum Schousboe ex Bornet & Flahault, N. punctiforme (Kützing) Hariot and N. linckia Bornet ex Bornet & Flahault to the cultivation of Echinacea purpurea (L) Moench. The obtained results demonstrated that the application of these biofertilizers had a significant positive effect on several phenological and morphological parameters of the plants. An acceleration of the phenological phases of development was found, with a reduction of the vegetation period by up to 7 days in the variants with the application of algal biofertilizers. Also, a significant increase in leaf area, plant length and biomass production was observed in E. purpurea plants treated with algal biofertilizers compared to controls. The best results were obtained when applying a dose of 30 kg/ha of algal biofertilizer from the genus Nostoc. These results suggest that algal fertilizers from the genus Nostoc can be used as an ecological and efficient alternative for increasing the production of Echinacea purpurea crops. (L) Moench. in the specific conditions of the Republic of Moldova.

Key words*: Nostoc genus, Echinacea purpurea, algal fertilizers, growth.*

INTRODUCTION

Some of the most effective, accessible and harmless biofertilizers widely used in agriculture are those of algal origin (cyanobacteria as they are called today according to their functions are also algae). When applying algal biofertilizers, a positive impact on the soil is attested, manifested by: increasing the degree of loosening, maintaining moisture, improving soil structure, accumulating organic matter (including molecular nitrogen fixation), improving physicochemical properties, stimulating microbiological activity, reducing erosion and other positive effects (Dobrojan, 2024; Karthikeyan et al., 2007).

The use of *Nostoc* algae as biofertilizers in the cultivation of medicinal plants has been analyzed in numerous studies, which have highlighted the positive effects on plant development and their physiological state. Research has shown that the application of these algae can considerably improve the vegetative mass and physiological indicators of plants, due to their ability to fix atmospheric nitrogen and the bioactive compounds they contain and release into the soil. The use of *Nostoc* algae as fertilizers can be achieved both by direct application to the soil and to plants, contributing to a significant improvement in soil quality and plant health.

Research by Obana, Miyamoto, Morita, Ohmori and Inubushi highlighted the beneficial effects of administering the algae *Nostoc* sp. to the soil, within the cultivation of the species *Brassica rapa* var. *peruviridis*. Among the observed results are the increase in the carbon and nitrogen content in the soil, as well as the accelerated development of the plants. The algae also demonstrated a high tolerance to soil drying and salinity, which suggests its potential to contribute to the rehabilitation of degraded soils (Obana et al., 2007).

Chittapun and co-authors confirmed that the application of *Nostoc carneum* and *Nostoc commune* algae and chemical fertilizers at half the doses traditionally used in crop cultivation significantly increased seedlings and the number of grains per plant compared to the control without fertilizer (Chittapun et al., 2018). The same conclusion was reached by Purwani J., Pratiwi E., Sipahutar I A., Husnain who experimented with the combined application of *Chlorogloea* sp. and *Nostoc* sp. algae and 100% N in rice cultivation, which resulted in an increase in crop productivity by 14.75%, increased nutrient absorption and reduced N fertilizer application rates by 25-50% (Purwani et al., 2021).

The combined administration of the algae *Anabaena* sp., Anabaena doliolum, *Nostoc carneum* and *Nostoc piscinale* in corn cultivation has shown an increase in corn yield by about 20-30% compared to variants without administration of algal fertilizers (Prasanna et al., 2016).

Foliar application of *Nostoc piscinale* biomass in corn cultivation in the V6-V7 phenological stage led to earlier plant development, an increase in the number of leaves, an increase in the chlorophyll content of the leaves, the yield of the grains by 6.5-11.5% and the protein content of them (Chittapun et al., 2017).

Research conducted by Santini and co-authors showed that foliar application of hydrolysates from the algae *Nostoc* sp., *Anabaena* sp., *Tolypothrix* sp., *Leptolyngbya* sp. and *Arthrospira* sp. to the cultivation of *Ocimum basilicum* L. plants led to plant growth they were effective in plant growth (up to 32%), plant number (up to 24%) and fresh weight (up to +26%) compared to the control variant where the algal fertilizer was not applied (Santini et al., 2022).

Algae of the *Nostoc* genus show promise in application as a fertilizer in the organic cultivation of medicinal plants, especially *Echinacea purpurea* (L) Moench. *Echinacea purpurea* (L) Moench is a perennial plant that is part of the *Asteraceae* family and is originally from North America. *Echinacea* appeared in Europe through co-colonizers and created new medical sensations. As early as 1871, the renowned German physiciane Meier patented an innovative preparation called "blood purifier" the basic component of which was *Echinacea.* In 1915, the immunostimulating effect of *Echinacea* in the treatment of tuberculosis, smallpox, viral diseases was officially demonstrated for the first time. Currently, echinacea preparations are popular throughout the world. According to the World Health Organization, echinacea preparations occupy the first place in the USA and European countries, surpassing even the most well-known medicines such as ginseng and Tibetan mumiyo. *Echinacea* has tonic properties, is an antimicrobial, antiviral, immunoregulatory, antiradiation agent, accelerates the healing of gingivitis, skin ulcers, boils, burns, wounds, urinary tract infections and vaginal mycorrhiza. *Echinaceae* is not toxic and does not cause side effects. According to experts, the plant is the most promising for cultivation, and its production will increase significantly from year to year (Buric et al., 1998; Șevcenco, 1999; Șașco & Șașco, 2009; Teleuţă et al., 2008).

Echinacea purpurea has long been cultivated in Germany, France, the USA and recently in the Republic of Moldova, Romania, Ukraine, Belarus and the European part of the Russian Federation (Berti et al., 2002; Zagumennicov 2011; Hasanova, 2003).

Echinaceae purpurea (L.) has an increased nectar-honey potential during the summer-autumn months. When installing beehives on the *Echinacea* plantation, a nectar production of 60-100 kg/ha, or 75-125 kg/ha of honey can be obtained. At the same time, the plant also has a beautiful decorative appearance due to the large inflorescences and the rich range of colors of the ligulate and tubular flowers, for which it is cultivated and appreciated (Florea & Pașa, 2006). Currently, in the Republic of Moldova a significant plantation of *Echinacea purpurea* (L.) Moench. cultivated in a controlled manner is managed by Melnic who researches and tends to effectively utilize the plant for phytotherapeutic purposes.

Thus, we set ourselves the goal of investigating the morpho-physiological effects attested to *Echinacea purpurea* (L.) Moench. as a result of the application of algal biofertilizers from the genus *Nostoc*.

MATERIALS AND METHODS

In the given experiments, the cyanophyte algae *Nostoc gelatinosum* Schousboe ex Bornet & Flahault, *N. punctiforme* (K ützing) Hariot and *N. linckia* Bornet ex Bornet & Flahault, which are stored in pure culture in the collection of the LCȘ "Algologie V. Șalaru", were used. The experiments in question were carried out in the open field on the lands administered by the GŢ

"Melnic Cristin Victor", which are located in the village of Dobrogea, Republic of Moldova. The research was carried out in the spring-autumn period. The following experimental batches were used in the experiments: 1 – administration of the combined live biomass of *algae N. gelatinosum* + *N. punctiforme* + *N. linckia* in the dose of 20 kg/ha; 2 - *N. gelatinosum* + *N. punctiforme* + *N. linckia* in the dose of 30 kg/ha; 3 - *N. gelatinosum* + *N. punctiforme* + *N. linckia* at a dose of 40 kg/ha; 4 – control variant where no algal biomass was administered. Each experimental variant was mounted on an area of 30 m, in three repetitions. Algae inoculation was carried out at the end of March 2024. During the experiments, phenological indicators, morphological changes and the amount of biomass at harvest of *Echinacea purpurea* (L.) Moench plants were monitored.

RESULTS AND DISCUSSIONS

The application of fertilizers, in optimal doses and concentrations, in the cultivation of *Echinacea purpurea* (L.) Moench. attests to a positive impact on the crop, manifested in particular, on morpho-physiological aspects (Iahtanigova & Culișova 2021).

It is worth mentioning that during the summer season of 2024, a heat wave with a deficit of precipitation was observed on the territory of the Republic of Moldova. The average air temperature for this season was 22.7-25.6ºC in the territory.

The meteorological conditions specific to the research year directly affected the development of algal biofertilizers, which was reflected in the results obtained.

However, the application of algal biofertilizers influenced the phenological phases of the *E. purpurea* culture experimented. Their effect was manifested starting with the stemming phase and up to fruiting. The most significant results were obtained in the variant with the application of algal biofertilizers at a dose of 30 kg/ha where the early appearance of the stemming phase was attested (2 days earlier compared to the control), budding (by 2 days), flowering (by 1 day) and fruiting by 2 days.

The total duration of the vegetation period of the culture in variant no. 2 was 57 days, and in the control group 64 days, which allows us to conclude that algal biofertilizers at a dose of 30 kg/ha ensure the acceleration of the phenological phases of the *E. purpurea* culture. Variant no. 3 manifested a medium impact on the acceleration of the phenological phases of *E. purpurea* by 4 days, and that with no. 1 did not attest to any specific phenological changes (Table 1).

Table 1. Phenological characteristics of *Echinacea purpurea* (L.) Moench. when administered with algal fertilizers

Phenological phases	Duration of the phenological phase, days			
	Variant 1	Variant 1	Variant 1	Variant 1
Increase	13	13	13	13
Stemming	16	14	15	16
Budging	13	11	12	13
Flowering	4	3	3	4
Fruiting	18	16	17	18
Duration of the growing season	64	57	60	64

In all the experimental variants with biofertilizer application, the leaf surface area of the leaves are larger compared to the control variant starting with the stemming phase and until the end of the flowering phase. The most significant results were attested in the variant with the administration of 30 kg/ha of algal biofertilizer where the leaf surface in the development phases from stemming to flowering was 0.9-2.4 cm^2 larger compared to the control variant. The lowest results were attested in the group with the administration of 20 kg/ha of algal biofertilizer where the leaf surface area of the researched plants was 0.27-0.62 cm^2 larger compared to the control (Table 2).

Table 2. Plant leaf surface at different ontogenetic stages of development

Development phases	Leaf area, cm^2			
	1	2	3	Control
Stemming	13.67	14.30	14.07	13.40
Budging	22.65	23.76	23.31	22.21
Flowering	30.72	32.51	32.21	30.10

It is worth mentioning that in the groups with the administration of algal biofertilizer, a dark green color of the plant leaves was observed compared to those in the control variant. The increase in leaf area indicates that the health of the plants is improved and that they have a greater capacity to resist some stress conditions.

The application of experimental algal fertilizers attested the increase in the height of the researched plants. In variants 2 and 3, practically similar heights of the researched plants were attested during the stemming phase (49.60±2.67 - 49.55±2.77 cm), these being 1.30-1.35 cm higher compared to variant 4 (control). The increase in the height of the plants in the variants with administration of algal fertilizers was attested also during the budding phases and until the end of the flowering of the plants. The most significant differences in the height of the plant stem between the variants with administration of biofertilizers and the one without administration were attested at the end of the flowering phase where the height of the plants in the groups with algae application was 1.90-4.49 cm higher than in the control groups (Figure 1). The results in question allow us to conclude that the application of algal biofertilizers has a positive impact on the growth of *E. purpurea* plants.

Figure 1. Changes in stem height in *Echinacea purpurea* (L.) Moench. different phenological growth phases, cm

From the data presented in table 3 it is observed that the application of algal biofertilizers has been shown to increase the biomass of *E. purpurea.* As in the case of the other analyzed indicators, the highest amount of biomass (11.02±0.44 t/ha) was shown in variant no. 2, being followed by that with no. 3 (10.98±0.46 t/ha) and 1 (10.21±0.41 t/ha). Thus, we find that when applying algal fertilizers from the genus *Nostoc,* in the investigated doses, in the directed cultivation of *E. purpurea* seedlings, the increase in plant biomass by 0.18-0.99 t/ha is shown, which means that the algae has a quantitatively positive influence on the crop.

Table 3. Changes in *Echinacea purpurea* (L.) Moench. biomass attested to the administration of algal fertilizers, t/ha

Experimental variants			
1	2	3	4
10.21±0.41	11.02±0.44	10.98±0.46	10.03±0.43

Species of cyanobacteria from the genus *Nostoc*, used as biofertilizers, generate a positive impact on both soil quality and the development of various plant species, significantly contributing to stimulating growth processes and increasing crop yields (Tadesse, 2022).

Previous research has shown that the application of *Nostoc piscinale* biomass in *Oryza sativa* L. crops stimulated plant growth and development processes, manifested by the elongation of the root system and shoots, as well as by increasing the chlorophyll content in their leaves (Go Oco et al., 2024).

The application of the biomass of the cyanobacteria *Nostoc carneum* and *Nostoc punctiforme* as a biofertilizer, in the cultivation of the species *Matricaria chamomilla* L., had a significant effect on the overall growth of the plant, including the development of the root system, as well as on the increase in the essential oil content. (Zarezadeh et al., 2020).

The use of the biomass of the cyanobacteria *Amorphonostoc punctiforme, Stratonostoc linckia* and *Anabaena variabilis* in cotton cultivation has shown a positive influence on plant development, contributing to increasing their productivity with values ranging between 23.3% and 37%. (Musaev & Umarova, 1967).

The application of the suspension of cyanobacteria *Anabaena torulosa, Nostoc calcicola, Nostoc ellipsosporum, Trichormus doliolum* and *Oscillatoria* sp. in the culture of the medicinal plant *Thymus vulgaris* L. revealed a favorable effect, manifested by the intensification of enzymatic activity, the increase in lignin content and the enhancement of plant resistance to biotic and abiotic stress (Rasuli et al., 2023).

Foliar administration of the cyanobacterium *Spirulina platensis* in the cultivation of

Amaranthus gangeticus, Phaseolus aureus and *Solanum lycopersicum* species demonstrated a beneficial effect, evidenced by increasing plant productivity, as well as by increasing the content of nitrogen (N), phosphorus (P) and potassium (K) in plant biomass (Gaia et al., 2021).

The research conducted by our team has highlighted similar results, which allows us to state that the application of fertilizers based on the studied *Cyanobacteria* stimulates the growth of the analyzed plants, as well as increases their biomass.

CONCLUSIONS

The research carried out has highlighted the positive effect attested to the administration of algal fertilizers consisting of the combination of the species *Nostoc gelatinosum* Schousboe ex Bornet & Flahault, N. *punctiforme* (Kützing) Hariot and *N. linckia* Bornet ex Bornet & Flahault in the cultivation *of Echinacea purpurea* (L.) Moench. The application of algal fertilizers has been attested to the acceleration of the phenological phases of development (by 1-2 days), the reduction of the vegetative period (by 4-7 days) the increase of the leaf area (by 0.27-0.62 cm^2), the length of the plants (by 1.90-4.49 cm) and the amount of biomass (by 0.18-0.99 t/ha) of *Echinacea purpurea* (L.) Moench.

The most significant results were attested to the administration of algal biofertilizers from the genus *Nostoc* at a dose of 30 kg/ha, which is recommended for application to the directed cultivation of *Echinacea purpurea* (L.) Moench.

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RHIZOSPHERE MICROBIOTA PROFILES ACROSS DIFFERENT WINTER WHEAT CULTIVARS

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Abstract

Global changes, driven by climate change and the growing human population, have necessitated the development of innovative solutions to maintain agricultural productivity and quality. This has often led to the adoption of intensive agricultural practices, which significantly alter soil properties, including its physical, chemical, and biological characteristics. Soil microbiota are key mediators of essential soil processes, climate regulation, and plant health, influencing nutrient cycling, organic matter decomposition, and overall soil fertility. Plant roots serve as the primary source of nutrients for soil microbiota, releasing organic exudates into the soil. These exudates create a dynamic rhizosphere environment that attracts microorganisms, which, in turn, contribute to plant health by providing nutrients, releasing hormones, and neutralizing toxins. This study aimed to analyse the rhizosphere microbiome associated with three winter wheat (Triticum aestivum L.) cultivars Glosa, PG102, and Miranda. Soil samples were collected during different growth stages to assess the composition and variation of bacterial and fungal communities. Microbiological analyses revealed temporal and cultivar-dependent shifts in microbial abundance. The total number of bacteria was highest in autumn, decreased during winter, and increased again towards the flowering stage. Gram-positive bacteria followed a similar trend, with minor cultivar-specific deviations. Fungal community composition also varied over time. These results suggest that microbial communities are influenced by both environmental conditions and wheat genotype, highlighting the potential role of cultivar selection in shaping rhizosphere microbiota.

***Key words**: microbiota community structure, soil microbiota, winter wheat, rhizosphere.*

INTRODUCTION

Soil represents a complex natural system composed of consolidated mineral and organic components, located on the surface of the lithosphere (Bardgett, 2005). It serves as the primary medium for terrestrial vegetation development and supports the diversity of organisms that carry out their biological and ecological cycles within it (Hillel, 2007). The properties and characteristics of soil are the result of biotic and abiotic components and the interactions between these components (Kumar et al., 2020). At the soil level, the zone where some of the most important processes take place is the rhizosphere. The term rhizosphere was first introduced by Hiltner in 1904 to define the region of soil directly influenced by plant roots and their associated microbial communities (Berg & Smalla, 2009). Due to the significant role of plant-microorganism interactions in the rhizosphere, understanding the factors that shape microbial communities in this habitat is essential (Kent & Triplett, 2002). These factors include root exudates, soil composition, moisture, temperature, biotic interactions, and the specific plant species cultivated, all of which contribute to determining the structure, diversity, and functionality of microbial populations (Huang et al., 2014; Hawkes et al., 2007; Brimecombe et al., 2000). The plant species plays a crucial role, as different species produce distinct root exudates and create unique microenvironments that influence microbial community composition and activity (Hernández-Cáceres et al., 2022). It has been demonstrated that rhizosphere microorganisms directly influence plant growth by producing phytohormones (e.g., auxins, gibberellins) and enhancing nutrient uptake (Kurepin, 2014).

Winter wheat (*Triticum aestivum* L.) is one of the most important cereal crops worldwide, playing a crucial role in global food security. Different winter wheat cultivars are selected based on environmental tolerance, disease resistance, yield, and grain quality. While genetic improvement of winter wheat cultivars often focuses on visible traits, their impact on the rhizosphere microbiome remains less explored. In wheat crops, microbial interactions play a crucial role in improving plant health and productivity. Beneficial microbes facilitate the solubilisation of essential nutrients such as phosphorus and nitrogen, making them more available to wheat roots. Additionally, certain microorganisms contribute to stress tolerance by protecting plants from pathogens and environmental stressors, such as drought and soil salinity. Understanding and harnessing these microbial communities can lead to more sustainable wheat production, reducing dependence on chemical fertilizers and promoting healthier soil ecosystems (Mahoney et al., 2017). The aim of this study is to compare the rhizosphere microbiome profiles associated with different winter wheat cultivars (Glosa, PG102, Miranda). By analysing the diversity and composition of microbial communities, the study seeks to identify specific variations that may be influenced by plant genotype.

MATERIALS AND METHODS

The research sites were established at the Agralmixt S.A. farm, located in north-eastern Romania, Iasi County, Andrieseni village (47°34'9" N, 27°20'38" E), at an altitude of 60 meters above sea level. The region experiences a temperate continental climate, characterized by an average annual air temperature of 9.5°C and a total annual precipitation of 520 mm (Gafencu et al., 2023a).

During the studied period (September 2023 - August 2024), the average air temperature was 13.52°C, indicating an increase compared to the region's multiannual average. This average temperature can significantly impact ecological and agricultural processes in the area, directly affecting the growth rate of crops and other agricultural activities. Additionally, temperature fluctuations throughout the year, especially during the warmer months, may influence precipitation patterns and the water requirements of plants (Table 1). The data shows significant temperature fluctuations throughout the winter wheat vegetation period. January recorded the lowest temperatures (-18.12°C), while July had the highest (46.95°C). Precipitation was highest in June (117.4 mm) and lowest in August (12.0 mm), with a total annual precipitation of 515.5 mm.

Table 1. Monthly air temperature (average, minimum, and maximum) and atmospheric precipitation recorded during the winter wheat vegetation period in 2024 at the S.C. Agralmixt S.A. farm, weather station 00001CED

Month	Average temperature (°C)	Minimum temperature (°C)	Maximum temperature (°C)	Atmospheric precipitation (mm)
August 2023	24.29	10.75	39.89	**12.0**
*September 2023**	*19.46*	*7.16*	*33.98*	*23.2*
October 2023	13.60	-4.02	33.87	23.0
November 2023	6.07	-8.78	21.74	37.4
December 2023	1.89	-7.32	15.83	**12.0**
January 2024	**-1.31**	**-18.12**	12.44	24.4
February 2024	6.19	-11.22	25.08	29.8
March 2024	7.29	-9.42	34.23	40.0
*April 2024**	*15.03*	*-0.71*	*34.31*	*43.8*
*May 2024**	*18.62*	*1.24*	*37.06*	*65.8*
June 2024	24.47	10.31	40.12	**117.4**
July 2024	**26.68**	10.19	**46.95**	86.2
Annual average (°C) Total sum (mm)	**13.52**			**515.5**

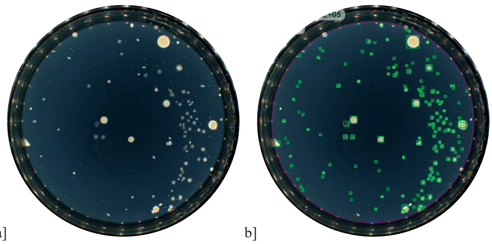
*Months in which soil samples were collected

Soil samples were collected from chernozem soil cultivated with winter wheat under conventional agricultural practices, including the use of synthetic pesticides and fertilizers. Soil sampling was conducted three times during the winter wheat growing season: [1] the first sample was taken in the fall of 2023, after crop sowing and emergence of wheat plants, [2] the second sample was taken in April 2024, and [3] the third sample was taken in May 2024, during wheat flowering. In this study, three winter wheat varieties were examined: Glosa, PG102, and Miranda, each with its unique agronomic traits. Glosa is a widely used variety, prized for its high yield potential and strong disease resistance, making it suitable for a wide range of environmental conditions. PG102 is known for its adaptability to various climates and its resilience to environmental stresses such as drought and extreme temperatures. Miranda stands out for its exceptional grain quality and durability, often selected for its superior performance across different soil types and climatic conditions. Within each analysed plot, soil samples were collected separately for each tree cultivar (Glosa, PG102, and Miranda) from 20 randomly selected points. The soil was sampled from around the plant roots at a depth of approximately 7-8 cm (Figure 1). After collection, samples were transported to the Microbiology Laboratory at the Iași University of Life Sciences, where they were stored overnight at 4°C, dried at room temperature, and sieved before microbiological analysis.

Figure 1. Soil sampling in the rhizosphere of wheat plants

To determine the total number of bacteria in the soil, expressed as colony-forming units (CFU) per gram of dry soil, the plate culture method was employed, using serial dilutions before plating (Gafencu et al., 2024). The Potato Dextrose Agar (PDA) medium (Scharlau, Spain, 39 g/L) was used for bacterial cultivation (Gafencu et al., 2023b). To selectively enumerate Gram-positive bacteria, Streptomycin (35 mg/L) was incorporated into the PDA medium. The antibiotic was thoroughly mixed into the medium post-autoclaving (15 minutes at 121°C) at approximately 48°C. For filamentous fungi assessment, the PDA medium was supplemented with Rose Bengal (35 mg/L) to limit the spread of fast-growing moulds (Smith & Dawson, 1944).

For each sample, successive dilution methods were applied. To assess bacterial and fungal populations, 1 mL of suspension from the 10^{-3} and 10^{-4} dilutions, respectively, was transferred to a Petri dish, followed by the addition of 17 mL of culture medium at 48°C. The contents were homogenized using orbital movements before solidification. The Petri dishes were then incubated at 28°C (Gafencu et al., 2021). After 24 hours, bacterial colony number were determined for both PDA and Streptomycin-supplemented PDA media using the Scan® 1200 automatic colony counter (Figure 2). The total bacterial count per gram of soil was calculated by multiplying the obtained values by the inverse of the dilution factor. Results were expressed as CFU×10^5 g^{-1} dry soil.



a] b]

Figure 2. Determination of the number of bacterial colonies grown on the culture medium (a] before counting; b] after counting)

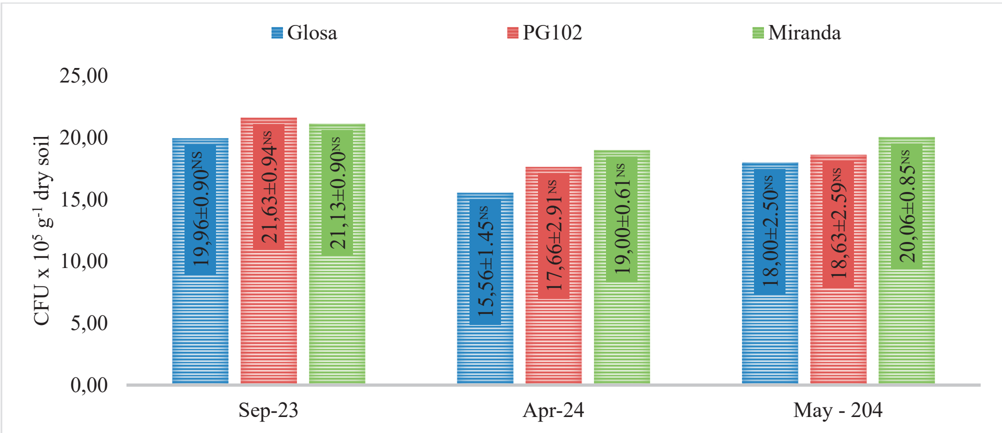
Filamentous fungi were evaluated after five days, and identification was based on morphological characteristics (Figure 3) following established taxonomic references (Gilman, 1957; Barnett, 1960; Ellis & Ellis, 1985; Seifert & Gams, 2011; Guarro et al., 2012). Fungal species that did not form spores within this period and could not be identified were categorized as "*Other species*".

Figure 3. Fungal colonies grown on PDA medium supplemented with Rose Bengal

Statistical analysis of the collected data was performed using IBM SPSS Statistics 26.

RESULTS AND DISCUSSIONS

The cultivation technology was similar for all three studied wheat varieties. The preceding crop was sunflower. After harvesting the sunflower crop, soil preparation included shredding plant residues, soil scarification, and seedbed preparation. Chemical fertilizers were applied in moderate amounts, ensuring good nutrient availability. Additionally, during the vegetation period, pesticides were used to prevent and control pathogens, pests, and weeds, thereby supporting the optimal development of the wheat crop. The results obtained from analysing the influence of wheat plants on the total number of bacteria in the soil show that in autumn, the total bacterial number reached its highest values. During winter, the total number of bacteria decreased, with the lowest values recorded in April. Towards the end of the vegetation period, the total number of bacteria increased but did not reach the value observed in autumn (Figure 4).



NS – P > 0.05 * – P ≤ 0.05 ** – P ≤ 0.01 *** – P ≤ 0.001

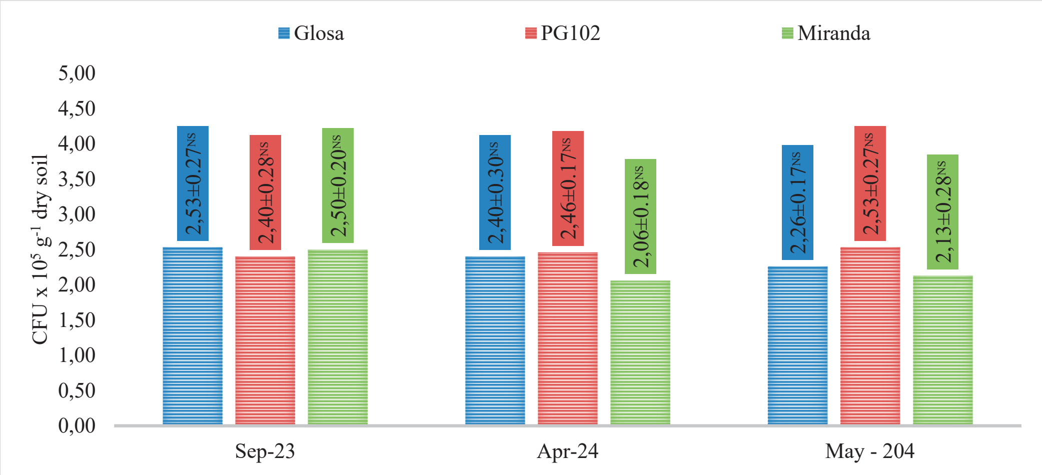
Figure 4. Total number of soil bacterial communities under the influence of winter wheat cultivar

This trend was observed in all three wheat cultivars studied. The highest total number of bacteria in the soil was observed for the PG102 wheat cultivar, in autumn, while the lowest values were recorded in the rhizosphere of the Glosa cultivar in April. For the Miranda cultivar, the total number of bacterial colonies in the soil showed the smallest differences across the three sampling periods.

Figure 5 presents the recorded results regarding the number of Gram-positive bacteria. A similar trend was observed in this particular bacterial group. The highest values were recorded in the fall of 2023. By April, the number of Gram-positive bacteria had slightly decreased, followed by an increase up to the wheat flowering stage.

For the Glosa cultivar, the number of Gram-positive bacteria decreased from 2.53±0.27 CFU × 10^5 g^{-1} dry soil in the fall to 2.40±0.30 CFU × 10^5 g^{-1} dry soil in April. However, by May, a further reduction was observed, reaching 2.26±0.17 CFU × 10^5 g^{-1} dry soil. In contrast, a different pattern was observed for the PG102 cultivar, where the number of Gram-positive bacteria in the soil increased from fall to May, rising from 2.40±0.28 CFU × 10^5 g^{-1} dry soil to 2.53±0.27 CFU × 10^5 g^{-1} dry soil.

By analysing the total number of bacteria and the number of Gram-positive bacteria in the rhizosphere of the studied wheat cultivars, it is evident that bacterial communities reached their peak after crop establishment, declined over the winter, and gradually increased until the flowering stage. However, when examining the Gram-positive bacterial community specifically, slight deviations from the general trend were observed, suggesting potential shifts in bacterial community structure within the wheat rhizosphere. These variations could be attributed to the influence of the specific cultivar studied.



NS – P > 0.05 * – P ≤ 0.05 ** – P ≤ 0.01 *** – P ≤ 0.001

Figure 5. Number of Gram-positive soil bacterial communities under the influence of winter wheat cultivar

The diversity of fungi in the rhizosphere of the studied wheat cultivars exhibited variations in fungal community composition throughout the growing season, reflecting the influence of seasonal conditions and cultivar-specific factors (Figure 6).

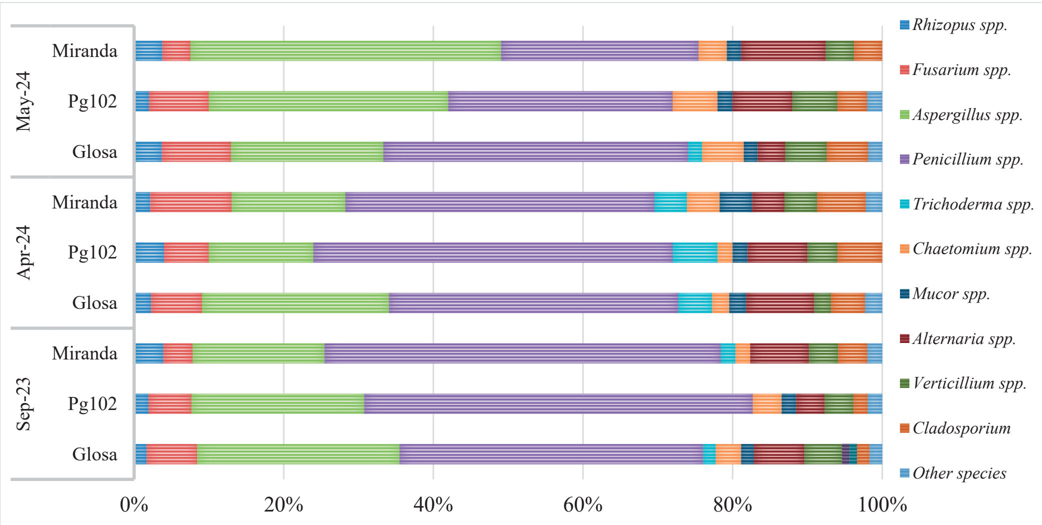


Figure 6. Results on the influence of winter wheat cultivar on the structure of fungal communities in the rhizosphere

Comparing the data from September 2023 to May 2024, notable changes were observed in the abundance of different fungal genera. *Aspergillus* spp. showed a significant increase in the Miranda cultivar (from 17.6% to 41.4%), suggesting a high adaptability to spring conditions. In contrast, *Penicillium* spp., which was dominant across all samples, decreased in Pg102 and Miranda, indicating possible shifts in microbial competition within the soil. *Trichoderma* spp., a genus known for its beneficial effects on plant health, was absent in Pg102 in both autumn and May but present in April, suggesting a seasonal influence or an association with wheat's developmental stage. Additionally, *Alternaria* spp. showed a significant increase in the Miranda cultivar (from 7.8% to 11.3%), indicating a potential preference for certain environmental conditions. These fluctuations in fungal communities suggest that the structure of the rhizosphere microbiome is influenced by both environmental factors and cultivar-specific characteristics, which may have implications for soil health and crop productivity.

CONCLUSIONS

The study demonstrates that winter wheat cultivars influence the structure and dynamics of microbial communities in the rhizosphere. The total number of bacteria was highest after crop establishment, declined during winter, and recovered towards the end of the vegetation period. PG102 exhibited the highest bacterial abundance in autumn, while Glosa recorded the lowest values in April, suggesting cultivar-dependent variations in microbial support. The composition of Gram-positive bacteria showed minor fluctuations, indicating a relatively stable subpopulation within the broader microbial community. Fungal diversity exhibited seasonal changes, with increases in *Aspergillus* spp. and *Alternaria* spp. in Miranda, while *Penicillium* spp. declined in PG102 and Miranda, suggesting shifts in fungal competition and adaptation to environmental conditions. These results underline the importance of plant genotype in shaping soil microbial ecosystems, which can have implications for soil fertility, nutrient availability, and plant health.

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AGRONOMICALLY VALUABLE SOIL AGREGATS WITH INOVATIVE CULTIVATION TILLAGE

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Abstract

The final process of mechanical impact of the working soil tillage tools is the achievement of a certain aggregate composition regarding the size of the fractions. The object of the study is Innovative working bodies and the certain values of soil fragmentation. The indicator of agronomically valuable soil is determined by the fraction with sizes from 1 to 25 mm. The data are processed at three different indicators regarding the speed of the machine-tractor unit, at two different soil terrains. The experiment was carried out with a tillage mill with non-standard, disk working bodies with a horizontal axis of rotation. The percentage of soil fragmentation into aggregates is determined for each experiment, by taking 5 soil samples at equal distances along the length of the experimental field. The results after the experiment show that with innovative disks at certain indicators such as soil type, humidity and speed, optimal values of the soil aggregate composition are reached.

Key words*: soil tillage machines, agronomically valuable soil, percentage of soil fragmentation.*

INTRODUCTION

The main purpose of soil tillage machines is to cultivate the soil in a way that provides the best opportunity for the development of the cultivated crop (Delibaltova et al., 2024; Mitkov, 2023). Another important vocation is to preserve soil fertility and create equal conditions for the germination and emergence of seeds from agricultural crops. Last but not least, of course, the economic aspect of the operation must meet the minimum for reducing production costs and increasing income from the cultivated crop. As a natural resource, soil is a product of long-term and extremely complex processes that have been occurring for millennia. It occupies the surface layer of the earth and is at a depth of 20-50 cm (in rare cases it reaches 1-1.5 m). It is strongly influenced by changes in external conditions, changing its state to a large extent due to its structure. The importance of soil from an economic point of view is determined by its generalized characteristic fertility, which is its ability to supply plants with the necessary nutrients, air and water. The change in its properties is due to its structural structure and all the impacts to which it is subjected (Bileva et al., 2022; Manhart & Delibaltova, 2022). The soil as a composition is a complex system. Its properties change with every passing moment. Its technological properties depend on the ratio between the liquid and gaseous phases in the soil (Hristova et al., 2022). The necessary soil treatments are determined primarily by agro-technical expedients, which are widely used in all countries with developed and mechanized agriculture. This treatment system also has a number of disadvantages. The main economic disadvantage is that a lot of energy, labor and resources are spent on the large number of treatments (for trench crops up to 10-12 per year). The main agrotechnical disadvantages are that with repeated passes of themachinery, the physical properties of the soil deteriorate, it quickly compacts, and maintaining it in a loose state when there are no developed crops facilitates water and wind erosion. All this has necessitated the search for ways to optimize soil cultivation in order to maintain high soil fertility while significantly reducing cultivation costs. By using high-performance machinery and properly combining shallow cultivation with periodic deep cultivation. The creation of innovative machinery with the aim of reducing cultivation, or the so-called Regenerative Agriculture, is environmentally friendly. It is a system of principles and practices that seek to restore and improve the entire ecosystem of the farm from the point of view of sustainability, including improving human health and economic prosperity. This is a method of agriculture that places a serious priority on soil health and improves the quality of the resources it uses (soil, water, biodiversity, etc.). Improving and restoring both the ecosystem and the soil to its natural fertile state begins with stopping degradation and saving soils from erosion. Therefore, Regenerative Agriculture is based on Conservation Agriculture, which has been practiced, developed and disseminated for over 60 years on large-scale industrial farms. Conservation Agriculture is an agricultural system that promotes minimal soil disturbance (i.e. no tillage), maintenance of permanent soil cover and diversification of plant species. The aim is to improve biodiversity and natural biological processes above and below the ground surface, which contribute to increased efficiency of water and nutrient use and improved and sustainable crop production. After a number of studies, Innovative active working bodies lead precisely to Regenerative Agriculture, reduced tillage and incorporation of all types of preparations, preservation of in the soil. For the conduct of the study, and not only for growing any crop, the type of soil or the so-called soil analysis is of utmost importance (Bakhtin, 1969). In modern agriculture, new information technologies, Geographic Information Systems - GIS, are increasingly used. Geographical Information Systems (GIS) consists of various components, starting with the incorporation of geographical data from remote sensing sources or maps and is then converted into a computer-readable form (Baniya, 2008). For establishing sustainable plants cultivation all collected data is transformed into spatial data for using GIS application (Malczewski, 2004).

MATERIALS AND METHODS

The research aims to:
Investigate innovative working bodies with active drive, for surface soil cultivation, combining the kinematics of a soil tillage machine with a horizontal axis of rotation and the horizontal displacement of the soil by a disk working body, to obtain agronomically valuable (Figure 1).
Regenerative Agriculture. To achieve the goal, it is necessary:

- Design and manufacture of new working bodies.
- Assembly of the working bodies to the horizontal shaft, with a certain angle of operation.
- Soil analysis.
- Conducting experiments.
- Data processing.
- A schematic diagram of the arrangement of the working bodies relative to the shaft is given. Both models are made with a different profile of 65G steel.

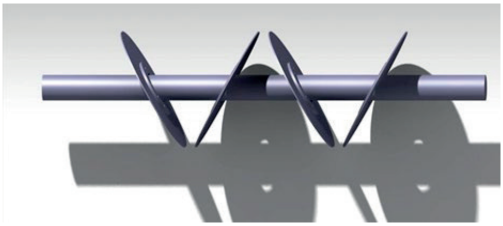

Figure 1. Disk with a horizontal axis of rotation and the horizontal displacement

Where the experiments were carried out Perushtitsa background: on plowing and the soil is a representative of TGP, heavy clay soil with a content of 50% physical clay.
The machine is an aggregated TK-80 with an active machine whose working width is Bp-0.76 m, PTO speed 540 min-1, at a speed of movement of the unit V = 2.5; 4.4; 7.5 km/h, and a set processing depth a = 12 cm, and the humidity is from W = 11% to W = 26%.
Through laboratory tests using the Kaczynski method (pipette method) it was established that the soil is a representative of TGP (heavy clay soil) with a content of 50% physical clay. 3. The percentage of soil fragmentation into aggregates is determined for each experiment, by taking 5 soil samples at equal distances along the length of the experimental bed in the following way: a box without a bottom measuring 400 x 230 x 230 mm is driven in. The bottom is inserted under it and the box with the soil is removed. The samples taken are left indoors, where they are dried to an air-dry state and divided into fractions through sieves with openings of 1 and 25 mm. The permissible deviation must be within ± 5%. degree of saturation - 70% of the soil aggregates should be from 1 to 25 mm in size, and aggregates up to 1 mm in size should be below 30% and the

soil surface should be level with an allowable ridge height of 20% of the processing depth.

The fractions are weighed with an accuracy of 1 g and their percentage composition is determined.

The indicator of soil erosion hazard is characterized by the fraction with a size of up to 1 mm (Figure 2).

The indicator of agronomically valuable soil is determined by the fraction with sizes from 1 to 25 mm.

The studies of the aggregate composition in soil background plowing are based on speed and moisture and are carried out using a regression Equations are derived after processing the data describing the fragmentation of the three soil fractions: up to 1 mm; from 1 to 25 mm and over 25 mm.

The following formulas calculate the soil fragments and are grouped into 3 levels: three soil fractions: up to 1 mm; from 1 to 25 mm and over 25 mm.

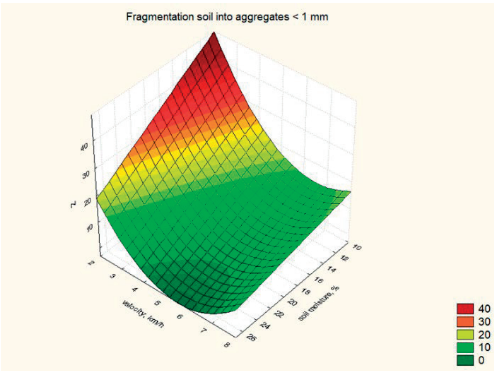

Figure 2. Fragmentation soil into aggregates < 1 mm

Aggregate composition up to 1 mm

Function=110.52-2.21*W-25.25*V+1.77*V^2+0.21*W*V [%]

The graph shows that the soil moisture is a significant factor, with its increase in aggregates < 1 mm decreasing and the agronomically valuable structure increasing by % from 1 to 25 mm in the speed range V of 2.5 km/h (Figure 3).

Aggregate composition between 1 mm-25 mm

Function=-18.57+1.02*W +31.26*V -3.62* V^2 [%]

The graph shows that at a speed of 4.4 km/h and soil moisture W 22.1%, the agronomically valuable structure from 1 to 25 mm is the best, with a high % amount of soil.

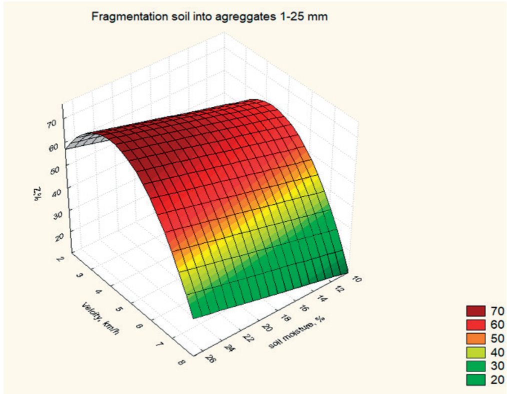

Figure 3. Fragmentation soil into aggregates 1-25 mm

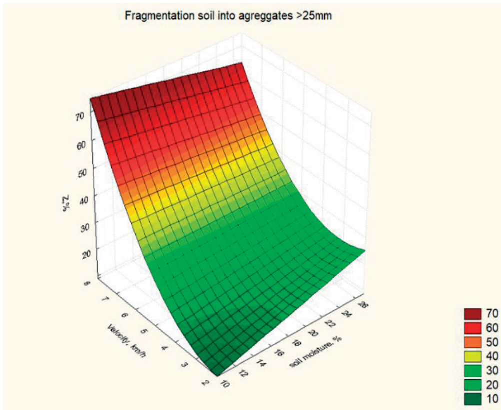

Figure 4. Fragmentation soil into aggregates > 25 mm

Aggregate composition above > 25 mm

Function=1.51*W-3.92*V+1.76*V^2*0.27*W*V [%] The graph shows that at a speed of 7.5 km/h and increasing soil moisture, the fractions with a size of >25mm decrease, but not significantly (Figure 4).

Methodology is based on using GIS platforms and application for analysing to present the results. All transformation actions of collected data are made by QGIS software and appropriate spatial data filters. The results are thematic maps of studied data.

RESULTS AND DISCUSSIONS

The main tasks of soil cultivation are many and varied, but can be synthesized in the following form:

1. After soil cultivation, to have agronomically valuable soil aggregates as a size.

2. Compliance with a given depth of cultivation.
3. To create a certain ratio between the solid, liquid and gaseous phases.
4. To give the soil surface and the cultivated layer the necessary microrelief.
5. To create the necessary ratio between the size of the aggregates inside the solid phase.
All processing requirements are of essential importance, but the leading factor is the size of the soil aggregates. The profile of an already cultivated area depends on the working bodies that have passed a given area. Each machine has a different profile according to its geometric shape (Figure 5).

Figure 5. Disk with a horizontal axis of rotation and the horizontal displacement mounted on tractor

A determining factor for agricultural machines is the material of manufacture. Recommended materials for durable coatings of details, assemblies and elements of agricultural machinery are indicated and their areas of use are indicated (Penyashki et al., 2003).The cattle livestock has continuously decreased from 5,381 thousand heads in the year 1990 to 2,680 thousand heads in the year 2010, as a result of the dissolution of the agricultural units and of the fact that many old cattle have been slaughtered because of their low production (Zahiu et al., 2010).
Working bodies with which the experiments were carried out (Figure 6).
In the case of soil tillage bodies, the wedge is the basis of the device. It represents a spatial body limited by an unlimited number of surfaces that make a certain angle with each other. The intersection points of these surfaces are lines that play the role of cutting edges when interacting with the soil, respectively, they are subdivided depending on the type and number of their working surfaces. The development of modern agriculture is unthinkable without complex mechanization of all production processes.

Figure 6. Working bodies

Industrialization includes the development of new machine systems, the improvement of a given technology when growing a given crop, the provision of soil tillage machines with high operational reliability and a significant increase in labor productivity (Dimov, 1979). It has been established (Dallev, 2015) that with different types of agricultural machines on the same soil we have a different final type of cultivated area, a different fraction of the soil. And all this again depends on all indicators of the soil type. Soil cultivation with active working bodies is gaining extraordinary development, both from an agronomic and economic, as well as from an ecological point of view.
When cultivating the soil, we have a mechanical impact of the working bodies of agricultural machinery, in order to achieve a certain structure under the agrotechnical conditions for growing a given crop. for a certain time, on a certain volume of soil. In this study, we classify the size of the fragmentation of soil aggregates in the soil under consideration, with a new type of active working bodies. The requirements for soil cultivation are quite diverse and of different nature and aspect. The area of soil samples is above to be equal during the various terrains. Soil background plowing at the specified humidity and speed. In the present analysis, they are:

- Fractions with sizes of agronomically valuable soil aggregates from 1 to 25 mm;
- Good profile of the already cultivated area;
- Minimization of treatments.

The experiments were carried out in the area of Perushtitsa, city of Plovdiv region, Bulgaria (Figure 7).

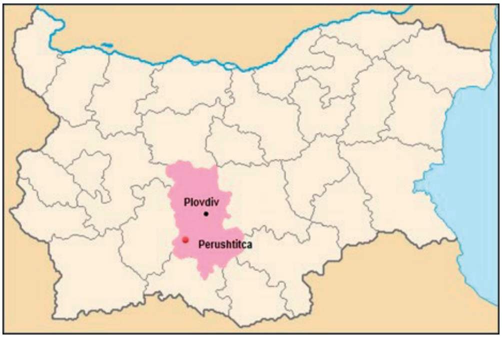

Figure 7. Studied area-Perushtitsa city, Plovdiv region, Bulgaria

All information about the region of study is collected from different scientific, experimental and literary sources. The results are gathered, analysed and presented by thematic maps. The region of Perusthitsa city covers 4871.6 ha, including 2298.9 ha land using area.

The topography of different regions creates a complex mosaic of topoclimates changes from 150 m to 800 m. The relief is various from plane to hilly. The Perushtitza Village belongs to the temperate continental climate zone. However, topographic setting causes to have great variation in climatic condition between the valley basin and the surrounding hilly area. The topography of different regions creates a complex mosaic of topo climates.

By its large resources possibilities, commands and tools, this Geographical Information Systems has a power to action with huge amount of information.

All conditions and factors, which influenced on soil aggregates, are presented by thematic maps (Figures 8-10). Agriculture database activities as collecting, organizing, transforming, analyzing and presenting the results of the studied area by GIS modeling.

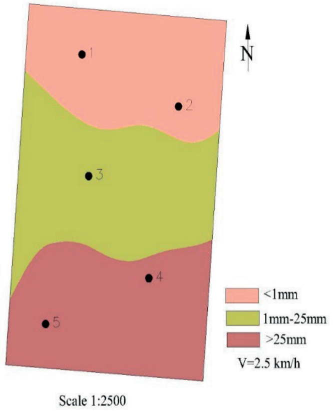

Figure 8. Map of Soil aggregate composition with speed V = 2.5 km/h

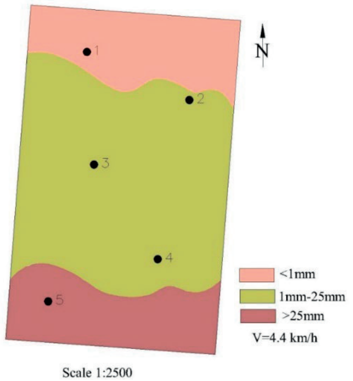

Figure 9. Map of Soil aggregate composition with speed V = 4.4 km/h

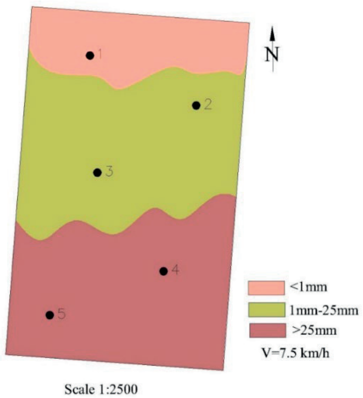

Figure 10. Map of Soil aggregate composition with speed V = 7.5 km/h

CONCLUSIONS

The unification shows that with discs 1 we have a balanced and percentage satisfactory crushing of this size fraction on both soil backgrounds under the specified criteria. The deduction from the analysis of disks 1 on the soil background plowing shows that agronomically valuable soil with fraction sizes of 1-25 mm is obtained at a speed of 4.4 km/h and humidity W 18.9-25.7%. The resulting soil aggregates are of great importance in the cultivation of any agricultural crops. From the placement of the seed in the soil, the planting material to the development of the given crop. The size of the soil aggregates after processing is final and of essential importance.

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LAWFUL ANATOMICAL TRANSFORMATION OF THE BASIC PHYSICAL PARAMETERS OF ARABLE CHERNOZEM LAND IN THE AREA BETWEEN THE PRUT AND NISTER RIVER

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Abstract

Through the perspective of the biophysical concept and the priority role of the interdetermined and interdependent processes of humus formation and accumulation, as well as aggregation-structuring in the realization of the chernozem process of solification, materialized in the pedofunctional system [bioenergetic system]↔[aggregate system], the basic physical parameters of chernozems are the indices of the structural-aggregate state. The changes in the composition and functioning of the humic system, induced by the inclusion of agricultural chernozems in the agricultural circuit, led to the establishment of an evolution trend of the aggregate system, indispensable and dependent on the tillage system. At the hierarchical level of the microaggregate, the evolution of the structural-aggregate indices is manifested by the increase in the content of microaggregates > 0.01 mm. At the hierarchical "aggregate" level, the evolution processes of the aggregate system materialize in the stable tendency of regeneration of the aggregation-structuring process and its intensification, as the degree of soil disturbance. At the same time, however, the 5-year period is insufficient to ensure a unidirectional trend of stabilization (hydrostabilization) of the aggregate structure, the main limiting factor being the insufficient humus content.

***Key words**: physical parameters, soils, structural-functional, typogenetic, chernozem.*

INTRODUCTION

From the perspective of the concept of chernozem evolution under agroecosystem conditions, the meaning and intensity of chernozem typogenetic processes are determined by the physical state of the soil responsible for the pedogenetic and pedofunctional regimes of the soils (Jigău, 2023a; Jigău et al., 2023b).
Despite this fact, both in the agricultural systems practiced in the last century and at present, the physical properties of the soils have been and are still neglected, with the emphasis placed on mineral fertilization and soil tillage. At the same time, research in the field of soil physics has had a predominantly agronomic-utilitarian character through the relations in the "soil-plant" system, their management being reduced to the "optimization" of the physical state through soil tillage, despite the fact that multiple research in the field has shown that these and the effects induced by them play one of the main roles in modifying the physical properties of the soils and the pedogenetic and pedofunctional regimes determined by them (Canarache, 1990).
At the same time, more recent research has shown that none of the technologies practiced in contemporary agriculture provide the necessary conditions for a unidirectional regeneration of chernozem typogenetic processes and, respectively, the expanded reproduction of the chernozem pedogenetic process, although the so-called "conservative tillage systems" involve some pedoregenerative elements. In this concept, the present research involves the identification and evaluation of the evolution laws of the basic physical parameters under the conditions of various tillage systems in order to improve and optimize them in a pedoregenerative sense (Shaxson, 1998; Nortcliff, 1998; Karlen et al., 1997).

MATERIALS AND METHODS

The theoretical framework of the present research is provided by the Law of Ecological Determinism, according to which chernozem pedogenesis in the space between the Prut and the Dniester is the product of the interdependent and interdetermined evolution of the landscape and soil in its basic quality of the landscape and involves its examination only within the concrete landscape conditions. The methodological framework is provided by the Law of Genetic Determinism and involves the examination of chernozem pedogenesis only through the prism of its dependence, exclusively, on the granulometric composition (inherited from the parent rock) and the content and composition of the humic system of the soil (Jigău et al., 2022).

In this sense, from the perspective of the bio-physical concept of pedogenesis, it represents a complex of processes of integration of abiotic and biotic matter materialized in the modeling of the mineral component in accordance with the needs of the soil biota. Through this prism of ideas, namely, the physical properties of the mineral substrate are subjected to the highest degree of pedogenetic transformation by the soil biota, which implies the conclusion that chernozems represent a natural model of pedogenetic formation with an optimal structural-functional composition - a standard for modeling soil matter through the integration of biotic and abiotic matter and materialized in the physical state of the soil.

Through this prism of ideas, the physical state of the soil is the product of multiple interactions between the abiotic components (solid, liquid, gaseous) and the biotic one, which involve two interdependent and interacting stages: a) the formation of the physical system (soil system) through the interaction of the physical phases; b) the formation of the soil ecosystem (biophysical system) through biogenic modeling mechanisms of the physical system. This implies the conclusion that the basic physical parameters are to be evaluated in interdependent and interdetermined interactions within the biophysical system.

In this context, the present research involves: a) the identification of the basic physical parameters that characterize the physical state of the soil: b) the identification of the meaning of the intensity of the processes of modification of the basic physical parameters and the laws of their evolution.

The achievement of the proposed objectives involves the identification of integral functional parameters easily accessible for evaluation that reflect the soil reaction to intercalated inputs induced by agrogenesis and climate change manifested in the meaning and intensity of chernozem typogenetic processes (humus formation-accumulation; carbonate migration, soil mass aggregation-structuring).

In this sense, our previous research has shown that the basic integrating parameter of the meaning and intensity of chernozem typogenetic processes are the humus state and soil structure (Kuznetsova et al., 2011; Kholodova et al., 2019; Semenov et al., 2020).

In our research for the evaluation of the meaning and intensity of the processes of evolution of the structural-aggregate state, the structural-aggregate parameters were used as follows:

> 10 mm – the amount increases during plowing, their content indicates the degree of degradation of the structural-aggregate state. The higher the content of aggregates > 10 mm, the higher the degree of degradation of the structure;

10-2 mm – an increase in their amount indicates the realization of the processes of regeneration of the chernozem structure in soils; a decrease in their amount indicates the degradation of the structure;

2-1 mm – is characterized by relatively stable amounts during vegetation and indicates its compensatory reproduction;

1-0.25 mm – aggregate reserve – in conditions of regeneration of meso-and macroaggregates the amount of the fraction;

1-0.25 mm is reduced as a result of its consumption during the formation of aggregates;

1-10 mm an increase in its content indicates a low intensity of the realization of the processes of regeneration of the aggregate structure in soils;

< 0.25 mm – its quantity is reduced during plowing and increases when aggregate layers are regenerated.

Our more recent research has shown that under conditions of agrogenetic pedogenesis and soil humus deficiency in arable chernozems with coagulation, root and coprolitic aggregation-structuring processes, a specific compensatory-mechanical mechanism is realized caused by the increase in the degree of contrast of thermal and hydrothermal regimes with the formation of aggregates > 5 mm. This process occurs more intensively in the middle and lower segments of the profile. In the upper segment under conditions of deficient humus content (< 3.5%), the increase in the content of aggregates > 5 mm is determined by mechanical processes induced by soil tillage.

For the reasons specified in our research, the mechanical processes of aggregate formation > 5 mm were united in a separate group of metastructuring processes and for their quantitative evaluation the ratio > 5 mm: < 5 mm is used. The higher the specified ratio, the more intensive the metastructuring process of the aggregate structure (Jigău et al., 2022).

Laboratory research included: determination of humus content - TINAO method; determination of the composition of the humic system - I. V. Tiurin method; determination of the structural-aggregate composition - Savviniv method (dry fractionation); and aggregate stability (fractionation in water) - Savvinov method.

RESULTS AND DISCUSSIONS

With reference to the current state of arable chernozems and their evolution through the prism of the Law of the priority role of the processes of formation-accumulation of humus and those of aggregation-structuring of soil matter, we consider that the current degradative trend of the chernozem process of solification is determined by the disruption of the interdependent and interdetermined functionality of the pedofunctional system [bioenergetic system]↔[aggregative system] under agrogenic conditions.

The primary factor with direct impact, which determines the disruption of the functionality of the pedofunctional system [bioenergetic system]↔[aggregative system] is the negative transformation of the structural-aggregative organization of chernozems which leads to quantitative and qualitative changes in the process of formation and accumulation of humus, as well as its localization in the restructured structural aggregates under conditions of agrogenic pressure (Jigău et al., 2022).

The degradation of the structural-functional organization of soil organic matter, materialized in the total loss of hydrostability by structural aggregates > 5 mm, leads to a significant modification of the process of humus formation-accumulation in structural aggregates and creates premises for the intensification of humus mineralization processes. As a result, the chernozems in the region are in the critical phase of bioenergetic degradation materialized in soil overcultivation (Table 1).

Table 1. Evolution of the composition of the bioenergetic system of typical moderately humiferous chernozems under conditions of agrogenic overcultivation

Maintenance mode	Total organic matter content	Composition of the bioenergetic system, % of total organic matter content		
		Humus	humified organic matter	Humic substances extracted in 0.1n NaOH
Forest strip (47 years)	5.84	78.8	12.8	8.4
Fallow land 16 years	5.39	76.1	13.7	10.2
Lightly overcultivated arable land	3.68	92.8	1.4	5.3
Moderately overcultivated arable land	3.08	94.8	0.9	4.3
Heavily overcultivated arable land	2.36	96.2	0.7	3.1

The amount of non-humified organic matter is reduced in poorly cultivated chernozems by 9 times, in moderately overcultivated ones by 14 times, and in strongly overcultivated ones by 18 times. As a result, during the vegetation period, the amount of organic phytonutrients (extracted in 0.1n NaOH solution) formed in the process of decomposition of organic residues is reduced by 1.4 times in poorly overcultivated chernozems, by 1.9 times in moderately overcultivated ones, and by 2.7 times in strongly overcultivated ones.

Therefore, the degree of exposure of inert humus to mineralization processes significantly increases, which entails the disaggregation of the soil mass and the intensification of the humus mineralization process.

The biohydrothermal and bioaerohydric environment that was established in chernozems after their inclusion in the agricultural circuit led to changes in both the course of the humification process and the composition of their humic system (Table 2).

Table 2. Composition of the humic substance system of typical moderately humiferous chernozems under overcultivation conditions

Maintenance mode	Total C, %	C humic acids, %				C fulvic acids					C hydrolyzed residue, %	Cah/ Caf
		Ah 1	Ah2	Ah3	∑	Af1a	Af1	Af 2	Af3	∑		
Forest strip (47 years)	2.68	11.8	27.5	3.9	43.2	1.9	6.7	15.2	2.8	26.5	30.3	1.63
Fallow land 16 years	2.39	10.7	26.5	8.0	45.2	1.9	5.5	11.9	5.0	24.3	30.5	1.86
Lightly overcultivated arable land	1.98	14.0	19.7	6.2	39.9	6.6	7.9	10.0	4.8	29.3	30.8	1.37
Moderately overcultivated arable land	1.70	14.7	17.8	4.6	37.1	7.6	8.6	10.9	4.4	31.4	31.4	1.18
Heavily overcultivated arable land	1.32	14.9	18.1	4.6	37.6	7.8	8.8	9.8	4.9	31.3	31.1	1.21

The data in Table 2 show that in arable chernozems a pedogenetic environment was established that was much different from that characteristic of uncultivated soils, which led to the modification of both the course of the humification process and the composition of the humic system. In this sense, in the composition of the humic system, the content of the mobile humic acid fraction (Ah1) that forms compounds with monovalent cations (Na+K+) and mobile forms of R2O3 increased, as well as the Ah3 fraction that forms stable complex compounds with R2O3 and clay minerals.

Under the newly formed biohydrothermal conditions, the synthesis of “aggressive fractions” of fulvic acids (Af1a, Af1) proceeds more intensively. The total content of fulvic acids increased by about 1.2-1.3 times and the Cah:Caf ratio decreased to 1.37-1.18, values uncharacteristic of typical moderately humic chernozems. At the same time, the carbon content of the unhydrolyzed residue practically does not change. This allows us to consider that the changes in the composition of the humic system of arable chernozems are determined by the changes in the mechanisms of the humification process at the current stage of evolution of arable chernozems within the framework of anthropogenic-natural chernozem pedogenesis. At the same time, from the data presented in Tables 1 and 2, we note that the biohydrothermal and bioaerohydric environment with a lower degree of oxidation, induced by the substitution in the fallow regime of the pronounced non-percolative exudative-transpirative water regime (characteristic of overcultivated chernozems) with the non-percolative deductive-transpirative water regime (characteristic of overcultivated chernozems) contributed, over a short period of time (16 years), to the restoration of the total organic matter content up to about 92% compared to the forest strip (Table 1) and to the transformation of the "aggressive fractions" of humic substances (Ah1, Af1a, Af1) into more stable humic substances (Ah2, Ah3) (Table 2) which contribute to the regeneration of aggregation-structuring processes and the restoration of the aggregate system.

This implied the conclusion that the transfer of overcultivated soils into fallow is the only procedure that can lead to the restabilization of the chernozem process in them (Sharkov & Antipina, 2022).

At the same time, however, the cited authors mention that under the conditions of "market agriculture" this procedure can only be used on a small scale in areas where for certain reasons it is not appropriate to cultivate agricultural crops. At the same time, several authors argue that the technologies practiced in contemporary agriculture cannot ensure the reproduction of humus reserves to quantities corresponding to the bioclimatic potential of the region (Kerschen, 1992).

In this context, it is imperative to evaluate the humus formation-accumulation-stabilization potential of current agricultural technologies in order to manage the basic physical parameters responsible for reproducing the chernozem process of pedogenesis.

Our research during 2011-2015 in an experimental plot in the North Moldovan plain showed that "alternative" soil tillage systems have different potential for humus formation-storage-stabilization (Table 3).

Table 3. Composition of the humic system of typical moderately humiferous chernozem depending on the tillage system (average data 2011-2015) (layer 0-50 cm), % of total C

Humic components	Maintenance mode				
	Forest strip	Plowing	Chisel work	Shallow work	No-till
Total C, %	2.58	1.98	2.00	2.03	2.04
C mobile humic substances	8.76	4.46	7.02	9.12	10.36
C water-soluble humic substances	0.85	0.80	0.82	0.83	0.5
C humic acids	39.64	36.94	38.06	38.33	38.11
C fulvic acids	20.18	26.90	23.82	22.11	20.38
C strable humus	30.57	30.90	30.28	29.61	30.30
Cah:Caf	1.96	1.37	1.60	1.73	1.87

From the data presented in this report, we find that, compared to uncultivated soils, all evaluated technologies have a lower potential for humus formation-storage-stabilization. At the same time, the comparative analysis of the impact of plowing and alternative tillage systems (chisel tillage, shallow tillage (Mini-till with mulch), No-till) shows that the latter contribute to the regeneration of the humus formation-storage-stabilization process in the soil. An informative parameter in this regard is, first of all, the content of mobile humic substances, which exceeds in the case of shallow tillage by 1.04 times and in the case of No-till by 1.18 times their content in uncultivated soils (forest strips).

Compared to the plowing variant, their content is 2.04 times higher in the shallow tillage variant and 2.32 times higher in the No-till variant.

In the case of the chisel tillage system, the content of mobile humic substances is lower than in uncultivated soils but is 1.57 times higher than in the plowing variant.

Under conditions of “alternative systems” of soil cultivation, the humification process proceeds with the formation, predominantly of humic acids. As a result, the Cah: Caf ratio increases from 1.37, the plowing variant, to 1.60 (chisel cultivation), 1.73 (shallow cultivation) and 1.87 (No-till).

The specified values allow us to consider that within the “alternative systems” of soil cultivation, a different biohydrothermal and bioaerohydric environment is created for the humification process. At the same time, the practically identical values of the content of non-hydrolyzed residue allow us to consider that the changes attested in the composition of the humic system are determined, exclusively, by the environment induced by the cultivation systems.

Based on the above, we can conclude that the bioenergetic system of overcultivated arable chernozems is receptive to agrotechnological measures for its restoration.

At the same time, however, to ensure the stability of this trend and more efficient realization of the biopedoclimatic and agrotechnology potential, agrobiotechnological measures adapted to the specific landscape conditions are necessary.

Changes in the composition and functioning of the bioenergetic system of soils entail changes in the composition and functioning of the aggregate system.

Table 4. Microaggregate composition of typical moderately humiferous chernozem under various tillage systems (average data 2011-2015)

Tillage system	Depth, cm	Aggregate diameter, mm. Microaggregate content, %							
		1-0.25	0.25-0.05	0.05-0.01	0.01-0.005	0.005-0.001	< 0.001	> 0.01	< 0.01
Ploughing	0-50	2.5	36.4	51.3	5.6	2.6	1.6	90.2	9.8
Chisel tillage	0-50	3.9	36.1	50.8	5.3	2.5	1.7	90.5	9.5
Mini-till with mulch	0-50	4.1	33.0	54.3	5.4	2.4	1.6	90.5	9.5
No-till	0-50	4.8	33.2	54.3	4.8	1.9	1.0	92.3	7.7

At the hierarchical level "microaggregate" these are manifested in the increase in the content of microaggregates > 0.25 and 0.05-0.01 mm. The increase in the content of microaggregates > 0.25 mm is achieved at the expense of those with a diameter of 0.005-0.001 and < 0.001 mm. This allows us to consider that the microaggregation of the soil mass is supported by the modifications in the humification process specified above. The intensity of the microaggregation process increases in the order: chisel work → work → superficial → No-till, similar to the increase in the share of humic acids in the composition of the humic system (Table 5).

Table 5. Structural-aggregate composition of the agrogenic layer of typical moderately humitic chernozems in various tillage systems (average data 2011-2015), layer (0-50 cm)

Tillage system	Depth, cm	Aggregate diameter, mm. Microaggregate content, %						Structuring coefficient
		> 10	10-0.25	10-1	3-1	1-0.25	< 0.25	
Ploughing	0-50	26.8	71.1	62.0	51.2	11.4	2.1	2.5
Chisel tillage	0-50	20.3	77.4	65.0	54.0	10.9	2.5	3.4
Mini-till with mulch	0-50	16.2	81.6	66.8	55.2	11.6	2.4	4.4
No-till	0-50	14.8	81.9	67.0	56.2	10.6	1.7	4.5

In the hierarchical "aggregate" nickel, the evolution processes of the aggregate system materialize in the stable tendency of regeneration of the aggregation-structuring process within which a series of laws are achieved:

- reduction of the content of aggregates > 10 mm;
- increase to excellent values of the content of agronomically valuable aggregates (0.25-10 mm);
- increase of the content of 10-1 mm aggregates responsible for the pedofunctional regimes (hydrothermal, aerohydric, oxidation-reduction, biological);
- increase of the content of chernozem aggregates (3-1 mm) responsible for the hydrophysical properties of the soils;

In all the evaluated variants, the decisive role in the formation of the agronomically valuable structure belongs to the 10-1 and 3-1 mm aggregates. At the same time, however, the content of 10-1 mm aggregates under chisel work conditions in the 0-50 cm layer is higher than under plowing by 3%, and in the superficial and No-till variants by 4.8% and, respectively, 5%. The same pattern is achieved in the case of the 3-1 mm aggregate content, which compared to plowing is higher by 2.8%, 4.0% and, respectively, 5%. Both in the case of the 10-1 and 3-1 mm aggregate content, the aggregate profile of the agrogenic layer is divided into two substrates with different intensity of their formation mechanisms. The "ploughing" variant is characterized by the maximum degree of stratification of the agrogenic layer. In the case of the "shallow tillage" and "No-till" variants, the degree of stratification is insignificant and implies the conclusion that homogeneous conditions for the functioning of the biophysical system are created throughout the entire thickness, which indicates the regeneration of the aggregation-structuring process to the same extent.

The structuring coefficient decreases in the order No-till > shallow tillage > chisel tillage > plowing.

The evaluated technological systems differ significantly in their impact on the degree of aggregate stability. The total content of hydrostable aggregates increases in the following order: chisel tillage > No-till > plowing > shallow tillage (Table 6).

Table 6. Aggregate stability of the agrogenic layer of typical moderately humus-rich chernozems under various working conditions (average data 2011-2015)

Tillage system	Depth, cm	Aggregate diameter, mm. Microaggregate content, %						Hydrostability criterion, %
		5-3	3-2	2-1	1-0.5	0.5-0.25	<0.25	
Ploughing	0-50	-	2.4	16.9	17.2	31.7	33.4	429
Chisel tillage	0-50	-	4.7	20.4	16.6	31.4	25.8	440
Mini-till with mulch	0-50	-	3.2	19.3	16.1	26.4	33.7	366
No-till	0-50	-	9.4	30.8	12.7	13.8	30.2	250

At the same time, in the plowing, chiseling and shallow tillage variants, the major share in the composition of hydrostable aggregates belongs to the 1-0.25 mm aggregates, the content of which is, respectively, 48.9%, 48.0% and 42.4%. In the No-till variant, their content is 31.5%, and the content of hydrostable aggregates >1 mm is 44.1%, which is about 2.3 times higher than in plowing, 1.8 times higher than in chiseling and 2 times higher than in shallow tillage.

In the composition of hydrostable aggregates > 1 mm, the major share belongs to the 2-1 mm aggregates. Their average content in the 0-50 mm layer decreases in the order: No-till (30.8%) > chiseling (20.4%) > shallow tillage (19.3%) > plowing (16.9%). An analogous regularity is also observed in the case of 3-2 mm hydrostable aggregates: No-till (9.4%) > chisel work (4.7%) > shallow work (3.2%) > plowing (2.4%). At the same time, in the order plowing < chisel work < shallow work < No-till, a stable trend of increasing hydrostability of 5-3 mm aggregates is outlined.

The quantitatively mentioned regularities are correlated with the quantitative regularities, induced by the evaluated work systems, in the content and composition of the bioenergetics system, which allows us to consider that the increase in the hydrostability of structural aggregates is supported by the positive quantitative and qualitative changes occurring in the composition of the bioenergetics system and, respectively, in the interdependent and interdetermined functioning of the pedofunctional system [bioenergetics system]↔[aggregate system].

At the same time, however, the quantitative and qualitative parameters of aggregate stability imply the conclusion that the period of 5-7 years is insufficient to ensure a unidirectional trend of stabilization of the aggregate structure, and the main factor with limiting impact is the small amount of humus produced following the transformation-humification of organic residues produced by cultivated agrophytocenoses.

Another limiting factor is the advanced degree of physical degradation of the agrogenic layer, which ensures an internal pedogenetic environment that is less favorable for promoting the humification process but more favorable for the mineralization of soil organic matter, including newly formed humic substances.

CONCLUSIONS

From the perspective of the biophysical concept of the chernozem pedogenesis process, further indications for evaluating the meaning and intensity of chernozem typogenetic processes under agrogenesis conditions are the parameters of the structural-aggregate state, materialized in the interdetermined and interdependent interaction of the pedofunctional system [bioenergetic system]↔[aggregate system].

Their application has highlighted the basic laws of interdependent and interdetermined evolution of the process of humus formation-accumulation and that of soil mass aggregation-structuring within various tillage systems.

Under plowing conditions in arable chernozems, a stable tendency of overcultivation is established, manifested in the interdependent and interdetermined degradation of the basic chernozem processes.

Within the framework of technologies with conservative elements of soil cultivation, with a lower degree of disturbance, in arable chernozems a stable tendency of regeneration of chernozem typogenetic processes is established, their intensity being increasing as the degree of disturbance is reduced. Their intensity, however, depends on the quantities of newly formed humus. This implies the conclusion that the management of chernozem pedoregenerative processes must be based on the sustainability of bioenergetic resources and the reduction of mechanical pressures on them.

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GEOSPATIAL MODELING AND REGIONAL ANALYSIS OF SOIL EROSION RISKS IN ALBANIA

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Abstract

Soil erosion poses a significant threat to Albania's environment and economy, impacting agricultural productivity, food security, public health, and infrastructure. This study conducts a regional assessment of soil erosion risks, identifying the most affected areas, underlying causes, and potential mitigation strategies. According to the World Bank, approximately 70% of Albania's land is affected by erosion, with an estimated soil loss of 20 tons per hectare per year, while only 10% remains minimally impacted. Over 60 million tons of sediment are transported to the Adriatic Sea annually, exacerbating land degradation and reducing agricultural sustainability. In regions such as Fier, where 80% of drainage systems are operational but half of the irrigation infrastructure is damaged, erosion-related flooding further deteriorates land productivity. This study integrates field measurements with geospatial analysis using the Revised Universal Soil Loss Equation (RUSLE) and Geographic Information Systems (GIS) to model erosion risks and propose effective land management strategies. The findings emphasize the urgent need for sustainable land use practices, afforestation efforts, and policy interventions to mitigate erosion and preserve soil resources for future generations.

***Key words**: sustainability, soil erosion, land degradation, GIS, land management.*

INTRODUCTION

Soil erosion refers to the detachment, transport, and deposition of soil particles caused by both natural and anthropogenic factors (Nan, Q.J., 2003). Accelerated erosion, which results from deforestation, overgrazing (Yassoglou et al., 1998), and inappropriate agricultural practices (Li, 2008), has been widely documented for its negative effects on soil productivity, biodiversity, and water quality (Wu et al., 2015). On a global scale, soil erosion is recognized as a major environmental issue that threatens sustainable development (Sartori et al., 2019).

To date, a large number of scientific studies on soil erosion have been published, focusing mainly on its impact on agricultural production (Lal, 1998), environmental quality, and ecosystem health (Pimentel, 1998; Duran et al., 2008), as well as comprehensive analyses of soil erosion at different research scales and regions (Wu et al., 2015). The Global Soil Partnership, led by FAO, estimates that approximately 75 billion tons of soil are eroded yearly, resulting in financial losses of up to $400 billion (GSP, 2017). This assessment of soil erosion dates back to 1993 when it was first reported by Myers (1993) and has been cited in several subsequent studies (Eswaran et al., 2001). Soil erosion has also been described as a major degradation process and identified as a key priority for action under the Soil Thematic Strategy of the European Commission (2006a; 2015).

Soil erosion, particularly topsoil loss, has been recognized as a warning sign of a global food crisis and has been recently reviewed (Kaiser, J., 2004). The challenge of feeding a growing population, with increased demand for livestock products, undoubtedly intensifies the pressure on fertile soils (Montanarella, 2015), further exacerbating the erosion problem. The Mediterranean region is especially prone to erosion due to its long dry periods followed by heavy, erosive rainfall storms, leading to significant soil loss. Erosion losses of 20-40 t/ha during storms, which may occur once every two or three years, are commonly recorded in Europe, with losses exceeding 100 t/ha during extreme events (Morgan, 1992). Albania is particularly vulnerable to soil erosion due to its heavy and prolonged rainfall throughout the year, particularly in autumn and winter, as well as its predominantly steep, hilly-mountainous terrain. This process is further accelerated by inappropriate land management (Grazhdani S. & Shumka S., 2007). Studies estimate that about 24% of the territory of Albania is affected by erosion, with annual soil losses reaching 37 t/ha (Zdruli & Lushaj, 2012).

Also, studies conducted on the sediment assessment of the Erzen and Vjosa watersheds (Kucaj, 2022; Lushaj & Kucaj, 2024) show that the river network transports more than 60 million tons of sediments each year (Laze & Kovaci, 1996).). In this study, in addition to the assessment of soil erosion with direct measurements, (at monitoring stations) the use of predictive models is necessary to assess soil loss based on the causal parameters of the studied areas. The objective of this paper was to evaluate soil erosion data collected from study areas in Albania, comparing it with soil loss estimates at measuring stations.

There are several models used to assess erosion risk, with the most widely used being the Universal Soil Loss Equation (USLE) (Wischmeier & Smith, 1978) and its improved version, the Revised Universal Soil Loss Equation (RUSLE), developed by Renard et al. (1997). With the advancement of geospatial technologies, such as Geographic Information Systems (GIS), spatial interpolation techniques, and an ever-increasing range of environmental data, soil erosion models are playing an increasingly important role in the design and implementation of soil management and conservation strategies (Panagos et al., 2015).

MATERIALS AND METHODS

A combination of field monitoring and geospatial modeling was used to assess soil erosion risks. Field measurements were conducted through direct erosion assessments at selected monitoring stations across Albania. Albania is located in the southwestern part of the Balkan Peninsula, with most of the country covered by hills and mountains and an average altitude of 708 m. This study focuses on visible soil erosion, its assessment in specific areas (such as plots or fields), and the associated deposits (measured by the mass of eroded soil in each plot). The primary goal is to collect and use environmental data that can be applied to other studies of an environmental nature, such as biotope inventory, atmospheric pollution, water pollution, soil erosion, or soil quality. The surface erosion assessment stations in Albania are located in Kallmet (Lezha), Qafshul (Librazhd), and Vithkuq (Korca), as shown in Figure 1.

Experimental stations (4 plots at each monitoring station) with different vegetation covers, the same width between stations, and consistent variants within stations, as well as a slope of 12%, are used to measure the rate of erosion. At the end of each plot, containers are placed to collect the amount of eroded soil material for each variant. For the development of this study, we present the results of an in-depth review of the scientific literature on soil erosion modelling by colleagues. Periodic assessments and measurements of erosion in plots are valuable tools for studying its flow and erosion dynamics, as well as for comparing the relative differences between vegetation cover, land use, and agricultural practices.

The assessment is repeated three times per year, in the periods January-May, June-September, and September-December, with results calculated for the entire year. The collected sediments are weighed, dried, and converted to per-hectare values. The results are expressed in tons/ha/year. The main characteristics (pedological, chemical, organic, topographical, etc.) of the soils at the measurement sites were determined through field studies.

Geospatial analysis was performed using the Revised Universal Soil Loss Equation (RUSLE) (Renard et al., 1997), applied using GIS tools to estimate soil loss in different regions. The impact of factors on soil surface erosion from a quantitative point of view was calculated using the Universal Soil Loss Equation (USLE), using the formula: $A = R \times K \times LS \times P \times C$

The factors influencing the erosion rate according to this model include: rainfall erosivity (R factor), soil erodibility (K factor), land cover and management practices (C factor), slope and length (LS factor), and support or conservation practices (P factor).

Land cover data in the studied areas was obtained from the Copernicus Climate Service and the State Geospatial Information Authority (ASIG). Data on rainfall amounts, as well as minimum, maximum, and average temperatures for the period 2020-2023 (IGJEO, 2024), soil moisture and its conditions, vegetation types, and other relevant factors were evaluated to measure the erosion rate.

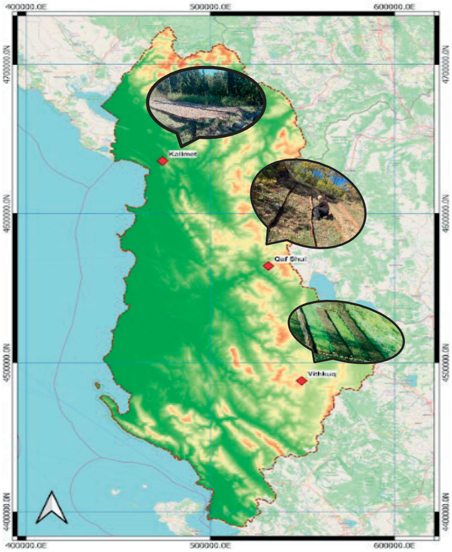


Figure 1. Monitored areas/surface erosion stations in Albania

RESULTS AND DISCUSSIONS

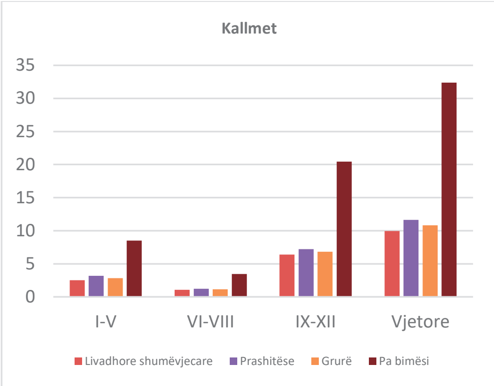
Evaluation of pedological, topographic, and climatological indicators for the monitoring station

Obtaining and evaluating soil erosion (surface erosion) results obtained at the erosion monitoring station in Kallmet-Lezha for the period 2018-2022 (Figure 2).

Figure 2. Erosion monitoring station Kallmet-Lezhë

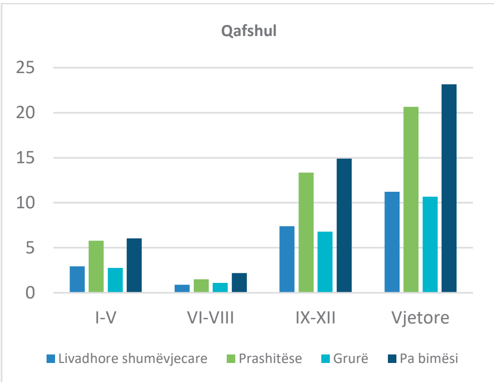
Below are graphical representations of the amount of soil eroded at the Kallmet, Qafshul, and Vithkuq erosion monitoring stations, based on data from the four plots over the five-year analysis period (2018-2022).

From the assessments and monitoring, soil loss was highest in the plot without vegetation (fallow plot), with an average of 32.4 tons/ha/year, and in the perennial meadow plot, with a loss of 9.96 tons/ha/year at the Kallmet-Lezhë station (Graph 1).



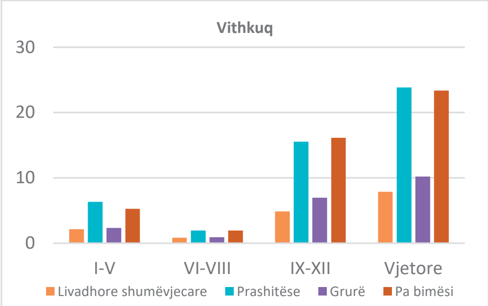
Graph 1. Graphical presentation of erosion for the Kallmet-Lezhë station for the five years 2018-2022

This monitoring point represents the areas of Lezhë, Shkodra, and the surrounding regions, extending to the vicinity of Durrës and Tirana. For the Qafshul - Librazhd station (Graph 2), soil loss was highest in the plot without vegetation, with an average of 23.14 tons/ha/year, followed by the fallow plot, with an average of 20.64 tons/ha/year.



Graph 2. Graphical presentation of erosion for the Qafshul station for the five years 2018-2022

This monitoring point represents the areas of Librazhd, Elbasan, and the surrounding regions, extending to the vicinity of Prrenjas and Burrel.



Graph 3. Graphical presentation of erosion for the Vithkuq-Korça station for the five years 2018-2022

From the assessments and monitoring conducted at the Vithkuq-Korçë station (Graph 3), soil loss was highest in the fallow plot, with an average of 23.84 tons/ha/year, followed by the plot without vegetation, with an average of 23.36 tons/ha/year. The perennial meadow plot had a loss of 7.88 tons/ha/year. This monitoring point represents the areas of Korçë, Voskopoja, and Erseka. The Kallmet study area, located in the Lezhë district, lies below the Vela mountain range in the Shkodër-Lezha plain, also known as Zadrima. Due to its geographical location and predominantly low relief, Lezhë experiences a mild Mediterranean climate. This climate is characterized by hot and dry summers and mild, wet winters in the lowlands and city areas, while the mountainous regions experience wet and cold winters. The average annual temperature for the district is 15°C, with January averaging around 7°C and July reaching 24-25°C. The average annual rainfall is between 1500-2000 mm (Climatic Bulletin, IGJEO, 2023). The average number of days with rainfall above 10 mm varies between 45 and 50 days. The soils are shallow, consisting of Cambisols and Gleysols textures.

Rainfall Erosivity Factor (R)

The rainfall erosivity factor (R) measures the erosive force of rainfall and runoff. Rainfall of mild intensity may cause minimal erosion, while heavy annual rainfall can result in significant soil loss. The R factor is estimated by considering the erosive effects of storms and plays a crucial role in calculating soil loss. The rainfall erosivity factor (R) is determined using the following equation:

$$R = I_{30}\,(9.28P - 83.83)/1000$$

where:

I30 = 75 mm/hour (Wischmeier & Smith, 1978).

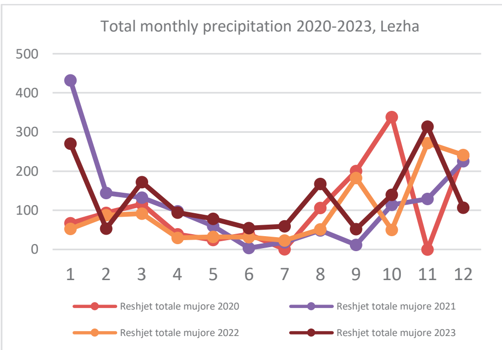
P is the average annual precipitation (in mm) over the past few years.

The "R" factor is the most important element of the Universal Soil Loss Equation. This factor, which largely determines the potential risk of soil erosion, is primarily influenced by the intensity and duration of precipitation. The latest annual precipitation data have been provided by the Institute of Geosciences.

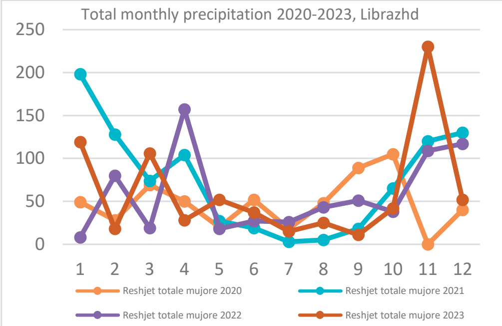
Data on total monthly precipitation for the period 2020-2023 are presented in Graphs 4-6. The calculated R factor values, shown in Table 1, demonstrate a high correlation with the average annual precipitation recorded at the meteorological stations nearest to the experimental stations.

Table 1. Value of the "R" factor

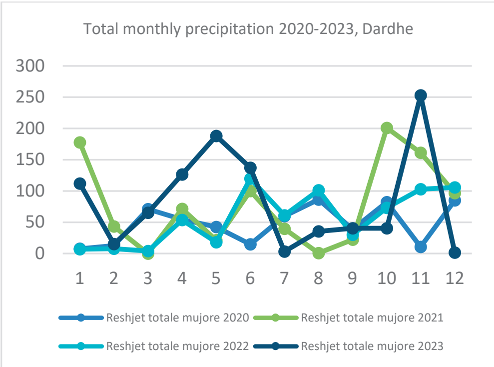
Name	Coef. R	Average precip. mm	Days with precipit.>10 mm	Days with snow
Kallmet	71.3	1346.1	35-45	0-5
Vithkuq	40.1	800.4	25-45	> 100
Qaf-Shul	35.6	721.9	45-55	> 100



Graph 4. Total monthly precipitation for the period 2020-2023 for the Lezhë study area



Graph 5. Total monthly precipitation for the period 2020-2023 for the Librazhd study area



Graph 6. Total monthly rainfall for the period 2020-2023 for the Dardhe study area

Soil erodibility factor (K)

The soil erosion factor (K) is the degree of susceptibility of soil particles to erosion per unit of the rainfall erosivity factor (R). K factor values (Foster et al., 1981) were calculated using the pedological values of the soils from the study area taken as part of the assessment of this factor, using the following equation:

$$100\,K = 2.8 * 10 - M1.14\,(12 - 0M) + 4.3 * 10 - 3\,(S - 2) + 3.3 * 10 - 3\,(P - 3)$$

To assess the impact of soil composition on erosion, as the value of the “K” factor in the Universal Soil Loss Equation, data on the particle size composition of the soils of the monitored areas were used. The K factor values calculated for each soil type indicate that Cambisol soil is more fragile due to its high clay content, and less sensitive to water detachment and transfer, as shown in Table 2.

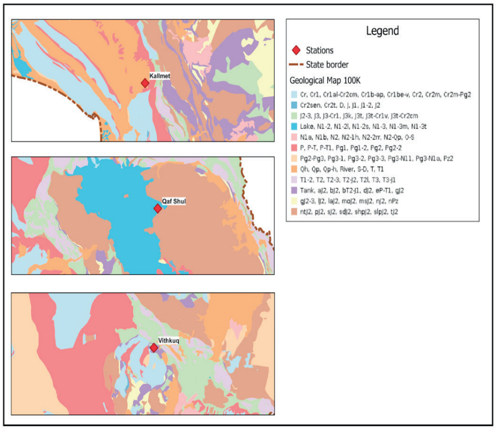


Figure 3. Geological age map of the study areas

Table 2. Value of the “K” factor and some ground indicators at the experimental stations

Experimental Areas	Texture in %			Soil Erosion Class	Coef. K
	Sand	*Silt*	*Clay*		
Lezhë (Kallmet)	46.5	27.6	25.9	2 (medium sensitivity)	0.35
Librazhd (Qaf-Shul)	45.8	28.4	25.8	2 (medium sensitivity)	0.32
Korçë (Vithkuq)	47.28	26.48	26.24	2 (medium sensitivity)	0.38

Topographic Factor Determining the influence of slope and length of steep slope (“LS” Factor)

The slope length factor LS is calculated using the following equation:

$$LS = (0.065 + 0.0456*\lambda + 0.0065*\lambda^2\ (\lambda/22.1)^{0.5}$$

Based on our assessments, the study areas were classified into 10 different elevation and slope classes, ranging from 0 to 84.21. The dominant elevation values are represented by the first four classes, as 80% of the study areas have a slope of up to 20 degrees. The LS factor map shows that the maximum LS factor values occur in the steepest areas, close to watercourses (Figure 4). Data on LS factor values for the erosion monitoring areas are presented in Table 3.

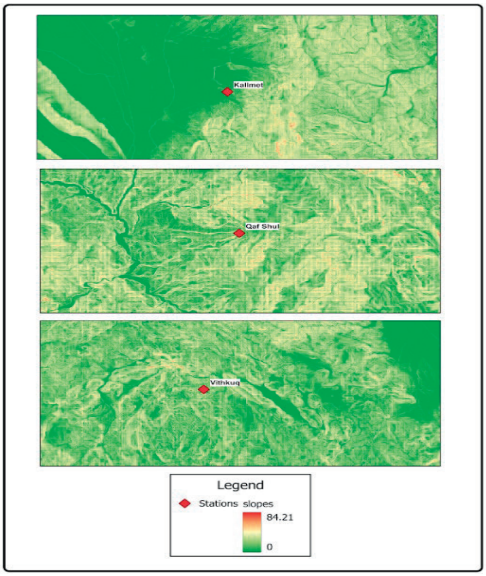


Figure 4. Map of LS Slope in the study areas

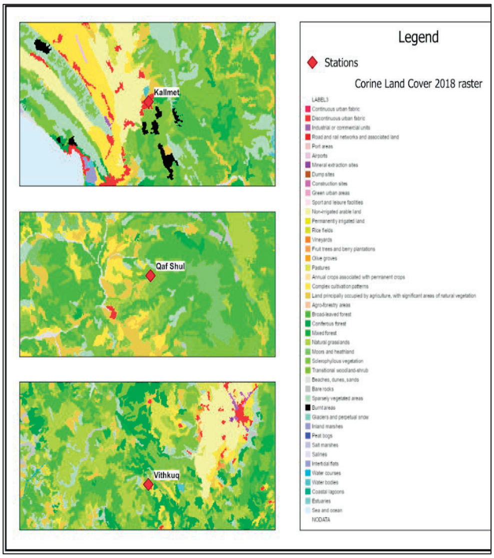


Figure 5. Land cover map in the study areas

Table 3. "LS" factor values

Monitoring areas	Slope length x (m)	Slope S (%)	LS Factor
Kallmet	10	9	0,527
Qaf-Shul	10	30	5,011
Vithkuq	10	20	1,152

Determining the impact of vegetation cover (factor "C")

Factor C is calculated based on the equation:

$$C = A/R \times K \times LS \times P$$

Starting with the erosion values measured in the monitored areas, we calculated the effect of plant cover on erosion by comparing the amount of soil eroded in a vegetated area to the soil eroded in unplanted land (considered as 1 unit). This ratio is used to calculate the "C" factor in the Universal Soil Loss Equation (U.S.L.E.). The data obtained from these calculations are presented in Table 4.

Table 4. Value of the factor "C"

Name	Kallmet	Qaf-Shul	Vithkuq	Averadge
Plot planted with meadow (Alpha-alpha) alpha	0.36	0.37	0.42	0.38
Plot planted with wheat	0.64	0.41	0.46	0.51
Plot planted with corn	1.20	1.22	0.92	1.13
Plot without vegetation (fallow)	1.00	1.00	1.00	1.00

For all plots planted with meadow vegetation, the soil receives almost the same level of protection. Planting with crops also provides excellent protection. The protective ability in the eastern and southern mountainous regions, where winter precipitation is mainly in the form of snow, is significantly higher than in the coastal lowland regions, where precipitation during the autumn-winter period is more intense and primarily in the form of rain.

Table 4 shows the influence of plant cover factors for each area. Autumn precipitation, which coincides with the early stages of plant development, affects the coefficient values of these plants in the regions mentioned above (Figure 5).

Assessment of potential soil erosion risk according to the "Corine" method

To estimate soil loss, data for each rainfall event that caused erosion during the period 2018-2022, obtained from measurements in the study area, were used. They were compared with the corresponding erosion values, using basic statistics and indices based on statistical estimates. This method helped create a detailed and reliable assessment of erosion risk for each study area.

Soil erodibility index

Soil erodibility refers to the susceptibility of soil to erosion. Based on the chosen methodology, the erodibility index (EI) is calculated with the following formula:

IE (Erodibility Index) = Texture x Depth x Rock Content

Soil texture is divided into 12 groups and classified according to the size of the soil particle diameter, which is further divided into fractions based on the proportion they occupy in the total soil composition. Soils with particles smaller than 0.002 mm are classified as Clay (C), those with a diameter between 0.002 and 0.05 mm are classified as Silt (S), and those with particles ranging from 0.05 to 2.00 mm are classified as Sand (S). The determination of soil texture is made using the American Soil Triangle, where each side of the triangle represents a specific soil texture.

In the Kallmet area, soils with a sandy-silty texture dominate. Soil depth was estimated by analyzing the depth of the soil profile (from the surface to the parent material). Soils with a depth of up to 25 cm are highly susceptible to erosion, while those with a depth ranging from 25 cm to 75 cm have a medium sensitivity to erosion. The Kallmet study area has a soil profile depth of 68 cm, classifying it as having medium erosion sensitivity. The content of stones is another factor, estimated based on the percentage of the soil surface covered by stones. This is classified into two levels: up to 10% and over 10%. Based on this, the soil sensitivity to water erosion is assessed in two classes.

The resulting Soil Erodibility Index falls within the range of > 0 to 3, indicating low sensitivity.

Soil Erosivity Index

Erosivity is a second element for determining the potential risk of soil erosion. This index is a result of climatic conditions, where rainfall intensity is considered the main determinant of erosivity and as a consequence of the use of the USLE method.

Erosivity Index = Fournier Index x Bagnouls-Gaussen Index

The Fournier Index (FI) is an analysis of total monthly and average annual precipitation and is expressed in the formula:

$$FI = \sum (P_i^2 / P_{annual})$$

Data on total monthly and average annual precipitation for the period 2020-2023, used to estimate the Fournier Index for the Albanian territory, were provided by the Institute of Geosciences, Department of Meteorology. The result falls within the FI limits of 60-90, indicating low sensitivity to erosion. To further strengthen the assessment of erosivity, the Bagnouls-Gaussen Drought Index (BGI) is also used. This index considers not only precipitation but also the average monthly temperatures. It is expressed by the following formula:

$$BGI=\sum(2T-P) *K$$

The Bagnouls-Gaussen index values for the Kallmet area survey are described as "dry".

Slope steepness index

The topographic factor is the third element in determining the potential risk of surface soil erosion. In the USLE methodology, this factor reflects the influence of slope angle and length, with the rate of soil erosion increasing as slope steepness increases, while the volume of erosion grows with slope length.

The slope angle, expressed as a percentage, ranges from 5% to 15%, indicating a mild level of erosion risk.

Land cover index

This index is considered one of the most important elements in erosion modeling, as it is the only factor on which humans have a direct influence. Table 5 shows the ratio of land cover to erosion, a ratio that was used in the project methodology according to the corresponding index.

Table 5. Vegetation cover assessed according to erosion sensitivity

Name	Erosion	Type of vegetation cover
Plot planted with meadow (Alpha-Alpha)	Full protection	Meadow, dense protective vegetation
Wheat plot	Partial protection	Wheat, medium impact cover
Corn plot	Partial protection	Corn, medium impact cover
Plot without vegetation (fallow)	Unprotected	Cultivated land, bare land

In this table, "Partial protection" is used for plots planted with wheat and corn, as these plants do not provide complete protection against erosion, but can affect its reduction to a certain extent. For the plot without vegetation (fallow plot), the category "Unprotected" is assigned, as this land is bare and has a high risk of erosion.

CONCLUSIONS

Integrating field data with geospatial modeling provides a comprehensive approach to identifying high-risk areas and developing targeted soil conservation strategies. The study on soil loss from erosion at three stations over the period 2018-2022 reveals the following findings:

1. The level of erosion shows significant differences between types of vegetation cover, seasonal periods, and terrain. Specifically, at the Vithkuq station, during the September-December period, soil loss in the variant without vegetation cover is 16.14 tons/ha/year, accounting for 37% of the annual soil loss.
2. At the same stations, in the same period, soil loss in the variant with perennial meadows is 6.4 tons/ha/year, representing 35.7% of the annual soil loss.
3. In the September-December period, soil loss in the variant with vegetation plants is 7.22 tons/ha/year, or 37.9% of the annual soil loss.
4. Similarly, during the September-December period, soil loss in the variant with wheat plants is 6.81 tons/ha/year, or 36.9% of the annual soil loss.

Using the USLE and CORINE models, soil loss was estimated for the study areas in Kallmet, Albania. The most critical months for four main land use categories were also identified. The quantitative results (t/ha) helped determine the most vulnerable sites, critical periods, and the impact of land use on erosion. Based on the implementation of these models, soil loss in the study areas, depending on vegetation cover, was found to be moderate for wheat and corn cover, high in areas without vegetation cover, and relatively lower in areas with perennial vegetation cover. Regarding erosion monitoring in the study areas, assessments were conducted every 3-4 months over the 5-year study period from January 2018 to December 2022. These data revealed that the risk of erosion ranged from moderately high to high across the study areas. In nearly all four plots at the monitoring stations, significant erosion-related problems were encountered, with recent years showing a more dominant emergence of this phenomenon due to changing climatic conditions.

The USLE and CORINE models proved to be highly adaptable, effectively integrating alternative model-supported algorithms with the available datasets. They also demonstrated their efficiency in this study by providing all the expected results in a clear, systematic, and consistent manner. Key recommendations include implementing sustainable soil management practices, such as encouraging the use of cover crops and improved tillage techniques to reduce soil loss. Strengthening erosion control infrastructure, including the enhancement of irrigation and drainage systems, is also essential. Additionally, reinforcing national soil conservation policies and promoting incentives for sustainable agricultural practices are crucial steps. Finally, establishing long-term monitoring stations and advancing GIS-based erosion models will improve risk assessments and mitigation strategies.

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PHOSPHORUS BALANCE IN LONG-TERM EXPERIMENTS ON THE LEACHED CHERNOZEM WITH DIFFERENT FERTILIZATION SYSTEM

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Abstract

The article presents the results of the phosphorus balance assessment in long-term experiments on leached chernozem during 1991-2020 yrs. at the level of agricultural plants, crop rotation and fertilization system. It was established that the phosphorus balance in the control variant (without fertilizers) was negative throughout the research period, on average constituting minus 30.5 kg/ha. The application of phosphorus fertilizers in doses of 45 kg/ha on the field with mineral application system it almost equilibrated the phosphorus balance, with a negative balance, on average over 30 years, from minus 1.3 to minus12.9 kg/ha annually. Organic-mineral fertilizers led to an increase in the productivity of field crops and a decrease in the negative phosphorus balance in the crop rotation, on average by 19.7-36.1 kg/ha compared to the unfertilized variant. At the growing of winter wheat, corn for grains, sunflower and leguminous crops on leached chernozem, the application of phosphorus fertilizers at a dose of 45 kg/ha compensated the phosphorus deficit, the balance becoming equilibrated or positive.

Key words*: phosphorus balance, field crops, fertilization level, leached chernozem.*

INTRODUCTION

The nutrient cycle changes in the soils of the Republic Moldova are very large, as a result of the drastic decrease of fertilizers application, as well as changes in the structure of sown agricultural crops (Andrieș, 2011; 2016; Leah et al., 2013; Lungu, 2024).

The extent of these changes and their impact on present and future agriculture is not known today. The last round of agrochemical mapping of soils (in order to know the nutrient content in soils) was carried out in 1990 by the State Agrochemical Service (Burlacu, 2000). An agrochemical mapping of soils at the current stage cannot be carried out, the reason being the lack of a specialized structure, as well as the very high costs for it.

The nutrient balance can serve as an indirect alternative method for assessing the state of soil fertility in agriculture, and at the same time a less expensive one (Lixandru et al., 1990; Zagorcea, 1989; Лунгу, 1992). The dynamic determination of the nutrient balance state in the soil highlights the quantitative changes in the income and consumption items when growing agricultural crops. The first assessment of the balance of biophilic elements and humus in Moldovan agriculture was carried out in 1990s and covered the period 1965-1990 (Andries, 2013; Zagorcea, 1989; Лунгу, 1992; Лях, Т. & Лях Н, 2012).

The agroecological and economic importance of the phosphorus balance lies in the fact that it is a scientific criterion for establishing the forecast of the level of agricultural production, as well as the need for fertilizers for it.

Of great scientific and practical importance is the study of the phosphorus balance in long-term fertilizer field experiments. They make it possible to carry out an objective scientific assessment of the main items of nutrient input and consumption, since all calculations are carried out on the analytical material accumulated over the years (Lixandru et al., 1990; Madjar & Davidescu, 2008).

The main objective of this study is to determine the phosphorus balance in leached chernozem in long-term experiments depending on the fertilization system and fertilizer doses applied to field crops over a period of 30 years.

MATERIALS AND METHODS

The studies were carried out within the long-term experimental station of Institute of Pedology, Agrochemistry and Soil Protection "Nicolae Dimo" (Ivancea commune, Orhei district), founded in 1964 on loamy-clayey leached chernozem. The humus content in the arable layer of soil - 3.4%; pHH_2O - 6.8; $\sum Ca^{2+} + Mg^{2+} = 37.4$ me/100 g soil. Since 2000, the station has been registered in the European Database of long-term experiments on soil organic matter - EuroSOMNET (Andries, Lungu, Leah, 2014; Uwe Franko et al., 2002).

The evaluation of the phosphorus balance in experiments was carried out during the period 1991-2020. The following field crops were cultivated in rotation during this period: winter wheat and barley, corn for grains, sunflower, leguminous crops (peas, beans, soybeans, alfalfa). The research was carried out on three fields with the following fertilization systems: *Field 1* – mineral fertilizer system; *Field 2* – organic-mineral system (mineral fertilizers are applied on the basis of 60 t/ha of manure associated with plant residues); *Field 3* – organic-mineral system (mineral fertilizers are applied on the basis of plant residues).

Organic fertilizers (manure) were applied in the autumn of 1990, 1995 and 2005 at a dose of 60 t/ha, chemical fertilizers (NPK*) after the preceding crops, annually, during the basic soil work, in the periods of 1985-1995 and 2006-2020 (*K* - is not applied in from 2010*). In 1996-2005, their post-action was studied.

The levels of mobile phosphorus in the soil were within the limits of 1.5-4.5 mg/100 g of soil (*Macighin method - extracted in a 1% ammonium carbonate solution in a ratio of 1:20, pH-9*). These were maintained by compensating for the export of phosphorus from the preceding crop at a dose of 45 kg/ha.

The nitrogen (N) doses on the $P_{3.0}K_{60}$ basis were: for winter wheat, corn for grains and alfalfa - 0, 30, 60, 90, 120, 150 kg/ha in active substance (a.s); barley, sunflower and leguminous crops - 0, 30, 45, 60, 75, 90 kg/ha a.s. On the basis of mobile phosphorus ($P_{1.5\text{-}4.5}$) the nitrogen doses were: for winter wheat and corn - N_{120}; alfalfa - N_{60}; barley and sunflower - N_{45}; peas, beans and soybeans - N_{30}.

The phosphorus balance (BP_2O_5) was calculated on fertilization systems and crops according to the formula: $BP_2O_5 = (P_2O_5\ input - P_2O_5\ export)$, where: P_2O_5 *input* - the phosphorus input with mineral and organic fertilizers; P_2O_5 *export* is the phosphorus export with the harvest and secondary production (Donos, Andrieş, 2001; Методические. ...., 1989).

RESULTS AND DISCUSSIONS

Field 1: *Mineral fertilizer application system on the leached chernozem.* The phosphorus balance values in crop rotation on the Field 1 is presented in Table 1.

Table 1. Phosphorus balance in the mineral fertilizer application system, kg/ha (Field 1)

No.	Variant	Average over periods						
		1991-1995	1996-2000	2001-2005	2006-2010	2011-2015	2016-2020	**1991-2020**
1	Control (unfertilized)	-58.7	-21.9	-29.5	-27.7	-29.2	-28.4	**-32.6**
2	Fond - 0	-	-	-	-	-	-	**-**
3	$N_{30\text{-}120}P_{1.0\text{-}1.5}K_{60}$	-39.1	+19.1	+11.7	+11.9	+8.3	+8.0	**+3.3**
4	$N_{30\text{-}120}P_{1.5}K_{60}$	-51.8	+16.7	+9.4	+8.9	+1.4	+1.3	**-2.4**
5	$N_{30\text{-}120}P_{2.0}K_{60}$	-57.8	+15.7	+8.3	+4.1	-2.1	-5.9	**-6.3**
6	$N_{30\text{-}120}P_{2.5}K_{60}$	-60.9	+14.8	+8.0	+0.4	-4.7	-9.0	**-8.6**
7	$N_{30\text{-}120}P_{3.0}K_{60}$	-62.7	+14.8	+7.7	-2.0	-6.8	-10.7	**-10.0**
8	$N_{30\text{-}120}P_{3.5}K_{60}$	-64.2	+14.3	+6.4	-2.9	-8.3	-10.1	**-10.8**
9	$N_{30\text{-}120}P_{4.0}K_{60}$	-63.9	+13.6	+5.6	-1.6	-7.1	-8.8	**-10.4**
10	$N_{30\text{-}120}P_{4.5}K_{60}$	-63.7	+14.1	+5.2	-3.0	-6.6	-9.4	**-10.6**
11	$P_{3.0}K_{60}$	-47.3	+17.0	+6.9	+6.8	+5.3	+3.3	**-1.3**
12	$N_{30}P_{3.0}K_{60}$	-54.1	+15.4	+7.1	+3.4	-2.4	-2.4	**-5.5**
13	$N_{45\text{-}60}P_{3.0}K_{60}$	-63.1	+13.2	+5.5	0.0	-6.3	-7.3	**-9.7**
14	$N_{60\text{-}90}P_{3.0}K_{60}$	-66.0	+11.5	+6.9	-2.1	-7.2	-9.1	**-11.0**
15	$N_{75\text{-}120}P_{3.0}K_{60}$	-72.5	+10.7	+4.8	-2.5	-8.1	-10.1	**-12.9**
16	$N_{90\text{-}150}P_{3.0}K_{60}$	-72.8	+11.1	+4.7	-1.7	-6.8	-8.7	**-12.4**
17	$N_{30\text{-}120}P_{3.0}K_{120}$	-63.8	+13.0	+5.3	-2.6	-7.4	-9.9	**-10.9**
18	$N_{30\text{-}120}P_{3.0}K_{60}+Zn_{10}$	-67.4	+12.9	+3.6	-2.2	-7.0	-13.2	**-12.2**

As a research result, it was established that the average phosphorus balance in the period 1991-1995 (5 years) on the Field 1, where alfalfa was cultivated, is deeply negative.

During the years in the control variant, where since 1964 no fertilizers have been applied, the phosphorus balance is negative ranging from -21,9 to -58,7 kg/ha, the average for the period 1991-2020 being minus 32,6 kg/ha.

The application of mineral fertilizers in crop rotation led to a decrease in the negative balance, from minus 39,1...-72,8 kg/ha to a equilibrated and even positive balance of +19,1 kg/ha (Table 1).

Therefore, the annual average phosphorus balance on the field with a mineral fertilizer application system for 1991-2020 period is almost equilibrated, from positive (+3.3) to negative (-1.3...-12.9 kg/ha).

Field 2: *Organic-mineral system (mineral fertilizers was application on the basis of 60 t/ha of manure associated with plant residues)*. The phosphorus balance on the Field 2 in crop rotation on the soil are presented in Table 2.

Table 2. Phosphorus balance in the organic-mineral fertilizer application system, kg/ha (Field 2)

No.	Variant	Average over periods						
		1991-1995	1996-2000	*2001-2005	2006-2010	*2011-2015	*2016-2020	**1991-2020**
1	Control	-31.2	-24.4	-31.2	-28.0	-29.3	-36.4	**-30.1**
2	Fond (60 t/ha manure + vegetable residue)	-4.8	+4.4	-35.8	-5.5	-35.1	-41.8	**-19.8**
3	Fond+$N_{30-120}P_{1.0-1.5}K_{60}$	-10.0	+1.8	+8.4	-10.8	+6.8	-7.1	**-1.8**
4	Fond+$N_{30-120}P_{1.5}K_{60}$	-11.0	-1.3	+6.9	-16.4	+4.1	-14.1	**-5.3**
5	Fond+$N_{30-120}P_{2.0}K_{60}$	-12.2	-3.0	+6.5	-19.6	+2.5	-17.0	**-7.1**
6	Fond+$N_{30-120}P_{2.5}K_{60}$	-12.7	-3.5	+4.3	-21.8	+1.2	-18.4	**-8.5**
7	Fond+$N_{30-120}P_{3.0}K_{60}$	-13.9	+4.4	+3.5	-22.9	+0.9	-19.1	**-7.9**
8	Fond+$N_{30-120}P_{3.5}K_{60}$	-12.3	-4.2	+3.9	-24.0	+0.2	-18.1	**-9.1**
9	Fond+$N_{30-120}P_{4.0}K_{60}$	-13.2	-6.3	+3.9	-23.9	-0.2	-18.6	**-9.7**
10	Fond+$N_{30-120}P_{4.5}K_{60}$	-14.2	-4.4	+3.7	-24.3	-0.4	-18.9	**-9.8**
11	Fond+$P_{3.0}K_{60}$	-8.0	+0.7	+6.6	-19.7	+6.7	-2.6	**-2.7**
12	Fond+$N_{30}P_{3.0}K_{60}$	-12.4	-1.5	+6.2	-22.2	+4.0	-7.5	**-5.6**
13	Fond+$N_{45-60}P_{3.0}K_{60}$	-14.8	-3.3	+3.5	-25.8	+0.1	-16.2	**-9.4**
14	Fond+$N_{60-90}P_{3.0}K_{60}$	-14.4	-4.8	+2.9	-24.4	-0.3	-19.3	**-10.1**
15	Fond+$N_{75-120}P_{3.0}K_{60}$	-12.8	-5.4	+2.0	-23.7	+0.7	-18.8	**-9.7**
16	Fond+$N_{90-150}P_{3.0}K_{60}$	-9.4	-5.3	+4.9	-21.4	+2.1	-17.5	**-7.8**
17	Fond+$N_{30-120}P_{3.0}K_{120}$	-15.2	-4.8	+3.4	-23.4	-0.8	-19.1	**-10.0**
18	Fond+$N_{30-120}P_{3.0}K_{60}$+Zn_{10}	-15.1	-4.7	+3.2	-24.6	+0.7	-18.1	**-9.8**

**Post-action period of manure greater than 5 years.*

On the Field 2 was established that in the control variant, approximately 24-36 kg/ha of phosphorus is annually exported from the soil with the crops, the average for 1991-2020 years being 30.1 kg/ha (Table 2). The application of manure in dose of 60 t/ha in the autumns of 1990, 1995 and 2005 associated with plant residues for the next 5 consecutive years led to a decrease in the negative phosphorus balance by approximately 22-29 kg/ha. During the 2001-2005, 2011-2015, 2016-2020 of post-action of manure, the phosphorus balance became negative, almost equalling with the unfertilized variant.

Manure in a dose of 60 t/ha maintained a equilibrated phosphorus balance over a 5-year period. The mineral phosphorus fertilizers administration in doses of 45 kg/ha on the basis of manure led to an increase in field crop yields, while also reducing the negative phosphorus balance to -3...-10 kg/ha, on average over 30-year period (Table 2).

Therefore, the role of organic fertilizers in maintaining a equilibrated phosphorus balance is essential in the fertilization system of agricultural crops.

Field 3: *Organic-mineral fertilization system (mineral fertilizers were administered on the basis of plant residues)*. As a research result, it was established that on the Field 3 of the rotation in the control variant, approximately 23-38 kg/ha of phosphorus is annually exported with the crops, the average for the years 1991-2020 being 28.9 kg/ha. On the plant residues basis, due to the higher yields, the phosphorus balance was negative throughout the research period, the average being 4,1 kg/ha higher than on the control variant (Table 3). The mineral fertilizers application with phosphorus in doses of 45 kg/ha compensated this deficit, the phosphorus balance becoming almost equilibrated and even positive in some years. Mineral fertilizers on the plant residues basis led to an increase in the crop productivity of rotation and a decrease in the negative phosphorus balance, on average by 23.8-36.1 kg/ha compared to the unfertilized variant (Table 3).

Table 3. Phosphorus balance with the organic-mineral fertilizer application system, kg/ha (Field 3)

No.	Variant	Average over periods						
		1991-1995	1996-2000	2001-2005	2006-2010	2011-2015	2016-2020	**1991-2020**
1	Control	-30.8	-24.0	-38.5	-26.9	-29.7	-23.3	**-28.9**
2	Fond (vegetable waste)	-34.6	-28.7	-44.2	-31.4	-32.4	-26.6	**-33.0**
3	Fond+$N_{30-120}P_{1.0-1.5}K_{60}$	+5.3	+10.4	-2.2	+9.4	+8.0	+12.2	**+7.2**
4	Fond+$N_{30-120}P_{1.5}K_{60}$	+3.9	+8.3	-5.1	+6.9	+5.0	+5.6	**+4.1**
5	Fond+$N_{30-120}P_{2.0}K_{60}$	-1.5	+7.2	-8.8	+4.3	+0.6	+0.8	**+0.4**
6	Fond+$N_{30-120}P_{2.5}K_{60}$	-4.3	+5.6	-14.6	+1.6	-3.1	-3.0	**-3.0**
7	Fond+$N_{30-120}P_{3.0}K_{60}$	-5.2	+5.6	-15.3	+0.1	-4.4	-4.7	**-4.0**
8	Fond+$N_{30-120}P_{3.5}K_{60}$	-2.7	+5.6	-16.7	-0.2	-4.2	-6.1	**-4.1**
9	Fond+$N_{30-120}P_{4.0}K_{60}$	-6.5	+5.2	-17.3	+0.9	-3.6	-5.0	**-4.4**
10	Fond+$N_{30-120}P_{4.5}K_{60}$	-2.0	+4.7	-17.6	+1.0	-3.8	-5.8	**-3.9**
11	Fond+$P_{3.0}K_{60}$	-0.2	+9.3	-10.3	+6.1	+6.3	+10.6	**+3.6**
12	Fond+$N_{30}P_{3.0}K_{60}$	-6.3	+6.8	-12.2	+1.9	+1.9	+3.9	**-0.7**
13	Fond+$N_{45-60}P_{3.0}K_{60}$	-7.3	+5.7	-16.8	-0.2	-2.1	-1.3	**-3.7**
14	Fond+$N_{60-90}P_{3.0}K_{60}$	-6.0	+4.7	-17.3	-1.3	-4.4	-3.9	**-4.7**
15	Fond+$N_{75-120}P_{3.0}K_{60}$	-7.2	+3.3	-16.2	-0.6	-4.6	-5.0	**-5.1**
16	Fond+$N_{90-150}P_{3.0}K_{60}$	-5.6	+3.6	-14.9	-0.7	-3.0	-3.1	**-4.0**
17	Fond+$N_{30-120}P_{3.0}K_{120}$	-7.7	+5.4	-14.4	-1.1	-4.5	-5.8	**-4.7**
18	Fond+$N_{30-120}P_{3.0}K_{60}$+Zn_{10}	-6.8	+5.4	-16.9	+0.3	-4.5	-5.4	**-4.7**

Winter wheat. It was established that in the control variant, the 24-35 kg/ha of phosphorus is annually exported from the soil with winter wheat (Table 4).

Table 4. Phosphorus balance when growing winter wheat on leached chernozem, kg/ha

No.	Variant	Winter wheat (average by periods)						
		1991-1995	1996-2000	2001-2005	2006-2010	2011-2015	2016-2020	**1991-2020**
1	Control	-31.1	-24.2	-35.5	-33.0	-27.0	-26.8	-29.3
2	Fond*	-16.5	-10.1	-26.5	-17.3	-23.5	-23.2	-19.9
3	$N_{120}P_{1.0-1.5}K_{60}$	-13.3	+4.0	+6.9	-1.3	+9.9	+4.0	+2.2
4	$N_{120}P_{1.5}K_{60}$	-17.0	+1.1	+6.4	-4.6	+5.7	-4.8	-1.9
5	$N_{120}P_{2.0}K_{60}$	-19.0	-0.2	+4.1	-9.8	+1.9	-10.9	-5.5
6	$N_{120}P_{2.5}K_{60}$	-20.9	-1.8	+2.9	-13.9	-1.1	-14.9	-8.2
7	$N_{120}P_{3.0}K_{60}$	-21.2	-2.7	+1.0	-16.1	-2.8	-16.1	-9.6
8	$N_{120}P_{3.5}K_{60}$	-21.3	-3.3	-1.1	-17.4	-3.5	-16.2	-10.4
9	$N_{120}P_{4.0}K_{60}$	-21.4	-4.1	-1.2	-17.1	-2.9	-15.6	-10.3
10	$N_{120}P_{4.5}K_{60}$	-21.2	-4.3	-1.5	-16.9	-2.9	-16.4	-10.4
11	$P_{3.0}K_{60}$	-11.1	+4.4	+3.4	-6.4	+9.1	+4.0	+1.2
12	$N_{30}P_{3.0}K_{60}$	-19.6	+0.9	+2.8	-10.8	+2.9	-3.8	-4.2
13	$N_{60}P_{3.0}K_{60}$	-21.5	-2.1	-0.2	-15.2	-0.1	-11.2	-8.1
14	$N_{90}P_{3.0}K_{60}$	-21.1	-5.6	-0.5	-17.5	-3.4	-15.2	-10.4
15	$N_{120}P_{3.0}K_{60}$	-18.8	-7.6	+1.1	-17.3	-3.0	-16.1	-10.2
16	$N_{150}P_{3.0}K_{60}$	-17.0	-8.5	+3.1	-15.6	-1.3	-14.2	-8.8
17	$N_{120}P_{3.0}K_{120}$	-22.2	-4.1	-0.6	-16.6	-3.3	-17.1	-10.6
18	$N_{120}P_{3.0}K_{60}$ + Zn_{10}	-21.5	-4.3	+0.4	-15.5	-3.5	-19.7	-10.8

***Note:** Field 1: Fond - 0; Field 2: Fond - 60 t/ha manure + plant residues; Field 3: Fond - plant residues.

The average of phosphorus balance for 1991-2020 was 29.3 kg/ha. The phosphorus balance improvement was observed on the field with manure in dose of 60 t/ha. The application of organic and mineral fertilizers on the leached chernozem on average over 30 years led to a reduction in the negative balance by 18.5-31.5 kg/ha compared to the control variant. Phosphorus fertilizers in doses of 45 kg/ha did not fully compensate for this deficit with the obtained crops, the balance becoming equilibrated or positive only in the variants with lower yields. On average in crop rotation during the 1991-2020 on fertilized variants, phosphorus fertilizers led to a decrease in the negative P_2O_5 balance by 21 kg/ha compared to the control variant (Table 4).

Corn grains. As a research result, it was established that on the control variant, approximately 29-41 kg/ha of phosphorus is annually exported from the soil with corn, the average for 1991-2020 was 33.8 kg/ha. The application of organic and mineral fertilizers with phosphorus during the research years led to a decrease in the negative phosphorus balance by 18.9-43.7 kg/ha compared to the control variant. Phosphorus fertilizers in doses of 45 kg/ha practically compensated this deficit, the balance becoming equilibrated and even positive in some variants. On average over 30 years on the leached chernozem during corn cultivation, the negative phosphorus balance decreased by 29.6 kg/ha compared to the unfertilized variant (Table 5).

Table 5. Phosphorus balance when growing corn for grain on the leached chernozem, kg/ha

No.	Variant	Corn grains (average over periods)						
		1991-1995	1996-2000	2001-2005	2006-2010	2011-2015	2016-2020	**1991-2020**
1	Control	-33.9	-29.2	-31.7	-29.7	-40.8	-37.7	**-33.8**
2	Fond*	-22.8	-8.2	0	-38.1	-30.3	-26.6	**-21.0**
3	$N_{120}P_{1.0\text{-}1.5}K_{60}$	-2.5	+5.1	+13.0	+2.6	-7.0	-4.1	**+1.2**
4	$N_{120}P_{1.5}K_{60}$	-3.0	+2.8	+11.8	-0.9	-12.5	-6.9	**-1.5**
5	$N_{120}P_{2.0}K_{60}$	-8.2	+1.4	+11.2	-1.0	-15.3	-10.3	**-3.7**
6	$N_{120}P_{2.5}K_{60}$	-9.8	+0.3	+12.0	-0.8	-16.7	-11.8	**-4.5**
7	$N_{120}P_{3.0}K_{60}$	-12.0	+11.6	+11.5	-1.7	-16.0	-13.6	**-3.4**
8	$N_{120}P_{3.5}K_{60}$	-7.2	+0.5	+11.4	+0.1	-16.9	-13.9	**-4.3**
9	$N_{120}P_{4.0}K_{60}$	-12.9	-4.1	+11.9	+5.8	-16.3	-13.2	**-4.8**
10	$N_{120}P_{4.5}K_{60}$	-8.6	+0.5	+10.6	+5.9	-15.7	-13.6	**-3.5**
11	$P_{3.0}K_{60}$	-6.1	+3.2	+9.5	+0.2	-6.4	-2.4	**-0.3**
12	$N_{30}P_{3.0}K_{60}$	-11.3	+2.2	+10.3	-1.2	-11.8	-5.7	**-2.9**
13	$N_{60}P_{3.0}K_{60}$	-14.2	+0.8	+10.2	-1.9	-17.7	-11.2	**-5.7**
14	$N_{90}P_{3.0}K_{60}$	-12.9	+0.5	+11.1	-1.0	-16.8	-13.3	**-5.4**
15	$N_{120}P_{3.0}K_{60}$	-15.0	-0.4	+10.4	-1.9	-17.2	-14.1	**-6.4**
16	$N_{150}P_{3.0}K_{60}$	-12.7	+0.4	+10.0	-0.9	-15.7	-13.5	**-5.4**
17	$N_{120}P_{3.0}K_{120}$	-14.4	+1.0	+10.0	-0.7	-17.2	-14.0	**-5.9**
18	$N_{120}P_{3.0}K_{60}+Zn_{10}$	-14.2	+0.4	+10.5	0	-16.6	-13.8	**-5.6**

***Note:** Field 1: Fond - 0; Field 2: Fond - 60t/ha manure + plant residues; Field 3: Fond - plant residues.

Sunflower. It was established that with the sunflower harvest on the control variant, approximately 17-35 kg/ha of phosphorus was exported from the soil annually, the average for the 1991-2020 being 26.3 kg/ha (Table 6). The application of mineral fertilizers with phosphorus in doses of 45 kg/ha largely compensated this deficit in the researched variants, the phosphorus balance being equilibrated and positive throughout the research period (Table 6).

Thus, we can consider that the dose of 45 kg/ha of P_2O_5 for sunflower can ensure a phosphorus balance in soil at the equilibrated state

***Legumes** (peas, soybeans, beans).* Based on the research, it was established that in the unfertilized variant, approximately 14-25 kg/ha of phosphorus was annually exported from the soil with legumes, on average over the period 1991-2020, obtaining a negative balance -minus 20.6 kg/ha of phosphorus (Table 7).

Table 6. Phosphorus balance in sunflower cultivation on leached chernozem, kg/ha

No.	Variant	Sunflower (average over periods)						
		1991-1995	1996-2000	2001-2005	2006-2010	2011-2015	2016-2020	**1991-2020**
1	Control	-35.0	-17.1	-35.5	-17.3	-27.3	-25.3	**-26.3**
2	Fond*	-25.0	+6.9	-22.2	-12.8	-24.7	-16.2	**-15.7**
3	$N_{45}P_{1.0-1.5}K_{60}$	-4.2	+18.3	+5.5	+24.2	+11.8	+12.9	**+11.4**
4	$N_{45}P_{1.5}K_{60}$	-3.4	+14.6	+4.6	+22.8	+10.1	+5.2	**+9.0**
5	$N_{45}P_{2.0}K_{60}$	-6.4	+12.4	+2.2	+20.8	+7.4	-0.3	**+6.0**
6	$N_{45}P_{2.5}K_{60}$	-7.6	+12.9	+1.0	+18.8	+4.1	-3.4	**+4.3**
7	$N_{45}P_{3.0}K_{60}$	-8.8	+12.0	+0.6	+17.5	+2.2	-4.4	**+3.2**
8	$N_{45}P_{3.5}K_{60}$	-7.1	+13.6	+1.0	+16.6	+1.2	-4.1	**+3.5**
9	$N_{45}P_{4.0}K_{60}$	-9.3	+12.6	+0.3	+16.1	+1.6	-3.3	**+3.0**
10	$N_{45}P_{4.5}K_{60}$	-9.0	+13.0	+0.3	+15.5	+1.2	-4.2	**+2.8**
11	$P_{3.0}K_{60}$	-8.7	+16.5	+2.3	+20.3	+7.5	+9.2	**+7.9**
12	$N_{30}P_{3.0}K_{60}$	-12.0	+14.8	+1.5	+16.8	+4.7	+3.0	**+4.8**
13	$N_{45}P_{3.0}K_{60}$	-10.8	+13.1	+1.1	+17.3	+1.7	-3.3	**+3.2**
14	$N_{60}P_{3.0}K_{60}$	-9.7	+12.2	+0.3	+16.4	+1.0	-3.2	**+2.8**
15	$N_{75}P_{3.0}K_{60}$	-7.1	+13.3	0.0	+17.7	+1.4	-3.1	**+3.7**
16	$N_{90}P_{3.0}K_{60}$	-3.8	+13.4	-0.2	+16.6	+2.1	-1.8	**+4.4**
17	$N_{45}P_{3.0}K_{120}$	-11.7	+12.5	+1.0	+16.7	+1.0	-3.0	**+2.8**
18	$N_{45}P_{3.0}K_{60}+Zn_{10}$	-11.3	+12.9	-0.4	+17.3	+1.9	-2.3	**+3.0**

***Note:** Field 1: Fond - 0; Field 2: Fond - 60 t/ha manure + plant residues; Field 3: Fond - plant residues.

Table 7. Phosphorus balance when growing legumes on leached chernozem, kg/ha

No.	Variant	Legumes (average over periods)						
		1991-1995	1996-2000	2001-2005	2006-2010	2011-2015	2016-2020	**1991-2020**
1	Control	-13.9	-19.7	-25.3	-22.1	-	-22.1	**-20.6**
2	Fond*	-13.8	-5.7	-18.0	-8.8	-	-	**-11.6**
3	$N_{30}P_{1.0-1.5}K_{60}$	+31.4	+17.6	+14.4	+17.8	-	+21.8	**+20.6**
4	$N_{30}P_{1.5}K_{60}$	+31.2	+16.0	+12.3	+16.5	-	+14.5	**+18.1**
5	$N_{30}P_{2.0}K_{60}$	+31.2	+15.1	+11.9	+14.4	-	+11.0	**+16.7**
6	$N_{30}P_{2.5}K_{60}$	+31.2	+14.6	+5.2	+11.1	-	+10.1	**+14.4**
7	$N_{30}P_{3.0}K_{60}$	+28.1	+14.3	+7.9	+9.6	-	+9.3	**+13.8**
8	$N_{30}P_{3.5}K_{60}$	+28.8	+38.4	+9.0	+9.0	-	+11.4	**+19.3**
9	$N_{30}P_{4.0}K_{60}$	+28.7	+13.8	+7.8	+8.8	-	+11.9	**+14.2**
10	$N_{30}P_{4.5}K_{60}$	+28.8	+13.6	+8.9	+8.9	-	+12.9	**+14.6**
11	$P_{3.0}K_{60}$	+30.8	+15.1	+10.8	+13.7	-	+13.9	**+16.9**
12	$N_{30}P_{3.0}K_{60}$	+30.4	+13.8	+10.0	+10.5	-	+13.1	**+15.6**
13	$N_{45}P_{3.0}K_{60}$	+28.6	+12.9	+7.6	+8.3	-	+12.0	**+13.9**
14	$N_{60}P_{3.0}K_{60}$	+29.5	+12.7	+7.7	+8.9	-	+10.5	**+13.9**
15	$N_{75}P_{3.0}K_{60}$	+29.8	+11.5	+8.8	+10.2	-	+11.1	**+14.3**
16	$N_{90}P_{3.0}K_{60}$	+29.5	+12.1	+9.6	+10.8	-	+12.8	**+15.0**
17	$N_{30}P_{3.0}K_{120}$	+28.6	+12.1	+7.7	+8.5	-	+11.9	**+13.8**
18	$N_{30}P_{3.0}K_{60}+Zn_{10}$	+29.8	+13.0	+8.0	+9.1	-	+11.4	**+14.3**

***Note:** Field 1: Fond - 0; Field 2: Fond - 60 t/ha manure + plant residues; Field 3: Fond - plant residues.

The negative balance in the unfertilized variant was maintained throughout the entire period of legume cultivation. Organic and mineral fertilizers led to a decrease in the negative phosphorus balance on average from 9.0 kg to 41.2 kg/ha compared to the unfertilized variant. The application of mineral fertilizers with phosphorus in doses of 45 kg/ha compensated for the phosphorus deficit, the phosphorus balance being positive throughout the entire research period (Table 7).

Thus, we can consider that the dose of 40-45 kg/ha of P_2O_5 for leguminous crops for grains can ensure a equilibrated phosphorus balance.

Lucerne. As a result of the investigations, it was established that the phosphorus balance when growing alfalfa on leached chernozem during the period 1991-2010 largely depended at the production level, which in turn was influenced by weather conditions and ranged from negative -72,8 kg/ha to positive +15,0 kg/ha. On the control variant, approximately 21-59 kg/ha of phosphorus was annually exported from the soil with the green mass harvest, the average was 36.1 kg/ha (Table 8).

On the variants with plant residues (Fond*) the phosphorus balance was negative by approximately 6-7 kg/ha, but higher yields were obtained compared to the unfertilized variant. The application of manure in the autumn of 2005 in dose of 60 t/ha (Fond*) increased the harvests level and reduced the negative phosphorus balance by 22 kg/ha compared to the control variant. On average, the negative phosphorus balance on leached chernozem, during the investigation period, on the fertilized variants reduced by 8-26 kg/ha compared to the control variant (Table 8). Therefore, the role of organo-mineral fertilizers in maintaining a equilibrated phosphorus balance is important in the alfalfa fertilization.

Table 8. Phosphorus balance when growing alfalfa on leached chernozem, kg/ha

No.	Variant	Lucerne (average over periods)						
		1991-1995	1996-2000	2001-2005	2006-2010	2011-2015	2016-2020	**1991-2020**
1	Control	-58.7	-21.2	-37.5	-27.0	-	-	**-36.1**
2	Fond*	-	-27.0	-44.8	-5.0	-	-	**-25.6**
3	$N_{60}P_{1.0-1.5}K_{60}$	-39.1	+15.0	-4.7	-11.0	-	-	**-10.0**
4	$N_{60}P_{1.5}K_{60}$	-51.8	+11.0	-9.0	-17.0	-	-	**-16.7**
5	$N_{60}P_{2.0}K_{60}$	-57.8	+9.6	-11.8	-20.7	-	-	**-20.2**
6	$N_{60}P_{2.5}K_{60}$	-60.9	+7.5	-17.6	-22.8	-	-	**-23.5**
7	$N_{60}P_{3.0}K_{60}$	-52.7	+6.7	-17.9	-24.1	-	-	**-22.0**
8	$N_{60}P_{3.5}K_{60}$	-64.2	+7.2	-18.9	-25.1	-	-	**-25.3**
9	$N_{60}P_{4.0}K_{60}$	-63.9	+7.2	-20.1	-24.8	-	-	**-25.4**
10	$N_{60}P_{4.5}K_{60}$	-63.7	+6.7	-20.4	-25.4	-	-	**-25.7**
11	$P_{3.0}K_{60}$	-47.3	+11.0	-13.3	-20.2	-	-	**-17.5**
12	$N_{30}P_{3.0}K_{60}$	-54.1	+8.0	-15.2	-22.9	-	-	**-21.1**
13	$N_{60}P_{3.0}K_{60}$	-63.1	+7.5	-19.9	-27.1	-	-	**-25.7**
14	$N_{90}P_{3.0}K_{60}$	-66.0	+6.1	-19.9	-25.4	-	-	**-26.3**
15	$N_{120}P_{3.0}K_{60}$	-72.5	+6.7	-20.7	-25.0	-	-	**-27.9**
16	$N_{150}P_{3.0}K_{60}$	-72.8	+7.5	-20.9	-23.1	-	-	**-27.3**
17	$N_{60}P_{3.0}K_{120}$	-63.8	+7.2	-16.8	-24.8	-	-	**-24.6**
18	$N_{60}P_{3.0}K_{60}+Zn_{10}$	-67.4	+6.7	-20.4	-26.4	-	-	**-26.9**

***Note**: Period 1991-1995: Fond - 0; Period 1996-2005: Fond - plant residues; Period 2006-2010: Fond - 60 t/ha manure + plant residues.

CONCLUSIONS

It was established that the phosphorus balance on the leached chernozem in the three fertilization systems during 1991-2020 period ranged from negative to positive. In the control variant the phosphorus balance was negative throughout the throughout period, with average minus 30.5 kg/ha.

The phosphorus fertilizers application in doses 45 kg/ha on the field with the mineral application system equilibrated the phosphorus balance, on average during 1991-2020 period, from minus 1.3 to -12.9 kg/ha annually.

On the fond of manure in dose of 60 t/ha associated with plant residues, phosphorus mineral fertilizers in doses 45 kg/ha led to an increase in field crop yields, while also reducing the negative phosphorus balance, on average over a 30-year period from -2.7 to -10.1 kg/ha annually.

The phosphorus balance on the organic-mineral system with mineral fertilizers application, phosphorus in doses of 45 kg/ha, on the background of plant residues, on average over the period 1991-2020, was eqiulibrated (-0.7… -5.1 kg/ha) and even positive (+0.4…+4.1 kg/ha), thus reducing the negative balance to a minimum.

At the cultivating the winter wheat, corn for grains, sunflower and leguminous crops on leached chernozem, the phosphorus fertilizers application in dose of 45 kg/ha compensated the phosphorus deficit, the balance becoming equilibrated or positive. For alfalfa, the compensation of the annual export of phosphorus with green mass over 5 years of growing, the dose of 300-350 kg/ha P_2O_5 at the soil basic tillage before the sowing of the culture is sufficient.

Organic-mineral fertilizers led to an increase in the field crops productivity and a decrease in the negative phosphorus balance in the rotation, on average by 19.7-36.1 kg/ha compared to the control variant. The role of organic-mineral fertilizers in maintaining a equilibrated phosphorus balance is essential in the fertilization system of agricultural crops.

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RESEARCH ON THE REALIZATION AND OPTIMIZATION OF EQUIPMENT FOR SUSTAINABLE SOIL BIOREMEDIATION

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Abstract

The mobile equipment developed and tested by INMA Bucharest performs sustainable in-situ soil bioremediation, including a dosing device for biocomposites obtained from recycled resources, based on slag, dolomite, grape marc and wine yeast. The optimization consisted in determining the optimal combination between the speed of the biocomposite dosing devices and the working speed of the equipment. To simulate the dosing and distribution process of biocomposite materials, Altair EDEM™ software, was used. In order to validate the theoretical simulation method, the results obtained from the simulation were compared with the experimental results which was carried out according to SR ISO 5690-2:1995. The relative error between the results obtained by simulation and experimental had small values and it could be concluded that the theoretical simulation method has a good predictive capacity. The quantities of biocomposite that the equipment can distribute per hectare at two working speeds indicated in the current regulations were calculated and the optimal combination between the speed of the dosing devices (28 rpm) of the biocomposite materials and the working speed of the equipment (1.5 m/s) was determined.

Key words: *biocomposites, dosing device, mobile equipment.*

INTRODUCTION

Soil health is essential for sustainable agriculture. Soil is essential for life, providing nutrients, water and oxygen and supporting plants. It is a non-renewable resource. An assessment of the state of soils in the EU found that around 60-70% of them are in poor health due to current management practices (*European Commission, Retrieved from https://commission.europa.eu/index_en*).
Excessive use of nutrients, including manure, on agricultural land in the EU also has a negative impact on water quality and biodiversity (*EU Mission Soil Deal for Europe, 2022. Retrieved from https://mission-soil-platform.ec.europa.eu/living-labs/lighthouses*; *European Court of Auditors, 2023. Retrieved from www.eca.europa.eu*).
Soil or sediment remediation depends on several factors, such as the type of soil, its physical properties, the nature of the contaminants, the possibility of their isolation, the degree of handling required, and the costs involved (Wuana et al., 2011).
The traditional methods available for soil remediation can be grouped into three categories, namely chemical, physical, and biological methods, the latter being carried out either in the polluted place (*in situ*) or outside it (*ex situ*) (Sales da Silva et al., 2020).
These methods are widely used to treat contamination with heavy metals and other toxic substances. Although effective in some cases, they have a high cost and a negative impact on the environment. Also, these methods do not restore soil fertility, but only focus on removing contaminants. In addition, they do not contribute to the circular economy and have a high carbon footprint.
Biological methods can significantly alter soil chemical properties by adding chemicals and nutrients to stimulate microbial growth. Also, in situ soil washing techniques or land use restrictions can lead to groundwater contamination (Dermon et al., 2008).
A simpler and more economical solution, especially for land intended for horticultural and agricultural use, is to cover the surfaces with a superficial layer of clean or

uncontaminated soil (Khan et al., 2018; Yang et al., 2021).

Bioremediation refers to the use of microorganisms to degrade contaminants that pose risks to environmental quality and human health. Phyto- and bioremediation have recently been intensively studied because they are ecofriendly, are able to quickly remove various contaminants and have a relatively lower cost compared to pre-existing techniques (Soleimani, 2014; Kumar et al., 2018).

On the Romanian and international market, competition in the field of soil remediation comes mainly from two directions: traditional physico-chemical methods and alternative bioremediation solutions developed by other companies or research institutes.

In this context, the partners within the CeSoh complex project funded by the PNRR (National Recovery and Resilience Plan) have developed innovative and emerging bioremediation solutions, which will significantly contribute to the ecological restoration of soils, by valorizing waste from various industries (metallurgical/iron and steel, construction materials, viticulture, etc.) and obtaining biocomposites with potential for use in the remediation of soils contaminated with potentially toxic elements (www.cesoh.ro). The project responds to an urgent need for cost-effective and efficient solutions for the regeneration of soils affected by industrial and agricultural activities.

In this study, the optimization of mobile equipment for *in situ* soil bioremediation carried out by INMA Bucharest, consisted in determining the optimal combination between the speed of the biocomposite materials dosing devices and the working speed of the equipment, so that the quantities applied to the soil comply with the regulations in force and the doses currently used in agricultural practice. For this purpose, the behavior of the biocomposite material in the dosing and distribution process was simulated, using the Altair EDEMTM software, and the data were validated experimentally.

MATERIALS AND METHODS

Recycled materials such as slag, dolomite, grape pomace and wine yeast were used to obtain biocomposites. The advantages of using recycled materials are the low costs and the ecological character of the obtained biocomposites. While traditional competition focuses on short-term and often destructive solutions, biocomposites offer a holistic solution, which not only eliminates contaminants, but also restores the structure and health of the soil.

Following the granulometric analysis of the material obtained by mixing the components, it was found that it falls into granulometric class 4, with grain sizes between 1.7 and 4 mm (SR ISO 5690-1, Annex A). These physical parameters are important for the design of mobile equipment for in-situ soil bioremediation and especially for the design of dosing and distribution devices.

Since the amount of biocomposite material obtained in the laboratory at this stage of the CeSoh complex research project was insufficient to perform dosage and distribution tests with mobile equipment for in-situ soil bioremediation, a commercially available Smart mineral amendment was chosen, which has characteristics similar to those of the Biocomposites made within the project (Figure 1).

Figure 1. Amendment used to verify the operation of the biocomposite dosing and distribution device

Smart Minerals is generally used to improve the physical structure of the soil, but also to degrade harmful chemical elements, balance pH, improve biological properties, helping to promote healthy plant growth.

Within the CeSoh project, the team of the partner INMA Bucharest designed, built and tested a mobile equipment for in-situ soil bioremediation, by applying solid or liquid biocomposites, which is presented in Figure 2.

The mobile equipment for in-situ soil bioremediation consists mainly of a frame with rubber wheels, on which a spur roller is mounted that creates alveoli on the soil surface, three devices for spreading granulated biocomposites, a smooth roller for covering the biocomposites and compacting the soil, a 100 l granulated biocomposites hopper, three spiral-type dosing devices with propellers driven through a chain transmission by an ECM070/030U electric gear motor powered by 12 V and an installation (optional) for administering liquid biocomposites (100 l tank, ARAG ProFlo 12V pump, filter, nozzle, hoses).

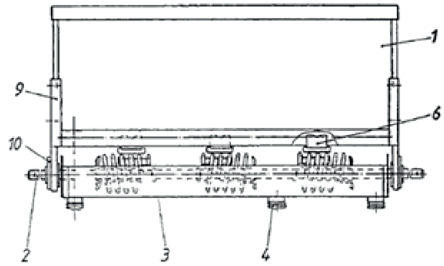
Figure 2. Mobile equipment for in-situ soil bioremediation - experimental model

For this study, a complex design and simulation process was developed, starting with the creation of the biocomposite hopper - chute - dosing devices assembly, using the SolidWorks modeling software. The SolidWorks program was chosen due to its ability to generate precise and detailed models, facilitating the design of the assembly under study.

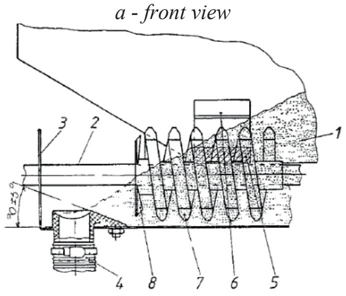
The biocomposite hopper *1* has a prismatic shape with flow angles for all types of biocomposites, powders, granules (vermicular, spherical, cylindrical fragments) or pills (prepared in spherical form with a weight of 0.2-0.3 grams). It is equipped with screens to prevent the biocomposites from agglomerating and a sieve to prevent lumps or other hard materials from entering the hopper when feeding, which could damage or prevent the operation of the equipment. The biocomposite hopper is provided at the bottom with slots *5* for feeding the dosing devices, which can be closed by adjustable shutters *6*. Below these slots are the dosing devices *7* mounted on the shaft *2* and the chute *3* provided with some discharge openings *4*, positioned at a slope of 30° ± 5°, to limit the free flow of the biocomposite. The dosing devices are of the auger type equipped with uniforming propellers *8* (Figure 3 a).

The biocomposite in the hopper *1* flows through the slots *5* between the spirals of the auger *7* into the chute *3*, creating a cone of material. When the shaft *2* rotates, the fertilizer cone is taken up by the spirals of the auger under the action of gravitational force and friction forces and moved towards the discharge mouth *4*, the propeller *8* achieving the uniformity of the material flow rate. The material discharged through the discharge mouth *4* is replaced by free flow, within the limit of the natural slope angle, by the material in the hopper *1*, so that during operation the cross-section of the working enclosure in the area of the dosing device is occupied in a proportion of about 40% with material that will be distributed in full (Figure 3 b).

The amount of biocomposite distributed depends on the speed of the shaft *2*, driven by a chain transmission by the electric gear motor ECM070/030U.



a - front view



b - section through a dosing device

Figure 3. Object of the study: biocomposite hopper – chute - dosing device assembly:
1 - hopper, 2 - shaft, 3 - chute, 4 - outlet, 5 - slot, 6 -sluice, 7 - auger dosing device, 8 - propeller, 9 - support, 10 - bearing

To simulate the dosing and distribution process of biocomposite materials, Altair EDEM™

software, a simulation program based on the discrete element method (DEM), was used.

The discrete element method was also used by Sun et al. (2023) to conduct a phenomenological analysis and numerical investigation of the particle motion characteristics influenced by structural feature parameters of the groove wheel-type fertilizer discharge device. After optimization, the discharge CV was reduced from 91.54% to 31.48%, and the uniformity was improved by 60.06%.

The influence of the different fertilizer discharge parameter combinations on fertilizer discharging performances of the spiral fertilizer applicator was analyzed by Zhang et al. (2023). Furthermore, an EDEM simulation model was built and the fertilizer discharge mechanism was explored. The impact of the fertilizer discharge parameter combinations on the discharging performances was examined from both macroscopic and microscopic perspectives.

This method is particularly useful in simulating the motion and interaction of small particles, such as biocomposites. Figure 4 shows the 3D model of the biocomposite hopper made in SolidWorks, which was subsequently converted to an .stl file and imported into the Altair EDEM™ simulation software.

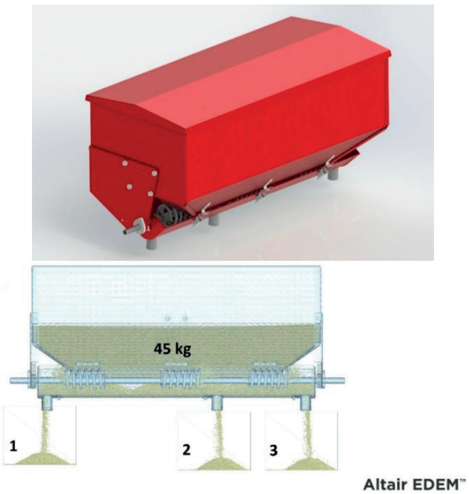
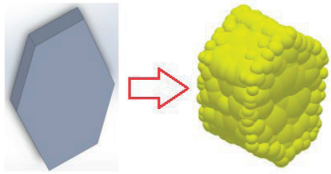


Figure 4. 3D model of the biocomposite hopper-chute-dosing devices assembly and simulation of the dosing and distribution process of the fertilizer material

Simulation in Altair EDEM™ allowed us to analyze with great accuracy the behavior of the material as it is transported through the chute and flows through the vertical tubes into the three collection boxes 1, 2 and 3. This approach provided us with relevant data on the uniformity of the transverse distribution of the designed equipment.

Within the simulation, the first step consisted of defining the materials from which the components of the studied assembly are made: S275JR for the biocomposite hopper and chute, 56Si17A for the auger-type dosing devices.

The next step was to create a detailed 3D CAD model of the smart mineral particle and import it into Altair EDEM™ (Figure 5). This approach allowed us to generate particles with similar characteristics, thus optimizing the accuracy of the simulation of their distribution.



3D model (SolidWorks) *simulation model (EDEM)*

Figure 5. Mineral particle used in the simulation

Next, we realized the interaction between the biocomposite hopper-chute-dosing devices assembly and the smart mineral particles, to ensure an accurate simulation of their behavior. Subsequently, we created a polygon and a factory, in which we filled the biocomposite hopper with a quantity of 45 kg of material. After filling the hopper, we virtually modified the three sluices, allowing the material to flow into the chute and be distributed in the three tubes. Finally, we gave the dosing devices a circular motion, with preset speeds of 20, 24 and 28 rotations per minute, which will also be used in the experimental research.

RESULTS AND DISCUSSIONS

The simulation of the fertilizer dosing process had as its main purpose the theoretical determination of the degree of non-uniformity of the transverse distribution (over the working width of the equipment). The simulation program automatically provided the quantities of fertilizer collected in the three virtual boxes, based on which the absolute mean, standard deviation and coefficient of variation were calculated using Excel. The acceptability condition is if Cv<10%.

The degree of non-uniformity of the fertilizer distribution across the working width of the equipment was highlighted by the coefficient of variation (*Cv*), calculated with the relation (1):

$$C_v = \frac{S}{\bar{x}} \times 100, \% \qquad (1)$$

where: S is the standard deviation, which was calculated with the relation (2):

$$S = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(x_i - \bar{x})^2}, \text{g} \qquad (2)$$

where: n is the number of dosing devices, n=3;
x_i - the average amount of fertilizer collected from each dosing device;
$\bar{x}$- absolute average (average quantity collected at all dosing devices), calculated with the relation (3):

$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i, \text{g} \qquad (3)$$

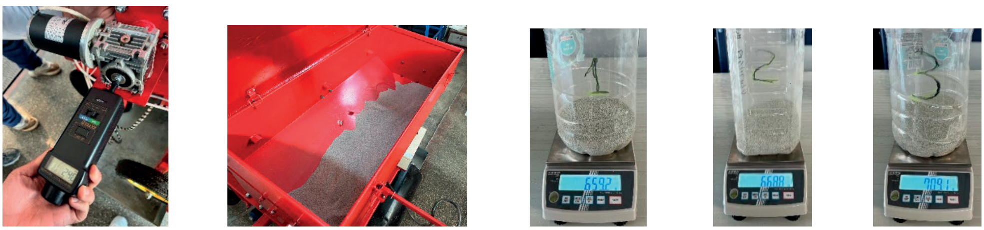
The results obtained from the simulation of the fertilizer material dosing process are presented in Table 1.

Table 1. The degree of non-uniformity of the transverse distribution (Coefficient of variation) determined theoretically (by simulation)

Speed (rpm)	Dosing device no.	Average amount of fertilizer collected from each dosing device, x_i (g)	Absolute average, $\bar{x}$ (g)	Standard deviation, S (g)	Coefficient of variation, C_v (%)
20	1	480	472	18.36	3.89
	2	451			
	3	485			
	Average total quantity (g)	1416			
24	1	642	650	29.82	4.59
	2	625			
	3	683			
	Average total quantity (g)	1950			
28	1	764	759	38.74	5.10
	2	718			
	3	795			
	Average total quantity (g)	2277			

Experimental research was carried out according to the test method regulated by the SR ISO 5690-2:1995.

The tests were carried out by driving the dosing devices at speeds of 20, 24 and 28 rpm, measured with the EXTECH Instruments tachometer, collecting and weighing the fertilizer distributed by each device with the KERN electronic balance (Figure 6).



speed measurement *fertilizer level in the hopper* *weighing samples*

Figure 6. Aspects during experimental research

Each test was carried out simultaneously on the three dosing devices of the equipment, in five repetitions. The time required for each test was 30 seconds. The average of the five samples from each test was calculated and an average value of the amount of fertilizer collected at each dosing device was obtained. The experimentally obtained values for the coefficient of variation (C_v) are presented in Table 2.

Table 2. The degree of non-uniformity of the transverse distribution (Coefficient of variation) determined experimentally

Speed (rpm)	Dosing device no.	Average amount of fertilizer collected from each dosing device, x_i (g)	Absolute average, $\overline{x}$ (g)	Standard deviation, S (g)	Coefficient of variation, C_v (%)
20	1	619	636	32.97	5.18
	2	615			
	3	674			
	Average total quantity (g)	1908			
24	1	700	713	36.29	5.09
	2	685			
	3	754			
	Average total quantity (g)	2139			
28	1	792	791	66.51	8.41
	2	724			
	3	857			
	Average total quantity (g)	2373			

In order to verify and validate the theoretical simulation method, the results obtained from the simulation were compared with the experimental results. A first observation is that, in both cases, the coefficient of variation is below the preset upper limit of 10%. A value close (8.41%) to the upper limit of 10% was observed in the case of experiments at the speed of 28 rpm of the dosing devices, which indicates a possible inappropriate behavior of the fertilizer at high speeds. As can be seen in Table 3, the relative error between the results obtained by simulation and experimental has small values, so we can conclude that the theoretical simulation method has a good predictive capacity, and can be used to predict other qualitative indices of mobile equipment for in-situ soil bioremediation, such as the degree of non-uniformity of distribution on the soil surface.

Table 3. Comparison between the values of the coefficient of variation obtained by simulation and experimentally

Speed (rpm)	Coefficient of variation, C_v (%)		Relative error (%)
	Values obtained from simulation	Experimentally obtained values	
20	3.89	5.18	1.29
24	4.59	5.09	0.5
28	5.10	8.41	3.31

For the two working speeds (1.5 m/s and 2.5 m/s) recommended by SR ISO 5690-2:1995 and using the experimental data in Table 2 for the average total amount of biocomposite collected from the dosing devices at the three speeds of the dosing devices, the theoretical amounts of biocomposite (Q_{tot}) that the equipment can distribute per hectare were calculated, with the relationship (4).

$$Q_{tot}(\frac{kg}{ha}) = \frac{Q_i\,(g)}{S_i(m^2)} \times 10 \qquad (4)$$

where: Q_i is the total average amount of biocomposite collected from the dosing devices for each of the three speeds, in grams, calculated with the relation (5):

$$Q_i = \sum_{i=1}^{3} x_i, \text{ g} \qquad (5)$$

S_i is the area covered by the equipment during t=30 s, in m^2.

Thus, in 30 seconds, the equipment with a working width of 1.4 m, at an average speed of 1.5 m/s (5.4 km/h), would travel 45 linear meters, covering an area equal to 63 m^2. At an average speed of 2.5 m/s (9 km/h) the equipment would travel 75 linear meters, covering an area equal to 105 m^2.

The results obtained for the theoretical quantities of biocomposite that the equipment can distribute per hectare are presented graphically in Figure 7.

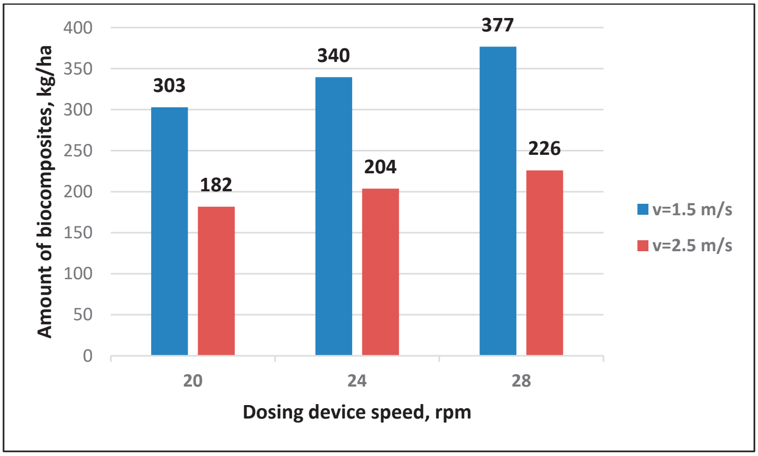


Figure 7. Evolution of the amount of biocomposites applied per hectare depending on the speed of the dosing device, for two working speeds of the equipment

Analyzing the graph in Figure 7, it is observed that the closest value of the amount of biocomposite that the equipment can distribute per hectare (377 kg/ha) to the value indicated in SR ISO 5690-2:1995 for this type of fertilizer was obtained for the dosing device speed of 28 rpm and the equipment working speed of 1.5 m/s. At the same time, it was observed that the range of amounts of biocomposite that the equipment can distribute per hectare is between 182 kg/ha and 377 kg/ha, completely covering the amounts currently used in agricultural practice and specified in the SR ISO 5690-2:1995.

CONCLUSIONS

In this study, a complex design and simulation process was developed, starting with the realization of the biocomposite hopper - chute - dosing devices assembly of the mobile equipment for in-situ soil bioremediation, using the SolidWorks modeling software. To simulate the dosing and distribution process of biocomposite materials, the Altair EDEM™ software was used, a simulation program based on the discrete element method.

In order to verify and validate the theoretical simulation method, the results obtained from the simulation were compared with the results obtained experimentally, for the degree of non-uniformity of the transverse distribution highlighted by the coefficient of variation.

Since the relative error between the results obtained by simulation and experimentally had small values, it can be concluded that the theoretical simulation method has a good predictive capacity, and can be used to predict other qualitative indices of mobile equipment for in-situ soil bioremediation.

At the same time, using simulation in Altair EDEM™, iterative adjustments to the 3D model in SolidWorks can be made, testing improvements. This iterative design and testing process will allow the gradual optimization of the components of the mobile equipment for in-situ soil bioremediation, ensuring the efficiency and reliability necessary for use in real field application conditions.

The theoretical quantities of biocomposite that the equipment can distribute per hectare at two working speeds indicated in the current regulations were calculated and the optimal combination between the speed of the dosing devices (28 rpm) of the biocomposite materials and the working speed of the equipment (1.5 m/s) was determined.

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CHARACTERISATION OF PEDOGENIC CARBONATES IN CALCOCAMBISOL AT A LOCATION IN THE DINARIC PART OF CROATIA

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Abstract

Pedogenic carbonates are secondary carbonate deposits and are a constitutional part of many soils. We analysed a 95 cm deep Calcocambisol soil profile at a location in the Dinaric part of Croatia. Here, pedogenic carbonates are more abundant in the deeper part of the profile (>23 cm), and their amount and size increase with depth corresponding to soil properties along the profile. These pedogenic carbonates are spherical to irregular in shape and can be classified as nodules. Microscopical analysis of these nodules shows that dissolution and re-precipitation of carbonate take place in situ, without considerable movement through the soil profile. The growth of the nodules starts from multiple centres of nucleation, and their internal structure is a result of spatial and temporal environmental conditions in the soil matrix during carbonate precipitation. The inclusion of noncarbonate particles and preservation of the original soil structure confirm the replacive nature of nodule growth. Furthermore, the internal structure of nodules reveals multiple stages of calcite precipitation, indicating seasonal or event-based precipitation of carbonate.

***Key words**: pedogenic carbonates, calcite nodules, Calcocambisol, Croatia, Dinarides.*

INTRODUCTION

Pedogenic carbonates (PC) are secondary carbonate precipitates in soils and are most commonly a result of chemical weathering processes of carbonate rocks. They are a constitutional part of many soils (West et al., 1988; Chesworth, 2008), with a direct impact on the soils hydraulic properties (Castellini et al., 2019) and erodibility (Panagos et al., 2014), and have a high potential of carbon sequestration (Egli et al., 2021).

The formation of PC depends on different abiotic and biotic factors but is predominantly controlled by environmental factors temperature and the amount of precipitation (Borchardt & Lienkaemper, 1999), as well as soil moisture and carbon dioxide concentration. PC can be formed in a wide range of climates (Amit et al., 2011) but are more characteristic for soils in areas with higher temperatures where evapotranspiration exceeds precipitation (Royer, 1999). Thus, PC are commonly found in many soils in Mediterranean climates (Jiménez-Ballesta et al., 2023), such as Terra rosa (Domínguez-Villar et al., 2022), Calcic luvisols (Rovira & Vallejo, 2008), Cambisols and Regosols (Jiménez-Ballesta et al., 2023).

Pedogenic carbonates are formed in soils from geogenic (i.e. from soil parent material or allogenic particles), biogenic (i.e. from carbonates formed within or released by animals and plants), or older PC (Zamanian et al., 2016). They differ in morphology, shape, size, density, porosity and amount of incorporated impurities. Thus, according to the size of the carbonate crystals forming them, PC can be classified as micrite (<5 μm-long carbonate crystals), microsparite (between 5 and 20 μm long), and sparite (>20 μm long) (Becze-Deàk et al., 1997). As well, PC can form different morphologies, such as: earthworm biospherolits, rhizoliths, hypocoatings (pseudomycels), nodules, coatings on clasts, calcretes, and laminar caps (Zamanian et al., 2016).

Studies of PC have been conducted worldwide, especially in carbonate areas (e.g. Cerling & Bowman, 1989; Driese & Mora, 1993; Naiman et al., 2000; Wagner et al., 2012; Jiménez-Ballesta et al., 2023; Bayat et al., 2023; Fu et al., 2024), but there is still a limited number of studies on this topic done in Dinaric karst area (Brlek & Glumac, 2014; Bensa et al., 2021; Domínguez-Villar et al., 2022).

Calcocambisol is one of the most common soil types developed over carbonates in Dinaric karst area (Pilaš et al., 2016; Hasan et al., 2020). Here, Calcocambisols mainly form by weathering and transformation processes observed in carbonate rocks of different ages (Škorić et al., 1985; Husnjak, 2014). Furthermore, Calcocambisol formation is as well influenced by relief characteristics, resulting in its high spatial variability (Vrbek & Pilaš, 2007a; 2007b) and resulting in the development of soils of various depths (Bogunović et al., 2009).

Calcocambisols are silty clayey to clayey soils having favourable soil-water relations due to stable granular and angular structure (Husnjak, 2014; Švob et al., 2021). Furthermore, it has an acidic (Škorić et al., 1985; Miloš & Maleš, 1998) to an alkaline reaction (Bogunović et al., 2009; Miloš & Bensa, 2014) depending on the presence of the carbonates. The production potential of these soils varies and is influenced by numerous factors, including rockiness, skelet content, slope, elevation, and soil depth. If present in the soil, PC can present a problem, affecting soil properties and potentially impacting plant growth.

North Dalmatian Plain is a vast levelled carbonate surface located in the Dinaric karst region (Figure 1). It is dominantly built up by carbonate Upper Cretaceous to the Oligocene lithologies intercalated with marly sediments and occasional bauxites and coal beds (Mamužić, 1971; Ivanović et al., 1978; Velić and Vlahović, 2009). Here, Calcocambisols are a common soil type (Čolak & Martinović, 1973), occasionally characterized by the presence of PC within the soil profile. Thus, the aim of this study is to characterize PC developed in Calcocambisol soil profile at a location within the North Dalmatian Plain.

MATERIALS AND METHODS

The study site (43°47’39" N, 16°00'08" E; 200 m asl) is located in the southeast part of the North Dalmatian Plain (Croatia) within the southern part of Krka National Park (Figure 1 A). The carbonate bedrock at the study site is composed of Eocene foraminiferal limestones and carbonate conglomerates (i.e. Promina Beds) (Ivanović et al., 1977; Brlek et al., 2014), intensively faulted and folded forming structures having Dinaric orientation (i.e., NW-SE) (Figure 1 B). Study site is characterised by discontinuous Calcocambisol soil, typically up to < 0.3 meter thick, with rock exposures covering up to 50% of the terrain (Švob et al., 2021; Bensa et al., 2021). Although, regionally Calcocambisols form shallow soil cover (Vrbek & Pilaš, 2007a), locally in areas where rock fissures occur (as a result of the karstification process) soil depth may extend up to 1 meter (Bogunović et al., 2009; Švob et al., 2021). The study area is characterized by a Mediterranean (Csa) type of climate with dry and hot summers and mild, rainy winters (Filipčić, 1998), and local meteorological conditions are being recorded since 2019 in a station installed in a proximity of study site. Here the mean annual temperature during 2019-2023 period was 14.9°C, while the annual amount of precipitation during this period was 1060 mm with both having clear seasonal pattern.

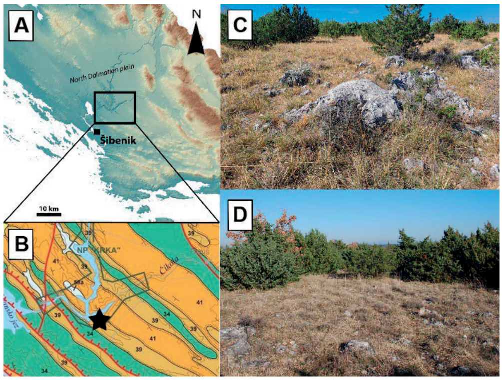

Figure 1. Location of the studied site: A. Location of the study site in the Adriatic part of Croatia; B. Geological map of the study area (after CGI, 2009); 58a: Quaternary sediments, 41: Eocene-Oligocene detrital carbonates (including conglomerates) 39: Eocene foraminiferal limestones, 34: Cretaceous limestones; C. and D. Natural vegetation and soil cover at study site

Vegetation of study site is characterised by fragmented evergreen oak (Quercus ilex) and flowering ash forest (Fraxino orni-Quercetum ilicis H-ić/1956/1958) (Medak & Perić, 2007) degraded to the garrigue state (Figure 1 C, 1 D). Due to reduced amount of biomass production, this type of vegetation has a minimum impact on soil morphology (Vrbek & Pilaš, 2007a).

To investigate soil properties, a 95 cm deep soil profile was dug out and soil sampling was performed along vertical axis of the profile, at 5 cm intervals. Sampling comprised of collection of soil samples in a non-disturbed state using 100 cm3 cylinders, as well as of collection of bulk soil samples. Soil properties were investigated and described following FAO recommendations (2006). Field capacity (FC) was determined by the gravimetric method with core samples, (ISO 11461:2001). Subsequently, the same samples were used for gravimetric determination of bulk density (BD) post-drying at 105°C, adhering to the methods (ISO 11272:2017). According to ISO 11508:2017, the particle density (PD) was measured in water using a 100-mL pycnometer, and according to Danielson & Sutherland (1986), total porosity (TP) and air porosity (AP) were calculated.

Bulk soil samples were air-dried, followed by separation of carbonate particles (i.e. skelet and pedogenic particles), while the rest of the sample was ground and sieved using a sieve with a 2 mm mesh size (ISO 11464:2006). Particle size distribution analysis was done using the pipette method, with wet sieving and sedimentation after dispersion with sodium-pyrophosphate (Na4P2O7, c = 0.4 M) (ISO 11277:2009).

Soil pH values were measured using a combined glass electrode in a 1:5 (v/v) suspension of soil in water and soil in KCl solution (c=1M) according to ISO 10390:2005. The humus content was analyzed by acid potassium-dichromate (K2Cr2O7, c=0.4M) digestion, following the method of Tjurin (JDPZ, 1966), and soil organic carbon (SOC) content was calculated by dividing the content of humus by 1.724 (the Van Bemmelen factor). Total carbonate content was determined by applying the modified volumetric method (ISO 10693:1995). All soil analyses were performed in the Laboratory of the Department of Soil Science, at the Faculty of Agriculture, University of Zagreb.

The analysis of PC within soil samples comprised of particle classification, morphometry, and microscopic analyses. PC were examined using binocular lenses, polarizing petrographic microscope and Scanning Electron Microscope (SEM) at CENIEH (Burgos, Spain). Thus, in order to eliminate the surrounding clay, PC were briefly rinsed in tap water (using an ultrasonic bath). Those to be inspected using SEM were dried overnight in an oven at 50 ˚C and let to cool down in a desiccator until they were gold coated before their analysis, while others were embedded in epoxy resin before being cut and mounted in thin sections to be observed using a polarizing petrographic microscope.

RESULTS AND DISCUSSIONS

The studied soil profile consists of the following mineral horizons: A-Bw-Bk-R (Figure 2). The A horizon (0-7 cm) is characterized by dark brown colour (7.5 YR 4/4) indicating organic matter accumulation. It features a granular structure and common plant roots. The underlying cambic Bw horizon (7-23 cm) is reddish brown (5 YR 4/4) characterized by the increased clay content, noticed by the feel. It has an angular blocky structure and very few to few plant roots. The Bk horizon (23-95 cm) is characterized by the accumulation of PC, mostly in the form of nodules. Furthermore, rock fragments ranging in size from fine gravel (2-6 mm) to gravel (6-20 mm) also occur, Figure 2. Their abundance can be classified as many (15-40%) according to FAO (2006). The R horizon at the bottom of the soil profile refers to the hard, non-weathered rock underlying the soil profile - parent material.

Bulk density (BD) values of the studied soil increased with depth from 1.11 g cm^{-3} to 1.67 g cm^{-3} (Table 1). The lowest BD value was measured in topsoil (0-5 cm) and can be attributed to the highest SOC content of 4.70% (Table 3) and granular structure (Håkånsson & Lipiec, 2000). The increase of BD values with depth suggests soil compaction occurring in the Bw and Bk horizons, which implies restrictions in water movement and root growth. This compaction is linked to higher clay content and an angular blocky structure (FAO, 2006). Particle density (PD) values varied across the profile (2.44-2.62 g cm^{-3}), Table 1, with the lowest values measured within the first 10 cm of depth. The individual values of PD point to differences in SOC content and mineralogy (Hollis et al., 2012).

The highest field capacity (FC) values (42.70% and 41.75%; Table 1), were measured in topsoil horizon (up to a depth of 10 cm) and can be attributed to the accumulation of humified organic matter (Bensa et al., 2021). Across the soil profile, the average FC value was 35.02%, while the lowest values were measured in the deeper parts of the profile (28.86% at 75-80 cm and 29.13% at 50-55 cm depth). These low values can be attributed to the high content of sand particles (Švob et al., 2021), as indicated in Table 2.

Total porosity (TP) values show variation throughout the soil profile, with an average value of 43.43%. Air capacity (AC) values also varied across the soil profile, from 1.03% recorded at a depth of 85–90 cm to 14.03% at a depth of 10-15 cm (Table 1). Very low AC values at the bottom of the soil profile (> 70 cm) are linked to low TP values and high BD values. The measured physical properties of the studied soil are consistent with the previous findings for Calcocambisol in this area (Čolak & Martinović, 1973; Miloš & Maleš, 1998; Švob et al., 2021).

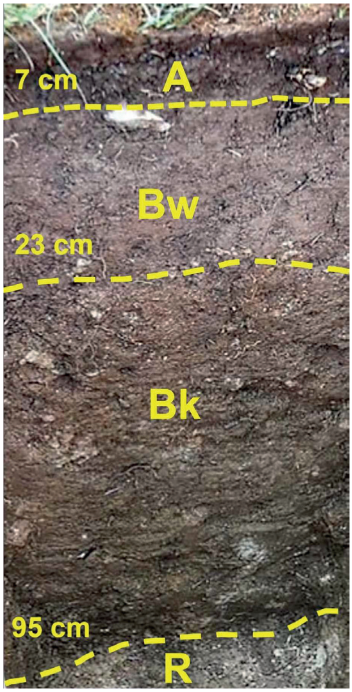

Figure 2. Studied soil profile with indicated soil horizons

Table 1. Basic physical properties of the investigated soil profile

Soil depth (cm)	Bulk density (g cm^{-3})	Particle density (g cm^{-3})	Field capacity (%)	Total porosity (%)	Air capacity (%)
0-5	1.11	2.44	42.70	54.41	11.71
5-10	1.18	2.44	41.75	51.50	9.75
10-15	1.13	2.48	40.37	54.40	14.03
15-20	1.23	2.51	39.48	51.05	11.57
20-25	1.23	2.54	39.65	51.41	11.76
25-30	1.39	2.56	33.92	45.66	11.74
30-35	1.41	2.56	35.46	44.79	9.33
35-40	1.48	2.58	33.42	42.48	9.06
40-45	1.48	2.58	33.32	42.84	9.52
45-50	1.48	2.58	32.97	42.83	9.86
50-55	1.55	2.62	29.13	40.61	11.48
55-60	1.60	2.59	30.59	38.11	7.52
60-65	1.58	2.61	31.29	39.55	8.26
65-70	1.55	2.60	32.00	40.27	8.27
70-75	1.63	2.58	34.88	36.88	2.00
75-80	1.67	2.60	28.86	35.79	6.93
80-85	1.66	2.57	31.73	35.53	3.80
85-90	1.58	2.56	37.43	38.46	1.03
90-95	1.58	2.57	36.35	38.56	2.21

Results of particle size distribution analysis (Table 2) show that clay particles are the most abundant soil fraction throughout the entire soil profile, having the highest values in the Bw horizon (5-30 cm). The average clay content throughout the profile is 48.3%. This is followed by the silt fraction, with an average value of 35.8 %, with a predominance of fine silt in the upper part of the soil profile (0-30 cm), while the coarse silt fraction dominates in the lower part of the soil profile. The sand fraction has the lowest proportion, with an average of 18.9%, and increases with depth, especially coarse sand at the bottom of the soil profile (> 75 cm).

These data are comparable to previous results for Calcocambisols in this area (Čolak & Martinović, 1973; Miloš & Maleš, 1998; Švob et al., 2021).

Soil texture varies through the profile: silty clay in topsoil (0-5 cm), clay at depths 5-50 and 65-95 cm, and clay loam at depths 50-60 cm, affecting soil water content (Figure 3). Determined textural classes are typical for Calcocambisol (Škorić et al., 1985; Husnjak, 2014).

Table 2. Basic physical properties of the investigated soil profile

Depth (cm)	Coarse sand 2.0-0.2 mm	Fine sand 0.2-0.063 mm	Coarse silt 0.063-0.02 mm	Fine silt 0.02-0.002 mm	Clay < 0.002 mm
0-5	3.9	7.2	15.5	28.8	44.6
5-10	2.4	5.0	14.5	24.5	53.6
10-15	3.2	5.5	12.0	23.4	55.9
15-20	2.4	5.2	10.9	20.0	61.5
20-25	2.0	5.3	14.6	19.9	58.2
25-30	3.9	5.1	15.9	17.4	57.7
30-35	6.5	6.8	20.5	14.5	51.7
35-40	8.3	7.0	20.5	16.3	47.9
40-45	7.5	8.8	21.7	16.3	45.7
45-50	9.8	8.7	23.3	18.2	40.0
50-55	7.2	14.5	23.7	16.9	37.7
55-60	7.2	16.3	24.4	16.1	36.0
60-65	7.5	15.4	21.2	15.4	40.5
65-70	7.5	8.3	19.1	14.7	50.4
70-75	10.5	7.1	19.2	14.3	48.9
75-80	16.4	7.3	18.6	13.6	44.1
80-85	13.8	7.1	17.0	15.8	46.3
85-90	13.2	6.9	16.6	15.8	47.5
90-95	13.2	8.1	14.8	15.1	48.8

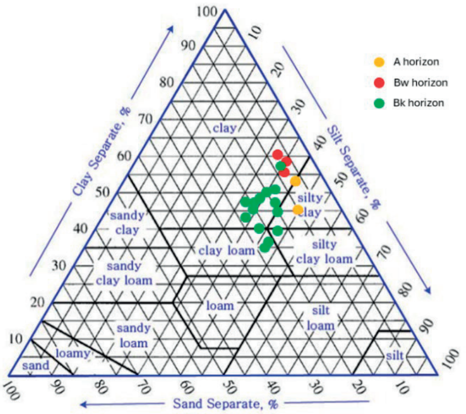

Figure 3. Distribution of soil samples on texture triangle (FAO, 2006)

The analysis of soil chemical properties reveals the lowest $pH_{(KCl)}$ values (Table 3) at the top of the soil profile that gradually increase with depth. The soil reaction is acidic in the A and Bw horizons (up to a depth of 25 cm) as a result of organic matter decomposition and the presence of humic acids, and is alkaline in the Bk horizon (> 25 cm), due to the presence of carbonates. Carbonates appear at a depth 15-20 cm (1.68%) and their amount increases with depth up to 37.38% measured at the 55-60 cm, followed by decrease up to 26.04% at the bottom of the soil profile, Table 3. The accumulation of organic matter in the topsoil (0-15 cm) leads to an increased SOC content (3.60-4.70%) reinforcing the dissolution of carbonate particles and consequently resulting in the absence of carbonates. With depth, SOC content decreases, reaching a minimum of 0.47 % at the depth of 90-95 cm (Table 2).

At the same time, pH values increase indicating that the acidic environment has been neutralized by the dissolution of carbonates, allowing the formation of PC. Measured chemical soil properties are in accordance with the results of previous studies of Calcocambisol in the area (Miloš & Maleš, 1998; Švob et al., 2021; Bensa et al., 2021).

Table 3. Chemical properties of the investigated soil profile

Soil depth (cm)	pH (H_2O)	pH (KCl)	Total carbonates (%)	SOC (%)
0-5	6.61	5.80	-	4.70
5-10	6.49	5.62	-	3.69
10-15	6.40	5.45	-	3.60
15-20	7.14	6.47	1.68	2.55
20-25	7.59	6.94	4.20	2.09
25-30	7.73	7.12	4.03	1.97
30-35	7.82	7.25	11.76	1.61
35-40	7.93	7.35	19.74	1.46
40-45	7.81	7.37	20.16	1.61
45-50	7.94	7.47	29.82	0.98
50-55	8.08	7.42	36.12	0.80
55-60	8.12	7.54	37.38	0.66
60-65	8.13	7.43	30.24	0.66
65-70	8.14	7.42	25.20	0.65
70-75	8.20	7.40	28.14	0.68
75-80	8.24	7.42	31.08	0.60
80-85	8.11	7.45	29.82	0.59
85-90	8.23	7.42	28.14	0.59
90-95	8.19	7.39	26.04	0.47

Understanding of the morphology and formation process of secondary carbonate deposits (i.e. pedogenic carbonates) is essential to address various aspects related to soil formation in arid and semiarid regions (Zamanian et al., 2016). The final morphology of these deposits is a result of the interaction of various factors: soil texture, hydrological regime, salt concentration, soil erosion, and concentration of soil CO_2 (Wieder & Yaalon, 1982; Sobecki & Wilding, 1983; Domínguez-Villar et al., 2022).

Analysis of the studied soil profile shows that PC are more abundant in the deeper part of the profile (>23 cm), and their amount and size increase with depth (Figure 2 and Table 3) corresponding to soil properties along the profile. The upper part of the soil profile (< 20 cm) is characterized by rich biological production leading to higher SOC content, and more acidic environment (i.e. lower pH values; Table 3) favouring carbonate dissolution and resulting in the absence of PC. This corresponds to "*perdescendum*" model of PC formation (Zamanian et al., 2016) where carbonate in the topsoil is dissolved, leached to the subsoil and re-precipitated; and is commonly considered to be one of the main processes of PC redistribution and accumulation in soil horizons (Machette, 1985; Royer, 1999). On the other hand, results of soil analyses showed that physical soil properties vary along the soil profile (Table 1 and Table 2), and in combination with temperature and amount of precipitation certainly affect currently active dissolution and re-precipitation of PC along the soil profile. Thus, this corresponds to *in situ* model of PC formation (Zamanian et al., 2016) where dissolution and re-precipitation of carbonate takes place without considerable movement through the soil profile and redistributes carbonates within the horizon (Monger & Adams, 1996; Rabenhorst & Wilding, 1986; West et al., 1988).

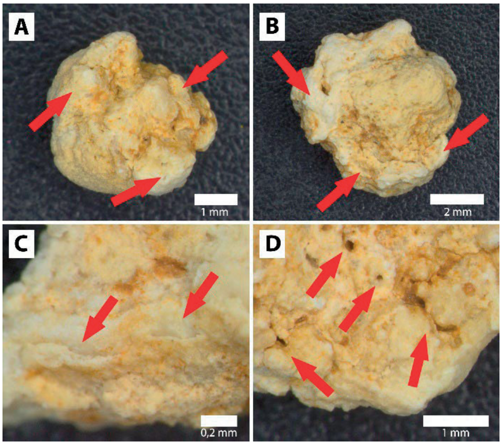

Figure 4. Pedogenic carbonate nodules from the studied soil profile analysed using binocular lenses: A. Typical semispherical calcite nodule formed by agglomeration of several nodules (indicated by red arrows); B. Irregular calcite nodule. Red arrows indicate to different constitutional parts of the nodule; C. Surface of the nodule. Red arrows indicate layers - coatings of sparite calcite attached to the nodule surface, indicating event based or seasonal carbonate accumulation due to different environmental conditions; D. Detail of pitted and hummocky nodule surface. Red arrows indicate dissolution features

Pedogenic carbonate particles from the studied soil profile are mostly spherical to irregular in shape, with diameters up to 64 mm (Figure 4), and can be classified as nodules (Barta, 2011). Nodules are commonly defined as roughly equidimensional pedofeatures that are not related to natural surfaces or voids and do not consist of single crystals (Stoops, 2003) and are usually formed *in situ* by impregnation of soil matrix with $CaCO_3$ (Durand et al., 2010), reworked to varying degrees, or inherited from the parent material (Verrecchia & Trombino, 2021).

The majority of studied nodules from this location have sharp outer boundaries (Figure 4) and their morphology is often characterized by bulbous protrusions (Figures 4 A and 4 B) that indicate the agglomerative nature of their formation (i.e. inclusion of surrounding particles during nodule growth).

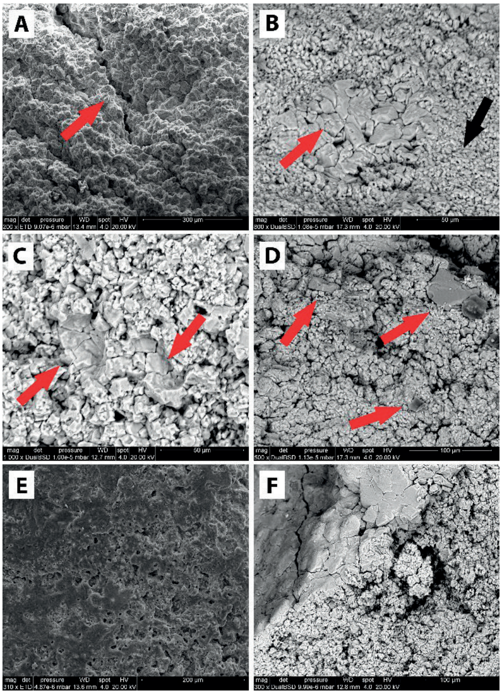

Figure 5. Pedogenic carbonate nodules from the studied soil profile analyzed using a scanning electron microscope: A. Microrunnel (i.e. dissolutional channel) on the surface of the nodule; B. Areas with micritic (black arrow) and microsparitic (red arrow) calcite; C. Casts at the surface formed by detachment of nodule components; D. Detail of the surface of carbonate nodule showing incorporated clay particles (red arrows); E. Pitted surface of the nodule indicating dissolution process; F. Edge of the carbonate nodule having surface flattened by displacive growth

Analyses of nodules using binocular lenses (Figure 4) and SEM (Figure 5) showed that surfaces of the nodules often display pitted and hummocky morphologies and features characteristic of the carbonate dissolution process. Thus, features such as dissolution pits (Figures 4 D and 5 E) and microrunnels (i.e. dissolutional channels; Figures 4 D and 5 A) are commonly observed. The carbonate dissolution process takes place not only at the surface of the nodule, but as well along voids and cavities (Krklec et al., 2013), causing the decrease of nodule cohesion and can result in detachment and removal of its components, as can be observed in Figure 5C. Furthermore, closer inspection of calcite grains surfaces reveals a lack of sharp crystal edges, reinforcing the presence of an active dissolutional process, characteristic for carbonate areas (Krklec et al., 2016; 2018; 2021; 2022; 2024).

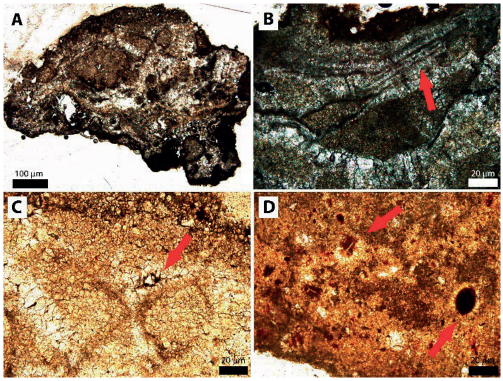

Figure 6. Pedogenic carbonate nodules from the studied soil profile analysed using polarizing petrographic microscope: A. Irregular nodule showing incorporation of various surrounding soil and carbonate particles; B. Edge of nodule showing incorporated particle with multiple stages of micrite and microsparite precipitation; C. Internal nodule structure showing the transition between micrite and microsparite calcite. Red arrow indicates larger sparite crystals formed within empty pore space; D. Detail of carbonate nodule showing the inclusion of different particles. Red arrows indicate grains surrounded by calcite that possibly served as centers of nucleation

On the other hand, analysis of nodules cross sections using polarizing petrographic microscope (Figure 6) shows internal structure of the nodules and provides additional information on carbonate precipitation and formation of these PC particles. Studied carbonate nodules are composed of calcite (micrite, microsparite and sparite) and contain inclusions of other particles (e.g., quartz crystals or soil particles). Their internal structure shows that there is no unique center of nodule nucleation, but rather carbonate precipitation starts around multiple nucleuses and progresses outwards in all directions (Figures 6 A and 6 D). The transition between zones dominated by micrite to those dominated by microsparite is commonly gradual (Figures 6 C and 6 D), although in some cases opposite can be observed (Figures 6 A and 6 B). This distribution of micrite and microsparite crystals within nodules is a result of spatial and temporal environmental conditions in the soil matrix during carbonate precipitation. Larger sparite crystals are commonly developed in cracks or occur as larger pore infillings.

Furthermore, some nodules have features such as carbonate coatings (Figures 4 C and 6 B) where layers of micrite and microsparite interchange, usually parallel to the surface, indicating different stages of calcite precipitation. Thus, these coatings and the internal structure of nodules (interchange of micrite and microsparite) shows that studied nodules do not grow continuously but are formed in multiple stages and/or generations, indicating different pedogenetic phases (Durand et al., 2010; Verrecchia & Trombino, 2021). This episodic carbonate precipitation is driven by seasonal variations in soil moisture and CO_2 levels (Domínguez-Villar et al., 2022), highlighting the role of environmental fluctuations in nodule growth dynamics.

As previously mentioned, carbonate nodules contain inclusions of particles (e.g., quartz grains) inherited from adjacent soil matrix (Figure 5 D, 6 A and 6 D). Some of those particles serve as centres of nodules nucleation, while others are included in nodule structure during its growth. Thus, the internal structure of the nodule preserves the original structure of the soil, even though it has been replaced by calcite. This replacive nodule growth (Colinson & Mountney, 2019) can be as well observed at the edges of the nodules where reaction rims show that the process of replacement has not reached completion (Figure 6 A, 6 B and 6 D). On the other hand, in rare cases, some nodules show evidence of displacive growth (Figures 5 E and 5 F). In this case during nodule growth host sediment is pushed aside and little or none is incorporated into within the nodule (Colinson & Mountney, 2019). Thus, displaced clay particles at the edge of the nodule prevent growth of calcite crystals in that direction, resulting in the formation of flat surfaces at the edge of nodule. (Figures 5 E and 5 F). It should be pointed out that although there is evidence of both, replacive and displacive growth of nodules, the replacive type of nodule growth is the dominant one, confirming *in situ* formation of these PC.

CONCLUSIONS

We studied a 0.95 m deep Calcocambisol soil profile located in the southeast part of the North Dalmatian Plain (Croatia). It consists of A-Bw-Bk-R mineral horizons, containing PC particles in deeper parts (> 23 cm). According to morphology (i.e. spherical to irregular in shape), these PC are classified as nodules. Microscopical analysis of these nodules showed that their formation takes place in situ, starting from multiple centres of nucleation, while inclusion of noncarbonate particles and preservation of the original soil structure confirms replacive nature of nodule growth. The final morphology and structure of a nodule is a result of multiple stages of precipitation and dissolution, indicating seasonal or event-based precipitation of carbonate.

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EARTHWORM CASTS AS INDICATORS OF SOIL SUSTAINABILITY IN A SILVOARABLE ECOSYSTEM FROM WESTERN ROMANIA

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Abstract

The research aimed to characterize a silvoarable ecosystem from western Romania through an indirect biological indicator of soil sustainability: the earthworms' casts. The ecosystem consisted of two components: a no-tilled woody perennial plant - Euro-American hybrid poplar trees (Populus deltoides x Populus nigra) and a conventional tilled agricultural crop – the rapeseed (Brassica napus L.) - hybrid LG Architect. The earthworms' casts have been identified as belonging to two ecological groups: epigeic and anecic earthworms in the poplar plantation and anecic earthworms in the rapeseed plantation. For both categories, the analyzed parameters of the earthworm casts were pH, organic matter (humus), total nitrogen, plant-available phosphorus, and plant-available potassium. The values of these chemical parameters were higher for the earthworm casts collected from the rapeseed crop than those collected from the poplar plantation. The results showed statistically significant differences ($p<0.05$) between certain analyzed cast characteristics (total N, plant-available P, plant-available K) of the two plant sub-systems and the same characteristics of the surrounding soil, suggesting that the differences arise from the microhabitat ecological conditions provided by the establishing of the silvoarable system.

***Key words**: agroforestry, intercropping, canola, Oligochaeta, Lumbricidae.*

INTRODUCTION

The classical indicators of soil sustainability that are generally more often addressed in studies regarding the sustainability of human ecosystems, and especially the sustainability of agricultural ecosystems are those physical (soil texture, bulk density, porosity, water infiltration, soil erosion etc.), chemical (pH, organic matter content, nutrient concentrations etc.) and biological (soil biodiversity and activity at different taxonomical units) (Phillips et al., 2020). Earthworms are largely monitored in the assessment of the agricultural ecosystems, including in the agroforestry ecosystems, predominantly by counting, biomass weighing, and species diversity (Iordache, 2012; Phillips et al., 2020; Chaudhary and Ghaley, 2025; Reis et al., 2025). The earthworm casts are less approached in the studies regarding the sustainability of the anthropogenic ecosystems, and in Romania these are newly proposed in this field (Iordache et al., 2021; Iordache, 2023; Iordache et al., 2023). Earthworm casts are indirect biological indicator of earthworms activity in soil which are recommended based on solid and concrete scientific arguments (Norgrove and Hauser, 2016; Iordache et al., 2024; Lejoly et al., 2024; Dinh et al., 2025). Even so, this indicator remains insufficiently addressed both for the conventional and widely practiced agricultural systems and for the alternative ones. Thus, in the non-conventional agricultural ecosystems and systems, this indicator is addressed even less often, and therefore there is a lack of data at this level. In Romania, the silvoarable ecosystems are rarely and less researched, and the contribution of earthworms within this type of ecosystem remained unstudied until present. In the last years, several attempts to understand the earthworm contribution in several types of anthropic ecosystems of Romania have been addressed, and also the study of earthworm casts (Iordache et al., 2021; Iordache, 2023; Iordache et al., 2023). Nevertheless, this indicator remains insufficiently studied within Romanian agricultural ecosystems, despite the globally acknowledged role of earthworms in such human-influenced environments, as highlighted by other research in the field (Lalthanzara et al., 2011; Hmar and Ramanujam, 2014; Mulia et al., 2021; Reis et al., 2025). This study intended to chemically characterize and compare the earthworm casts sampled from two component systems (a herbaceous crop and a woody plantation) of a silvoarable ecosystem from Romania, but also aimed to compare the chemical composition of the earthworm casts with the chemical composition of the adjacent soil, in order to reveal the chemical parameters which show differences, and thus to emphasize the contribution of earthworm casts to the assessment of the studied silvoarable ecosystem.

MATERIALS AND METHODS

The study was conducted in the year 2023 and the study site was a silvoarable ecosystem located in the Western side of Romania, in Timiș County (45.45418°N, 20.90334°E). Several chemical soil parameters widely used as indicators for soil sustainability were studied, namely: pH, the content of organic matter (soil humus), the content of total nitrogen, the content of plant-available phosphorous and the content of plant-available potassium. These parameters have been pursued both in earthworm casts and in the adjacent soil, as earthworm casts are soil transformed by these organisms by ingestion and digestion processes and afterwards excreted. According to earthworm ecological classification using the criteria of their feeding and activity ecology (Bouché, 1977), in this research, only the casts found at the soil surface, susceptible to be produced by the epigeic and anecic earthworm species were chosen to be studied. The susceptibility resides from the exploitation specificity of the researched silvoarable system and its components: a woody plantation of Euro-American hybrid poplar tree (*Populus deltoides* × *Populus nigra*) and a rapeseed crop (*Brassica napus* L.), LG Architect hybrid intercropped by the poplar plantation. These ecosystems were subjected to varying levels of exploitation at the soil level: the poplar plantation remained undisturbed for a continuous period of eight years, while the rapeseed crop underwent conventional tillage practices. Thus, in the poplar plantation, the analyze of the earthworm cast depositions at soil surface, revealed the predominance of epigeic earthworms, which are characteristic to undisturbed soils and live in the topsoil organic layer, mostly in the first 10 cm, covered by the litter and other decaying vegetal matter. In contrast, the rapeseed crop is characterized by tilled and disturbed soil, which creates unfavorable living conditions for epigeic species. However, the earthworms presence has been noted in this agroecosystem too, based on the casts deposited at soil surface and identified, by their shape and size, as belonging to anecic species. The anecic earthworms burrow deeply into the soil profile, but the great part of their feeding and casting activity takes place in the first 30 cm of soil profile and at the soil surface. These reasons led to the decision to collect the earthworm casts from the soil surface in both rapeseed crop and in the poplar plantation. In the present study, only the chemical composition of earthworm casts is presented, along with a comparison to the chemical composition of the adjacent soil. The latter has been previously reported for the top 30 cm of soil in the poplar plantation and the top 10 cm in the rapeseed crop, with both depth ranges being justified in the same prior study (Mazăre and Iordache, 2023). The earthworm casts analyses have been performed by OSPA Timiș County (Office for Pedological and Agrochemical Studies) using the following methodology: for pH (SR 7184-13:2001-PS-03); for humus (STAS 7184/21-82-PS-01); for the total N (STAS 7184/2-85-PS-08); for the plant-available P (STAS 7184/19-82-PS-02); for the plant-available K (STAS 7184/18-80-PS-06). The soil from the studied silvoarable ecosystem is vertisol (World Reference Base for Soil Resources, 2022). The statistical analysis has been performed using the IBM SPSS 28.0.0.0. software.

RESULTS AND DISCUSSIONS

The chemical compositions of earthworm casts sampled within the studied silvoarable ecosystem are presented in Table 1, alongside the chemical composition of the adjacent soil for the two subsystems (hybrid poplar plantation and rapeseed crop) of the silvoarable ecosystem (Mazăre and Iordache, 2023).

Table 1. Chemical composition of earthworm casts in the two sub-systems of the silvoarable ecosystem

Values of soil and earthworm casts parameters in *Populus* spp. (hybrid poplar) plantation (mean values)					
Substrate type	pH	Humus (%)	Total N (%)	Plant available P (ppm)	Plant available K (ppm)
Topsoil (0-30 cm) (Mazăre and Iordache, 2023)	6.36 ±0.16	1.71 ±0.63	0.13 ±0.10	4.72 ±0.81	104.66 ±18.71
Earthworm casts	6.53 ±0.27	1.93 ±0.87	0.15 ±0.017	17.26 ±3.56	131.33 ±13.61
Values of soil and earthworm casts parameters in *Brassica napus* (rapeseed) crop (mean values)					
Substrate type	pH	Humus (%)	Total N (%)	Plant available P (ppm)	Plant available K (ppm)
Topsoil (0-10 cm) (Mazăre and Iordache, 2023)	6.42 ±0.24	2.07 ±0.65	0.12 ±0.0057	18.78 ±15.61	124 ±15.87
Earthworm casts	5.91 ±0.22	2.16 ±0.20	0.17 ±0.03	101.56 ±52.64	237 ±91.06

It could be observed that the values of the analyzed chemical parameters of the earthworm casts are higher in the rapeseed crop than in the poplar plantation (Figure 1). This situation could be due to several factors, but any interpretation should consider that earthworm casts are in fact soil ingested (even not solely) and excreted, so the initial composition and fertility of the ingested soil is a direct factor which influences the final excreted cast. In our study, the rapeseed crop underwent conventional tillage practices, so it was chemically fertilized, and this could be a reason why higher values of the chemical factors were found in the earthworm casts sampled here than in the poplar casts. Another factor susceptible to be responsible for the mentioned result may be the different rate of decomposition of the organic matter input in the rapeseed crop versus in the poplar plantation (Heyn et al., 2019). Thus, in the poplar plantation, although the input of organic matter (leaf litter contribution, root decaying)is higher than in the rapeseed crop, its decomposition and turnover into mineral nutrients may be slower. Therefore, the biogeochemical cycle in the rapeseed soil may be faster than in the poplar soil. Another important factor which may differentiate the chemical composition of earthworm casts is the food type (C/N ratio) ingested by earthworms which ends in excreted casts, because in the rapeseed crop there were collected the casts of the anecic earthworm species, while in the poplar plantation the great part of the collected casts belonged to the epigeic species. The ratio C/N is higher in the casts of epigeic earthworms than in the casts of the anecic species (Zi et al., 2024), and the same in our study (Table 1).

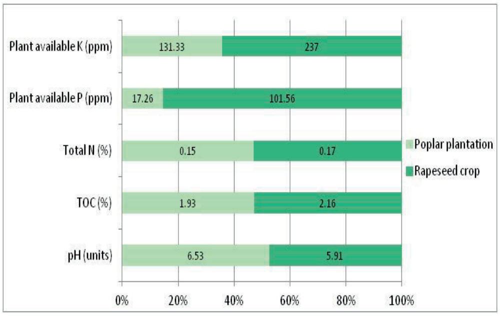

Figure 1. Chemical composition of earthworm casts in the two sub-systems of the studied silvoarable ecosystem

The statistical analysis (Paired Samples *t*-test) showed significant differences only for the soil pH and soil content of plant-available P between the earthworm casts of the two plant sub-systems (Table 2). However, higher values in the chemical composition are observed in the rapeseed casts compared to the poplar casts (for instance, the plant-available K content in the rapeseed casts is 80.46% higher than that in the poplar casts). Therefore, the lack of statistical significance is considered to be due to other factors that may influence the statistics, without undermining the validity of the results obtained (Botinelli et al., 2010; Chen et al., 2021; Iordache et al., 2024).

Table 2. Comparison (Paired Samples *t*-test) of the chemical composition of earthworm casts in the two sub-systems of the silvoarable ecosystem

Crt.no.	Comparison of chemical composition of earthworm casts (poplar versus rapeseed)	t	df	Significance ($p<0.05$)
1.	$pH_{poplar} - pH_{rapeseed}$	10.169	2	0.005
2.	$Humus_{poplar} - Humus_{rapeseed}$	-0.447	2	0.349
3.	$Total\ N_{poplar} - Total\ N_{rapeseed}$	-2.000	2	0.092
4.	$P_{poplar} - P_{rapeseed}$	-2.654	2	0.059
5.	$K_{poplar} - K_{rapeseed}$	-2.315	2	0.073

An interesting situation can be observed for the content of plant-available P both in the soil and in the earthworm casts sampled from the poplar plantation as compared to the same parameters analyzed in rapeseed. The content of this nutrient was very low in the poplar soil as compared to the rapeseed soil. According to the Egner-Riehm method (1960), this content corresponds to a medium supply of soil with plant-available P, while the content found in the rapeseed soil is considered to correspond to a very well supplied soil. This consistent difference may a result of the fertilization practices applied in the agricultural plot or even the result of crop rotations during the cultivation technologies, but it could also be the effect of some possible consequences resulted from the silvoarable establishment. A similar situation was previously reported by Yemadje et al. (2023) in an intercropped oil-palm agroforestry system, where levels of phosphorus (P) and potassium (K) were higher in soils from cropped fields compared to palm fallow soils. These results were attributed both to fertilization practices and to the substantial nutrient uptake by the woody oil palm plants. However, the demands of Euro-American hybrid poplar tree (*Populus deltoides* × *Populus nigra*) for P content in soil are met, because it requires moderate to high values of P in soil, and the necessities are high especially in the earlier stages of its development and growth (Singh and Sharma, 2007; Salehi et al., 2022).

For the comparative analysis of earthworm casts (poplar versus rapeseed), the differences between the chemical component parameters were statistically significant only for pH and plant-available P which suggest the contribution of the epigeic and anecic earthworms in the nutrient dynamics of the silvoarable ecosystem of these elements when the rapeseed and the poplar are associated as sub-systems of an silvoarable ecosystem. Thus, the pH slightly decreased in the rapeseed casts as compared to the poplar casts, and the plant-available P increased in the rapeseed cast by approximately 5.88 times than in the poplar casts (Figure 1). The higher value of the pH of earthworm casts sampled from the poplar soil could be an effect of the higher amount of decaying litter in the poplar plantation which determine changes in the pH of the topsoil and also is consumed by the epigeic and anecic earthworms as food together with topsoil particles. The genus *Populus* was earlier shown to increase the topsoil (0-40 cm) pH as effect of litter contribution (Ciadamidaro et al., 2014) as provider of organic matter.

The comparison of the chemical composition of earthworm casts collected from the rapeseed agroecosystem related to chemical composition of the surrounding (adjacent) soil showed higher values of the parameters in the earthworm casts (Figure 2), excepting the value of cast pH, which is lower than the pH of the adjacent soil. The slight decrease of the pH of earthworm casts related to the surrounding soil has been previously reported in other studies as result of microbiological complex interactions occurred during the food processing in earthworm gut (Yang et al., 2024).

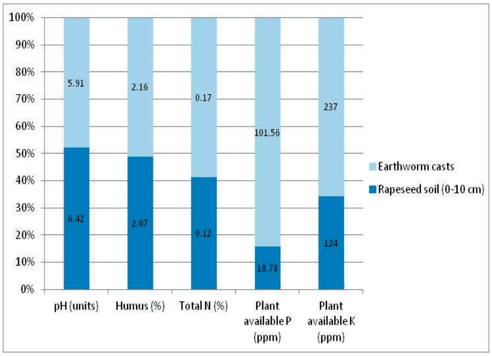

Figure 2. Chemical composition of earthworm casts related to the adjacent soil in the rapeseed crop

The differences between casts and the adjacent soil regarding the chemical composition are consistent especially for the contents of plant-available P and K in the rapeseed crop, respectively by 5.4 and 1.9 times greater in earthworm casts than in the adjacent soil, but these differences are not entirely supported by the statistical analysis (Paired Samples *t*-test), which showed significant differences only for the content of total N (Table 3). Thus, the content of total N was found to be 41.66% higher in earthworm casts than in the surrounding soil.

Table 3. Comparison (Paired Samples *t*-test) of the chemical composition of earthworm casts related to the adjacent soil in the rapeseed crop

Crt.no	Comparison of chemical parameters (adjacent soil vs earthworm cast)	t	df	Significance (p<0.05)
1.	$pH_{soil} – pH_{cast}$	1.952	2	0.095
2.	$Humus_{soil} – Humus_{cast}$	-0.190	2	0.434
3.	Total N_{soil} – Total N_{cast}	-2.982	2	0.048
4.	$P_{soil} – P_{cast}$	-2.485	2	0.065
5.	$K_{soil} – K_{cast}$	-2.442	2	0.067

Although the statistical analysis does not provide conclusive support for the increases observed in other nutrients, it does not undermine the clear potential of earthworm casts as a source of nutrient enhancement within the studied silvoarable system, benefiting both its components (rapeseed and poplar) (Figures 2 and 3). However, when the earthworm casts and the adjacent soil are compared, the variability of their nutritive elements is high, and therefore there is no generality and consistency regarding certain nutrients that would always be increasing in the earthworm casts as compared to the adjacent soil, while others would be decreasing, because the variability of factors that can lead to very heterogeneous results is high, as well as their range. For example, other studies on agroforestry systems showed significant differences between earthworm casts and its adjacent soil for K and P, while no significant differences were found for N (Hmar & Ramanujam, 2014).The absence of statistical significance in the achieved results does not diminish their relevance, as the variability of soil chemical parameters within anthropogenic ecosystems is influenced by multiple factors. These include the environmental heterogeneity, sampling limitations and inherent soil variability, all of which can contribute to the lack of statistical assurance (Iordache, 2023).

A similar situation was found in poplar: significant differences (Paired Samples *t*-test) between the chemical composition of earthworm cast and the chemical composition of the adjacent soil were found only for plant-available P and K (Figure 3 and Table 4).

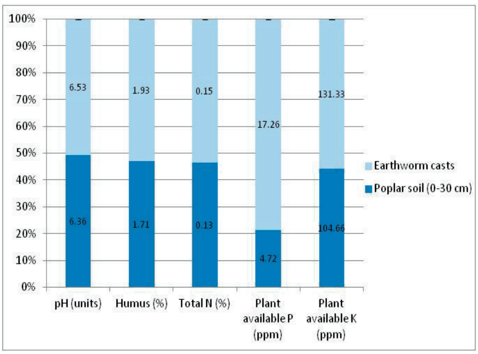

Figure 3. Chemical composition of earthworm casts related to the adjacent soil in the poplar soil

In the case of poplar soil, all the analyzed chemical parameters of earthworm casts were higher than the same parameters recorded for the adjacent soil, including the pH (Figure 3). Higher pH values of the earthworm casts than those of the adjacent soil has been previously found in the agroforestry systems (Hmar and Ramanujam, 2014) and could be the result of the *Populus* litter ingestion by earthworms, which accelerates the turnover process of nutrients within the biogeochemical cycles than does the litter decomposition (mineralization) unmediated by earthworms, by fragmenting the litter and enhancing thus complex microbial interactions with its ingestion (Yang et al., 2023).

Table 4. Comparison (Paired Samples *t*-test) of the chemical composition of earthworm casts related to the adjacent soil in the poplar plantation

Crt. no	Parameter comparison (soil vs earthworm cast)	t	df	Significance (p<0.05)
1.	$pH_{soil} - pH_{cast}$	0.695	2	0.279
2.	$Humus_{soil} - Humus_{cast}$	0.552	2	0.318
3.	Total N_{soil} – Total N_{cast}	2.646	2	0.059
4.	$P_{soil} - P_{cast}$	7.621	2	0.008
5.	$K_{soil} - K_{cast}$	2.915	2	0.050

In order to understand the relationship between the chemical parameters characterising the earthworm casts, statistical correlations (Pearson, Kendall, Spearman, p<0.05) were performed both for the casts sampled in poplar plantation and in the rapeseed crop (Table 5).

Table 5. Relationship (Pearson, Kendall and Spearman correlations, p<0.05) between chemical parameters of earthworm casts

Earthworm casts - Rapeseed crop					
			Nt	P	K
Pearson Correlation	pH	Correlation Coefficient	-	0.998	-
		Significance (p<0.05)	-	0.038	-
Kendall Correlation, Spearman Correlation	pH	Correlation Coefficient	1,000	1,000	1,000
		Significance (p<0.05)	.	.	.
	Nt	Correlation Coefficient	-	1,000	1,000
		Significance(p<0.05)	-	.	.
	K	Correlation Coefficient	-	1,000	-
		Significance (p<0.05)	-	.	-
Earthworm casts - Poplar plantation					
			Humus	K	
Kendall Correlation, Spearman Correlation	pH	Correlation Coefficient	1,000	1,000	
		Significance (p<0.05)	.	.	
	Humus	Correlation Coefficient	-	1,000	
		Significance (p<0.05)	-	.	

There were found positive correlations between cast pH and casts' humus, total N, plant-available P, and plant-available K. These types of relationship have been earlier identified within earthworm casts, although in other anthropogenic ecosystems (Iordache et al., 2023). For the earthworm casts sampled in the rapeseed crop, the soil pH was statistically significant correlated with the content of total N, plant-available P and plant-available K. Also, the content of total N was statistically significant correlated with the content of plant-available P and plant-available K, and the content of plant-available K was statistically significant correlated with the content of plant-available P. For the earthworm casts sampled in the poplar plantation, the soil pH was statistically significant correlated with the content of organic matter (humus) and with the content of plant-available K. Also, the content of organic matter (humus) was statistically significant correlated with the content of plant-available K.

There are many factors which can influence the great variability of earthworm casts pH, emphasized in various types of anthropogenic ecosystems, and its correlations with other chemical parameters of their composition: the most studied and frequent are the bioturbation of soil layers and its biochemical related factors determined by the native ecological group of earthworms and by the microbial interactions which characterize the earthworm activity within drilosphere (Wang et al., 2021; Ferlian et al., 2022). Interesting correlations were established by the plant-available K with two factors: plant-available P and total N (in rapeseed casts), but also with the humus in the poplar casts, which are consistent with other findings regarding the earthworm casts in the Romanian anthropogenic ecosystems (Iordache, 2023), revealing the complexity of the chemical interactions occurred inside the earthworm casts and their importance in the ecosystem biogeochemical cycles, and thus as indicator of soil sustainability.

CONCLUSIONS

The contents of the organic matter (humus), total nitrogen, plant-available phosphorus and plant-available potassium were higher in the earthworm casts collected from the rapeseed crop than those collected from the poplar plantation.

The chemical composition of earthworm casts related to chemical composition of the surrounding (adjacent) soil showed higher values of the parameters in the earthworm casts.

The results suggest the contribution of the epigeic and anecic earthworms to the nutrient dynamics in the studied silvoarable ecosystem established by associating the rapeseed crop and the hybrid poplar plantation.

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SUITABILITY OF LAND FOR FORESTRY USE IN GALAŢI COUNTY, TECUCI-MATCA AREA

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Abstract

In addition to being deficient in terms of the forestry sector, the studied territory presents pedoclimatic conditions less suitable for agricultural crops, because the rainfall is quantitatively reduced and the land is poor quality and prone to deflation. In order to reduce the effects caused by climate change, biodiversity conservation and implicitly the protection of agricultural land, it is intended to establish a forest on an area of 150.76 hectares, with forest species suitable from a pedoclimatic point of view. A complex pedological study was carried out, in different plots, by performing a soil profile and several control surveys, in order to establish the suitability of the land for forestry facilities. The soil type identified is typical arenosols, formed in the Tecuci Plain, due to the deposition of coarse and fine sand. The surface of land is a relatively flat configuration, loamy-sandy texture in the bioaccumulative horizon and sand on the depth of the soil profile, with contrasting distribution. For these pedoclimatic conditions with local specificity, the formula for afforestation with adapted species, with fast growth and low requirements in terms of soil trophicity was established.

Key words: *shelterbelts, windbreaks, mixed forest, species composition, typical arenosols.*

INTRODUCTION

The purpose of the forest protection curtains is to protect the agricultural lands against the prevailing wind, because by stopping or reducing it, the evaporation of water from the soil surface is reduced, the transpiration of plants is reduced and implicitly the conservation of water in the soil is achieved. At the same time, the capillary rise of the soil is reduced, maintaining a uniform layer of snow, preventing soil erosion, etc. besides these advantages, a specific microclimate is created, beneficial to cultivated plants, household centers and of course the restoration of the ecological balance.

By setting up protective forest curtains, it reduces about 4% of the land area but compensates by increasing production by up to 35% or more in some years.

A complete network of forest curtains (main and secondary), determined the reduction of wind speed by up to 50 %, the significant decrease of evapotranspiration and the conservation of water in the soil (Andreu et al., 2008).

Worldwide, Russia is considered the pioneer in combating extreme drought, since the first forest curtains with a protective role, were established in 1696 in southern Ukraine, planted at the ordinal of Tsar Peter the Great.

In 1883, 80 hectares with protective forest curtains were established, on the N-S direction, in the Kamennaya steppe (Vasilescu, 2004).

Based on the scientific assessment of the great scientist Dokuchaev, agroforestry systematically expanded in the steppe zones (Chendev et al., 2015).

These models of forest curtains have expanded in European countries, such as: Denmark, Germany, Italy, Hungary, Bulgaria, then in the USA, Canada, Japan, etc., but with much smaller dimensions (Vasilescu, 2004).

Afforestation as a forest management practice can be usefully applied to degraded lands unsuitable for agricultural crops to transform them into productive stands (Reyer et al., 2009; El-Beltagy, 2000).

In our country, the need to establish forest curtains was first mentioned by the great agronomist and politician I. I. de la Brad in 1866, who established the first forest curtains on the land of his farm in Roman, Neamt County, in the years 1870-1872 (Mușat et al., 2024).
Later, according to the same model, they were founded in 1880 in Mărculești, Ialomita County, then in Brăila County, in the period 1933-1937 on about 90 hectares (Vasilescu, 2004).
In 1960, the forest curtains protected a million hectares of land in Dobrogea and Bărăgan, and in 1961 it reached 7000 km of forest curtains that protected the fields and 1400 km that protected the communication routes (Costachescu et al., 2012).

MATERIALS AND METHODS

The studied area is a land area of 150.76 ha, framed in several soles with relatively flat relief, belonging to the Romanian Academy, located in the Tecuci-Matca area, Galati County. The lithological substrate in the studied area is represented by sandy eolian deposits of different geographical granulations, the area being framed in the Tecuci Plain (Figure 1).

Figure 1. Tecuci-Matca, Galati county

The research methods used in this study were those practiced according to the ICPA methodology, 1987, vol. I, II and III.
Soil analysis
Analysis methods used to determine chemical properties:
- Organic matter (humus): determined volumetrically by the wet oxidation method according to Walkley-Black, in the Gogoaşă modification – STAS 7184/21-82;
- CaCO3 (carbonates): gasometric method using the Scheibler calcimeter, according to SR ISO 10693:1998 (%);
- Nitrogen content was determined indirectly (by calculation) based on the humus content and the degree of base saturation.

$$IN = humus \times V / 100$$

- Accessible phosphorus (mobile P): according to the Egner-Riehm-Domingo method and dosed colorimetrically with molybdenum blue, according to the Murphy-Riley method (reduction with ascorbic acid);
- Accessible potassium (mobile K): extraction according to the Egner-Riehm-Domingo method and dosage by flame photometry;
- pH: determined potentiometrically, with a combined glass and calomel electrode, in aqueous suspension at a soil/water ratio of 1/2.5 - SR 7184/13-2001;
- Hydrolytic acidity - extraction with sodium acetate at pH 8.2;
- Sum of bases - Kappen Schoffield Chiriță method by extraction with 0.05 n hydrochloric acid.

Analysis methods used to determine physical properties
Determination of particle size fractions:
- pipette method for fractions ≤ 0.002 mm;
- wet sieving method for fractions 0.002-0.2 mm and dry sieving method for fractions > 0.2 mm;
- Bulk density (AD): method of metal cylinders of known volume (100 cm^3) at the current soil moisture (g/cm^3);
- Total porosity (PT): by calculation (% volume -% v/v);
- Aeration porosity (PA): by calculation (% volume -% v/v);
- Degree of compaction (GT): by calculation GT = [(PM – PT)/PMN] x 100 (% volume -% v/v), where: PMN – minimum total porosity required, varied depending on the clay content of the respective sample, is calculated with the formula PMN = 45+0.163 A (% volume -% v/v); PT = total porosity (% v/v); A – clay content (% g/g);
- Wilting coefficient (CO): by calculation, by multiplying the hygroscopicity coefficient by 1.5;
- Field water capacity (CC): by estimation based on texture and apparent density, according to "Methodology for Elaborating

Pedological Studies", ICPA, 1987, vol. I, page 101 (% by weight - % g/g).

RESULTS AND DISCUSSIONS

A soil profile and three surveys were carried out in different soils, which were morphologically and physico-chemically characterized, according to the guide for the field description of the soil profile and specific environmental conditions. (Munteanu et al., 2009).

Profile 1 Typical Arenosols (Figure 2)
Coordinates: *45.838295 - N and 27.479516 - E*
Rocks: *eolian deposits (sandy)*
Relief: *wavy landscape*
Use: *arable*
Groundwater: *over 10 m*

Figure 2. Profile 1 Typical Arenosols

Morphological characterization of the profile
Horizon Ao (0-34 cm), fine sandy loam, dark brown (10 YR 3/3 when wet and 10 YR 4/4 when dry), moderately developed grain structure, small and medium aggregates, moist, frequent fine roots, weakly adhesive, weakly plastic, moderately compact at the base, gradual wavy transition;
Horizon A/C (34-62 cm), medium loamy sand, light brown (10 YR 4/4 when wet and 10 YR 5/6 when dry), poorly formed grain structure, visible sand grains on the surface of the aggregates, rare fine roots, non-plastic, non-adhesive, clear wavy transition to the lower horizon;
Horizon C1 (62-104 cm), fine, yellowish-brown loamy sand (10 YR 4/6 when wet and 10 YR 6/6 when dry), unstructured, very friable, gradual transition;
Horizon C2 (104-126 cm), medium, yellowish loamy sand (2.5 Y 5/4 when wet and 2.5 Y 6/6 when dry),unstructured, very friable;
Horizon C3 (126-178 cm), coarse, olive-yellowish loamy sand (2.5 YR 4/6 when wet and 2.5 YR 6/6 when dry), unstructured, very friable.
The soil samples were analyzed physical and chemical, the results being shown in Table 1.

Table 1. Physical and chemical analyses for Typical Arenosols

Soil horizon	Ao	AC	C_1	C_2
Depth (cm)	0-34	34-62	62-104	104-146
Coarse Sand (2-0.2 mm)	14.6	27.1	24.6	31.8
Fine Sand (0.2-0.02 mm)	36.5	32.9	37.8	32.6
Dust (0.02-0.002 mm)	30.6	29.7	28.4	26.9
Clay (< 0.002 mm)	18.3	10.3	9.2	8.7
Texture	SF	UM	UF	UM
Soil reaction (pH)	5.36	5.84	6.28	7.14
Humus content (%)	1.78	1.42	0.53	0.12
Bulk density (g/cm^3)	1.31	1.54	1.58	1.51
Total porosity (%)	50	41	40	42
Degree of compaction (%)	non-compacted	mod. compacted	mod. compacted	slightly compacted
Nitrogen index (IN)	1,35	1.1	0.43	0.1
Base saturation (V%)	76	78	82	86
Mobile phosphorus (ppm)	16	11	7	-
Mobile potassium (ppm)	134	96	68	-
Wilting coefficient (%)	7.8	7.2	5.5	4.8
Field capacity (%)	14.2	13.1	10.1	8.7
Available water capacity (%)	6.4	5.9	4.6	3.9
Total water capacity (%)	38	27	25	28
Humus reserve (t/ha)	79	61	35	7.6

Secondary profile 1 Typical Arenosols (Figure 3)
Coordinates: *45.835706 - N and 27.485576 - E*
Rock: *eolian deposits (sandy)*
Relief: *wavy landscape*
Use: *arable*
Groundwater: *over 10 m*

Figure 3. Secondary profile 1

Morphological characterization of the secondary profile 1
Horizon Ao (0-22 cm), fine loamy sand, dark brown (2.5 Y 3/2 when wet and 2.5 Y 4/3 when dry), medium grain structure, poorly developed, wet, weak effervescence, frequent fine roots, non-adhesive, non-plastic, gradual transition to the lower horizon, hardpan at the base (20-26 cm);
Horizon A/C (22-48 cm), fine loamy sand, dark olive brown (2.5 Y 3/3 when wet and 2.5 Y 4/4 when dry), moderately developed grain structure, moist, frequent fine roots, non-adhesive, non-plastic, gradual transition to the next horizon;
Horizon C1 (48-86 cm), coarse, poorly structured, olive brown loamy sand (2.5 Y 4/3 when wet and 2.5 Y 5/4 when dry), moderately developed grain structure, moist, frequent fine roots;
Horizon C2 (86-115 cm), coarse, yellowish brown loamy sand (2.5 Y 5/3 when wet and 2.5 YR 6/4 when dry), poorly structured, sand grains visible on the surface of the aggregates, non-plastic, non-adhesive, clear transition to the lower horizon;
Horizon C3 (> 115 cm), coarse, yellowish brown sand (7.5 YR 4/6 when wet and 2.5 YR 7/6 when dry), unstructured, very friable, does not effervescent.
The soil samples were analyzed physico-chemically, the results being shown in Table 2.

Table. 2. Physical and chemical analyses for Typical Arenosols - Secondary profile 1

Soil horizon	**Ao**	**AC**	**C_1**	**C_2**
Depth (cm)	0-22	22-48	48-86	86-115
Coarse Sand (2-0.2 mm)	29.8	32.8	38.6	43.4
Fine Sand (0.2-0.02 mm)	31.6	30.1	31.4	30.3
Dust (0.02-0.002 mm)	30.4	29.8	28.7	25.6
Clay (< 0.002 mm)	8.2	7.3	1.3	0.7
Texture	UF	UG	NG	NG
Soil reaction (pH)	5.0	5.7	6.4	6.7
Humus content (%)	1.38	1.04	0.76	0.15
Bulk density (g/cm^3)	1.23	1.28	1.33	1.44
Total porosity (%)	54	52	50	46
Degree of compaction (%)	non-compacted	non-compacted	non-compacted	non-compacted
Nitrogen index (IN)	0.97	0.74	0.59	-
Base saturation V (%)	70	72	78	83
Mobile phosphorus (ppm)	21	17	13	7
Mobil potassium (ppm)	110	86	71	56
Wilting coefficient (%)	5,3	4.5	3,3	3.1
Field capacity (%)	7.95	6.75	4.95	-
Available water capacity (%)	2.65	2.25	1.65	-
Total water capacity (%)	44	41	37.5	-
Humus reserve (t/ha)	78.0	34.6	22.23	-

Secondary profile 2 Typical Arenosols (Figure 4)
Coordinates: *45.823452 - N and 27.500447 – E*
Rock: *eolian deposits (sandy)*
Relief: *undulating field*
Usage: *arable, wheat stubble*
Groundwater: *over 10 m*

Figure 4. Secondary profile 2

Morphological characterization of the secondary profile 2

Horizon Ao (0-24 cm) fine loamy sand, dark brown (10 YR 4/3 when wet and 10 YR 5/4 when dry), medium grain structure, poorly developed, revan, frequent fine roots, non-adhesive, non-plastic, diffuse transition to the lower horizon;

Horizon A/C (24-50 cm) medium loamy sand, light brown (10 YR 4/4 when wet and 10 YR 6/4 when dry), poorly developed grain structure, revan, frequent fine roots, non-adhesive, non-plastic, clear transition to the underlying horizon;

Horizon C1 (50-90 cm) coarse loamy sand, unstructured, yellowish brown (2.5 Y 4/3 when wet and 2.5 Y 5/4 when dry), moist, rare fine roots;

Horizon C2 (90-130 cm) coarse, unstructured, friable, yellowish loamy sand (2.5 Y 5/3 when wet and 2.5 YR 6/4 when dry), clear transition to the lower horizon;

Horizon C3 (> 130 cm) coarse, pale yellow sand (2.5 Y 6/4 when wet and 2.5 YR 7/4 when dry), unstructured, very friable, very weak effervescence at the base;

Soil samples were analyzed physicochemically, the results being shown in Table 3.

Table 3. Physical and chemical analyses for Typical Arenosols-Secondary profile 2

Soil horizon	Ao	AC	C_1	C_2
Depth (cm)	0-24	24-50	50-90	90-130
Coarse Sand (2-0.2 mm)	20.9	28.7	36.6	42.1
Fine Sand (0.2-0.02 mm)	37.5	32.6	26.8	24.9
Dust (0.02-0.002 mm)	30.7	29.4	28.5	25.3
Clay (< 0.002 mm)	10.9	9.3	8,1	7.7
Texture	UF	UM	UG	UG
Soil reaction (pH)	5.8	5.9	6.2	6.9
Humus content (%)	1.67	1.12	0.62	0.18
Bulk density (g/cm^3)	1.3	1.38	1.39	1.42
Total porosity (%)	52	51	50	44
Degree of compaction (%)	non-compacted	non-compacted	non-compacted	slightly compacted
Nitrogen index (IN)	0.97	0.74	0.59	-
Base saturation V (%)	74	75	79	84
Mobile phosphorus (ppm)	16	11	9	5
Mobile potassium (ppm)	134	126	101	86
Wilting coefficient (%)	5.3	5.0	4.3	3.7
Field capacity (%)	9.6	9.0	7.8	-
Available water capacity (%)	4.3	4.0	3.5	-
Total water capacity (%)	40	37	35	-
Humus reserve (t/ha)	52	40	34	-

Secondary profile 3 Typical Arenosols (Figure 5)

Coordinates: *45.82023 - N and 27.487188 – E*

Rock: *eolian deposits (sandy)*

Relief: *undulating field*

Usage: *arable, rape stubble*

Groundwater: *over 10 m*

Figure 5. Secondary profile 3

Morphological characterization of the secondary profile 3

Horizon Ao (0-32 cm), medium loamy sand, dark yellowish brown (10 YR 4/4 when wet and 10 YR 5/4 when dry), medium grain structure, poorly developed, wet, frequent fine roots, non-adhesive, non-plastic, gradual transition to the lower horizon;

Horizon A/C (32-64 cm), fine loamy sand, light yellowish brown (10 YR 5/3 when wet and 10 YR 6/6 when dry), poorly structured in the upper half, moist, frequent fine roots, non-adhesive, non-plastic, clear transition to the underlying horizon;

Horizon C1 (64-96 cm) coarse, unstructured, yellowish loamy sand (2.5 Y 5/4 when wet and 2.5 Y 6/4 when dry), unstructured, mineral grains of micaceous sand in its mass;

Horizon C2 (96-115 cm), coarse, pale yellow loamy sand (2.5 Y 6/3 when wet and 2.5 YR 7/4 when dry), unstructured, non-plastic, non-adhesive, diffuse transition to the lower horizon;

Horizon C3 (115-135 cm), coarse, grayish yellow sand (5 Y 6/2 when wet and 5 Y 7/3 when dry), unstructured, very friable, very weak effervescence at the base.

The soil samples were analyzed physicochemically, the results being shown in Table 4.

Table 4. Physical and chemical analyses for Typical Arenosols-Secondary profile 3

Soil horizon	**Ao**	**AC**	**C_1**	**C_2**
Depth (cm)	0-32	32-64	64-96	96-135
Coarse Sand (2-0.2 mm)	21.7	16.8	41.1	45.8
Fine Sand (0.2-0.02 mm)	35.8	42.7	21.4	20.6
Dust (0.02-0.002 mm)	31.2	30.8	29.2	26.2
Clay (< 0.002 mm)	11.3	9.7	8.3	7.4
Texture	UM	UF	UG	UG
Soil reaction (pH)	5.6	5.9	6.7	6.9
Humus content (%)	1.58	0.97	0.63	0.17
Bulk density (g/cm^3)	1.28	1.31	1.44	1.49
Total porosity (%)	54	52	50	47
Degree of compaction (%)	non-compacted	non-compacted	non-compacted	non-compacted
Nitrogen index (IN)	1.2	0.75	0.5	0.14
Base saturation V (%)	76	78	80	84
Mobile phosphorus (ppm)	17	11	9	7
Mobile potassium (ppm)	137	116	91	76
Wilting coefficient (%)	6.1	5.7	4.2	4.0
Field capacity (%)	11.1	10.3	7.6	7.3
Available water capacity (%)	5.0	4.6	3.4	3.3
Total water capacity (%)	42	39	35	31
Humus reserve (t/ha)	64	40	29	9.8

Into account the current pedoclimatic conditions in the studied areas, based on the criteria presented in the M. O. of 14.02.2022, the main forest species recommended for afforestation were established and presented.

The soil type is represented by typical arenosoil, very poorly supplied with humus and nutrients, non-adhesive, prone to wind erosion, therefore very easily shattered by the wind, which is why special technologies are recommended to stabilize the sands.

In this situation, it is recommended to reduce the wind speed by setting up forests or forest curtains, on the contour of the soils, establishing tree-wine plantations and maintaining them in pastoral regime (grassed), so that later, interventions according to technology to be carried out without difficulty.

The station sheet for this area was drawn up, according to Table 5.

Table 5. Stationary unit sheet, Tecuci-Matca area, Galaţi County

1. Unity and form of relief: plain 2. Configuration of land: horizontal 3. Slope: 2-5 % 4. Exposition X 5. Altitude: 90-100 m 6. Vegetation: segetal herb												
7	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*
Horizon	***Depth (cm)***	***Humus (%)***	***Texture clase***	***Colour***	***Scheletal***	***Structure***	***humidity***	***Compaction***	***pH***	***Efervescence***	***Soluble salt***	***concretions***
Ao	*0-34*	*1.78*	*Fine sandy loam*	*Dark brown*	-	*Graine moderately structured*	*Ue1*	*Non-compacted*	*7.66*	-	-	-
AC	*34-62*	*1.42*	*Medium clay sand*	*Light brown*	-	*Graine light structured*	*Ue1*	*Moderately compacted*	*7.92*	-	-	-
C1	*62-104*	*0.53*	*Fine clay sand*	*yellowish*	-	*unstructured*	*Ue 1*	*Moderately compacted*	*8.41*	-	-	-
C2	*104-126*	*0.12*	*Medium clay sand*	*yellowish*	-	*unstructured*	*Ue 1*	*Light compacted*	*7.24*	-	-	-
20. Material parental: wind deposits				*24. Zonal and local climate: Continental temperate, specific of plain*						*28.Tipe and subtipe of soil: Typical arenosoil*		
21.Morphological depth: 126 cm				*25. Groundwater: >10,0 m*						*29. Humus of type: mull calcic*		
22.Fiziological depth: 34 cm				*26. Hydrological regime and humidity; H 1 unpercolative*						*30. Proposal for works: - establishement of forest plantation - energic mobilization of soil*		
23. Wind erosion: moderately				*27.Character of floods: unenviable*								
Stational groupe: GS 79				*Composition of afforestation: 75 Sc (Fr.p, Fr.î) + 25 Ml (Gl, Dd)*								
Observation: Several condition of station (dry-arid climate, soil uncarbonated), natural regeneration occurs with difficulty												

CONCLUSIONS

The studied territory belongs to the urban area of Tecuci, Galați County, geographically included in the Tecuci Plain, characterized by a temperate continental climate, specific plain relief, with plateaus and gently sloping slopes, with the groundwater at over 10 m.

The area taken into study within TC Tecuci, Galați County, is 150.75 ha., arable land, from which over 40 soil samples were collected (in natural and modified settlement).

The purpose of the work was to know the properties of the soil in order to use it judiciously and evaluate the suitability of the land for forestry.

The soil cover in the studied area is consistent with the physical-geographical conditions of the area, with a single type of soil with a zonal character being identified (typical arenosols & molic).

The parental material is predominantly made up of aeolian deposits.

The texture of this type of soil is generally sandy (coarse) with a banded appearance, throughout the depth of the soil profile.

The main limiting factor of production potential is the poor rainfall during the growing season, sandy texture, low humus content, poor structuring, etc.

Recommendation:

- stabilization of the land (bioaccumulative horizon) by establishing fast-growing and lush crops, in order to enrich it with organic matter, stimulate the activity of microorganisms and restore the soil structure;
- protecting the land with a layer of mulch on the surface, with an anti-erosion role or avoiding carrying out land mobilization works in the fall.

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MORPHOLOGICAL IDENTIFICATION AND BIOCHEMICAL ANALYSIS OF RHIZOBACTERIAL DIVERSITY IN CHILLI AND THEIR ANTAGONISTIC ACTIVITY AGAINST *Colletotrichum jasminigenum* SPTD17

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Abstract

Anthracnose, a drastic fungal disease caused by Colletotrichum spp. is a significant constraint to chilli production all over the world, resulting in substantial yield losses across all major chilli-producing areas. Management of plant diseases using biocontrol agents is one of the best methodologies that may reduce the use of synthetic chemical based formulations. A total of fifteen rhizospheric bacteria were isolated from chilli fields. Preliminary morphological identification was done followed by scanning electronic microscope and then subjected for biochemical characterization. Antagonistic activities of all isolates were evaluated in vitro against Colletotrichum jasminigenum SPTD17. Based on the morphological and biochemical characterization six isolates categorized to Bacillus, two Lysinibacillus, one Paenibacillus, five Pseudomonas and one Acinetobacter genera were identified. Seven isolates (PS1, PS4, PS6, PS8, PS9, PS10 and PS11) showed strongest antagonistic activity and more than 80% zone of inhibition against Colletotrichum jasminigenum SPTD17. The results of this study suggest that chilli rhizosphere bacterial diversity can be resource for biocontrol of chilli anthracnose pathogens; further research may encourage the molecular characterization and PGRP applications of rhizospheric bacterial biocontrols.

Key words*: biocontrol, morphological characterization, scanning electron microscopy, biochemical characterization, antagonistic bacteria.*

INTRODUCTION

The rhizosphere contains a diverse range of microorganisms, with bacterial community composition influenced by various factors, including plant species and variety, plant growth stage and phenology, environmental stress and disturbance, occurrence of disease, pests and soil management practices (Marschner et al., 2011). The role of plant growth promoting rhizobacteria (PGPR) has emerged as a viable and eco-friendly strategy for enhancing plant growth, mitigating stress, and fostering sustainable agriculture practices, thereby providing a promising alternative to synthetic agrochemicals (Gupta & Pandey, 2019). As an ecological, efficient, and ecologically benign method of managing crop plant diseases, biological management is quickly gaining popularity. Kumar & Thirumalaisamy (2016) stated that the broad analysis of the biocontrol literature reveals a focus on soil borne pathogens relatively than foliar infections, with later receiving a more favorable response.

Rhizobacterial communities regulate key processes in ecosystem functioning, such as nutrient cycling, decomposition, and carbon sequestration, eventually influencing ecosystem productivity and flexibility (Kones et al., 2020). Soil represents the most productive reservoir of microbial life, surrounding a vast array of taxa, including fungi, actinomycetes, nematodes and protozoa, with bacterial populations showing the greatest richness and diversity (Yao et al., 2016). PGPR employ beneficial effects on host plants through two preliminary mechanisms among which one is plant growth promotion, which enhances plant development, another one is productivity, and biological disease control, which reduces the impact of plant pathogens (Kumar et al., 2011).

Paul & Frey (2023) revealed that biochemical examines contributes a fundamental approach in microbial identification based on their specific biochemical responses and metabolic processes. The bacterial identification is facilitated by the analysis of various biochemical and physiological characteristics, including proteolysis, lipolysis, glycolysis, enzymatic activity, and substrate utilization patterns (Franco-Duarte et al., 2019). Optimizing the utilization of PGPRs necessitates the development of efficient strategies for their isolation and characterization from rhizospheric soil, which will enable the formulation of crop-specific biofertilizers, tailored to meet the unique requirements of various crops (Muthukumar et al., 2021). The bacterial antagonistic mechanisms are involved in competition for space or nutrients, antibiosis for enrichment of plant, root growth, for introduction of plant inactivation and resistance to the enzymes of pathogens (Alam et al., 2021). The escalating environmental degradation resulting from climate change and modern agricultural practices underscores the need for innovative solutions.

The ultimate identification of these bacterial species demands the employment of molecular-based methodologies; with PCR – mediated gene amplification being a basic technique, providing exceptional specificity and sensitivity (Erlich, 1989). The application of contemporary techniques, including DNA sequencing and MALDI-TOF mass spectrometry, is indispensible for advancing microbiological research; however, these practices demand specialized technical expertise and substantial financial assets (Assis et al., 2011). A comprehensive understanding of PGPRs imposes the examination of conventional identification and characterization methods, including morphological, microscopic, and biochemical approaches, which will be systematically discussed in the present study. The present study was aimed to identify rhizobacterial strains exhibiting biocontrol potential against a *Colletotrichum jasminigenum* SPTD17 pathogen of chilli anthracnose. The forthcoming view is to develop a dual-purpose inoculum exhibiting biofertilizer and biocontrol potential for profitable use in nutrient and integrated disease management for defendable agronomy.

MATERIALS AND METHODS

Isolation of Fungal pathogen

Colletotrichum jasminigenum SPTD17 was used in this study, which has been isolated from anthracnose infected chilli fruit samples.

Isolation of fifteen rhizobacteria

A total of 17 rhizospheric soil samples were collected from different fields of chilli growing areas of Karnataka (Table 1). The soil samples were brought to the laboratory in sterile polyethylene bags and stored at 4 °C for the isolation of bacteria. Soil bacteria were isolated using serial dilution method from collected soil samples. Suspended 1 g of soil in 100 ml of sterile distilled water then kept on rotary shaker at 120 rpm for 10 min. Then it was diluted with distilled water (1:9 ratios) up to 10^7 fold. 100 µl aliquots from 10^{-3} to 10^{-7} dilutions were spread on King's B agar and nutrient medium and slightly spread by a sterile glass spreader (Somasegaran & Hoben, 1994). Then all the plates were incubated 3 days at 35 °C, all morphologically different colonies were picked and purified on nutrient and King's B agar plates by repeated sub-culturing. For the further confirmation all the isolates were subjected to other morphological and biochemical tests.

Table 1. Collection and geographical locations of soil samples collected from major chilli growing areas of Northern Karnataka

District	Village	Verity
Haveri	Agadi	Sangro
	Aladakatti	Syngenta 5531
	Hombaradi	Mailari
	Maranbeed	Tejashwini
	Krishnapur	Byadagi dabbi
	Teggihalli	Vaso
	Hedigonda	Jwala
Gadag	Shiratti	Byadagi dabbi
	Kurtakoti	Byadagi kaddi
Dharwad	Nelagudda	Devanur dabbi
	Kusugal	Delax
	Shiraguppi	Byadagi dabbi
	Annigeri	Devanur dabbi
Vijayanagar	Harapanhalli	Guntur
	Somalapur	Byadagi kaddi
Bellari	Kudligi	Byadagi dabbi
Raichur	Kochigudda	Guntur

Morphological characterization and Scanning Electronic Microscopic analysis of all fifteen rhizobacteria

The bacterial colonies obtained from the chilli rhizosphere underwent supplementary analysis, employing microscopic, were further analyzed by Scanning Electronic Microscopy (SEM), to elucidate their characteristics. Microscopic slide preparation involved a conventional Gram's staining technique, incorporating crystal violet as the primary stain and Safranin as the counterstain (Becerra et al., 2015). Prior to scanning electron microscopic analysis, the sample preparation was done by following a standard methodology, where 1 ml of overnight grown pure cultures of bacteria was washed with IX PBS (pH-7.4) incubated for 12 hrs after adding 2% of 1 ml glutaraldehyde. Additionally, samples were centrifuged for 10 min at 7000 rpm, then pellet was washed in sequence using 10-100% of ethanol and centrifuged, lastly 50 ml of 100% ethanol was added to pellet and drop of sample was smeared on a coverslip then stored in desiccators for overnight then examined in a ZEISS scanning electronic microscope at 15 kV.

Characterization of rhizospheric bacteria based on biochemical features

Biochemical assays including Gram's staining test (Fawole & Oso, 2004), mobility test (Oyeleke & Manga, 2008), Casein hydrolysis test (Muthukumar et al., 2021), indole test (MacFaddin, 1980), Hydrogen sulphide test (Cowan & Steel, 1970), Citrate test (Simmons 1976), Denitrification test (Seldin et al., 1984), methyl red test (McDevitt, 2009) Voges-Phoskaeuer (McDevitt, 2009), Oxidase test (Cheesbrough, 2006), Urea test (James & Sherman, 1992) and fermentation tests were performed using the procedure given by Fawole & Oso (2004). The bacterial colonies resulting from the sequential assays were systematically observed, recorded and archived for subsequent analysis and data interpretation (Talaiekhozani, 2013; Mahmud et al., 2023).

Screening of rhizospheric bacterial isolates for *in vitro* antagonistic activity against *C. jasminigenum* SPTD16

Bacterial isolates were tested for their antagonistic activity against the *Colletotrichum jasminigenum* SPTD17 using dual-culture method (Sakthivel & Gnanamanickam 1987). A fungal disc (≈5 mm^2) was placed at one side of the plate containing potato dextrose agar (PDA). Rhizobacterial isolates were streaked 3 cm away from plugged fungal disc. Plates inoculated with the fungal discs and control was maintained without the bacterial sample. Zone of inhibitions around the isolates were measured after 5 days of incubation at 28±2 °C and zone of inhibition was recorded by measuring the clear distance between margin of the test fungus and antagonistic bacteria. The colony diameter of the pathogen in control plate was also recorded. All strains were tested in triplicates and tests were carried out twice for each isolates with one control for maintaining only pathogen. The inhibition percent was calculated by using the following equation given by Ji et al. (2014).

$$Inhibition\ (\%) = \left[1 - \left(\frac{Fungalgrowth}{Control\ growth}\right)\right] \times 100$$

Data Analysis

Statistical parameters such as mean, standard deviation and analysis of variance were performed at P=0.05 using three replicate values in IBM SPSS Statistics version 20.00

RESULTS AND DISCUSSIONS

Identification of rhizospheric bacteria based on morphological, SEM and biochemical features

All the rhizospheric bacteria were successfully isolated and characterized on the bases of morphological, microscopic and biochemical features. The total of fifteen rhizobacteria were isolated and subjected to macro and microscopic studies by examining distinct colony colour, shape, texture, margin, elevation, opacity, and cell shape and pigmentation. The colonies of the isolates showed diverse range of variations in their shapes and sizes, either from circular to undulate or irregular to punctured, and size may be large or small. PS1, PS3, PS5 and PS12 showed rough colony texture whereas, rest all isolates possessed smooth. Colony margins were varied, some isolates showed entire to wavy and some were undulate to muciod.

Elevation was varied from umbonate, convex, raised, to flat. Opacity of the cells varies from opaque to translucent. Further Gram's staining test was carried out using standard protocol by Fawole and Oso (2004). Among all the 15 isolates, 9 isolates were Gram +ve, whereas the rest were Gram –ve. The cell shapes of bacterial isolated varied from rod, long rod to cylindrical rod. PS6, PS9, PS10 and PS14 showed yellowish green, PS8 showed brownish yellow and remaining 10 isolates showed white pigmentation. Table 2 explains the details of colony and cell morphology of rhizobacteria from different chilli fields of Karnataka.

For the morphological characterization, fifteen rhizospheric bacterial isolates were inoculated on nutrient agar and King's B agar plates, incubated for 24 h at 37±2 °C in incubator. The features such as shape, size, margin, elevation, texture, and opacity were observed and recorded. The microscopic characterization by Gram's staining and motility test were also performed. Fifteen rhizobacterial isolates were subjected to biochemical analysis, where bacterial isolates showed a diverse range of features which helps in identifying the bacteria up to genus level. PS7 was non-motile whereas, remaining all the isolates were motile, Casein hydrolysis was present in all the isolates except PS7. Indole test was negative in all the isolates. 7 isolates such as PS1, PS3, PS5, PS9, PS12, PS14 and PS15 showed positive hydrogen sulphide test. In present study all the isolates had citrate activity, which means all the 15 isolates can utilize the citrate as carbon source (Harold, 2002). Citrate utilization test was the indicated by the growth of bacteria, followed by an alkaline pH (Cappuccino & Sherman, 2002). The isolates tested negative for Methyl Red test except 4 isolates PS1, PS3, PS12 and PS15. For Voges-Proskaeur (VP) tests, deep red coloration indicated a positive result, in present analysis 5 isolates were found VP positive (PS3, PS4, PS11, PS12 and PS15). 11 isolates such as PS1, PS3, PS4, PS5, PS6, PS7, PS8, PS10, PS11, PS13 and PS15 showed positive result to denitrification test.

The population size of denitrifying bacteria were commonly observed in soils exceed the expected carrying capacity based on denitrifying respiration alone, suggesting that these microorganisms must employ additional metabolic strategies to sustain their growth (Murray et al., 1989). 2 isolates PS5 and PS7 were reported to be oxidase negative. 7 isolates such as PS1, PS2, PS3, PS7, PS12, PS13 and PS15 showed positive urease test. In acid fermentation activity 2 isolates such as PS4 and PS12 showed negative glucose fermentation, 2 isolates PS5 and PS11 showed negative fructose fermentation, 2 isolates such as PS5 and PS11, showed positive Mannitol test and 7 isolates PS1, PS2, PS3, PS5, PS7, PS13 and PS15 showed positive sucrose fermentation test (Table 3). In present study, identified isolates by microscopic and biochemical studies were further exposed for SEM analysis where distinct morphological features were comprehensively observed. The length and width of the bacterial cells recorded and ranges between 1 µm to 2 µm, comparable results were detected by Hagen et al. (1968) and Ibrahim et al. (2021). Figure 1 shows SEM images of representative members from the each genus of rhizospheric bacteria.

Table 2. Morphological and microscopic characteristics of rhizospheric bacterial isolates of chilli field soil

Codes of isolates	**Colony morphology**					**Microscopic observation**			**Pigmentation**	**Identification based on morphology**
	Colony size	**Colony shape**	**Colony texture**	**Margin**	**Elevation**	**Gram's test**	**Opacity**	**Cell shape**		
PS1	Large	Circular	Rough	Entire	Umbonate	Positive	Opaque	Rod	White	*Bacillus* sp.
PS2	Punctured	Circular	Smooth	Entire	Convex	Positive	Opaque	Rod	White	*Lysinibacillus* sp.
PS3	Irregular large	Circular	Rough	Wavy	Raised	Positive	Opaque	Long rods	White	*Bacillus* sp.
PS4	Irregular large	Circular	Smooth	Undulate	Convex	Positive	Translucent	Rod	White	*Paenibacillus* sp.
PS5	Large	Undulate	Rough	Wavy	Flat	Positive	Opaque	Rod	White	*Bacillus* sp.
PS6	Irregular large	Circular	Smooth	Undulate	Convex	Negative	Translucent	Cylindrical rod	Yellowish green	*Pseudomonas* sp.
PS7	Small	Circular	Smooth	Muciod	Slightly raised	Negative	Opaque	Rod	White	*Acinetobacter* sp.
PS8	Irregular large	Circular	Smooth	Undulate	Convex	Negative	Translucent	Cylindrical rod	Brownish yellow	*Pseudomonas* sp.
PS9	Irregular large	Circular	Smooth	Undulate	Convex	Negative	Translucent	Cylindrical rod	Yellowish green	*Pseudomonas* sp.
PS10	Irregular large	Circular	Smooth	Undulate	Convex	Negative	Translucent	Cylindrical rod	Yellowish green	*Pseudomonas* sp.
PS11	Irregular large	Circular	Smooth	Entire	Flat	Positive	Opaque	Rod	White	*Bacillus* sp.
PS12	Irregular large	Circular	Rough	Wavy	Raised	Positive	Opaque	Long rods	White	*Bacillus* sp.
PS13	Punctured	Circular	Smooth	Entire	Convex	Positive	Opaque	Rod	White	*Lysinibacillus* sp.
PS14	Irregular large	Circular	Smooth	Undulate	Convex	Negative	Translucent	Cylindrical rod	Yellowish green	*Pseudomonas* sp.
PS15	Small	Circular	Smooth	Entire	Raised	Positive	Opaque	Rod	White	*Bacillus* sp.

Table 3. Biochemical characterization of rhizospheric bacterial isolates of chilli field soil

Name of the test	**Rhizospheric bacterial isolates**														
	PS1	PS2	**PS3**	**PS4**	**PS5**	**PS6**	**PS7**	**PS8**	**PS9**	**PS10**	**PS11**	**PS12**	**PS13**	**PS14**	**PS15**
Motility	M	M	M	M	M	M	NM	M	M	M	M	M	M	M	M
Casein hydrolysis	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
Indole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydrogen sulphide	+	-	+	-	+	-	-	-	+	-	-	+	-	+	+
Citrate utilization	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Methyl red (MR)	+	-	+	-	-	-	-	-	-	-	-	+	-	-	+
Voges-proskaeur (VP)	-	-	+	+	-	-	-	-	-	-	+	+	-	-	+
Denitrification	+	-	+	+	+	+	+	+	-	+	+	+	-	-	+
Oxidase	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+
Urease	+	+	+	-	-	-	+	-	-	-	-	+	+	-	+
Acid fermentation															
Glucose	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+
Fructose	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+
Mannitol	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-
Sucrose	+	+	+	-	+	-	-	-	-	-	-	-	+	-	+
Identification based on biochemical test	*Bacillus* sp.	*Lysinibacillus* sp.	*Bacillus* sp.	*Paenibacillus* sp.	*Bacillus* sp.	*Pseudomonas* sp.	*Acinetobacter* sp.	*Pseudomonas* sp.	*Pseudomonas* sp.	*Pseudomonas* sp.	*Bacillus* sp.	*Bacillus* sp.	*Lysinibacillus* sp.	*Pseudomonas* sp.	*Bacillus* sp.

*+ indicates present, - indicates absent result

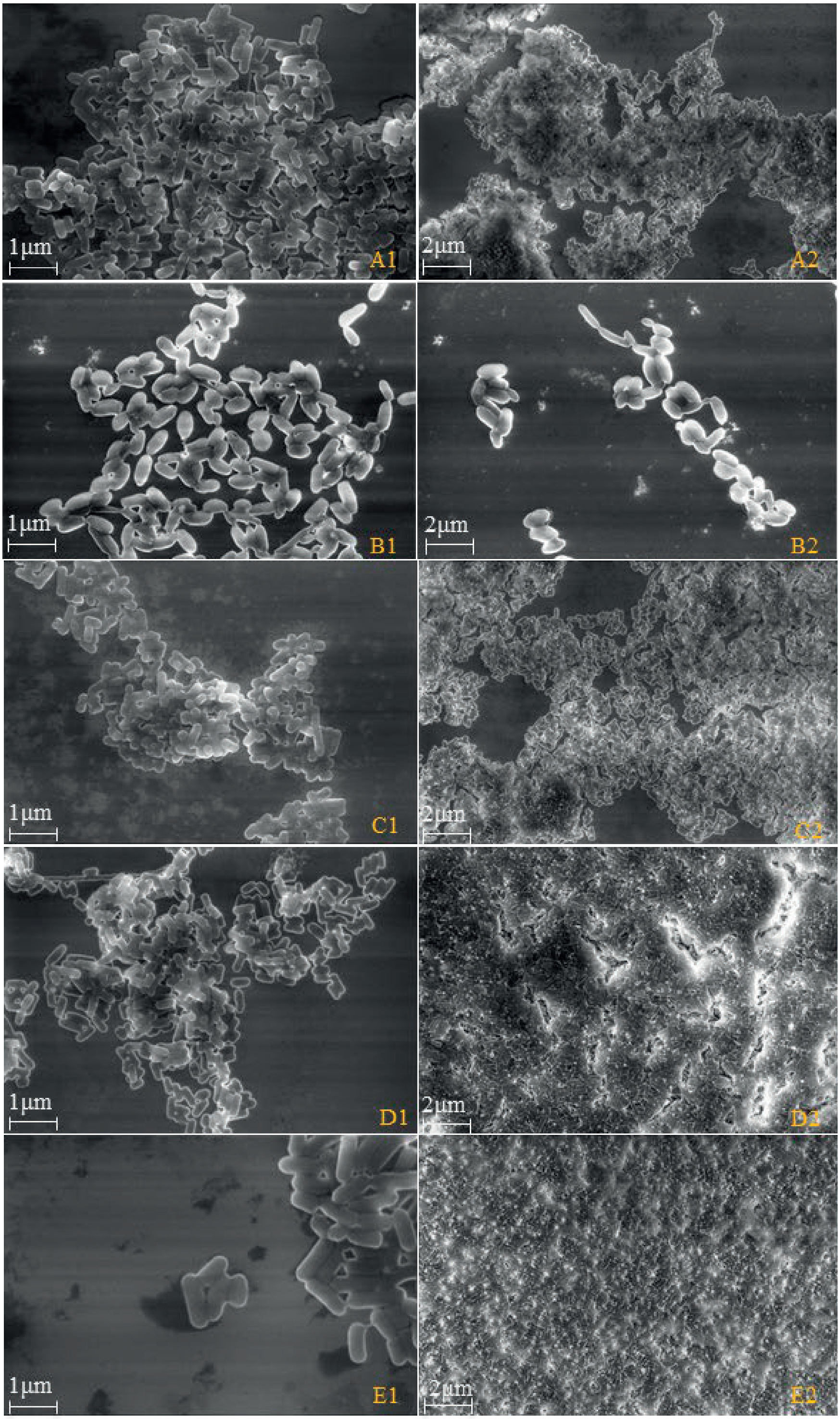

Figure 1. SEM images of representative bacterial genus, PS1 (A1-1 μm, A2-2 μm) - *Bacillus* sp.; PS2 (B1-1 μm, B2-2 μm - *Lysinibacillus* sp.; PS4(C1-1 μm, C2-2 μm) - *Paenibacillus* sp.; PS7 (D1-1 μm, D2-2 μm) - *Acinetobacter* sp.; PS8 (E1-1 μm, E2-2 μm) - *Pseudomonas* sp.

In-vitro antagonistic potential of rhizospheric bacteria against _Colletotrichum jasminigenum_ SPTD17

A substantial number of *Bacillus* species from rhizosphere soil was detected by Panigatti et al. (2025).

According to Rosch et al. (2002) bacteria belonging to these groups are widely distributed in the soil. Results revealed that the majority of organisms obtained from screening included *Bacillus*, *Lysinibacillus*, *Paenibacillus*, *Acinetobacter* and *Pseudomonas* sp.

Particular rhizobacterial strains possess inherent biocontrol properties, facilitating the elicitation of plant resistance and/or protection against a diverse array of plant pathogens (Compant et al., 2010).

There are a limited number of studies that have identified biocontrol agents possessing dual functionality, namely antagonistic activity against fungal pathogens and the ability to promote plant growth.

Present study was, therefore, designed to explore the potential of rhizosphere bacteria to control broad-host range fungal pathogen *Colletotrichum jasminigenum* SPTD17, with parallel stimulatory effects on the disease management.

There were 15 isolated and purified rhizospheric bacterial isolates from various regions of chilli growing areas of Karnataka.

In our investigation, we found that seven isolates designated as PS1 (93.33%), PS4 (86%), PS6 (90.66%), PS8 (92.66%), PS9 (87.33%), PS10 (91.66%) and PS11 (88.33%) were strongly inhibited the growth of *Colletotrichum jasminigenum* SPTD17 and they have been showed above 80% zone of inhibition those were grouped into a, whereas PS2 (71.6%), PS5 (77.33%), PS13 (70.33%) and PS14 (77.66%) were showed moderate zone of inhibition i.e., 70-80% under the group b, and PS3 (42.33%), PS7 (25.33%), PS12 (38.33%) and PS15 (40.66%) have been showed week activity with zone of inhibition 25-41% belong to c (Figure 2), and the bar graph showed different degrees of inhibition against *Colletotrichum jasminigenum* SPTD17 in dual culture assay was represented in Figure 3.

Bacillus strain has been demonstrated that decreased mycelial growth of pathogen *in vitro* and enhanced chilli seedling growth by Kumar et al. (2021).

Che et al. (2017) have been demonstrated that the antifungal VOCs produced by *Lysinibacillus* sp. FJAT-4748 were potentially useful as agents for controlling anthracnose caused by *Colletotrichum acutatum*. Darmadi et al. (2020) demonstrated that *Paenibacillus polymyxa* C1 and *Bacillus siamensis* C7B, were proven to possess strong antifungal activities against *Colletotrichum scovillei* in chilli.

Among the various rhizospheric bacteria, the bacteria belonging to *Pseudomonas*, which colonize roots of a wide range of crop plants, were reported to be antagonistic to soil borne plant pathogens.

Work done by Jisha et al. (2018) demonstrated that *Pseudomonas aeruginosa*, Ps 2 showed maximum inhibition of 93.41% whereas the other isolate Ps 3 showed 72.5% of inhibition against *Colletotrichum capsici* of chilli anthracnose after 5 days of incubation.

Several bacterial strains, including *Bacillus velezensis* and actinobacteria, have demonstrated significant antagonistic effects against *Colletotrichum capsici*, with some achieving up to 100% control efficacy (Yanti et al., 2023).

Work done by Sarmah et al. (2023) illustrated that in dual culture analysis against *Colletotrichum gloeosporioides* of chilli anthracnose, the endophytic bacteria, *B. velezensis* exhibited the highest inhibition (68.67%) against pathogen followed by *B. altitudinis* (52.89%), *B. cereus* (45.33%) and *B. mycoides* (65.33%).

Phenotypic characterization, based on biochemical and morphological attributes, may be insufficient for accurately assessing rhizosphere microbial diversity, due to potential homology among distinct species.

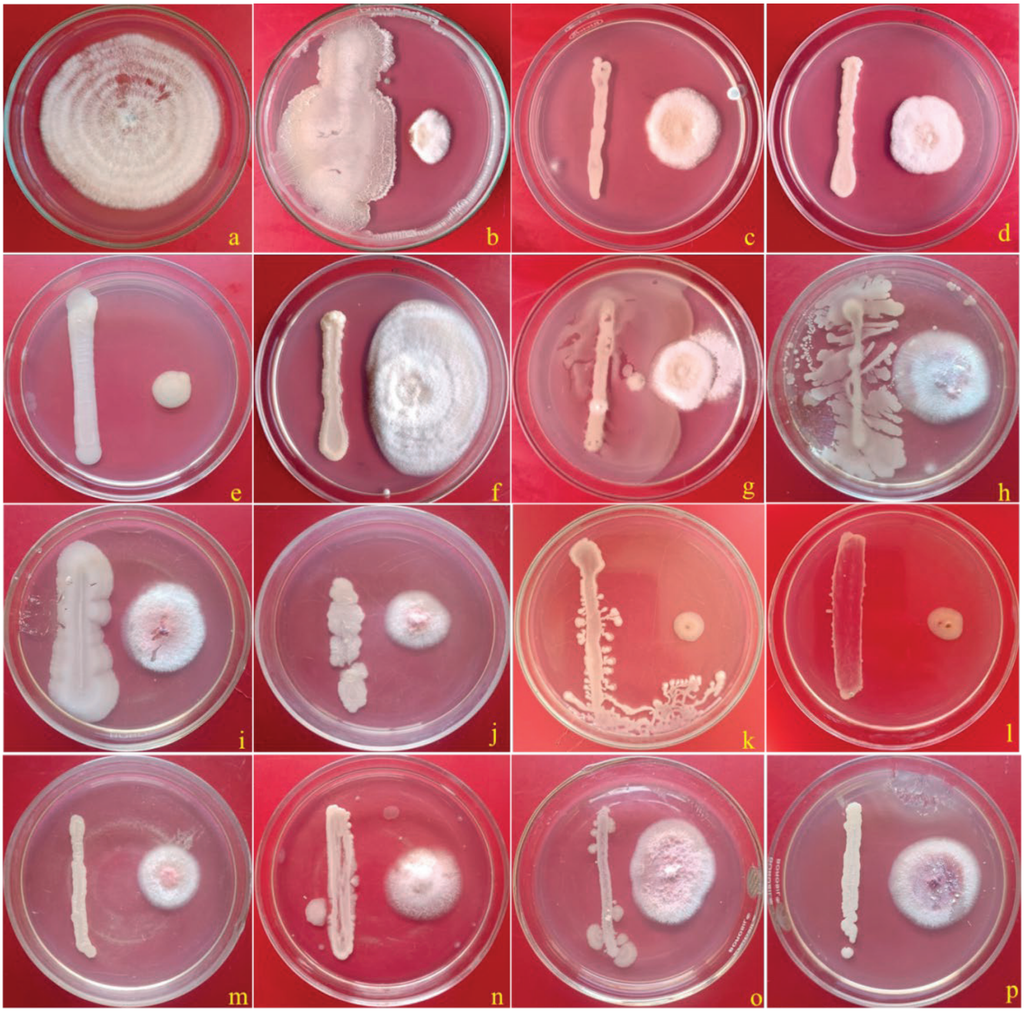

Figure 2. Antagonistic activity of 15 bacterial isolates against *Colletotrichum jasminigenum* SPTD17: control plate inoculated with only the pathogen (a) and fungal growth was inhibited towards the direction of inoculated bacteria (b-p); b-PS1, c-PS2, d-PS3, e-PS4, f-PS5, g-PS6, h-PS7, i-PS8, j-PS9, k-PS10, l-PS11, m-PS12, n-PS13, o-PS14, p-PS15

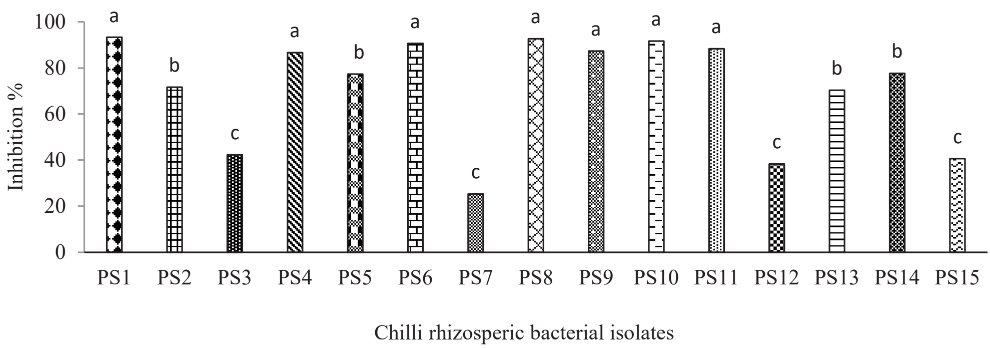

Figure 3. Inhibition percent of fifteen rhizospheric bacterial isolates against *Colletotrichum jasminigenum* SPTD17

Morphological identification of biocontrol agents is hindered by the paucity and homoplasy of distinguishing characteristics, which can be exacerbated by the occurrence of cryptic species, necessitating the adoption of molecular-based identification approaches (Kullnig et al., 2001). The application of molecular techniques, particularly those leveraging DNA analyses, is indispensable for elucidating the complex composition and functional dynamics of rhizosphere bacterial communities (Lagos et al., 2015). A polyphasic approach, combining morphological, molecular, and other characteristics, is essential for accurate species identification and assessing microbial diversity.

CONCLUSIONS

Rhizobacteria exhibit multifunctional plant-beneficial properties, including growth promotion and disease suppression, mediated by the elicitation of secondary metabolites with antimicrobial and plant-protective activities. The first and foremost step in biological control is the selection of effective antagonistic microbe. On the basis of this study, it is concluded that biochemical and morphological characterization of these isolates revealed the most abundant bacteria in the rhizosphere of chilli such as *Bacillus*, *Lysinibacillus*, *Paenibacillus*, *Acinetobacter* and *Pseudomonas* sp. The result of this study discloses that, in dual culture technique, bacterial antagonists strongly inhibited the spore germination and mycelial growth of the chilli anthracnose pathogen. The integration of biochemical, morphological and DNA-based approaches is necessary for a comprehensive understanding of rhizospheric microbial diversity, as DNA-evolution. Molecular characterization and screening of the isolated bacteria will be based methods provide critical insights into phylogenetic relationships and microbial performed to elucidate their biocontrol importance and facilitate the development of innovative biocontrol manufacture technologies.

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CORRELATION BETWEEN HEAVY METALS IN SOIL AND *Lotus corniculatus* IN A STUDY ACHIEVED IN COPȘA MICĂ

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Abstract

Heavy metals pollution is a global issue in the whole word. All soils contain heavy metals, but their concentrations usually are very low. According to the literature, bird's-foot trefoil (Lotus corniculatus) is used as adsorbent of heavy metals from soil. The study was achieved in Copșa Mică area, known for a long time in the top of the most polluted cities in Europe, due to the emissions from two factories. In article it is described the correlation between total forms and DTPA extractable forms and concentrations in Lotus corniculatus plants of cadmium, lead, copper and zinc. In conclusion, cadmium was most easily absorbed of Lotus corniculatus, while lead was identified as having the lowest accumulation in Lotus corniculatus, so it can be used for the removal of some heavy metals from soil.

Key words: *soil, heavy metals, Lotus corniculatus, Copșa Mică.*

INTRODUCTION

Because of the human population rapid growth, heavy metal pollution issues are increasing everywhere in the whole world due to the industrialization and urbanization (Bae et al., 2016). One of the major problematic pollutants are heavy metals like cadmium, lead which represents a significant threat for public health. Heavy metals are a continual source of pollution and are not biodegradable (Borda & Sparks, 2008). An alternative, less expensive with high efficiency for removal of heavy metal from soil is phytoremediation. *Lotus corniculatus*, commonly known as bird's-foot trefoil, is a leguminous plant often studied for its role in soil remediation, especially in heavy metals soil pollution/contamination. This plant is known to tolerate various environmental stresses, including the presence of heavy metals in the soil, making it a research priority of phytoremediation studies (Moussa et al., 2012).
Lotus corniculatus absorbs heavy metals from the soil through its root system. Once absorbed, the metals are translocated to various parts of the plant, including the leaves and stems (Escaray et al., 2012). The plant can accumulate metals in different tissues, but it is not a hyperaccumulator. It may accumulate metals like copper (Cu), zinc (Zn), cadmium (Cd), and lead (Pb) in moderate amounts, depending on soil conditions (Pichtel et al., 2000).
The main objective of this study is to evaluate the contamination/pollution degree with heavy metals of the meadows from Copșa Mică of analyzing total and mobile forms and the concentration of heavy metals in *Lotus coniculatus* which is a plant that can accumulate heavy metals.

MATERIALS AND METHODS

The research study has been achieved in Copșa Mică area. From the meadows situated in this area were collected 14 soil samples and 14 plant samples representing *Lotus corniculatus*, 6 samples are from Axente Sever, 1 sample from Valea Viilor, 1 from Copșa Mică, 4 from Târnava and 2 from Micăsasa. In Figure 1 is presented the location of the meadows in the study area in Copșa Mică. Soil samples were collected from surface (0-20 cm). The total concentration of cadmium, lead, zinc and copper were determined in the soil samples of acid digesting in a microwave digestion system, then dosing of atomic absorption spectrometry (Vrînceanu et al., 2022).

Figure 1. The location of the meadows in the study area in Copșa Mică (Google Earth)

The concentration of cadmium, lead, zinc and copper extractable in DTPA were determined in the soil samples of extraction method using diethylenetriaminepentaacetic acid, then dosing of atomic absorption spectrometry.

The plant samples were digested also with nitric acid in a microwave digestion system. The concentration of cadmium, lead, zinc and copper were measured of using atomic absorption spectrometry.

In Table 1 is presented the distance from the basket located in Copșa Mică of the meadows from the studied area

Table 1. The distance from the basket located in Copșa Mică of the meadows from the studied area

	Meadows code	Distance from the basket (m)
1	PP1AS	530
2	PP3AS	1160
3	PP10AS	2770
4	PP12AS	1900
5	PP15AS	2180
6	PP16VV	4010
7	PP19CM	2760
8	PP20TV	4510
9	PP22MS	4220
10	PP23MS	4459
11	PP34AS	5134
12	PP35TV	5180
13	PP36TV	3636
14	PP37TV	5368

RESULTS AND DISCUSSIONS

Lotus corniculatus may contribute to soil improvement beyond just metal uptake. As a plant, it fixes nitrogen, improving the nitrogen concentration of the soil. This can be especially beneficial in soils where heavy metal contamination has led to low nutrient levels (Moussa et al., 2022).

Table 2 presents the concentrations of total heavy metals (cadmium, lead, zinc and copper) in soil samples from the meadows in Copșa Mică area along with the reference values of the total cadmium, lead, zinc, copper concentrations in soil for sensitive use of land according with Order 756/1997 to evaluate the contamination / pollution degree.

As it can be observed, total cadmium concentration in soil varies between 0.38 and 48.29 $mg \cdot kg^{-1}$, with an average value of 8.65 $mg \cdot kg^{-1}$ and a coefficient of variation of 143.1% with the highest values registered near the basket from Copșa Mică. The intervention threshold is exceeded in 7 points from the studied area, 5 samples being collected from Axente Sever, 1 sample from Copșa Mică and 1 sample from Târnava. The alert threshold is exceeded in 2 points, 1 from Târnava and 1 from Micăsasa.

Table 2. The concentration of total heavy metals (cadmium, lead, zinc and copper) in soil samples from the meadows in Copşa Mică area and the reference values of the total cadmium, lead, zinc, copper concentrations in soil for sensitive use of land according with Order 756/1997

	Meadows code	Total Cd in soil (mg/kg DW*)	Total Pb in soil (mg/kg DW*)	Total Zn in soil (mg/kg DW*)	Total Cu in soil (mg/kg DW*)
1	PP1AS	48.29	955	2015	182
2	PP3AS	6.15	217	462	50
3	PP10AS	12.38	398	850	60
4	PP12AS	11.40	282	684	56
5	PP15AS	5.05	167	326	161
6	PP16VV	2.50	93	168	27
7	PP19CM	16.92	599	930	101
8	PP20TV	5.63	236	414	108
9	PP22MS	3.12	87	206	38
10	PP23MS	1.36	42	97	19
11	PP34AS	0.38	25	89	61
12	PP35TV	3.91	132	285	61
13	PP36TV	2.89	90	226	32
14	PP37TV	1.18	58	142	23
Minimum		0.38	25	89	19
Maximum		48.29	955	2015	182
Median		4.48	149.5	305.5	58.0
Geometric mean		4.42	151.0	330.5	55.8
Arithmetic mean		8.65	241.5	492.4	69.9
Standard deviation		12.38	258.8	515.3	50.5
Coefficient of variation		143.1%	107.2%	104.7%	72.2%
Normal values		1.0	20	100	20
Alert threshold		3.0	50	300	100
Intervention threshold		5.0	100	600	200

*DW - Dry Weight

Total lead concentration in soil varies between 25 and 955 mg·kg^{-1}, with an average value of 241.5 mg·kg^{-1} and a coefficient of variation of 107.2% and the highest values registered near the basket from Copşa Mică. The intervention threshold is exceeded in 8 points from the studied area, 5 samples being collected from Axente Sever, 1 sample from Copşa Mică and 2 samples from Târnava. The alert threshold is exceeded in 4 points, 2 from Târnava, 1 from Valea Viilor and 1 from Micăsasa.

Total zinc concentration in soil varies between 89 and 2015 mg·kg^{-1}, with an average value of 492.4 mg·kg^{-1} and a coefficient of variation of 104.7%. The intervention threshold is exceeded in 4 points from the studied area, 3 samples being collected from Axente Sever and 1 sample from Copşa Mică. The alert threshold is exceeded in 3 points, 2 from Axente Sever and 1 from Târnava.

Total copper concentration in soil varies between 19 and 182 mg·kg^{-1}, with an average value of 69.9 mg·kg^{-1} and a coefficient of variation of 72.2%. The intervention threshold is not exceeded for any sample. The intervention threshold and alert threshold in 4 points, 2 from Axente Sever, 1 from Copşa Mică and 1 from Târnava.

In Table 3 are presented the concentration of mobile forms of heavy metals, in soil samples from the meadows in Copşa Mică area.

The mobile concentration of cadmium varies between 0.23 mg·kg^{-1} and 33.5 mg·kg^{-1}, with an average value of 6.07 mg·kg^{-1} and a coefficient of variation of 141.7%. The mobile concentration of lead varies between 3.6 mg·kg^{-1} and 275.5 mg·kg^{-1}, with an average value of 88.9 mg·kg^{-1} and a coefficient of variation of 99.7%.

The mobile concentration of zinc varies between 7.1 mg·kg^{-1} and 336.3 mg·kg^{-1}, with an average value of 91.7 mg·kg^{-1} and a coefficient of variation of 119.0%.

The mobile concentration of copper varies between 1.59 mg·kg^{-1} and 23.41 mg·kg^{-1}, with an average value of 8.84 mg·kg^{-1} and a coefficient of variation of 68.9%. The highest concentration of mobile heavy metals are near the basket from Copşa Mică and are decreasing with the increasing of the distance from the pollution source.

Table 3. The concentration of heavy metals extractable form in DTPA (cadmium, lead, zinc and copper) in soil samples from the meadows in Copșa Mică area

	Meadows code	Cd_{DTPA} in soil (mg/kg DW*)	Pb_{DTPA} in soil (mg/kg DW*)	Zn_{DTPA} in soil (mg/kg DW*)	Cu_{DTPA} in soil (mg/kg DW*)
1	PP1AS	33.55	121.5	336.3	14.23
2	PP3AS	5.13	106.0	137.3	9.57
3	PP10AS	10.33	275.5	123.5	6.76
4	PP12AS	9.53	108.6	323.0	6.49
5	PP15AS	3.54	58.8	65.0	23.41
6	PP16VV	1.96	27.4	24.1	2.46
7	PP19CM	9.11	259.7	97.3	10.27
8	PP20TV	4.05	151.3	62.4	15.10
9	PP22MS	1.71	23.8	27.4	4.32
10	PP23MS	0.83	14.0	7.8	1.59
11	PP34AS	0.23	3.6	7.1	12.05
12	PP35TV	1.98	50.6	25.3	10.75
13	PP36TV	2.06	28.4	30.4	4.04
14	PP37TV	0.93	15.4	17.4	2.66
Minimum		0.23	3.6	7.1	1.59
Maximum		33.55	275.5	336.3	23.41
Median		2.80	55	46	8.17
Geometric mean		3.02	50.2	48.1	6.86
Arithmetic mean		6.07	88.9	91.7	8.84
Standard deviation		8.60	88.6	109.1	6.09
Coefficient of variation		141.7%	99.7%	119.0%	68.9%

*DW - Dry Weight

The concentration of heavy metals in *Lotus corniculatus* plant samples from the meadows in Copșa Mică area are presented in Table 4. The cadmium concentration in *Lotus corniculatus* plant varies between 0.10 mg· kg^{-1} and 2.5 mg·kg^{-1}, with an average value of 0.95 mg·kg^{-1} and a coefficient of variation of 83.2%.

Table 4. The concentration of heavy metals in *Lotus corniculatus* plant samples from the meadows in Copșa Mică area

	Meadows code	Cd_{plant} (mg/kg DW*)	Pb_{plant} (mg/kg DW*)	Zn_{plant} (mg/kg DW*)	Cu_{plant} (mg/kg DW*)
1	PP1AS	1.44	3.43	46	7.2
2	PP3AS	0.86	0.32	54	13.9
3	PP10AS	2.50	0.86	70	7.0
4	PP12AS	1.75	0.21	132	4.6
5	PP15AS	0.75	2.39	47	6.8
6	PP16VV	0.32	2.10	45	7.4
7	PP19CM	1.99	1.10	60	5.8
8	PP20TV	0.75	4.90	63	9.0
9	PP22MS	0.15	1.07	25	2.8
10	PP23MS	0.18	0.72	38	4.6
11	PP34AS	0.10	1.75	20	4.4
12	PP35TV	1.73	0.50	70	5.5
13	PP36TV	0.52	0.19	46	4.6
14	PP37TV	0.29	0.43	31	4.8
Minimum		0.10	0.19	20.0	2.80
Maximum		2.50	4.90	132.0	13.90
Median		0.75	0.97	46.5	5.65
Geometric mean		0.62	0.92	48.0	5.87
Arithmetic mean		0.95	1.43	53.4	6.31
Standard deviation		0.79	1.38	27.4	2.71
Coefficient of variation		83.2%	96.5%	51.3%	42.9%

*DW - Dry Weight

The lead concentration in *Lotus corniculatus* plant varies between 0.19 mg·kg^{-1} and 4.9 mg·kg^{-1}, with an average value of 1.43 mg· kg^{-1} and a coefficient of variation of 96.5%. The zinc concentration in *Lotus corniculatus* plant varies between 20 mg·kg^{-1} and 132 mg·kg^{-1}, with an average value of 53.4 mg· kg^{-1} and a coefficient of variation of 51.3%.

The copper concentration in *Lotus corniculatus* plant varies between 2.80 mg·kg^{-1} and 13.9 mg·kg^{-1}, with an average value of 6.31 mg·kg^{-1} and a coefficient of variation of 42.9%.

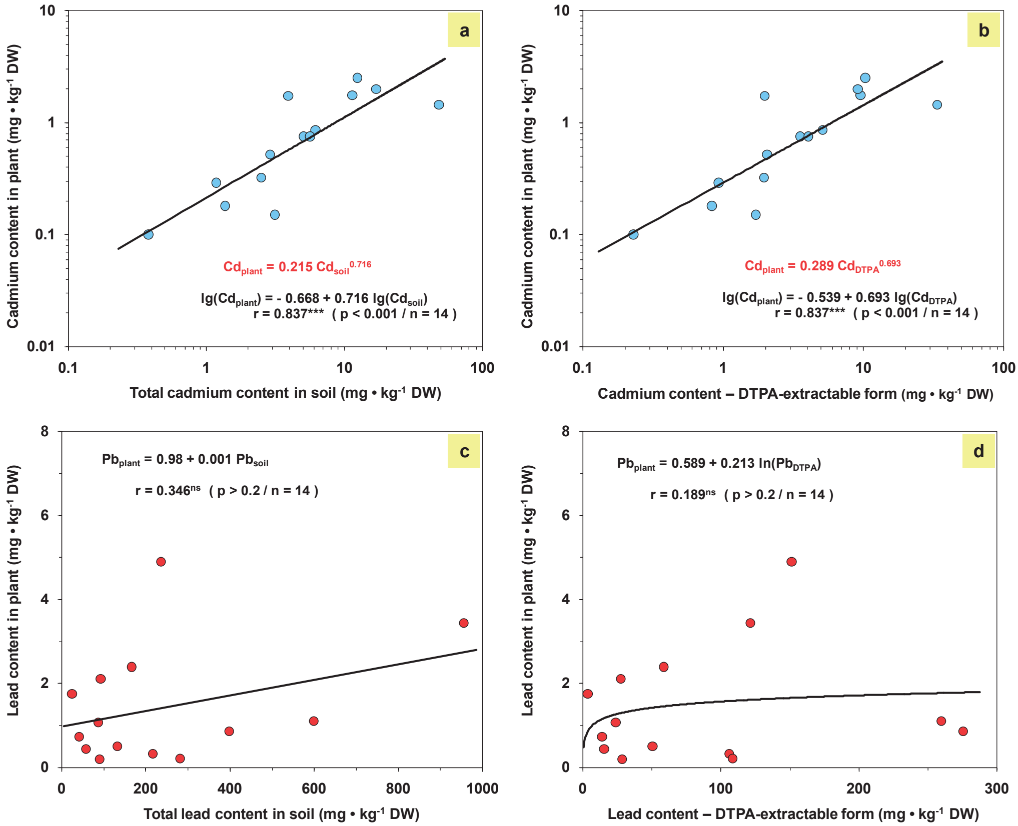

Figure 2. Regression curves that estimate the stochastic dependency between total cadmium concentration in soil (a), soil cadmium concentration – DTPA-extractable form (b), total lead concentration in soil (c), soil lead concentration – DTPA-extractable form (d) and cadmium/lead concentrations in *Lotus corniculatus* plants.

In Figure 2 are presented the regression curves that estimate the stochastic dependency between total cadmium concentration in soil (a), soil cadmium concentration – DTPA-extractable form (b), total lead concentration in soil (c), soil lead concentration – DTPA-extractable form (d) and cadmium/lead concentrations in *Lotus corniculatus* plants.

The value of the linear correlation coefficient between total cadmium concentration in soil and cadmium concentration in *Lotus corniculatus* plants is significant, r = 0.837*** (Figure 2a).

Also a significant linear correlation coefficient between cadmium concentration – DTPA-extractable form in soil and cadmium concentration in *Lotus corniculatus* plants have been found, r = 0.837*** (Figure 2b). In conclusion, this good correlation coefficient of cadmium proves that *Lotus corniculatus* can be a very good plant to remediate a moderate soil polluted with cadmium.

The regression curves that estimate the stochastic dependency between total lead concentration in soil and lead concentrations in *Lotus corniculatus* plants has a insignificant linear correlation coefficient with a value of r = 0.346^{ns} (Figure 2c).

Also a insignificant linear correlation coefficient between lead concentration –

DTPA-extractable form in soil and lead concentration in *Lotus corniculatus* plants have been found, r = 0.189^{ns} (Figure 2d). So, the low correlation coefficient of lead shows that *Lotus corniculatus* is not an efficient plant to remediate moderate soil polluted with lead.

CONCLUSIONS

In this study are presented the data regarding the accumulation of heavy metals (cadmium, lead, zinc and copper) in *Lotus cornicalatus* plants from the soil polluted which is known as a plant that can accumulate heavy metals. The highest correlation coefficient between total and mobile heavy metal and *Lotus corniculatus* was observed at cadmium (r = 0.837***). The translocation of heavy metals, especially of cadmium, in *Lotus corniculatus* plants is dangerous because of its ingestion of herbivores. It is important to note that the heavy metals that *Lotus corniculatus* plants accumulates may eventually enter the food chain.

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THE ALLUVIAL SOLS OF THE LOWER JIU FLOODPLAIN AND THEIR MAIN AGROPRODUCTIVE PROPERTIES

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Abstract

Geomorphologically, the lower Jiu floodplain, located south of the city of Craiova, covers an area of approximately 40.000 hectares and is more developed on the left side of the river. Based on the materials deposited during floods, it includes three zones: the littoral zone, situated near the watercourse, where coarse alluvial materials were deposited; the central zone, the most extensive, where medium-textured materials were deposited; the pre-tertiary zone, the lowest and farthest from the watercourse, where the deposited alluvial materials are the finest. The alluvial soils identified in the researched area are typical, mollic, gleyic, and salic. Typical alluvial soils are specific to the littoral zone, where soil formation processes are weakly differentiated, resulting in low fertility. Mollic alluvial soils are found in the central zone, where the genesis processes are more advanced, making these the most fertile soils of the floodplain. Gleyic and salic alluvial soils occupy the pre-terrace zone, where groundwater is shallow, leading to gleying and even salinization processes at the base of the soil profile.

Key words*: alluvial soils, floodplain, texture, structure, soil formation, fertility.*

INTRODUCTION

Sustainable management of natural and anthropogenic resources is a modern approach to ecosystem management, aiming to protect and promote biodiversity, as well as to ensure the long-term production of high-quality products. Thus, the identification and clear determination of the destination of each land area within the terrestrial space are essential for determining the ecological conditions and the specific role of a land in various activities (agricultural, forestry, socio-economic) (Nita et al., 2024). Among the factors and physical-geographical conditions that influence the environment in which plants grow and yield, soil plays an essential role, being, on the one hand, a complex indicator of the state of evolution of the characteristics that support plant growth and, on the other hand, a support for the influences of all other conditions and factors (Borcean et al., 1996; Feier-David et al., 2024). Numerous studies and research carried out at national level have highlighted the interdependencies between agricultural technology systems, the state of the environment, the level of economic development and the quality of life. Soil is an essential element for the food production on which life on this planet relies. The soil ecosystem provides various functional services, such as maintaining soil fertility, promoting ecosystem stability, and regulating climate change (Duka et al., 2024). Soil acts as an engineering environment, a habitat for soil organisms, a recycling system for nutrients and organic waste, a regulator of water quality, a modifier of atmospheric composition, and a medium for plant growth, making it a critical provider of ecosystem services (Dominati et al., 2010). The soil is used in agriculture, where it serves as the anchor and main nutrient base for plants. Globally, the arable area per capita in 1990 was 0.3 ha, in 2000, it decreased to 0.25 ha and it is estimated that in 2050, it will reach 0.15 ha and only 0.10 ha in 2150. Starting from this aspect, it is even more urgent to have a thorough knowledge of soil resources, their characteristics and through the innovative application of scientific technologies, humanity's requirements must be satisfied (Mușat et al., 2024). The expansion of the global population, which is driving an intensified development of agriculture, requires efficient use of soil, high-yielding varieties and hybrids, as well as the modernization of cultivation technologies to prevent damage to agricultural land. High temperatures and drought have continued to reduce production of all crop plants in many regions in recent decades. For this reason, irrigation is becoming increasingly important in agricultural activities. The interaction of factors reveals that irrigation and fertilization significantly increase yield compared to non-irrigated crops (Nițu et al., 2024a). These measures should be implemented at the level of large agricultural farms in order to achieve significant improvements in water use efficiency and crop productivity (Nițu et al., 2024b). In order to be able to feed the future world population, which is estimated to reach around 8 billion people, decisive action is needed to conserve soil resources, increase agricultural areas and, in particular, increase agricultural productivity (Bălan et al., 2024). Soil is an essential and valuable natural resource. It is extremely vulnerable to human activities, which can lead to its degradation and loss, both quantitatively and qualitatively. The rate of soil destruction considerably exceeds the rate of restoration, which makes it all the more important to limit the land areas affected by various degradation processes. The awareness of the value of soil as a limited and difficult to renew resource has led to the adoption of managerial practices designed to ensure its protection, conservation and improvement in the long term. Soil quality is defined by its chemical, physical and biological characteristics, which allow it to perform essential ecological functions (such as maintaining the global ecological balance, supporting local air circulation, storing water, regulating biological activities, providing nutrients, filtering and detoxifying), productive (supporting plant development) and protective functions. (Apostu et al., 2024; Bhattacharyya et al., 2017; Cherubin et al., 2016; Cojocaru & Abramov, 2023; Lazăr, 2010). When we talk about the natural fertility of a soil, we are referring to the fertility acquired by it during pedogenesis processes. The natural fertility of the soil can be diminished, by taking the soil in the crop or by the action of degrading, disruptive factors. However, the fertility of these soils can be improved, through good knowledge of their properties but also through the way in which various agropedo-ameliorative measures are applied (Popescu et al., 2024b). For a fertile soil, organic matter is very important, contributing to the improvement of its physical, chemical and biological properties (Bosch-Serra et al., 2023). Climate change in recent times represents a serious threat to agricultural activities. Their consequences, affect multiple aspects of life in all regions of the world, influence food security by reducing the production of cultivated species. The yields obtained from different plants grown in Romania are largely conditioned by the properties of the soils, the rainfall regime and the way in which the plants are provided with the appropriate nutrients (Iancu et al., 2024). In order to ensure good conditions for plant growth and fruiting, soil components must also be kept in optimal natural ratio, any disturbance of them causes a decrease in productive capacity (Popescu et al., 2024a). Knowing the characteristics of environmental factors, the physicochemical properties of the soil, are relevant aspects in establishing the soil production capacity in a given area (Popescu & Bălan, 2024). Sustainable land use is essential to maintain high production capacity and protect environmental quality (Zafiu & Mihalache, 2021; Bălan & Popescu, 2024). Soil quality is influenced by limiting factors, the same as those found in the creditworthiness system in Romania, but they manifest themselves differently depending on geomorphological, lithological, hydrological and climatic characteristics (Bălan & Popescu, 2024). The purpose of the present study was to identify the soils and determine the values of several soil parameters (physical, hydrophysical and chemical), in a meadow ecosystem (Figure 1), characterized by alluvial processes.

Figure 1. Aspects of the Jiu Meadow during the non-flood period

These processes are characterized by the deposition of the material brought by the watercourse, during overflows (Figure 2), which covers part of the surface of the meadow (Figure 3), leaving a layer of alluvium (Figure 4) when the waters recede, varying in thickness and texture, depending on the size of the flood and the nature of the rock from which the material comes.

Figure 2. Aspects of the Jiu Meadow during the overflow

Figure 3. Covering the old soil layer with a new one during floods

Figure 4. Alluvial material after the withdrawal of water from the waters

The area on which the lower Jiu meadow extends, south of the city of Craiova, is delimited to the east by the head of the Rojiște terrace on the line that connects the localities of Atârnați (Troaca) - south Romanești, Secui-Dobrești, Lișteava-Ostroveni and by the front of the Bârza terrace, between the localities of Dobrești-Sadova. To the west it is bounded by the edge of the Getic Piedmont, between the localities of Breasta-Podari, by the Sălcuța Field, between the localities of Podari-Drănic and the Danube terraces as follows: Perișoru Terrace - between the Drănic-Padea localities, Băilești Terrace between the Bârza-Valea Stanciului localities and the front of the Jiu Terrace (Teresa Malu Mare) between the Valea Stanciului-Zăval localities (Figure 5). The total area of the Jiu Meadow, south of the city of Craiova, is approximately 40,000 ha, with a greater development on the left side, being formed, from a geomorphological point of view, of three areas: the coastal area, which includes the areas in the immediate vicinity of the course of the Jiu River, with a variable width between 1.5 and 0.5 km and an altitude between 80 m, in the North and 30 m in the South. Coarse alluvial materials are deposited in this area. The central area, comprising the largest surfaces, is the most developed area of the meadow, where the alluvial materials with medium texture are deposited. In this area of the meadow, the most fertile soils are found. The pre-terrace zone is the area furthest from the watercourse, the lowest, depression and swampy area. In this area, the finest alluvial materials are deposited, the groundwater is found at a shallow depth, and meanders, swamps, or old riverbeds can be encountered. The soils found in this area are clayey, glazed and sometimes salty.

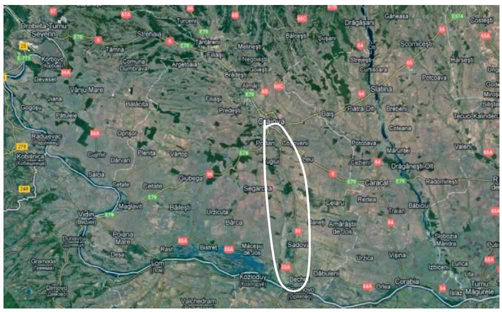

Figure 5. Lower Jiu Meadow

From a climatic point of view, the area is characterized by multiannual average temperatures of 10.8^{0}C (Figure 6) and precipitation of 532 mm (Figure 7). Solification materials are represented by alluvial deposits, with coarse to fine textures, by alluvial-proluvial and colluvial-alluvial deposits and by aeolian deposits.

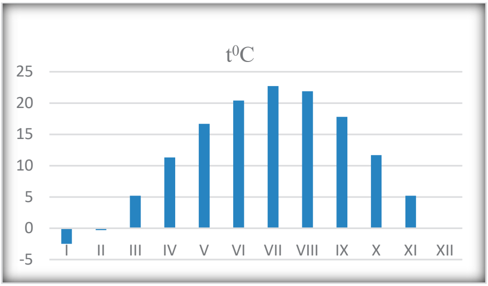

Figure 6. Average monthly temperatures

The natural vegetation specific to the Jiu meadow is very rich, represented by herbaceous species (mesophilic, hydrophilic and xerophilic) and by woody species of cvercinea.

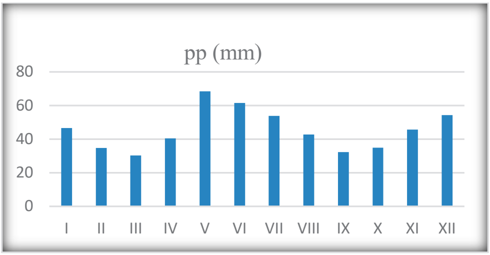

Figure 7. Average monthly rainfall

MATERIALS AND METHODS

The research carried out in the lower Jiu meadow is aligned with the current scientific and practical concerns, focused on the accumulation of knowledge about the physical-geographical conditions, which are essential for defining the quality of ecopedological resources. The paper provides essential information and methodological elements for the qualitative assessment of ecopedological resources, thus integrating into a broader framework of complex studies of natural resources. The physical and geographical conditions that influence the formation and evolution of the soil are briefly presented, emphasizing how the zonal particularities of the Jiu meadow determine a significant diversity of ecological conditions. Soil research was carried out to identify soil types and assess their fertility based on physicochemical characteristics. At the same time, soil samples were collected for laboratory analysis. In this regard, the natural conditions of soil formation in this area were studied, a study of the territory was carried out, during which the location points of the soil profiles were established, depending on the characteristics of the relief, so that each soil profile is as representative as possible for a larger area, according to the methodology established by the Research Institute for Pedology, Agrochemistry and Environmental Protection in Bucharest (ICPA Bucharest). After digging the soil profiles (Figure 8), they were studied in the field, from a morphological point of view (Munteanu & Florea, 2009).

Figure 8. Soil profile in the Jiu meadow

Soil samples collected from the field with natural soil structure and modified structure were prepared and analyzed in the laboratories of the Office for Pedological and Agrochemical Studies (OPAS) Dolj (Popescu, 2024). The analytical methods used to determine the physical properties were the following: The size fractions were determined by wet sieving, siphoning and pipetting, and the soil texture was established with the texture triangle;

- The bulk density was determined by the 100 cm^3 metal cylinder method;
- The density was determined by the pycnometer method;
- The total porosity was determined by calculation;

- The hygroscopicity coefficient was determined by drying the soil sample at 105^0C, after saturating it with water in a desiccator with water vapor, created by a 10% sulfuric acid solution;
- The wilting point, by calculation according to the value of the hygroscopicity coefficient;
- The field capacity, by the centrifugation method.

The interpretation of the results was carried out according to the methodology in force (Institute of Research for Pedology and Agrochemistry, ICPA, 1987). The analytical methods used to determine the chemical properties were as follows:
- The humus content was determined by the Walkley-Blak method;
- The pH value by the potentiometric method, in aqueous solution;
- $CaCO_3$ content by the gasometric method, using the Scheilbler calcimeter;
- The nitrogen content was determined by Kyeldahl method;
- Soluble potassium, by Egner-Riehn-Domingo method;
- Soluble phosphorus, by Egner-Riehn-Domingo method;
- Hydrolytic acidity, by Kappen method;
- The exchange capacity for bases, by Kappen method;
- The degree of saturation in bases, by calculation with the relationship V% = SB/Tx100.

The establishment of the suitability of the identified soils, for the main agricultural plants cultivated in the area, was achieved by the work of creditworthiness in natural conditions. The estimation of the productions that can be obtained on the identified soils was made with the help of the creditor notes, by multiplying them by the quantity of products per point of the creditworthiness note, for each plant, established according to the creditworthiness methodology in Romania.

RESULTS AND DISCUSSIONS

The soil cover in the lower Jiu meadow is presented in the form of a true mosaic, with very large variations on small surfaces, as a result of the complexity of natural factors and their influence on the solification process. The very large variation of the microrelief and microclimate also determines the modification of the other natural factors, which explains the diversity of soils encountered. It can be said that in the lower Jiu meadow, there is a combined action of all natural factors on the process of soil formation and evolution. However, the intensity of the action of natural factors is differentiated, with the water factor predominating, which is found at shallow depths and which determines more or less intense glazing processes. The water factor also intervenes in the alluvial processes so often encountered in the meadow and which are the basis for the formation of alluvial soils. Under the action of the multitude of general and especially local solification factors, in the lower Jiu floodplain, many soils have been formed, some occupying quite small areas while others occupy much larger areas. Considering the great diversity of the soils in the Jiu Meadow, they have been grouped into several classes. The class of Protosoils includes soils formed in the coastal and central areas of the floodplain, on alluvial materials, characterized by an undifferentiated or weakly differentiated profile, represented by typical alluvial soils, mollic alluvial soils, gleic alluvial soils, and entic alluvial soils. The hydrosol class includes soils that have been formed in conditions of permanent excess moisture, determined by the high level of the groundwater (0.5-1.5 m) or by the stagnation on the surface of water from floods, precipitations and coastal springs. They are mainly found in the pre-terrace zone and are represented by typical gleysols, chernozemic gleysols, and histic gleysols. The class of salsodisols includes soils with a soluble salt content higher than 0.1%. These soils appear as compact surfaces, mainly in the southern area (Rojiște-Dobrești-Sadova) and on smaller surfaces in the Podari - Livezi area. They are also found in the pre-terrace or central area and are represented by saline solonetz, alluvial solonetz, and solodic solonetz. This paper presents the characteristics of a typical alluvial soil, a mollic alluvial soil, and a gleic-saline alluvial soil.

The typical alluvial soil is found in the coastal meadow area, forming on coarse alluvial materials. The solification processes are poorly evidenced and the installation of vegetation is incipient.

For the characterization of this soil, the morphological, physical, hydrophysical and chemical properties of a soil profile executed near the city of Craiova, in the Romanești area, are presented.

Horizon Ao 0-15 cm, pale yellowish color 5Y5/4 in the wet state and whitish yellowish 5Y6/4 in the dry state, sandy-clayey, unstructured or granular very friable, loose, porous, rare herbaceous filiform roots, rare butterflies of mica white, does not effervescence, gradual transition;

Horizon AC 15-30 cm, faintly yellowish color 5Y5/3 in wet and whitish-gray state 5Y 5/6 in dry state, sandy-silt, unstructured, medium porous, medium compact, rare roots of phylloform plants, frequent grains of coarse quartz sand, revenge, weak effervescence, gradual passage.

Horizon C1 30-65 cm, whitish-gray 5Y 6/3 in wet state and dirty whitish 5Y 7/3 dry, sandy, unstructured, finely porous, compact, very rare threadlike roots, many grains of coarse sand, obvious effervescence, gradual passage;

Horizon C2 65-90 cm, whitish - dirty 5Y 7/3 in wet state and pale yellow 5Y8/3 in dry state, sandy, unstructured, medium porous, compact, very rare threadlike roots, very many grains of coarse sand, rare skeleton of 2-3 mm, obvious effervescence.

The particle size composition of the typical alluvial soil has a high coarse and fine sand content of over 85% and a very low dust and clay content of less than 15%. This particle size composition gives the soil a coarse, sandy or sandy-clayey texture to the soil over the entire profile (Table 1). Soil particle size composition is an indicator of soil sustainability, influencing several soil processes, including soil permeability (Zhao et al., 2024), water evaporation, pore space size and characteristics (Li et al., 2024), nutrient circuitry, stability of structural aggregates, and finally, soil fertility (Wang et al., 2022; Azaryan et al., 2022). In addition to influencing soil physicochemical properties, it has been established that soil particle size distribution influences several soil biological traits such as microbial biomass and decomposition (Iordache et al., 2024).

Table 1. Physical properties of typical alluvial soil

Horizon	Depth (cm)	Size fractions				Texture class	Bulk density (g/c...	Density (g/cm³)	Total porosity (%)
		Coarse	Fine sand,	Silt,	Clay,				
Ao	0-15	22.8	63.8	5.6	7.8	NL	1.29	2.68	51
AC	15-30	3,6	52.7	5,1	7.8	N-	1.37	2.69	49
C1	30-65	46.8	44.6	3.7	4.9	N	1.45	2.71	46
C2	65-90	69.6	23.8	4.1	2.5	N	1.47	2.71	46

As a result of the high content in coarse fractions and low in dust and clay, the typical alluvial soils retain very little water, the hydrophysical indices have low values, at the lower limit. The hygroscopicity coefficient oscillates between 0.8% and 2.2%, the field capacity between 3.2 % and 7.6% and the usable water capacity is less than 5% (Table 2).

Table 2. Hydro - physical properties of typical alluvial soil

Horizon	Depth (cm)	HC, %	WP, %	FC, %	AWC, %
Ao	0-15	2.2	3.2	7.60	4.4
AC	15-30	1.8	2.6	5.80	3.2
C1	30-65	1.1	1.6	3.90	2.3
C2	65-90	0.8	1.2	3.20	2.0

The chemical properties show that the soil has a low humus content, below 2%, has a weak alkaline reaction, pH 7.6-8.1, has a high degree of base saturation, over 90% and is poorly supplied with nutrients (Table 3).

The mollic alluvial soil is more commonly found in the central area of the floodplain, on land that is less frequently flooded. Under these conditions, the vegetation has developed better and the solification processes are more advanced. For the characterization of this soil, the morpho-physico-chemical properties of a soil profile, located in the area of Malu Mare, Dolj County, are presented.

Horizon Am 0-31 cm, has a very dark brownish-gray color 2.5Y 3/2 in the wet state and dark grayish brown 2.5 Y 4/2 in the dry state, clayey texture, structure of well-formed grainy aggregates with high stability, fine porous, compact environment, dense roots of filiform plants, rare butterflies of small white, cervotocin and rare coprolite, gradual passage;

Table 3. Chemical properties of typical alluvial soil

Horizon	Depth (cm)	Humus (%)	Total nitrogen (%)	Soluble P (mg at 100 g soil)	Soluble K (mg at 100 g soil)	pH in H_2O	CEC	HA	BS	BSD %
							(me at 100 g soil)			
Ao	0-15	2.11	0.098	2.8	8.9	7.6	0.7	7.9	8.6	92
AC	15-30	1.10	0.68	2.1	10.2	7.9	0.3	6.8	7.1	96
C1	30-65	0.38	0.023	1.4	9.6	8.1	-	6.4	6,4	100
C2	65-90	0.22	0.013	1.4	9.3	8.1	-	6.1	6.1	100

Horizon AB 31-57 cm has a dark brown-grey color (2.5Y 4/3) when moist and light olive brown (2.5Y 5/2) when dry, a loamy-clayey texture, with a structure of medium and large angular polyhedral aggregates, finely porous, generally compact, with few filamentous roots, rare cervotocines, and coprolites, with a gradual transition;

Horizon BC 57-72 cm, light olive-brown 2.5Y 5/2 in wet state and yellowish olive 2.5 Y6/6) in dry state, loamy-sandy texture, unstructured or crumbly lumpy by drying, finely porous, compact, very rare roots of filiform plants, frequent coarse quartziferous sand grains;

Horizon C 72-105 cm, light yellowish-brown colour 2.5Y 6/4 wet and grey open 2.5Y 7/2 in dry state, sandy-loamy texture, unstructured or lumpy friable by drying, finely porous, compact, very although grains of coarse sand and skeleton of 2-3 mm of quartziferous nature, rare spots.

From a textural point of view, this soil contains a much lower percentage of coarse and fine sand, less than 50% and a much higher percentage of clay, 46.6%. This particle size composition makes the soil texture clayey in the first horizons, as a result of the higher content of fine fractions. The soil also shows a more pronounced settlement over the entire depth of the profile (Table 4).

Table 4. Physical properties of mollic alluvial soil

Horizon	Depth (cm)	Size fractions				Texture class	Bulk density (g/cm^3)	Density (g/cm^3)	Total porosity (%)
		Coarse sand, %	Fine sand, %	Silt, %	Clay, %				
Am	0-31	3.9	24.3	25.3	46.5	A	1.34	2.69	50
AB	31-57	8.6	35.1	21.7	34.6	LA	1.41	2.70	48
BC	57-72	16.8	48.9	18.5	15.8	LN	1.47	2.71	46
Cc	72-105	24.6	57.2	10.7	7.5	NL	1.44	2.71	47

The presence of fine fractions, dust and clay, in a high percentage, over 50%, have determined the increase of the hydrophysical indices to values tending towards the upper limits. The value of the hygroscopicity coefficient, in the first Am horizon, has the value of 13.8%, the wilting coefficient has the value of 19.4%, the field capacity is 33.7% and the useful water capacity has increased to almost 15% (Table 5).

Table 5. Hydro-physical properties of mollic alluvial soil

Horizon	Depth (cm)	HC, %	WP, %	FC, %	AWC, %
Am	0-31	13.8	19.4	33.7	14.3
AB	31-57	9.2	13.6	25.1	11.5
BC	57-72	4.1	6.2	11.4	5.2
C	72-105	2.2	3.2	6.9	3.7

The chemical properties show that the soil is better supplied with humus, over 3% and has a higher reserve of nutrients, as a result of the richness in colloidal complex.

The soil reaction remains in the weakly alkaline range, with a pH value above 7.1 (Table 6).

The gleic-saline alluvial soil is widespread in the pre-terase area of the lower Jiu floodplain, where the finest alluvial materials were deposited by the water during overflows. In this area, the groundwater is found at a shallow depth and produces glazing and salinization processes at the base of the profile.

For the characterization of this soil, the properties of a profile located in the area of Rojiște, Dolj County are presented.

Table 6. Chemical properties of mollic alluvial soil

Horizon	Depth (cm)	Humus (%)	Total nitrogen (%)	Soluble P (mg at 100 g soil)	Soluble K (mg at 100 g soil)	pH in H_2O	CEC	HA	BS	BSD, %
							(me at 100 g soil)			
Am	0-31	3.13	0.170	28.8	112.6	7.1	2.3	36.8	39.2	94
AB	31-57	2.30	0.130	24.9	120.2	7.6	1.2	31.6	32.8	96
BC	57-72	0.65	0.037	16.1	104.4	7.8	0.6	15.9	16.5	96
C	72-105	0.26	0.016	8.5	80.8	7.7	0.3	7.9	8.2	96

Horizon Am 0-28 cm, very dark brown-gray (2.5Y 3/2) in the wet state and brown-gray (2.5Y 5/2) in the dry state, loamy-clayey texture, structure of medium and large granular aggregates with evident edges and corners, finely porous, moderately compact, frequent herbaceous and woody roots, cracked, intense biological activity, does not effervesce, gradual transition;

Horizon AB 28-41 cm, dark gray brown 2.5Y4/4) in the wet state and light gray brown 2.5Y6/2 in the dry state, clayey texture, medium and large angular polyhedral structure, fine porous, compact, woody and grassy roots, small and medium ferro-manganese concretions, weak effervescence, gradual transition;

Horizon BGo 41-80 cm, olive brown 2.5Y 4/4 in wet state and light olive brown 2.5Y 45/2 in dry state, clayey texture, columnar-prismatic structure, porous fine, very compact, medium and high frequent iron-manganese concretions, purple-gray spots and rust-brick veins due to the glazing process, obvious effervescence, gradual passage.

The particle size analysis of this soil indicates a high clay content, over 45%. The clayey and loamy-clay texture gives the soil a strong compaction throughout the entire profile (Table 7).

The hydrophysical indices have values that tend towards the upper limit, correlating well with the granulometric composition of the soil (Table 8).

The soil has a humus content of 2.7% and is poorly supplied with nutrients. The reaction becomes alkaline in depth, where the pH value increases to 8.7 (Table 9).

By conducting the land evaluation work under natural conditions, for the main agricultural crops practiced in the reference area (wheat, barley, corn, sunflower, potatoes, vegetables), it is observed that all the plants studied are best suited to mollic alluvial soil. From Table 10, it is observed that for typical alluvial soil, the land evaluation score for wheat cultivation is 20 points, corresponding to a favorability class IX, while for barley, it is 24 points, which places the soil in favorability class VIII.

High bonitation marks of 72 points, both for wheat and barley, respectively favorability class III, were obtained on mollic alluvial soil. The gleic-saline alluvial soil recorded for the two agricultural crops the lowest bonitation marks, 34 respectively 36, specific to the seventh grade.

Analyzing the favorability of the three soil units in the studied area for corn and sunflower crops (Table 11), it is found that these plants also record the highest values of bonitation marks, and the lowest favorability classes, also on the mollic alluvial soil.

Table 7. Physical properties of gleic-saline alluvial soil

Horizon	Depth (cm)	Size fractions				Texture class	Bulk density (g/cm^3)	Density (g/cm^3)	Total porosity (%)
		Coarse sand, %	Fine sand, %	Silt, %	Clay, %				
Am	0-28	4.8	31.9	17.4	45.9	LA	1.37	2.69	49
AB	28-41	4.5	32.6	15.3	47.6	A	1.43	2.70	47
BGo	41-80	3.1	29.3	17.2	50.4	A	1.49	2.71	45
CGsc	80-130	2.4	31.8	20.6	45.2	LA	1.47	2.71	46

Table 8. Hydro - physical properties of gleic-saline alluvial soil

Horizon	Depth (cm)	HC, %	WP, %	FC, %	AWC, %
Am	0-28	1.,4	18.5	35.1	16.6
AB	28-41	13.1	19.6	35.3	15.7
Bgo	41-80	14.2	21.1	36.6	15.5
CGsc	80-130	11.3	16.5	30.4	13.9

Table 9. Chemical properties of gleic-saline alluvial soil

Horizon	Depth (cm)	Humus (%)	Total nitrogen (%)	Soluble P (mg at 100 g soil)	Soluble K (mg at 100 g soil)	pH in H_2O	CEC	HA	BS	BSD %
							(me at 100 g soil)			
Am	0-28	2.7	0.140	6.9	11.9	7.2	1.9	28.1	30.0	94
AB	28-41	1.3	0.074	6.1	12.3	7.6	0.6	27.9	28.5	98
BGo	41-80	0.7	0.041	4.8	10.8	8.3	-	31.2	31.2	100
CGsc	80-130	0.2	0.011	3.6	10.9	8.7	-	35.4	35.4	100

Table 10. Suitability of soils for wheat and barley

Soil type	Wheat		Barley	
	Bonitation marks	Fertility class	Bonitation marks	Fertility class
typical alluvial soil	20	IX	24	VIII
mollic alluvial soil	72	III	72	III
gleic-saline alluvial soil	34	VII	36	VII

Table 11. Suitability of soils for maize and sunflower

Soil type	Maize		Sunflower	
	Bonitation marks	Fertility class	Bonitation marks	Fertility class
typical alluvial soil	18	IX	22	VIII
mollic alluvial soil	56	V	70	IV
gleic-saline alluvial soil	23	VIII	48	VI

Thus, corn is in the fifth class of favorability (56 points of bonitation mark) and sunflower obtains the fourth class of favorability, with 70 points of bonitation mark. The typical alluvial soil registers for these crops very low values of the bonitation marks, 18 points for corn and 22 points for sunflower, and the gleic-saline alluvial soil falls into the eighth class for the corn crop, which corresponds to 23 points for the bonitation mark and the fourth class for sunflower, with 48 points for the bonitation mark. Analyzing the potato and vegetable crops (Table 12), it can be seen that the bonitation marks and the favorability classes keep the same configuration as the previously analyzed crops, with the difference that on the mollic alluvial soil, the bonitation marks are ower (43) and the favorability classes higher (VIII), for both crops.

Table 12. Suitability of soils for potato and vegetables

Soil type	Potato		Vegetables	
	Bonitation marks	Fertility class	Bonitation marks	Fertility class
typical alluvial soil	20	IX	28	VIII
mollic alluvial soil	43	VI	43	VI
gleic-saline alluvial soil	10	X	29	VIII

On the typical alluvial soil, the potato obtains 20 points in bonitation mark and falls into the ninth class of favorability, and the vegetables have the bonitation marks of 28 points and fall into the eighth class of favorability.

The gleic-saline alluvial soil also registers low values of the bonitation marks for these crops. Potatoes have the lowest bonitation mark and the highest favorability class among all the plants analyzed, and vegetables fall into the eighth grade, with 29 bonitation mark points.

With the help of the bonitation marks and the productions on each point of the bonitation marks, the quantity of products that can be obtained at the main agricultural crops practiced in the reference area, under natural conditions, was estimated (Table 13).

Table 13. The agroproductive potential of the soils in the lower Jiu River floodplain for the main crops.

Cultivated plants	Typical Alluvial soil	Mollic Alluvial soil	Gleic-salinic Alluvial soil
	Estimated yields (kg/ha)		
Wheat	1.200	4.320	2.040
Barley	1.440	4.320	2.160
Maize	1.440	4.480	1.840
Sunflower	704	2.240	1.536
Potato	9.000	19.350	4.500
Vegetables	8.400	12.900	8.700

It is found that the highest agro-productive potential is held by the soft alluvial soil, which has also obtained the highest scores of natural bonitation marks and the lowest classes of favorability.

The lowest agro-productive potential is the gleic-saline alluvial, which obtained the lowest bonitation marks scores and the highest favorability classes.

CONCLUSIONS

The territory under study belongs to the lower floodplain of the Jiu River, south of the city of Craiova. The natural conditions, climate, relief, vegetation, geological substrate and hydrology, have determined the formation and evolution of soils with different profile development. The largest area is occupied by alluvial soils, soils of relatively low age. The alluvial units identified in the area are represented by the typical, mollic and gleic-saline subtypes.

Typical alluvial soils are found in the coastal area of the meadow, through the deposition of coarse alluvial materials, which have determined less intense solification processes. The mollic alluvial soils are found in the central area of the meadow, where the genesis processes are more advanced. The gleic-saline alluvial soils occupy the pre-terrestrial area, where the groundwater is found close to the soil surface, determining gleization and even salinization processes at the base of the soil profile.

The young soils in the Jiu floodplain have a very heterogeneous granulometric composition, ranging from sandy and sandy-loamy to clayey. They have good total porosity at the surface and low porosity in the lower horizons, indicating strong compaction at depth.

The hydrophysical indices are closely correlated with the soil texture and organic matter content, having lower values in typical alluvial soil and higher values in mollic alluvial soil and gleic-saline alluvial soil.

The humus content is low in typical alluvial soil (below 2%) and medium (2-3%) in mollic alluvial soil and gleic-saline alluvial soil, while the nutrient supply is poor for phosphorus and medium for potassium.

The reaction of the soils is neutral or alkaline, the pH value being generally higher than 7.

The fertility of the relatively low age soils in the Jiu meadow is different and is closely correlated with the physical, hydrophysical and chemical properties resulting from laboratory determinations. The best natural fertility is found in mollic alluvial soil, while at the opposite end is gleic-saline alluvial soil. On typical alluvial soil and mollic alluvial soil, all crop slopes are suitable with poor to medium results, whereas on gleic-saline alluvial soil, some plants are excluded from cultivation as they encounter unsuitable conditions for growth and fruiting. This category includes orchards and vineyards.

Estimating the agroproductive potential for the main agricultural crops practiced in the area (wheat, barley, corn, sunflower, potatoes, and vegetables), it was found that they can yield better production on mollic clayey alluvial soil, which also received the highest natural land evaluation scores and the lowest favorability classes. The lowest agroproductive potential is found in gleic-saline alluvial soil, which received the lowest land evaluation scores and the highest favorability classes. In addition to these agricultural plants that lend themselves to the alluvial soils of the Jiu meadow, tobacco benefits from appropriate soil and climate conditions (Iancu et al., 2009b). On all soils, it is recommended to apply fertilizers based on phosphorus, as chemical analyses have shown a poor supply of this element in the soils. The chemical fertilizers used every year, in the conditions of typical alluvial soils, have a positive influence on the plants grown on these soils, as a result of the sandy texture, high aeration porosity, low humification of the residual material and decomposition to mineralization (Iancu et al., 2009a).

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EVALUATION OF TRANSFORMATION OF THE QUALITY COMPOSITION OF ORGANIC MATTER AND PARTICLES SIZE DISTRIBUTION OF SOLUTION OF HUMIC ACID OF SODDY-PODZOLIC SOIL AFTER LONG-TERM APPLICATION OF VARIOUS FERTILIZATION SYSTEMS

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Abstract

Soil organic matter play crucial role in soil functions, physicochemical properties and structure stability, which determined main soil properties - fertility. The objective is to study transformation of the quality composition of organic matter of soddy-podzolic soil with the application of various fertilization systems in relation to deleted of nano-, micro-size particles distribution of alkali solutions of isolated humic acids (HAs) from soil. Via UV-visible spectroscopy and gel-permeation size-exclusion chromatograph, determined structural molecular complexity of isolated HAs and by dynamic light scattering and visual observing of dried drops of HA solutions was evaluate particles size of HA in solution at micro- and nano-level of secondary or colloid structural organization. Revealed relation between fraction compositions of organic matter that causing by fertilizer systems and molecular complexity with particles size distribution of isolated HA in alkali solution. We showed that under long-term application of mineral and organo-mineral fertilization systems significant increase ratio of small nanoparticles population and decrease of big microparticles in alkali HA solution of soddy-podzolic soil with highest degree of fulvatiozation.

***Key words**: soddy-podzolic soil, soil organic matter, fertilizers, humic acid, particles size distribution.*

INTRODUCTION

Under climate changes and escalating emissions of greenhouse gases from soils, more and more attention focused on soil organic matter (SOM) and its stabilization in soil under tillage, fertilizers or amendments. SOM play crucial role in soil functions, physicochemical properties and structure stability, which determined main soil properties - fertility (Angelico et al., 2023; Wu et al., 2024; Yang et al., 2020). The amount of SOM is therefore a very important parameter, but the qualitative parameters can decide extent on its quantity, and are more important in the characterization of changes in SOM transformation and deep processes of humification at molecular and nano-levels (de Aguiar, 2022; Fragouli, 2023).

The process of humification includes both the decomposition of plant materials with a high molecular weight (biodegradation), and the re-association of decomposition compounds products by means of polymerization \ condensation reactions, as well as molecular self-assembly and intermolecular aggregation that determine degree of structural randomness of particles size and molecular weights polydispersity of humic biomolecular associations. It mean that the polydispersity is due to the variety of possible variants of intermolecular re-associations and re-aggregations, which form humification products determined dynamics and stability of SOM (Beckett, 1987; Fragouli, 2023; Tombácz, 1999).

Humic substances (HS) main and most reactive component of SOM exist in the form of self-organized hydrophilic-hydrophobic micelles of aggregate particles of different sizes, which under certain conditions can undergo transformations, changing the balance of the quality of organic matter in soils (Angelico et al., 2023; de Aguiar, 2022; Wu et al., 2024). Molecular structural composition of HS effect on the functioning of soil organic carbon and sequestration of carbon in soil humic matter that was not always in an inert or stable sate (Drososa et al., 2020; Li et al., 2019; Song et al., 2014). In order to evaluating and monitoring of SOM quality and process of organic transformation need to have a more clear understanding of HS complex nature and their properties. In these case determine relations between parameters of SOM quality and directions of humification with structural characteristics and physico-chemical properties of isolated soil HS fractions is very important in modern humic chemistry and in evaluation of quality and stability of organic matter under soil perturbations (Escalona et al., 2023; Jiang et al., 2021; Jiménez-González et al., 2020; Olk et al., 2019a; 2019b; Vinci et al., 2021; Yang et al., 2020). Therefore, development and applications of new methodical approach play key role in humic chemistry improving and assessing of SOM into molecular and nano-level (Angelico et al., 2023; Jiménez-González et al., 2020; Jovanović et al., 2013; Olk et al., 2019a; 2019b;).

In the last decades evaluation of SOM quality significant transformation due to modern analytical methods and understanding of composition, structural organization and properties of soil HS fractions (humic\fulvic and humins). Like a molecular compositions, parameters of the secondary structural or colloid organization of HS, is an important indicators of SOM transformation, especially when caused by the impact of different land use (Escalona et al., 2023; Jiang et al., 2021; Jovanović et al., 2013; Wu et al., 2024).

Therefore, objectives of these study was explore the relationship between transformation of soddy-podzolic organic matter fraction composition under long-term fertilizer systems and nano-, micro-size particles distribution characteristics of solutions of isolated humic acid (HA) fraction evaluated via dynamic light scattering (DLS) and dry drop methods.

MATERIALS AND METHODS

The research was conducted in the laboratory of organic fertilizers and humus of the National Scientific Center «Institute for soil science and agrochemistry research named after O.N. Sokolovsky» in soil samples taken in a long-term field experiment on the territory of the Volyn State Agricultural Research Station of the Institute of Agriculture of Western Polissya NAAS of Ukraine, located in the village of Rokyny, Lutsk district, Volyn region, in the natural and climatic zone of Polissya. Soil samples was taken from soddy-podzolic soil of the field for the study of long-term use different fertilizers systems (60 years). Provided soil samples in triplicate repetition collected from 20 cm soil profile with variants: control (without fertilizers), mineral fertilizer ($N_{540}P_{510}K_{450}$), organo-mineral fertilizer (manure + $N_{540}P_{510}K_{450}$). Indexes of quality of SOM were determined using group and fractional analysis of soddy-podzolic soil samples by the method of Tyurin in the modification of Ponomarova and Plotnikova (DSTU 7828:2015).

Spectroscopic (UV-visible), gel-permeation methods of size-exclusion chromatograph (SEC) as well as DLS electrochemical technics (particle size diameter and polydispersity index, zeta potential) with visual observing of fractal aggregates in dried drop were applied to evaluate the differences in the behavior, molecular and colloid structural micro-organization of the Has in alkali media isolated from long-term fertilized soddy-podzolic soil.

Isolating of HA fractions from soil and preparing of study HA solutions. The isolation of the HA fraction that correspond to second HA-2 fraction at fractionation of humus by Tyurin method from the 0-20 cm layer of soddy-podzolic soil was carried out according to standard method of alkali-acid extraction. 100 g of prepared soil samples was stirred in 500 ml of 0.1 N NaOH for 24 h. After 24 h the slurry was centrifuged at 6000 rpm for 15 min to separate the supernatant containing the humic substances of humic and fulvic acid fractions from the mineral residues and humin fraction.

Concentrate HCl was added to the supernatant to acidify it to pH 1 for precipitation of HA fraction. After standing for 16 h, the suspension with humic acids was centrifuged at 6000 rpm for 15 min to obtain the HA precipitate. To remove mineral residues such as fine clay silicates, the precipitate was further dissolving in 0.1 N NaOH and periodic shaking for 2 h and performed HA re-precipitation. After that re-precipitating of HA was carry out by adding 6 M HCl to acidify the mixture to pH = 1, and allowing the suspension to settle for 16 h. This precipitated HA fraction was wash by distilled water and separated by centrifuging at 6000 rpm for 15 min, and dried for finely ground.

Solutions of isolated HAs fraction from soil samples were prepare by dissolving of 10 mg of isolated finely ground HA by 0.1. N NaOH in 100 ml flask with next stirring until complete dissolution. Prepared study solutions consisted of three alkali HA solu°tions from the soil with different fertilizers: HA solution from the soil without fertilizers; HA solution from the soil with mineral fertilizer; HA solution from the soil with organo-mineral fertilizer.

Size-exclusion chromatograph. To characterizing, the molecular-mass distribution of isolated HA solutions performed by SEC via permeation gel chromatography on column (2×40 cm) using Sephadex G-200 elution of humic fractions by distilled water with UV detection at 280 nm using UIT SFU-0170 spectrophotometer.

UV-visible spectra. To obtain UV-visible specters of absorption of HA solution used Hitachi spectrophotometer in the spectral range 200-600 nm. Measuring specters carry out in quartz cuvettes. Water solution of 0.1. n NaOH was used as a comparison standard when recording absorption spectra and removing alkali absorption in HA solutions. After placing the cuvettes with HA solution in a spectrophotometer under temperature control of 25°C waited for 5 minutes for a relative equilibrium of dynamic process in humic solution.

Dynamic light scattering (DLS) measuring. The particles size distribution (PSD) indexes and Zeta-potential (ZP) of HA solutions investigated by DLS via Zetasizer MAL1151890. Measuring triplicated and average values presented. DLS technique measures the diffusion of particles moving under Brownian motion, and a size distribution using the Stokes-Einstein relationship.

Drying drop methods of HA solutions. To obtain fractal micro-aggregates of HA solutions, the drying drop method (DDR) used which described in work (Song et al., 2014). A drop of HA solution with a volume of 3 μl without any pretreatment was placed for drying on a clean, fat-free, dry glass slide surface. The samples were left to dry under room conditions (27°C and humidity 55) and after 24 h examined visual morphology of obtained space-time organizations of fractal aggregates via a optic microscope at 100x with digital camera.

RESULTS AND DISCUSSIONS

Quality of organic matter of soddy-podzolic soil after long-term fertilizers. The transformation of organic matter of soddy-podzolic soil under long-term application of fertilization characterized by increasing of total organic carbon, fulvic and labile humic fraction but decreasing of mature HA fraction that lead to lowering of humification degree. In Table 1 represented results of indexes of quality of soddy-podzolic SOM.

Table 1. Indexes of quality of soddy-podzolic organic matter after long-term mineral and organo-mineral fertilizers

Indexes	Control soil without fertilizers	Mineral fertilizer (NPK)	Organo-mineral fertilizer (NPK + manure)
C_{total}, %	0.57	0.64	0.73
C_{HA}, % to C_{total}	47.4	35.9	35.6
Humification degree	Very high	high	high
C_{FA}, % to C_{total}	50.8	62.5	61.6
C_{HA}/C_{FA}	0.93	0.57	0.57
Humus type	Humat-fulvat	fulvat	fulvat
$C_{HA\text{-}1}$, % to total HA content	14.8	30.4	57.6
$C_{HA\text{-}2}$, % to total HA content	33.3	8.7	19.2

Revealed that fertilization increased of total soil organic carbon that associated with enhanced biotransformation activity of SOM as a result of entering in soil of fresh fertilizer source (NPK and manure) of transformation. Under long-term agricultural use of sod-podzolic soil under fertilization, the content of humus in the arable layer increased, but the degree of humification of organic matter changed from very high to high degree. Lowering of humification degree under fertilization of soddy-podzolic soil due to decreasing content of HA and increasing of fulvic fraction that lead to formation of fulvat humus state. Results indicate increasing of content of labile HA fraction (HA-1) in soddy-podzolic soil after fertilizer applications. Accumulation of labile humic fraction HA-1 is due to intense neo-formation of mobile HS fractions such as labile humic and fulvic acids cause by activation of biotransformation of fresh SOM. Showed that under organo-mineral fertilizer in soddy-podzolic soil form largest content of labile humic fraction (HA-1) which caused formation most mobile humus system compare to control soil without fertilizer and soil with mineral fertilizer. A decrease in the content of stable and polymerized mature humic fraction (HA-2) in the soil after the application of fertilizers was revealed compared to the control soil, especially on variants with mineral fertilizer. Results showed that increasing of total organic carbon in soddy-podzolic soil after fertilization occur by accumulation of labile HS fractions of HA and fulvic (FA).

SEC of HA solutions. Isolated HA fractions from soddy-podzolic soil correspond to stable mature humic fraction of soil (HA-2) that characterized by SEC and UV-visible spectroscopy revealed high molecular size and concentration of stable aromatic condensed benzoic structures in control soil without fertilizers and with highest total content of HA and stable mature fraction (HA-2). Increasing of molecular size and with high concentration of condensed aromatic structures promote formation more complex and stable HA structure that due higher hydrophobic-hydrophilic molecular heterogeneities (Debska et al., 2007; McAdams et al., 2018).

The obtained SEC profiles of molecular-mass distribution of isolated HA are mainly represent of peak of accumulation of heterogeneous low-weight molecular fraction (Figure 1).

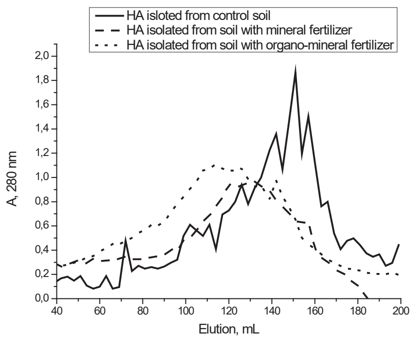


Figure 1. SEC profile of HA isolated from soddy-podzolic soil with fertilizers

The highest absorption at 280 nm of main peak observed in HA isolated from control soil without fertilizer. Application of fertilizers in soddy-podzolic soil decrease intensity of absorption of peak and broad the chromatogram that due to increase of molecular dispersity of HA. Revealed increasing of main chromatogram peak in HA from soil with organo-mineral fertilizer compare to HA from soil with mineral fertilizer.

Increasing absorption intensity at 280 nm of main SEC peak indicate higher concentration of heterogeneous low-weight stable (hydrophobic) aromatic structures that accumulate and under self-organization and intermolecular aggregation increase molecular size and complexity of hydrophobic-hydrophilic heterogeneities of soil HA (Kučerík et al., 2007; Piccolo et al., 1996). Higher molecular dispersity of HA is due to domination of small molecular size structures that spread in samples as result of weak intermolecular self-aggregation (Baigorri et al., 2007a, 2007b; Jovanovic et al., 2022). SEC results indicate decreasing of molecular size of isolated HA from soil with fertilizers as results of higher molecular dispersity and lower of molecular heterogeneity compare to HA from soil without fertilizer.

UV\visible spectra of humic solutions. On Figure 2 show UV-visible specters of absorbing HA solutions from the soddy-podzolic soil with fertilizers. Obtained specters are characterized by wide specters in the UV range that start to decrease monotonically with an increasing wavelength. The uniformity of the spectra indicates the similarity in general composition and structural organization of HA solutions. The main difference in obtained specters is expressed in intensity of absorption (A), the broad and tilt angle of the spectra line in the UV range. Absorption (A) in the UV range corresponds to various aromatic and phenolic-like structures. The most strong and high intensity of absorbing with broad spectra in the UV range corresponds to the HA solution from control soil without fertilizers, the lowest intensity indicates in the HA solution of soil with mineral fertilizer. Increasing UV absorption (at 254 nm) of HA solution occur to higher concentration of aromatic and phenolic humic structures that lead to stronger prosperity to form big sized complex humic aggregates. Obtained decreasing of UV-visible absorption of HA in solution is due to lowering the concentration of stable aromatic structures in isolated HA from soil with fertilizer. The increasing of UV-visible absorption observed in HA from soil with organo-mineral fertilizer.

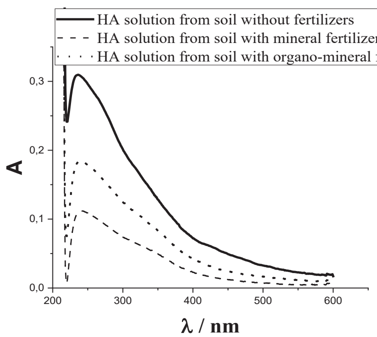


Figure 2. UV\visible specters of HA solutions from soil

Fertilizers promote to reduction of HA complexity and molecular heterogeneities of soddy-podzolic soil compare to HA from control soil. The reduction of HA complexity and molecular heterogeneities of soddy-podzolic soil was appear in decreasing of general molecular size and concentration of stable aromatic structures that promote self-organization of high molecular size associations with next intermolecular aggregation into big sized stable humic micellar aggregates structures in solution. The most simple humic molecular organization with low molecular heterogeneities and decreased molecular size was attributed to HA solution from soil with mineral fertilizer as result of fulvatization of humic fractions. However, organo-mineral fertilizer due to manure component as a source of fresh heterogenic aromatic structures leading to complication of HA structures by increasing the molecular size and concentration of stable aromatic structures of HA in solution.

Unfortunate the gel permeation SEC with spectroscopic methods to detailed evaluation of molecular size distribution of HS fractions is limited. The interesting findings in work (Tian et al., 2021) that HS fractions characterized by a very pronounced stable condensed aromatic structure associated with the highest molecular weight and size. Nevertheless, using DLS methods succeeded detailed characterization of PSD at nano- and micro-size distribution range of isolated HA in alkali solutions from soddy-podzolic soil after long-term application of fertilizer systems.

Particles size distribution of HA solutions. PSD reflect aggregation distribution character-ristics and secondary structural organization of HAs in solution. In Figures 3-5, the example of PSD by intensity, number and volume of HA solutions from soil with fertilizer systems shown. According to DLS measuring, results of intensity and volume size distributions of HA solutions had a tri-multimodal character in which three main peak highlight three population of particles size range: peak 1 range at 50-100 nm, peak 2 range at 150-2,000 nm, and peak 3 range at 2,000 nm to more than 1 µm.

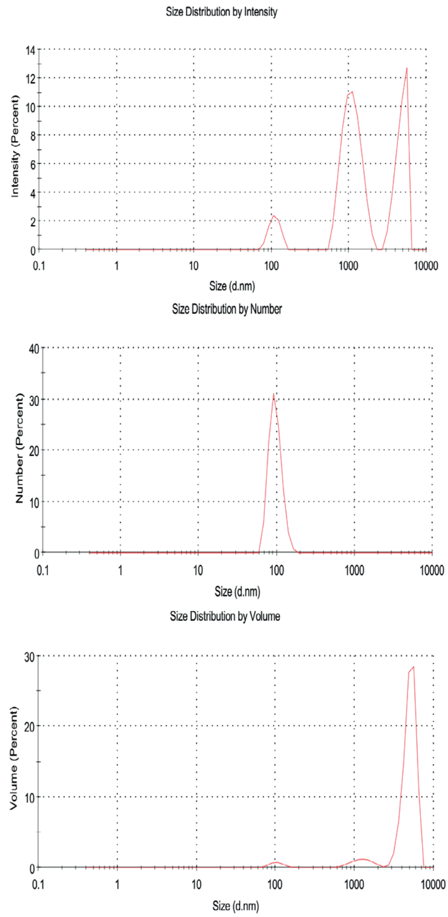


Figure 3. PSD by intensity, number and volume of HA solutions of control soil without fertilizers

fertilizers into the reduction of big sized particles aggregates.

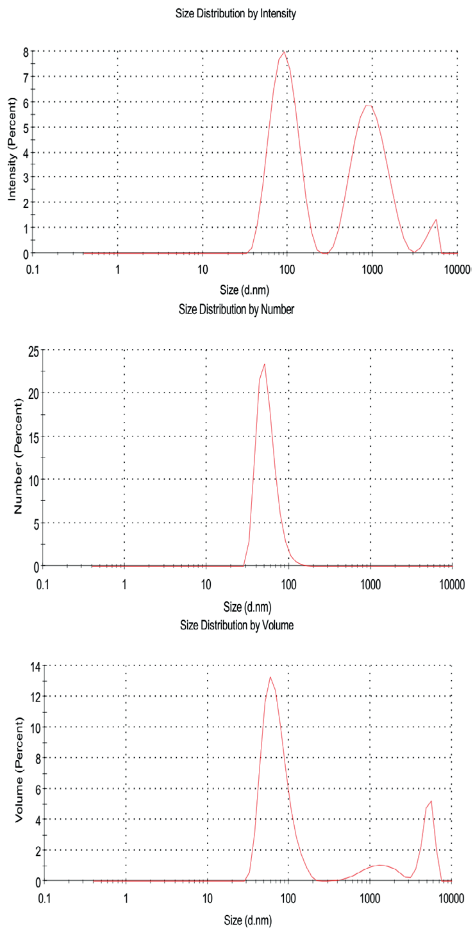


Figure 4. PSD by intensity, number and volume of HA solutions of soil with mineral fertilizer

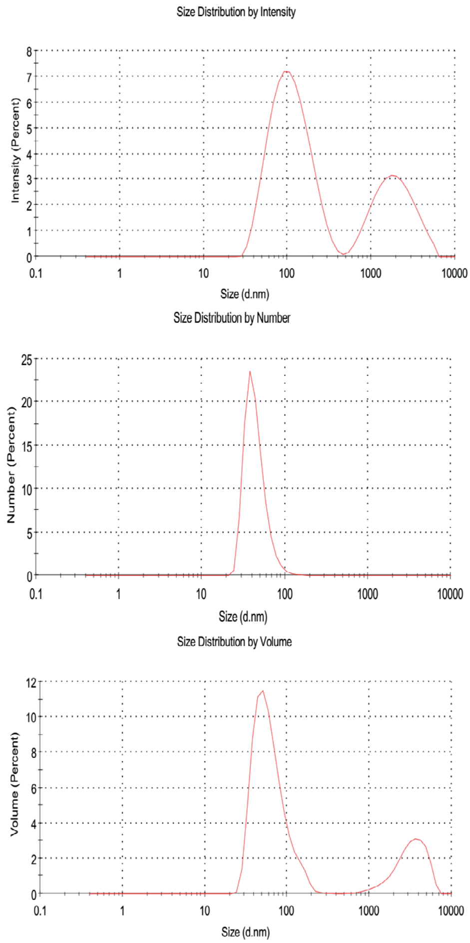


Figure 5. PSD by intensity, number and volume of HA solutions of soil with organo-mineral fertilizer

Tarasevich et al. who analyzed Aldrich HAs in alkali solution divided the same particles size population it into three main particles size fraction ranges called small nanoparticles (30-150 nm), sub-microparticles (200-1000 nm), and big microparticles (1.6-3 µm) with large macroparticles sub-fraction (3-5.6µm) (Dolenko & Trifonova, 2017; Tarasevich et al., 2013). Reveled significant increasing of peak intensity and volume of small-sized nano- and sub-microparticles and decreasing peaks of micro\macroparticles populations in HA solutions from soil with fertilizers compare to HA solution from control soil without fertilizer. The obtained results of re-distribution of PSD by intensity and volume testify significant re-arrangement of colloid or secondary structural organization of HA in solution from soil with

PSD by number of HA solutions from soil represent by monodisperse distribution of small sized nanoparticles populations. Observed shifting of PSD by number in more small nano-sized range of nanoparticles populations of HA solutions from soil with fertilizer systems (Figures 4 and 5). In HA solution from soil without fertilizer (Figure 3), the volume occupied by nano-particles was much lower but much higher in number in comparison with HA solution from soil with fertilizer systems (Figures 4 and 5). High content of macroparticles size population observed mainly in HA solution from control soil without fertilizer was due to micro- and macroparticles with a small volume of nanoparticles (~100 nm) population. On the contrary, in HA solution from soil with mineral fertilizer detected increasing the intensity and volume PSD of nanoparticles fraction (~100 nm) and decreasing of bigger microparticles population. Observed broadening of PSD peaks of sub-microparticles population in HA solutions from soil with fertilizers. Indicate broadening but decreasing PSD of intensity of overlapped peak of sub- and microparticles populations of HA solution from soil with organo-mineral fertilizer. In HA solution from soil with organo-mineral fertilizer also observed broadening peak of PSD by volume of bigger particles compare to HA solution from soil with mineral fertilizer (Figure 5).

The characteristics of particle sizes of study humic solutions listed in Table 2 that reflect colloid size organization of isolated HAs in solution.

Table 2. Indexes of Z-average size, PDI, particles size by volume and ZP of HA solutions of soil after long-term mineral and organo-mineral fertilizers

Samples	Z-average size (d. nm)	PDI	Peak 1 by volume, d. nm	Peak 2 by volume, d. nm	Peak 3 by volume, d. nm	ZP, mV
HA solution from control soil	1426	0.669	104,8	1255	4990	-25.1
HA solution from soil with mineral fertilizer	146.8	0.762	71,13	1373	5094	-30.3
HA solution from soil with organo-mineral fertilizer	117.0	0.671	64,81	overlapped	3367	-27.4

As can see (Table 2) fertilization of soddy-podzolic soil decrease the Z-average size (the average size diameters) of humic solutions but not be suitable parameter for humic size characteristics. Z-average particle sizes was determined from intensity, volume and number based DLS records, and represent as average of obtained records of multimodal distribution. Revealed increasing of polydispersity (PDI index) of HA solution from soil with mineral fertilizer that due to higher dispersity of humic system as result of nanoparticles population increasing. Decreasing of PDI observed in HA solution isolated from soil with organo-mineral fertilizer where revealed microparticles population increasing compare to HA solution from soil with mineral fertilizer. In general, the average diameter of the smallest nanoparticles and polydispersity in HA solutions from soil increased after fertilizer application that accompanied also with increasing of negative charge of ZP. In contrast, the humic solution from control soil without fertilizer contained mostly micro- and macroparticles with a diameter around 3-5 µm and lowers index of negative charge of ZP.

In our study, alkali HA solutions revealed relatively high numbers of small nanoparticles population with a diameter around 100 nm and less. Alkali solutions of HA lead to realis large number of smallest sized aggregate particles that was due to "open-folder" humic colloid organization in alkali media (Dolenko & Trifonova, 2017; Klucakova & Veznikova, 2017; Klucakova, 2018). On the basis the obtained PSD results, we used volume based DLS records in order to analyze PSD of study HA solutions from fertilized soil in detail. Volume based PSD of study humic solutions showed a higher volume of small nanoparticles with diameter less than 100 nm in HA solution from soil with fertilizers (Figure 6).

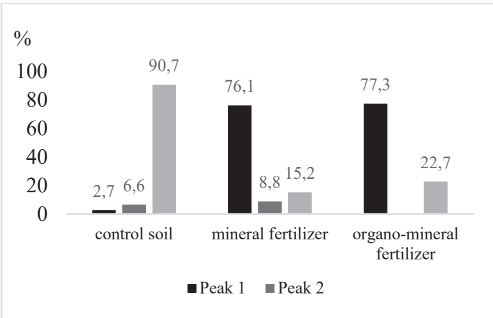
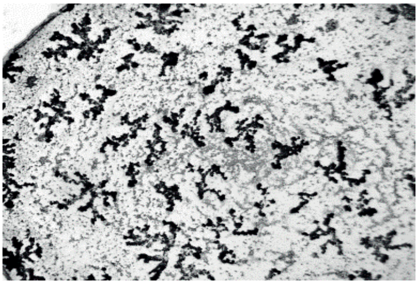


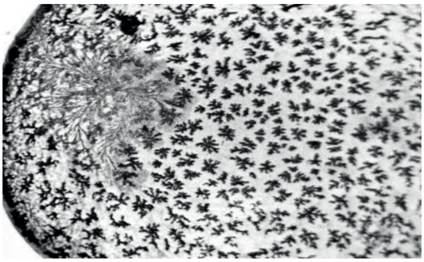
Figure 6. The intensity of peaks of PSD by volume of HA solutions of soil with fertilizers

The volume occupied by large particles populations in humic solution from control soil was much higher in comparison with humic solutions from soil with fertilizers. In control soil without fertilizer observed high volume of microparticles size population aggregates with diameter around 5 μm. Organo-mineral fertilizer promote to some high volume of bigger aggregates particles with a diameter around 3 μm compare to HA solution from soil with mineral fertilizer that mainly consist from small sized nanoparticles population.

Fractal aggregates after dried drop of HA solutions from soil. The method of dried drop of HA solutions was applied for the first time to visual evaluation of ability of fractal aggregates formation. Using a drying drop method of HA solutions from the soil with fertilizers obtained fractal aggregates that represented in Figure 7.



A



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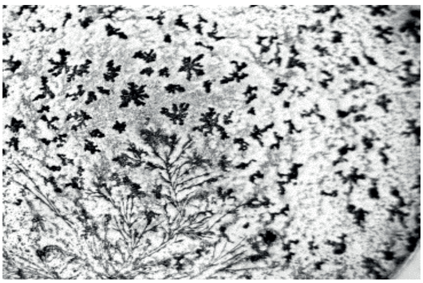

C

Figure 7. Self-organized fractal aggregates of dried drop of HA solution of control soil without fertilizer (A), soil with mineral fertilizer (B) and with organo-mineral fertilizer (C)

Visual characteristics of fractal aggregates after 24-hour drying of all studied HA solutions belong to the dendritic mass type morphology structure due to Brownian diffusion of particles by diffuse-limited aggregation (DLA) during dehydration self-organization of drops (Österberg et al., 1995; Rizzi et al., 2004). Obtained fractal aggregates of all studied HA solutions characterized by similar morphology type of fractals that indicates the universality of self-aggregation assembling of humic system. Assembling of fractal aggregates under dehydration of colloid solution is due to DLA (cluster-cluster DLA and reaction DLA) when one wandering colloid particle is attached to growth clusters and forms on a microscopic ordered space-time structure (Österberg et al., 1994). The form of this microscopic space-time aggregate structure takes place in the periphery as result of the removal of part of the solution from the center and locally depends on concentration and solution distribution on a glass surf. Visual observation revealed differences in size and number of self-organized fractal aggregates.

As one can see fractal aggregates of HA solution from the soil without fertilizer form most big on size fractal aggregates with low number and the smallest but with a large number of fractal aggregates corresponding to HA solution from soil with fertilizers. Visual detected that under self-organization dehydration of drop of HA solution from soil with mineral fertilizer increase the number of small fractal aggregates comparing even to number of fractal aggregates of dried drop of humic solution from soil with organo-mineral fertilizer.

CONCLUSIONS

Our study of transformation of soddy-podzolic organic matter fraction composition under long-term fertilizer systems was show differences in the structural molecular complexity and colloid particles size distribution between isolated HAs in alkali solutions via UV-visible, SEC, DLS and dried drop methods.

In general, fertilizer systems lead to increasing content of total organic carbon and carbon of labile HA and FA fractions caused by activation of biotransformation of fresh organic matter and intense neoformation of HS.

Nevertheless, observed increasing of total organic carbon and labile HA fractions of soddy-podzolic soil under fertilizers accompanied decreasing content of mature HA fraction due to high mineralization compare to control soil without fertilizers.

Revealed relation between fraction compositions of soddy-podzolic organic matter that causing by fertilizer systems and molecular complexity with particles size distribution of isolated HA in alkali solution. We showed that under long-term application of fertilizers significant increase ratio of small nanoparticles population, decrease of big microparticles in HA solution of soddy-podzolic soil as a results of neoformation of HS, accompanied of increasing content of labile HA and FA fractions.

According to our results, formation of big-sized microparticles of humic aggregates in alkali solution of isolated HAs of soddy-podzolic soil is due to high content of carbon and stable aromatic structures of total HA and mature HA fraction in soil.

Visual confirmed by drying drop methods of HA alkali solutions that long-term fertilizers lead to formation large number of small-sized fractal aggregates space-time structures. After drying drop of HA solution from soil with mineral fertilizer observed manly small fractal aggregates emerged from intermolecular self-aggregation of dominantly small nano-sized colloid particles.

We show that long-term mineral fertilizer of soddy-podzolic soil promote simplification of HAs molecular structure that in alkali solution behave as fulvic-like colloid structural organizations consist from mainly small and mobile nano-sized aggregate particles (less than 100 nm) that influence on mobility of SOM and weak accumulation of neoform labile HSs. However, manure of organo-mineral fertilizer increase the level of structural organization of isolated HA that promote formation of stable big-sized microparticles aggregates in alkali solution that due to stabilization of labile HA and increasing of soil organic matter maturity and quality.

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MICROBIOLOGICAL AND ENZYMATIC ACTIVITY OF TYPICAL CHERNOZEM UNDER DIFFERENT TILLAGE AND FERTILIZATION SYSTEMS

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Abstract

Studies (2021-2023) conducted on a typical low humus chernozem (Bila Tserkva, Ukraine) showed that in the arable layer, microbiota on starch-ammonium agar (SAA) is highest in non-fertilized plots under differentiated tillage and in fertilized plots under disc tillage. On meat peptone agar (MPA), the highest microbiota activity is observed under differentiated tillage across all plots. Microbiota on pectin-glucose agar with soil extract (PGASE), as well as nitrifying bacteria and actinomycetes, had the highest number under differentiated tillage, while the mineralization and pedotrophic coefficients had the lowest values. The lowest number of denitrifying bacteria and the highest number of nitrogen bacteria were recorded in the differentiated tillage. The activity of invertase, phosphatase dehydrogenases and polyphenol oxidases is higher, while catalase activity is lower under differentiated tillage compared to mouldboard and mouldboardless tillage. The coefficient of humus accumulation was higher under differentiated tillage and significantly lower under chisel and disking tillage. Increasing organic and mineral fertilizer rates with mouldboardless and disc tillage enhances the biological diversity of the arable layer.

***Key words**: fertilization, crop rotation, microbiota, enzymes, activity, productivity.*

INTRODUCTION

Soil monitoring in Ukraine is primarily focused on controlling agro-physical and agrochemical fertility indicators, and in some cases, assessing potential weed infestation. However, the concept of soil fertility requires consideration of its biotic parameters, not only in terms of quantity but also in relation to the structure of the biota (megafauna, macrofauna, mesofauna, microfauna, and microflora) (Prymak et al., 2023). This will ensure soil "health" through microbial diversity (Frunze N., 2015). In one gram of soil, the number of bacteria ranges from 300,000 to 95 million, and in some cases, up to 4 billion (Storchous, 2013) according to other researchers, this number ranges from 3.7 to 5.7 billion (Ostapchuk et al., 2016). Understanding the processes of microbial communities in agricultural soils is essential for developing optimal and energy-efficient agroecosystems (Shi et al., 2019).

The most important indicators of soil fertility are the abundance and structure of the microbiota, with enzymes playing a crucial role. Soil enzymes are the most stable component of its biological activity, as they can be adsorbed by soil particles after cell death. The primary source of enzymes in the soil is plant residues (Frunze, 2013). Soil microorganisms are the primary source of enzymes and play a crucial role in maintaining key nutrient cycles in the soil (C, N, P, S) by transforming them from organic matter (Aon et al., 2001).

In a broad sense, the enzymes present in the soil can be classified as intracellular and extracellular (Dick, 1994). The first category mainly includes enzymes associated with viable proliferating cells, while extracellular enzymes are those released into the surrounding environment through secretion or lysis, as well as active enzymes linked to dead cells and other non-living soil fractions (Sinsabaugh & Moorhead, 1994). The activity of soil enzymes is a key candidate for "sensors," as they integrate information, on the one hand, from the microbial state, and on the other hand, from the physico-chemical conditions of the soil (Aon et al., 2001).

Experiments suggest that soil biota depends not only on the ecological properties and tillage of the soil but also on the historical context, meaning the colonisation and extinction of certain species (Bender et al., 2016). The importance of historical contingency for community assembly has also been demonstrated in the secondary succession of vascular plants (Ejrnæs et al., 2006). Shafi et al. (2007) demonstrated that crop rotation systems increase the amount of nitrogen (N) available in the soil, thereby reducing the need for fertilisation, while promoting plant growth and health. Crop rotation, combined with long-term fertilisation, led to an increase in microbial diversity in the maize rhizosphere. A higher abundance of Acidobacteria, Actinobacteria, and Proteobacteria was found in fields where crop rotation was practised, compared to monoculture systems (Soman et al 2017).

The predominance of pathogenic or symbiotic microorganisms also depends on the physical properties of the soil: density, porosity, aeration, water-air and thermal regimes, as well as the systems of its cultivation (Prymak et al., 2022; Usharani et al., 2019).

Deep ploughing creates a shock state for the soil microbiota, with aerobic microflora being transferred to the anaerobic conditions of the lower part of the plough layer, while anaerobic microflora is, conversely, exposed to aerobic conditions. As a result, there is a significant decrease in the abundance of soil microbiota. This can be altered by using a non-inversion tillage system over 4-5 years. In most cases, with such soil cultivation, cellulose-degrading microbiota are more abundant in the upper part of the plough layer, while their numbers in the lower part remain the same or even lower (Nadtochii et al., 2010).

The activity of three enzymes (dehydrogenase, phosphatase, arylsulfatase) was higher in fields without tillage than in fields with conventional tillage (Bergstrom et al., 1998). No-tillage (NT) systems lead to an increase in the concentration of nutrients, organic matter, and pesticides on the soil surface (Dick, 1992). The activity of soil urease, acid phosphatase, and protease at a depth of 0–10 cm, as well as the activity of acid phosphatase, dehydrogenase, arylsulfatase, invertase, amylase, and urease at a depth of 0-7.5 cm, was significantly higher in the soil without tillage (NT) than with ploughing (Bolton et al., 1985).

The composition of the rhizosphere bacterial community can vary significantly under different soil cultivation methods. Rhizosphere bacterial communities under no-tillage conditions are found to be more stable than under plough tillage, which may be related to the relatively low disturbance of the soil and the unique environment of the rhizosphere under no-tillage conditions (Wang et al., 2020).

Mouldboard & mouldboardless (differentiated) tillage activates biological processes in typical chernozem, including the release of carbon dioxide and its assimilation by soil microbiota, cellulolytic, nitrification, and enzymatic activity. Under differentiated tillage, nitrifying bacteria prevail, while under disking (continuous shallow) tillage, ammonifying bacteria dominate (Voitovyk & Zhovtun, 2024).

The results of a long-term soil tillage experiment conducted on sandy soil in Lithuania showed that the use of sustainable soil cultivation can protect soils from biological degradation and maintain soil quality compared to conventional tillage. The intensity of soil tillage positively affected microbial substrate utilisation and urease activity, but negatively impacted dehydrogenase activity, the number of bacteria and fungi, and the Shannon microbial community diversity index. Higher total porosity stimulated higher enzyme activity; however, microbial activity negatively correlated with bulk density (Janušauskaite et al., 2013).

Legrand et al. (2018) assessed the impact of soil tillage on bacterial and fungal diversity and found an effect on community composition, along with a somewhat positive impact of reduced tillage on diversity indices, but not on saturation. Snell Taylor et al. (2018) noted that long-term management with no-tillage significantly increased the size of soil microbial communities while negatively affecting bacterial diversity. Sengupta A. & Dick W. A. (2015) concluded that higher soil tillage intensity leads to a lower number of dominant bacterial species.

Soil tillage prepares the soil through mechanical disruption and has a number of negative impacts on the soil environment of agricultural lands, causing degeneration and serious disruption of the soil ecosystem (Knowler & Bradshaw, 2007). In particular, prolonged soil tillage leads to negative consequences for soil health, such as nutrient loss and soil compaction (Foley et al., 2011). As an alternative, zero tillage, as a conservative practice for minimal soil disturbance, is widely used to increase and stabilise yields (Knapp & van der Heijden, 2018). Long-term implementation of zero tillage improves soil quality and the soil environment (Scopel et al., 2013). Zero tillage can increase the diversity of soil microbial communities (Wang et al., 2017; Mathew et al., 2012; Dong et al., 2017). Overall, enzyme activity in the soil increases with the minimisation of mechanical tillage. An active microbial community in the rhizosphere of winter rye plants with an intensive enzymatic complex is observed under strip-till cultivation (Vilnyi & Maklyuk, 2014).

Soil fertilisation is necessary to meet the growing demand for livestock feed and human food. However, fertilisation has both short-term and long-term effects on soil microbiota. This, in turn, can influence plant viability and growth (Kurzemann et al., 2020; Grabovskyi et al., 2024).

Mineral and organic fertilisers change the composition of the microeukaryote community, with only organic fertilisers having a dose-dependent effect on the microeukaryote community in the soil (Ding et al., 2019). However, compared to mineral fertilisers, organic fertilisers can retain a greater diversity of microbes in the soil environment (Ye et al., 2019).

The use of chemical fertilisers changes the abundance of microbial populations and stimulates their growth by adding nutrients. However, overall data showed that chemical fertilisers do not have a significant impact on the richness and diversity of bacteria and fungi. Instead, a large number of individual bacterial or fungal species were sensitive to fertiliser application, which was primarily explained by changes in the chemical properties of the soil caused by mineral or organic fertilisation. Among the negative effects of chemical fertilisation, a decrease in enzymatic activity has been highlighted in several studies, especially in soils that received the highest amounts of fertilisers, along with losses of organic matter (Dincă et al., 2022).

Based on the analysis of microbial communities from datasets of 64 long-term trials worldwide, it was concluded that the application of mineral fertilisers leads to a 15.1% increase in microbial biomass compared to non-fertilised control plots; additionally, the use of nitrogen (urea and ammonium fertilisers) can have a temporary or stable effect on increasing soil pH (Geisseler & Scow, 2014; Wang et al., 2020).

The application of nitrogen increases the potential nitrification speed but reduces nitrogen use efficiency and changes the beta-diversity of ammonia-oxidising bacteria (AOB), decreasing the relative abundance of *Nitrosospira* (nitrite-oxidising bacteria) and increasing the relative abundance of *Nitrosomonas* (which oxidise ammonia to nitrite) (Zou et al., 2022). On the other hand, the application of mineral and organic fertilisers significantly increased the species richness and alpha-diversity of AOB (Tao et al., 2017).

In general, organic and mineral fertilisers typically have a positive effect on numerous soil bacteria, the most common of which is *Azotobacter*. For example, the use of mineral fertilisers on semi-arid alfisols led to an increase in *Azotobacter* abundance (Cinnadurai et al., 2013). Similar results were obtained in maize crops (Adediran et al., 2003). Alternatively, intensive fertilisation with mineral nitrogen can negatively impact other specific bacterial groups (e.g., diazotrophs, beta-proteobacteria), which are important rhizospheric microbes with symbiotic N-fixing interactions with leguminous plants.

Mineral fertilisers have also been noted to have a negative impact on soil microbiota. Long-term use of mineral fertilisers leads to the loss of soil organic matter (SOM), particularly in arid and semi-arid regions or where monoculture practices (e.g., maize) are employed (Luo et al., 2015; Zhang et al., 2010). Prolonged use of mineral fertilisers can be detrimental, especially with high nitrogen fertiliser dozes, as it leads to increased gaseous nitrogen losses and deterioration of the soil's physical, chemical, and biological properties (Schjønning et al., 2018). It has also been found that mineral fertilisers cause a reduction in soil porosity and nutrient availability (Song et al., 2015). Additionally, mineral fertilisers have a significant effect on the abundance of microorganisms and the qualitative selection of entire soil microbial communities (Doran & Zeiss, 2000). Dangi et al. (2020) suggested that the use of organic fertilisers or amendments may potentially mitigate the harmful effects of inorganic fertilisers on the environment in agroecosystems, but they may also impact soil microorganisms, whose specific effects are not yet clearly defined. They found that soil amendments, such as biochar or the addition of other organic fertilisers over approximately two years, influenced microbial community biomass, composition, and yield.

Based on a review of the literature, the impact of specific soil cultivation and fertilisation technologies on agricultural crops has been established (Fernandez et al., 2019; Legrand et al., 2018; Huang et al., 2013; Mikanová et al., 2009; Mathew et al., 2012; Prymak et al., 2024; Grabovskyi et al., 2023). However, information on the microbiological condition of soils under different cultivation technologies and fertilisation systems is insufficient and fragmented.

The aim of our research was to determine the impact of basic soil tillage systems and fertilisation on the change in the microbial community and enzymatic activity of the plough layer of typical chernozem.

MATERIALS AND METHODS

The research was conducted in the experimental field of Bila Tserkva National Agrarian University in 2021-2023 on typical deep low-humus black soil in a stationary five-field crop rotation.

The scheme of the experiment included four systems (variants) of basic tillage (factor A) (Table 1) and four fertilizers (factor B) systems were studied: 0 - without fertilizers, 1 - 6 t/ha of manure + $N_{54}P_{48}K_{48}$, 6 t/ha of manure + $N_{92}P_{66}K_{90}$, 3 – 6 t/ha of manure + $N_{120}P_{92}K_{110}$.

Table 1. Systems of basic tillage in crop rotation

№ field	Crop	Tillage (factor A)			
		I	II	III	IV
		mouldboard (control)	mouldboardless	mouldboard & mouldboardless (differentiated)	disking (continuous shallow)
		Depth, cm			
1	Soybean	10-12	20-22	10-12	10-12
2	Winter wheat	6-8	6-8	6-8	6-8
	white mustard on green manure	10-12	10-12	10-12	10-12
3	Corn	25-27	25-27	25-27	10-12
4	Spring barley	10-12	10-12	10-12	10-12
	white mustard on green manure	10-12	10-12	10-12	10-12
5	Sunflower	25-27	25-27	25-27	10-12

Crop rotation fields were fully deployed in space and time. In the experiment, threefold repetition was placed completely on the area, plots of the first order (tillage system) – sequentially in one tier, the second (fertilizer rates) – sequentially in four tiers. The sown area of the elementary plot was 171 m^2 (9 x 19 m) and the accounting area was 112 m^2 (7 x 16 m). The area of each field is 7835.6 m^2 (76×103.1) and the total area under the experiment is 3.7 hectares. Cattle manure, ammonium nitrate, simple granulated superphosphate, and potassium salt were used as fertilizers. Microbiological and enzymatic activity of typical chernozem was determined by the methods Hrytsayenko et al., 2003, Gorodniy et al., 2005.

RESULTS AND DISCUSSIONS

The soil microbiota in areas with crop rotation consists of 76-93% bacteria, 9-25% actinomycetes, and 0.2-0.5% fungi (Table 2). The placement of organic and mineral fertilisers, as well as plant residues in the upper part of the plough layer (0-10 cm), under mouldboardless and disking tillage systems, facilitated the intensive development of microbiota. In the lower part of the plough layer (20-30 cm), this process was hindered due to the lack of energy material for the microbiota.

Table 2. Microbiota abundance in the plough layer (0-30 cm) of typical chernozem under different tillage and fertilisation systems, individuals/g of dry soil

Indicators	Soil cultivation systems (factor A)							
	mouldboard (control)		mouldboardless		mouldboard & mouldboardless (differentiated)		disking (continuous shallow)	
	Fertiliser systems (factor B)							
	without fertilizers	6 t/ha of manure + $N_{120}P_{92}K_{110}$	without fertilizers	6 t/ha of manure + $N_{120}P_{92}K_{110}$	without fertilizers	6 t/ha of manure + $N_{120}P_{92}K_{110}$	without fertilizers	6 t/ha of manure + $N_{120}P_{92}K_{110}$
Microbiota on SAA, mln	27.67	42.71	27.46	43.81	28.37	42.38	28.24	46.31
Microbiota on MPA, mln	11.81	14.12	11.21	13.45	12.94	15.06	10.94	13.29
Actinomycetes, mln	3.70	6.23	3.53	5.80	4.00	7.13	3.24	5.40
Fungi, ths.	12.42	17.20	14.71	21.33	13.22	18.77	15.15	22.04
Ammoniators, ths.	28.60	83.30	25.70	72.30	28.00	80.10	24.90	71.00
Cellulose-degrading bacteria, ths.	112.40	154.5	105.70	140.80	110.30	150.80	101.1	126.70
Denitrifiers, ths.	42.30	71.20	45.10	78.30	39.30	66.10	46.7	80.50
Nitrifiers, ths.	4.20	7.30	4.03	6.80	4.48	7.80	3.88	6.58
Microbiota on pectin-glucose agar with soil extract (PGASE), mln	19.68	33.79	15.26	23.42	21.63	35.47	15.87	26.36
Phosphorus-binding bacteria, ths.	30.30	41.30	29.00	39.6	29.80	40.5	28.40	38.70
Mineralisation ratio (SAA:MPA)	2.34	3.00	2.45	3.26	2.19	2.81	2.58	3.48
Pedotrophicity index (PGASE:MPA)	1.67	2.39	1.36	1.74	1.67	2.36	1.45	1.98
Azotobacter, colonies on soil plates	141	190	134	177	143	194	132	175

The number of microbiota in the 0-10 cm soil layer on meat peptone agar (MPA) and starch-ammonium agar (SAA) (which consume organic and mineral nitrogen, respectively), including actinomycetes, fungi, cellulolytic and nitrifying bacteria, was higher by 16% and 28%, 24%, 29%, 27%, and 25% under mouldboardless tillage, and by 53% and 86%, 34%, 43%, 41%, and 32% under disking tillage, compared to the control.

In the 20-30 cm soil layer, these values were lower by 37 and 10, 38, 33, 35, and 44% under chiselling and 39 and 6, 62, 41, 42, and 61% under disking compared to the control. Conducting deep ploughing during the crop rotation period in one field noticeably, and in two fields – significantly, reduces the heterogeneity of the arable layer based on these indicators.

In the arable layer of soil, the microbiota counts on SAA increased by 2.6% only on fertilized plots under chiseling compared to the control. The highest values were observed on unfertilized plots under differentiated soil tillage and on fertilized plots under disking. On MPA, the microbiota was highest under mouldboard-mouldboardless tillage and lowest under disking.

The coefficient of mineralization of soil organic matter on fertilized and unfertilized plots is higher by 5% and 9% under mouldboardless tillage and 10% and 16% under disking compared to the control. Under differentiated tillage, it is 6% lower than the control. Incorporating plant residues into the lower part of the arable layer of typical chernozem increases this coefficient in the 0-10 cm soil layer compared to disking. A narrower carbon-to-nitrogen ratio in humus increases the proportion of microbiota on SAA relative to its total count in the soil environment, ultimately raising the mineralization coefficient.

In the lower part (20-30 cm) of the arable layer of typical chernozem, the ratio of microbiota on SAA and MPA equalizes on the plots treated with mouldboard ploughing. Under mouldboardless and disking tillage, this ratio increases due to the significant reduction or absence of plant residues entering the lower part of the arable layer.

The number of actinomycetes in the arable layer on non-fertilized and fertilized plots is 5% and 7% lower under mouldboardless tillage, 12% and 13% lower under disking tillage, and 8% and 14% higher under differentiated tillage, compared to the control. The fungal microbiota count is higher in the mouldboardless tillage variants by 18% and 24%, in the differentiated tillage variants by 13% and 19%, and in the disking tillage variants by 22% and 28%.

The highest number of ammonifying bacteria was recorded in the arable layer under mouldboard & disking tillage; under mouldboardless, differentiated, and disking tillage, their count was 10% and 13%, 2% and 4%, and 13% and 15% lower, respectively, on non-fertilized and fertilized plots. A similar pattern was observed for cellulose-degrading bacteria, where the decrease was 6% and 9%, 2%, 10%, and 18%, respectively. Being typical representatives of the mineralization block of the soil microbiota, their numbers significantly respond to the mass and quality of plant residues. Their share in the upper, middle, and lower parts of the arable layer was 34%, 35%, and 31% under mouldboard & disking tillage; 45%, 34%, and 21% under mouldboardless tillage; 40%, 34%, and 26% under mouldboardless-mouldboard tillage; and 51%, 31%, and 18% under disking tillage.

Denitrifying bacteria in the arable layer were 8% and 12% higher under mouldboardless and disking tillage, respectively, and 7% lower under differentiated tillage compared to the control. For nitrifying bacteria, the opposite trend was observed: their count was 4-7% and 8-10% lower on chisel and mouldboardless-mouldboard tillage plots, respectively, and 7% higher under disking tillage compared to the control. A similar pattern, even more pronounced, was observed regarding the microbiota count on pectin-glucose agar with soil extract (PGAE). In this regard, the first tillage variant showed an advantage over the second and fourth variants by 22-31% and 19-22%, respectively.

The pedotrophic index, which characterizes the intensity of organic matter utilization in the soil, was almost at the same level under mouldboard & mouldboardless tillage and was 19-27% and 13-17% lower under chisel and disking tillage, respectively, compared to the control.

The population of *Azotobacter* in the plough layer, whose activity largely depends on the soil aeration, exceeded the control by only 1-2% under differentiated soil tillage, while under chisel and disc tillage, it was reduced by 5-7% and 6-8%, respectively.

Invertase activity depends on the fertilisation system, depth, and method of tillage of typical chernozem. The localisation of plant residues in the upper part of the plough layer under mouldboard and disc tillage increased this indicator by 8% and 12%, respectively, compared to ploughing at a depth of 25-27 cm. In the lower layers (10-20 and 20-30 cm), the opposite trend was observed: invertase activity was 11% and 21% higher in the ploughed plots. Overall, in the crop rotation, invertase activity in the plough layer was 5-7% lower under mouldboard and shallow tillage and 5-7% higher under mouldboard & mouldboardless tillage, compared to the control (Table 3).

Urease activity in the upper part of the plough layer was also higher under mouldboard and disc tillage by 11% and 16%, respectively, compared to the control. Overall, across the crop rotation, urease activity in the plough layer was nearly the same under mouldboard & mouldboardless and differentiated tillage, but significantly lower in the fertilised plots with mouldboard and disc tillage compared to the control.

Protease activity characterises the intensity of hydrolysis of organic compounds of a protein nature into peptides, which are subsequently broken down into amino acids. These amino acids undergo ammonification and nitrification, leading to the formation of plant-accessible forms of soil nitrogen. A certain proportion of amino acids condenses with oxidised forms of aromatic compounds, contributing to the formation of soil humus. Protease activity in different layers of the arable soil (0-10, 10-20, and 20-30 cm) varied across soil tillage options similarly to invertase and urease activity. Overall, across the crop rotation, the protease activity in the 0-30 cm soil layer under mouldboardless tillage slightly decreased, remained unchanged under differentiated tillage, and significantly decreased under disking, compared to the control.

Table 3. Enzymatic activity of tilthy soil under different tillage and fertilisation systems

Soil cultivation systems (factor A)	Fertiliser systems (factor B)	Invertase, mg of glucose per 1 g of soil per 24 hours	Urease, mg of N-NO_3 per 100 g of soil in 3 hours	Proteases, mg of amine nitrogen per 100 g of soil per 20 hours	Phosphatase, mg P_2O_5 per 100 g of soil per 48 hours	Dehydrogenases, Lenard optical density units	Catalase, ml of dry soil O_2 per 1 minute	Polyphenol oxidases (mg of purpurgalin per 100 g of soil in 30 minutes)	Peroxidases (mg of purpurgalin per 100 g of soil in 30 minutes)	Humus accumulation coefficient
mouldboard (control)	0	8.01	2.37	111	1.6	0.148	2.31	58	108	54
	1	9.23	3.31	141	2.7	0.291	2.58	81	117	69
	2	10.04	3.54	150	3.5	0.341	2.71	96	123	78
	3	10.83	3.78	158	3.8	0.360	2.78	107	132	81
mouldboardless (chisel)	0	7.49	2.24	108	1.7	0.142	2.45	51	102	50
	1	8.67	3.13	136	2.9	0.279	2.75	73	112	65
	2	9.47	3.34	144	3.7	0.325	2.89	88	121	73
	3	10.27	3.58	152	4.0	0.341	2.99	101	133	76
mouldboard &mouldboardless (differentiated)	0	8.42	2.39	110	1.6	0.163	2.23	66	113	58
	1	9.74	3.35	140	2.8	0.325	2.47	92	121	76
	2	10.62	3.57	151	3.7	0.385	2.58	105	125	84
	3	11.52	3.84	160	4.0	0.409	2.63	112	132	85
disking (continuous shallow)	0	7.50	2.23	104	1.5	0.136	2.55	50	104	48
	1	8.61	3.10	132	2.5	0.260	2.88	70	111	63
	2	9.33	3.30	142	3.3	0.310	3.04	85	120	71
	3	10.03	3.51	150	3.6	0.326	3.13	101	136	74
SD_{05}		0,52	0.15	7	0.4	0.017	0.17	6	8	4

Phosphatases, by catalysing the hydrolysis of organophosphorus compounds, orthoesters of alcohols, and phenols (detaching phosphate groups from organophosphates), facilitate the biochemical mobilisation of organic phosphorus, making it available to plants. The activity of phosphatases in the arable soil did not undergo significant changes across the tillage variants, although a trend of increased activity was observed on fertilised plots under chisel and differentiated tillage, while it decreased under disking.

Dehydrogenases catalyse the dehydrogenation of hydrogen from organic substances in the soil (carbohydrates, aromatic acids, humic acids, amino acids, alcohols, etc.), acting as an intermediate carrier of hydrogen. The detached hydrogen from carbohydrates and organic acids is transferred to organic compounds like quinones or to the oxygen in the air. The oxidation of these organic substances occurs both during mineralisation and humification. Dehydrogenase activity reflects the vitality of the microbiota and the content of humic substances that can be decomposed by it. The dehydrogenase activity under mouldboardless, differentiated, and disking tillage systems is higher in the upper part of the plough layer, while it is lower in the lower part by 13.6% and 23.0%, compared to the control. On fertilised plots, the dehydrogenase activity under disking tillage decreases significantly, whereas it increases significantly under differentiated tillage. Mouldboardless tillage, however, lags behind the control in this indicator.

Catalase activity, which reflects the intensity of soil respiration, is significantly higher in the plough layer under disking tillage compared to mouldboard-disking tillage. The difference increases with higher fertiliser doses: on unfertilised plots, it is 10%, and on fertilised plots with the highest dose, it is 13%. Under differentiated tillage, this indicator decreases insignificantly, while under chiselling tillage, it increases by 6-8%.

Polyphenol oxidases catalyse the oxidation of phenols to quinones. Through heteropolymer condensation of the latter with polypeptides and amino acids, primary humus compounds are formed under suitable conditions. Polyphenol oxidases and peroxidases are typically measured simultaneously. The latter catalyse the oxidation of polyphenols in the presence of hydrogen peroxide or organic peroxides, which are produced by microbial activity or the action of certain oxides. The intensity of humus mineralisation largely depends on reactions involving peroxidases, while humification is driven by polyphenol oxidases, as the latter significantly affect the conversion of aromatic organic compounds into humus components. Therefore, the intensity of humus formation is determined by the ratio of polyphenol oxidase activity to peroxidase activity, which is known as the humus accumulation coefficient. The activity of polyphenol oxidases was significantly lower in the second and fourth soil tillage treatments compared to the first. The differentiated tillage treatment exceeded the control by 5-14% for this indicator, with the difference being insignificant only at the highest fertilizer dose.

No significant changes in peroxidase activity were observed in the arable layer across the soil tillage treatments, but with the increase in fertilizer levels, it, like the activity of other enzymes, increased. However, on non-fertilized plots with mouldboard and disc tillage, peroxidase activity decreased by 2-6%, while it increased under differentiated tillage compared to the control.

The humus accumulation coefficient decreased by 6-7% and 7-11% for the second and fourth soil tillage treatments, respectively, and increased by 5-10% for the third treatment, compared to the control.

CONCLUSIONS

The use of deep ploughing throughout the crop rotation significantly reduces the heterogeneity of the arable layer in terms of microbiota population. Increasing organic and mineral fertilizer rates with mouldboardless and disc tillage enhances the biological diversity of the arable layer.

In the arable layer, microbiota on starch-ammonium agar (SAA) is highest in non-fertilized plots under differentiated tillage and in fertilized plots under disc tillage. On meat peptone agar (MPA), the highest microbiota activity is observed under differentiated tillage across all plots.

The highest population of microbiota on pectin-glucose agar with soil extract (PGASE), nitrifying bacteria, and actinomycetes was observed under differentiated tillage, while the mineralization and pedotrophic coefficients had the lowest values.

The highest fungal microbiota population was found under mouldboardless and disc tillage, while ammonifying and cellulolytic bacteria were most abundant under mouldboard & disc tillage. Under differentiated tillage, the lowest population of denitrifiers and the highest of *Azotobacter* was recorded.

The activity of invertase, phosphatases (on fertilised plots), dehydrogenases, and polyphenol oxidases is higher, while catalase activity is lower under differentiated tillage compared to mouldboard and mouldboardless tillage. The protease and urease activities in the arable layer are nearly at the same level for these tillage variants.

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RESEARCH ON THE EVALUATION OF SOIL RESOURCES SPECIFIC TO THE TERRACE AND FLOODPLAIN AREAS OF ROSEȚI COMMUNE, CĂLĂRAȘI COUNTY

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Abstract

The main objective of the research was to evaluate the morphological, agrophysical, and agrochemical characteristics of the soils in the terrace and floodplain areas of Roseți, Calarasi County. Environmental and geographical conditions influencing soil quality and its agricultural potential were also analyzed. The research also aimed to identify the characteristics that determine the adaptability/suitability of the land for various types of agricultural use.The studied area is characterized by a diversity of soils that support a variety of agricultural and ecological uses. In the terrace area, Chernozems and Phaeozems provide a suitable environment for cereal or leguminous crops, with a slightly alkaline pH (7.2-7.6), good to very good phosphorus (61-64 ppm) and potassium (160-400 ppm) supply, and a medium to good humus content (2.6-4.30%). In the floodplain area, the dominant soils are Alluvial soils, with a slightly alkaline pH (7.3-7.6) and a humus content of 2.69-3.5%, indicating a medium supply. These alluvial soils are in different stages of development but are relatively young, still evolving, and formed from alluvial deposits.

***Key words**: soil fertility, soil assessment, limiting factors.*

INTRODUCTION

Land evaluation is a fundamental field of natural resource management, playing a crucial role in ensuring the sustainable and efficient use of agricultural, forestry, and urban land. The importance of this activity has significantly increased in the context of globalization, climate change, and the growing demand for land for various economic uses.

According to the Food and Agriculture Organization of the United Nations (FAO, 1976), land evaluation is defined as "the process of estimating land performance in relation to specific uses, involving the analysis of environmental, social, and economic factors". This definition was later expanded by experts in the field, such as Rossiter (1996), who emphasized that "land evaluation is a systematic process of analyzing land potential based on soil, climate, and topographical characteristics to determine its most suitable use".

Land evaluation is not limited to the physical aspects of the soil but also includes socio-economic considerations. According to Dent and Young (1981), "land use decisions should not be based solely on the physical characteristics of the soil but also on economic, political, and social factors that influence the feasibility of implementing certain types of land use".

In the Romanian context, the land evaluation process is known as "bonitation" and plays an essential role in determining the productive capacity of agricultural soils. According to the methodology developed by ICPA Bucharest (1987), land is classified based on its pedological, agrochemical, and climatic characteristics to determine its suitability for various agricultural crops.

Another key concept in land evaluation is favorability, which, according to Țărău (2006), represents "the extent to which agricultural land meets the growth and development requirements of crops under normal climatic conditions and with the application of standard technologies". This definition is similar to that proposed by Klingebiel and Montgomery

(1961), who suggested using the term "favorability" to describe the overall performance of land in relation to specific uses. Therefore, land evaluation is not merely a technical process of soil analysis but a complex, interdisciplinary activity that contributes to the sustainable development of agriculture and land-use planning.

MATERIALS AND METHODS

Field Phase

This stage includes all activities carried out directly in the field, including:

- A general evaluation of the studied area;
- Soil profile analysis, correlated with natural factors and production conditions (the actual mapping process);
- Identification and description of soil morphological characteristics;
- Soil sampling for laboratory analysis.

To determine the qualitative properties of the soils, the following analyses were conducted:

- Determination of soil reaction (pH) in aqueous suspension (pH H_2O), using the potentiometric method at a soil-to-water ratio of 1:2.5;
- Analysis of mobile phosphorus content using the Egner-Riehm-Domingo method, based on ammonium acetate-lactate extract;
- Determination of mobile potassium content using the same method mentioned above;
- Analysis of humus content using the modified Walkley-Black oxidation method (Gogoasă modification);
- Calculation of the base saturation degree (V), by determining hydrolytic acidity and the sum of exchangeable base cations;
- Determination of granulometric fractions using the Kacinsky method.

Office Phase

During this stage, the following operations were performed:

- Identification and delimitation of soil territorial units (US);
- Classification of soils according to the Romanian Soil Taxonomy System (SRTS, 2012), at both higher levels (type, subtype) and lower levels (variety, granulometric species, family, and variant);
- Characterization of soil territorial units based on field observations and laboratory analysis results, in terms of morphological and physico-chemical properties;
- Evaluation of agricultural land quality using the methodology established by the Research Institute for Pedology and Agrochemistry (ICPA), for assigning soil fertility ratings and classifying land according to its suitability for different agricultural crops, as well as into specific quality classes for each type of agricultural use.

RESULTS AND DISCUSSIONS

The commune of Roseți is located in southeastern Romania, in Călărași County, and is characterized by a lowland plain relief consisting of floodplain areas and terrace surfaces (Rîpă et al., 2024).

Relief and Land Use

The floodplain area is situated near the Danube River, featuring low-lying terrain with nutrient-rich alluvial soils, but it is susceptible to periodic flooding. These lands are primarily used for agriculture, favoring crops such as rice, corn, and vegetables. To improve agricultural productivity, drainage works and hydrotechnical arrangements have been implemented.

The terrace area is located at a slightly higher altitude and features proxicalcaric chernozem and cambic phaeozem soils, known for their moderate to high fertility. This region is mainly dedicated to cereal crops such as wheat and barley, as well as viticulture and orchards. Due to its higher elevation, this land is less exposed to flooding risks, providing more stable conditions for agricultural exploitation.

Thus, the geomorphological diversity of the Roseți commune shapes a complex agricultural landscape, where the efficient use of land must be adapted to the characteristics of each area, ensuring the sustainable exploitation of resources.

Hydrology and Hydrography

The Frățești layers, present in the southern part of the Romanian Plain, give the area high permeability. In the Danube region and the Getic Plain, gravel deposits predominate, known for their highwater absorption capacity (porosity between 25-35%), while sandy formations dominate the central and eastern parts of the plain.

Due to this increased permeability, the area benefits from significant groundwater reserves. The groundwater level varies between 3 and 10 meters in the terrace area, and in some places, it exceeds 10 meters. The groundwater in the Frățești layers moves from west to east, with the Danube River as the primary drainage direction, also influenced by the local hydrographic network.

The flow rate of the aquifers ranges between 6 and 10 liters per second, but as they move further into the plain, it decreases to approximately 3 liters per second. The groundwater has low mineralization (between 0.7 and 1.7 g/L) and falls into the category of bicarbonate waters, which explains the absence of strong soil salinization phenomena in the area.

Climate

Climatic data collected from the Călărași meteorological station indicate a temperate-continental climate with the following characteristics:

- The average annual temperature is 11.2°C.
- The highest temperatures are recorded in July–August, reaching 22.8°C, while the lowest temperatures, down to -22°C, occur in January.
- At the end of March and the beginning of April, temperatures allow for the initiation of spring agricultural work.
- The first frosts appear in late September, while the last ones can be observed until early May.

Precipitation

The average annual precipitation is 497.9 mm, but in recent years, a downward trend has been observed.

- The highest amount of precipitation recorded within 24 hours occurred in June, when torrential rains sometimes caused the lodging of crops, especially wheat.
- During summer, moisture deficits frequently occur due to the torrential nature of rainfall. Rainwater tends to accumulate in microdepressions in the plain instead of gradually infiltrating the soil, leading to its rapid evaporation during heatwaves.

Predominant Winds

- *Crivăț* (from the Northeast) is the dominant wind, intensifying low temperatures in winter and early spring.
- *Austral* (from the Southwest) contributes to severe drought conditions in summer.
- *Băltăreț* (from the South), though less intense, brings precipitation, carrying moisture that condenses and generates moderate rainfall.

Vegetation

In the territory of Roseți, besides the steppe vegetation in the north, a specific type of vegetation develops in the area between the Danube and the Borcea Branch:

- Higher areas are covered by spontaneous forests, dominated by species such as: *Populus alba*, *Populus nigra*, *Salix alba*, *Salix fragilis* and *Tamarix ramosissima*.
- Between the high lands and the marshy areas, meadows with mesophilic and hydrophilic plants have formed, including: *Convolvulus arvensis*, *Setaria viridis*, *Centaurea cyanus*, *Cynodon dactylon*, *Papaver rhoeas* and *Plantago lanceolata*.

Profile 1 - Cambic Phaeozem

Latitude: N: 44°19'16"

Longitude: E: 27°27'56"

Major relief unit: Romanian Plain

Unit: Bărăganului Plain

Parental material: carbonate loessoid deposits

Groundwater: >10 m

Current use: Arable

Representative profile: 12

Morphological characterization (Figure 1)

Ap (0-19 cm): dusty clay, very dark gray brown (10 YR 3/1) wet, dark gray brown (10 YR 4/2) dry, small grain structure, good plastic, good adhesive, good compact, rare medium macropores, frequent thin grassy roots.

Am (19-40 cm): dusty loam, very dark grayish brown (10 YR 3/1) in wet state, dark brownish gray (10 YR 4/2) in dry state, small-medium grained well developed, friable, moderately plastic, moderately cohesive, moderately adhesive, medium-frequency macropores.

A/B (40-62 cm): dusty clay, dark gray brown (10 YR 3/2.5) wet, gray brown (10 YR 4/3) dry, well-developed subangular polyhedral, friable, moderately plastic, moderately

cohesive, loose, frequent medium macropores, frequent thin grassy roots, gradual transition.
Bv (62-113 cm): clay loam, dark brown (10 YR 3/3) wet, brown (10 YR 5/3) dry, medium-high polyhedral moderately developed, moderately cohesive, loose, medium frequent macropores.
B/C (113-156 cm): clay loam, dark yellowish brown (10 YR 4/3) wet, brown (10 YR 5/4) dry, large subangular polyhedral, poorly developed, friable, weak plastic, weak adhesive, weak compact, rare medium macropores.
Ck (156-180 cm): medium clay, dark yellowish brown (10 YR 4/4) wet, yellowish brown (10 YR 6/3) dry, loose, friable, moderately plastic, moderately adhesive, loose, macroporous medium rare, medium to strong effervescence.

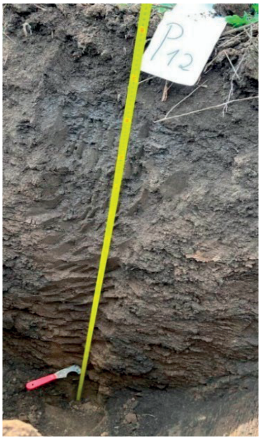

Figure 1. Cambic Phaeozem

n profile 1, from the Ap horizon to the B/C horizon, the soil reaction is neutral and down the profile up to 180 cm, the reaction is weakly alkaline. Mobile phosphorus has a value of 64 ppm and is well supplied to the soil. Mobile potassium having the value of 160 ppm, good supply (Table 1). The humus has a good supply and humus reserve is 251 t/ha, very high, and does not penalize the credit score. The soil texture is medium, dusty clay up to the A/B horizon, fine texture with a clay percentage greater than 32% in the Bv and B/C horizon, and medium texture in the Ck horizon. It is a profile with carbonates at 156 cm.
For the US 1 profile, evaluation marks were calculated for: wheat, barley, sunflower, peas and beans, 72 points were obtained, falling into the 2nd quality class and the 3rd favorability class. Maize and soybeans with 64 and 65 points and fall into the 2nd quality class and the 4th class of favorability. The potato crop obtained 45 points and is in the 3rd quality class and 6th favorability, and the beet crop obtained 50 points and is in the 3rd quality and 6th class of favorability. The depth of the hydrostatic level of the phreatic water is located at a very deep depth (> 10 m), contributing to the reduction of the evaluation marks with a coefficient of 0.8 for all crops. The evaluation marks for the eight crops is 64 points and falls into the 2nd quality class and the 4th favorability class (Table 2).

Table 1. The maine physical and chemical properties

Horizon	**Depth** (cm)	**Physical properties**				**Texture class**	**Chemical properties**				
		Coarse sand (2-0.2 mm)	Fine sand (0.2-0.02 mm)	Dust (0.02-0.002 mm)	Colloidal clay (<0.002 mm)		pH (H_2O)	$CaCO_3$ (%)	Humus (%)	P_{AL} (ppm)	K_{AL} (ppm)
Ap	0-19	1.23	29.30	37.80	31.67	LP	7.2	-	4.50	64	160
Am	19-40	1.23	30.80	37.24	31.73	LP	7.0	-	4.22	-	-
A/B	40-62	0.99	31.48	31.20	36.33	LP	6.9	-	3.74	-	-
Bv	62-113	1.26	30.86	31.22	36.66	LA	6.9	-	-	-	-
B/C	113-156	0.92	36.68	30.82	31.58	LA	6.9	-	-	-	-
Ck	156-180	1.23	29.30	37.80	31.67	LM	7.7	9.4	-	-	-

Table 2. Land Suitability for the main crops

Crop	**Tem 3C**	**Pre 4C**	**Gl 14**	**Stg 15**	**Sal/Alc 16/17**	**Text 23**	**Pol 29**	**Slo 33**	**Ls 38**	**HL 39**	**Flo 40**	**TP 44**	**$CaCO_3$ 61**	**pH 63**	**EV 133**	**HR 144**	**EM 181**	**EM**
Wheat	1	0.9	1	1	1	1	1	1	1	0.8	1	1	1	1	1	1	1	72
Barley	1	0.9	1	1	1	1	1	1	1	0.8	1	1	1	1	1	1	1	72
Maize	1	0.8	1	1	1	1	1	1	1	0.8	1	1	1	1	1	1	1	64
Sunflower	1	0.9	1	1	1	1	1	1	1	0.8	1	1	1	1	1	1	1	72
Potato	0.8	0.7	1	1	1	1	1	1	1	0.8	1	1	1	1	1	1	1	45
Sugar beet	0.9	0.7	1	1	1	1	1	1	1	0.8	1	1	1	1	1	1	1	50
Soybean	0.9	0.9	1	1	1	1	1	1	1	0.8	1	1	1	1	1	1	1	65
Peas/Beans	1	0.9	1	1	1	1	1	1	1	0.8	1	1	1	1	1	1	1	72

Note: average annual temperature-3C, average annual precipitation-4C, gleization-14, stagnogleization-15, salinization or alkalization-16/17, texture-23, pollution-29, slope-33, landslides-38, hydrostatic level-39, floodability-40, total porosity-44, total $CaCO_3$ content-61, soil pH-63, edaphic volume-133, humus reserve-144, surface soil moisture excess -181

Profile 2 - Proxicalcaric Chernozem
Latitude: N: 44°16'02"
Longitude: E: 27°28'07"
Major relief unit: Romanian Plain
Unit: Bărăganului Plain
Parental material: carbonate loessoid deposits
Groundwater: 4-5 m
Current use: Arable
Representative profile: 17

Morphological characterization (Figure 2)
Ap (0-15 cm): medium clay, very dark brown (10 YR 3/2) wet, dark grayish brown (10 YR 4/2) dry, small polyhedral structure, friable, weakly cohesive, weak plastic, weak adhesive, weak compact, loose, rare medium pores, rare thin grass roots, weak effervescence, coprolites, clear passage.
Am (15-45 cm): medium clay, very dark brown (10 YR 3/2) in wet condition, dark brown (10 YR 4/2) in dry condition, fine-grained medium-developed, friable, moderately cohesive, moderately plastic, moderately sticky, weak compact, loose, frequent medium pores, rare thin grassy roots, weak to medium effervescence, gradual transition.
A/C (45-125 cm): dusty clay, very dark grayish brown (10 YR 3/3) when wet, dark grayish brown (10 YR 4/3) when dry, polyhedral structure not developed, friable, moderately cohesive, good plastic, good adhesive, loose, frequent medium pores, rare grassy roots, medium effervescence, gradual transition.
Ck (125-150 cm): dusty clay, dark yellowish brown (10 YR 5/4) in wet state, yellowish brown (10 YR 7/3) in dry state, weakly eroded, weak, weakly plastic, weakly adhesive, moderately compact, poorly cemented, rare small pores, strong mass effervescence.
The pH increases in the depth of the profile from 7.6 to 8.0, the reaction being weakly alkaline. The supply of humus is average with values of 2.6%-1.82% in the first 55 cm (Table 3). Mobile phosphorus at the depth of 0-20 cm has a value of 61 ppm, which means that the soil is well supplied. And mobile potassium having a value of 400 ppm, very well supplied. The texture is medium, medium clay between 0-33 cm and from 33-125 cm it is medium texture, dusty clay.
The humus reserve is 155 t/ha, medium, from where it penalizes all crops by 0.9, lowering the evaluation marks.

Figure 2. Proxicalcaric Chernozems

Following the calculation of evaluation marks, 81 points were obtained for: wheat, barley, sunflower, peas and beans, falling into the 1st quality class and the 2nd favorability class. Maize and soybeans fall into the 2nd quality class and the 3rd class of favorability. The potato crop has 50 evaluation marks, being in the 3rd quality class and the 6th favorability class, as well as the beet crop, which ranks with 57 credit rating points, in the 3rd class of qualities and the 5th of favorability (Table 4).
The depth of the hydrostatic level of the phreatic water is located at medium depth (3-5 m), does not penalize the rating.
The evaluation marks for the eight crops is 72 points and falls into the 2nd quality class and the 3rd favorability class.

Table 3. The maine physical and chemical properties

Horizon	Depth (cm)	Physical properties				Texture class	Chemical properties				
		Coarse sand (2-0.2 mm)	Fine sand (0.2-0.02 mm)	Dust (0.02-0.002 mm)	Colloidal clay (<0.002 mm)		pH (H_2O)	$CaCO_3$ (%)	Humus (%)	P_{AL} (ppm)	K_{AL} (ppm)
Ap	0-20	0.55	37.33	30.92	31.20	LL	7.6	1.8	2.6	61	400
Am	20-33	0.59	38.57	29.80	31.04	LL	7.8	9.4	2.2	-	-
A/C1	33-55	0.66	33.24	36.29	29.81	LP	7.8	8.0	1.82	-	-
A/C2	55-88	0.44	33.60	35.20	30.76	LP	8.0	14.6	-	-	-
Cca	88-125	0.58	35.00	34.80	29.62	LP	8.0	14.8	-	-	-

Table 4. Land Suitability for the main crops

Crop	Tem 3C	Pre 4C	Gl 14	Stg 15	Sal/Alc 16/17	Text 23	Pol 29	Slo 33	Ls 38	HL 39	Flo 40	TP 44	$CaCO_3$ 61	pH 63	EV 133	HR 144	EM 181	EM
Wheat	1	0.9	1	1	1	1	1	1	1	1	1	1	1	1	1	0.9	1	81
Barley	1	0.9	1	1	1	1	1	1	1	1	1	1	1	1	1	0.9	1	81
Maize	1	0.8	1	1	1	1	1	1	1	1	1	1	1	1	1	0.9	1	72
Sunflower	1	0.9	1	1	1	1	1	1	1	1	1	1	1	1	1	0.9	1	81
Potato	0.8	0.7	1	1	1	1	1	1	1	1	1	1	1	1	1	0.9	1	50
Sugar beet	0.9	0.7	1	1	1	1	1	1	1	1	1	1	1	1	1	0.9	1	57
Soybean	0.9	0.9	1	1	1	1	1	1	1	1	1	1	1	1	1	0.9	1	73
Peas/Beans	1	0.9	1	1	1	1	1	1	1	1	1	1	1	1	1	0.9	1	81

Note: average annual temperature-3C, average annual precipitation-4C, gleization-14, stagnogleization-15, salinization or alkalization-16/17, texture-23, pollution-29, slope-33, landslides-38, hydrostatic level-39, floodability-40, total porosity-44, total $CaCO_3$ content-61, soil pH-63, edaphic volume-133, humus reserve-144, surface soil moisture excess -181

Profile 3 - Mollic Gleyic Alluviosol
Latitude: N: 44°11'38"
Longitude: E: 27°25'54"
Major relief unit: Romanian Plain
Unit: Bărăganului Plain
Parental material: carbonate loessoid deposits
Groundwater: 2-3 m
Current use: Arable
Representative profile: 22

Morphological characterization (Figure 3)
Ap (0-17 cm): medium clay, dark brown (10 YR 3/2) when wet, brown (10 YR 4/3) when dry, moderately developed granular structure, moist, slightly cohesive, slightly plastic, slightly adhesive, slightly compact, rare medium-sized macropores, dense thick grass roots, moderate effervescence, clear straight transition.
Am (17-30 cm): medium clay, brown (10 YR 3/2) when wet, pale brown (10 YR 4/3) when dry, small weakly developed angular polyhedral structure, moist, slightly plastic, slightly adhesive, slightly compact, frequent medium-sized macropores, moderate effervescence, gradual transition.
A/CGo (30-46 cm): medium clay, dark yellowish-brown (10 YR 3/4) with olive brown spots (2.5 Y 4/4) when wet, yellowish-brown (10 YR 5/4) with light olive brown spots (2.5 Y 5/4) when dry, moist, slightly compacted, slightly plastic, slightly adhesive, slightly compact, rare medium-sized macropores, strong effervescence, gradual transition.
CGo (46-106 cm): medium clay, yellowish-brown (10 YR 5/6) with frequent olive spots (5 Y 4/3) when wet, yellowish-brown (10 YR 5/8) with frequent olive spots (5 Y 5/3) when dry, weakly developed polyhedral structure, moist, medium plasticity, medium adhesiveness, medium compactness, 10% oxidation spots, strong effervescence throughout, gradual transition.
CGo (106-125 cm): medium clay, yellowish-brown (10 YR 5/8) with frequent olive spots (5 Y 5/6) when wet, brownish-yellow (10 YR 5/8) with frequent pale olive spots (5 Y 6/3) when dry, polyhedral structure, 6-10% oxidation spots, slightly plastic, slightly adhesive, medium compactness, strong effervescence throughout.

Figure 3. Mollic Gleyic Alluviosol

For the Mollic Gleyic Alluviosol with medium clay texture, the annual average temperature is very high - 11.5°C, while the annual average precipitation is low - 475 mm.
The pH values increase with depth, ranging from 7.6 to 8.0, indicating a slightly alkaline reaction. The humus supply is medium, with values between 3.50% and 1.92% in the first 46 cm (Table 5). Available phosphorus, at a depth of 0-17 cm, has a value of 63 ppm, meaning the soil is very well supplied. The potassium supply is good (196 ppm).

The texture is medium clay throughout the soil profile. The groundwater level is shallow (2-3 m), which contributes to a reduction in the evaluation marks. with a coefficient of 0.9 for crops such as wheat, barley, beans, potatoes, soybeans, peas, and other legumes.

The humus reserve is 170 t/ha, which is moderate, leading to a penalty for all crops, with a coefficient of 0.9, lowering the evaluation marks.

Evaluation marks were calculated for the eight crops: for wheat, barley, sunflower, peas and beans, 73 points were obtained, falling into the 2nd quality class and the 3rd favorability class. Maize is in the 2nd quality class with 72 credit rating points and in the 3rd favorability class. Soy falls in the 3rd quality class with 66 credit rating points and in the 4th favorability class. The potato crop is the most penalized, with 45 points falling into the 3rd quality class and the 6th favorability class, as well as the beet crop, which falls with 57 evaluation marks, into the 3rd quality class and the 5th favorability class.

The evaluation marks for the eight crops is 67 points and falls into the 2nd quality class and the 4th favorability class (Table 6).

Table 5. The maine physical and chemical properties

Horizon	Depth (cm)	Physical properties				Texture class	Chemical features				
		Coarse sand (2-0.2 mm)	Fine sand (0.2-0.02 mm)	Dust (0.02-0.002 mm)	Colloidal clay (<0.002 mm)		pH (H_2O)	$CaCO_3$ (%)	Humus (%)	P_{AL} (ppm)	K_{AL} (ppm)
Ap	0-17	0,16	43,08	31,264	25,50	LL	7.6	5.4	3.50	63	196
Am	17-30	0,09	46,58	27,636	25,70	LL	7.6	10.2	2.44	-	-
A/Go	30-46	0,13	53,07	20,706	26,10	LL	7.6	12.4	1.90	-	-
Go	46-106	0,120	57,06	16,12	26,70	LL	7.9	14.0	-	-	-
CGo	106-125	0,821	56,23	16,04	26,90	LL	8.0	20.0	-	-	-

Table 6. Land suitability for the main crops

Crop	Tem 3C	Pre 4C	Gl 14	Stg 15	Sal/Alc 16/17	Text 23	Pol 29	Slo 33	Ls 38	HL 39	Flo 40	TP 44	$CaCO_3$ 61	pH 63	EV 133	HR 144	EM 181	EM
Wheat	1	0.9	1	1	1	1	1	1	1	0.9	1	1	1	1	1	0.9	1	73
Barley	1	0.9	1	1	1	1	1	1	1	0.9	1	1	1	1	1	0.9	1	73
Maize	1	0.8	1	1	1	1	1	1	1	1	1	1	1	1	1	0.9	1	72
Sunflower	1	0.9	1	1	1	1	1	1	1	0.9	1	1	1	1	1	0.9	1	73
Potato	0.8	0.7	1	1	1	1	1	1	1	0.9	1	1	1	1	1	0.9	1	45
Sugar beet	0.9	0.7	1	1	1	1	1	1	1	1	1	1	1	1	1	0.9	1	57
Soybean	0.9	0.9	1	1	1	1	1	1	1	0.9	1	1	1	1	1	0.9	1	66
Peas/Beans	1	0.9	1	1	1	1	1	1	1	0.9	1	1	1	1	1	0.9	1	73

Note: average annual temperature-3C, average annual precipitation-4C, gleization-14, stagnogleization-15, salinization or alkalization-16/17, texture-23, pollution-29, slope-33, landslides-38, hydrostatic level-39, floodability-40, total porosity-44, total $CaCO_3$ content-61, soil pH-63, edaphic volume-133, humus reserve-144, surface soil moisture excess -181

Profile 4 - Calcaric Alluviosol
Latitude: N: 44°08'21"
Longitude: E: 27°27'00"
Major relief unit: Romanian Plain
Unit: Bărăganului Plain
Parental material: carbonate loessoid deposits
Groundwater: 4-5 m
Current use: Arable
Representative profile: 31

Morphological characterization (Figure 4)

Ap (0-19 cm): fine sandy loam, light brown (10 YR 3/2) when moist, brown (10 YR 4/3) when dry, structure destroyed by agricultural work, loose, weakly cohesive, slightly plastic, weakly adhesive, weakly compact, small macropores, moderate effervescence, clear transition.

Ao (19-38 cm): fine sandy loam, brown (10 YR 3/2) when moist, brown (10 YR 5/3) when dry, fresh, with thin grass roots, structureless, slightly plastic, weakly compact, rare small macropores, gradual transition.

A/Go (38-59 cm): fine sandy loam, pale brown (10 YR 4/4) when moist, yellowish brown (10 YR 5/4) when dry, with grayish brown spots (2.5 Y 5/2) when moist, light grayish brown (2.5 Y 6/2) when dry, polyhedral structure, rare thin grass roots, friable, moderately cohesive, slightly plastic, moderately adhesive, rare medium macropores, moderate effervescence, gradual transition.

Go1 (59-75 cm): fine loamy sand, dark yellowish brown (10 YR 4/4) when moist, yellowish brown (10 YR 5/4) when dry, with light gray spots (2.5 Y 6/4) when moist and olive yellow (2.5 Y 6/6) when dry, polyhedral structure, slightly plastic, weakly adhesive, moderately compact, weak effervescence.

Go2 (75-98 cm): sandy loam, dark brown (10 YR 3.5/3) when moist, brown (10 YR 4/3) when dry, medium-large subangular polyhedral structure, fresh, friable, moderately plastic, moderately adhesive, moderately compact, frequent medium macropores, 10% oxidation spots, moderate effervescence, rare thin grass roots.

CGo 1 (98-125 cm): coarse sand, yellowish brown (10 YR 6/5) when moist, yellowish (10 YR 6/6) when dry, slightly plastic, weakly adhesive, weakly compact, rare small macropores, moderate effervescence.

CGo 2 (125-150 cm): medium sandy loam, yellowish brown (10 YR 6/5) when moist, yellowish (10 YR 6/6) when dry, slightly plastic, weakly adhesive, weakly compact, rare small macropores, strong effervescence.

Figure 4. Calcaric Alluviosol

For the Calcaric Alluviosol with sandy loam texture, the average annual temperature is very high, reaching 11.5°C, while the average annual precipitation is low, at 475 mm.

The pH increases with soil depth, ranging from 7.3 to 7.8, indicating a slightly alkaline reaction. The humus supply is moderate, with values between 2.69% and 1.87% in the upper part of the profile. The mobile phosphorus content at a depth of 0-19 cm is 38 ppm, indicating a good phosphorus supply. The potassium supply is very good, reaching 360 ppm (Table 7).

The soil texture is sandy loam throughout the entire profile. The groundwater table is at a moderate depth (3-5 m), which does not affect the evaluation marks.

The humus reserve is 141 t/ha, classified as moderate, introducing penalties for all crops. The penalization coefficient is 0.9, reducing the evaluation marks.

Evaluation marks were calculated for the eight crops: in the case of wheat, barley, sunflower, peas and beans crops, 81 points were obtained, falling into the 1st quality class and the 2nd favorability class. Maize is in the 2nd quality class with 72 credit rating points and in the 3rd favorability class. Soya falls in the 2nd quality class with 73 credit rating points and in the 3rd favorability class. The highest penalties were recorded for the potato crop, with a score of 50 points, falling in the 3rd quality class and in the 4th class of favorability, as well as for the beet crop, which, with 57 credit points, falls into the 3rd class of quality and the 5th class of favorability. The evaluation marks, for the eight crops, is 72 points, the land falling into the 2nd quality class and the 3rd favorability class (Table 8).

Table 7. The maine physical and chemical properties

Horizon	Depth (cm)	Physical properties				Texture class	Chemical features				
		Coarse sand (2-0.2 mm)	Fine sand (0.2-0.02 mm)	Dust (0.02-0.002 mm)	Colloidal clay (<0.002 mm)		pH (H_2O)	$CaCO_3$ (%)	Humus (%)	P_{AL} (ppm)	K_{AL} (ppm)
Ap	0-19	1.81	64.43	15.16	18.60	LP	7.3	-	2.69	38	360
Ao	19-38	1.61	60.59	20.32	17.50	LP	7.4	1.8	2.16	-	-
A/Go	38-59	4.07	60.69	12.64	16.60	LP	7.5	9.4	1.97	-	-
Go1	59-75	1.46	76.62	13.52	10.40	LP	7.7	8.0	-	-	-
Go2	75-98	3.14	52.59	25.07	19.20	LP	7.6	14.6	-	-	-
CGo1	98-125	45.77	46.65	2.98	41.60	LP	7.8	14.8	-	-	-
CGo2	125-150	2.80	60.89	19.11	17.20	LP	7.8	-	-	-	-

Table 8. Land suitability for the main crops

Crop	Tem 3C	Pre 4C	Gl 14	Stg 15	Sal/Alc 16/17	Text 23	Pol 29	Slo 33	Ls 38	HL 39	Flo 40	TP 44	$CaCO_3$ 61	pH 63	EV 133	HR 144	EM 181	EM
Wheat	1	0.9	1	1	1	1	1	1	1	1	1	1	1	1	1	0.9	1	81
Barley	1	0.9	1	1	1	1	1	1	1	1	1	1	1	1	1	0.9	1	81
Maize	1	0.8	1	1	1	1	1	1	1	1	1	1	1	1	1	0.9	1	72
Sunflower	1	0.9	1	1	1	1	1	1	1	1	1	1	1	1	1	0.9	1	81
Potato	0.8	0.7	1	1	1	1	1	1	1	1	1	1	1	1	1	0.9	1	50
Sugar beet	0.9	0.7	1	1	1	1	1	1	1	1	1	1	1	1	1	0.9	1	57
Soybean	0.9	0.9	1	1	1	1	1	1	1	1	1	1	1	1	1	0.9	1	73
Peas/Beans	1	0.9	1	1	1	1	1	1	1	1	1	1	1	1	1	0.9	1	81

Note: average annual temperature-3C, average annual precipitation-4C, gleization-14, stagnogleization-15, salinization or alkalization-16/17, texture-23, pollution-29, slope-33, landslides-38, hydrostatic level-39, floodability-40, total porosity-44, total $CaCO_3$ content-61, soil pH-63, edaphic volume-133, humus reserve-144, surface soil moisture excess -181

Soil Suitability and Recommendations for Agriculture in Roseți Commune

Based on the obtained evaluation marks, the most favorable soils for agriculture are the Proxicalcaric Chernozem and Calcaric Alluviosol. The Cambic Phaeozem and Mollic Gleyic Alluviosol are more vulnerable to the influence of groundwater, which affects their suitability for certain crops. This geomorphological diversity highlights the need to adapt agricultural practices based on the specific characteristics of each soil to ensure a sustainable and efficient use of natural resources (Figure 5).

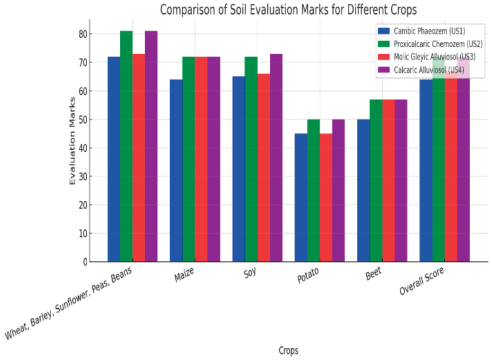

Figure 5. Comparison of soil evaluation marks for different crops

CONCLUSIONS

The studied area represents the terrace area of The Roseți commune is located in southeastern Romania, in Călărași County, benefiting from a lowland plain relief characterized by the presence of floodplain and terrace lands. The floodplain area is situated near the Danube River, containing fertile alluvial soils, which are prone to periodic flooding and are predominantly used for agriculture. The terrace area includes Cambic Phaeozem and Proxicalcaric Chernozem soils, which have moderate to high fertility, making them suitable for cereal crops, viticulture, and fruit growing.

Analyzing the four soil units in this area, significant differences can be observed regarding fertility, texture, and the influence of groundwater on agricultural land quality:

Cambic Phaeozem (US1) - The general evaluation marks is 64 points. This soil has good fertility, with a high humus reserve (251 t/ha). It is favorable for crops such as wheat, barley, and sunflower, but less suitable for potato and sugar beet, which score below 50 points. The groundwater level is over 10 meters, having a minimal impact on the soil.

Proxicalcaric Chernozem (US2) - This soil unit has the highest evaluation marks of 72 points due to its high fertility, providing optimal conditions for plant growth. It is suitable for most crops, with slight penalties for potato and sugar beet. The groundwater level is 3-5 meters, without negatively affecting the bonitation scores.

Mollic Gleyic Alluviosol (US3) - The general evaluation marks is 67 points, and the soil is well supplied with nutrients. However, the shallow groundwater depth (2-3 meters) penalizes evaluation marks for most crops. It is favorable for wheat, barley, and sunflower, but less suitable for potato and sugar beet.

Calcaric Alluviosol (US4) - The general evaluation marks is 72 points, and the soil has high natural fertility. However, the sandy texture negatively affects its water retention capacity, making it suitable for crops with moderate moisture requirements. Potato cultivation records the lowest evaluation marks, and sugar beet also has reduced suitability.

Recommended measures for soil quality improvement and increased crop yields:

- applying sulfur or gypsum amendments to correct alkaline reactions;
- using organic fertilizers to increase the humus reserve, improving soil structure;
- performing soil loosening operations to enhance soil permeability;
- ensuring an adequate drainage system to mitigate the negative impact of shallow groundwater levels.

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DEGRADED LANDS IN THE ABANDONED RICE FIELDS FROM BANAT REGION. CASE STUDY: DEGRADED SOILS IMPROVEMENT OF BEREGSĂU SETTLEMENT - COMTIM

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Abstract

In the Low Plaine from rivers Mureş - Timiş - Bârzava interfluve, during the 18th and 19th centuries has been existed about 14-15 rice - fields, for example at Banloc, Valcani, Sânnicolau Mare, Beregsău, Uivar, Otelec, Denta, Partoş, and other settlements. In the present time only the rice – field of Banloc functions, during of 124th years. The majority of rice - fields have been abandoned because of a wrong management, which has caused the water table to rise and the soils became gleyic - salic and/or sodic. We consider that the majority great areas with Gleysols and Solonetz, and also gleyic - salic - sodic soil types like Chernozems, Vertisols and Fluvisols which cover the Low Plaine, belongs to the abandoned rice - fields. There are present also, some aspects of the geological, geomorphological and the variation of the depth of ground water layer. Analytical data of the soil types dominantly for the rice - fields have been permited to establish the methods for improvement the former areas of rice - fields. The case study relates to the complex improvement measures for a plot of 53 ha which has been radically transformed really in a new soil type Anthrosols, much more productive.

Key words: *rice field, degraded lands, analyse improvement, fertility.*

INTRODUCTION

Beginning with the year 1718th ample hydrotechnics and land improvement works have been effectuated, with a maximum intensity in the years of 20th century, respectively during decades 70th.

The transformation in arable territory from the vast expanse swamps, has been development an advanced agricultural system with a great number of crops.

The process of intensification of land use can be observed by the area with rice fields, starting with the rice - field Banloc, in the year 1786, from the Italian cultivators (Beutura D., 2002).

The rice is a pretentious plant, with certain environmental conditions, especially for clime and soil. The minimum germination temperature is 11-12ºC, optimum for growth 28-30ºC, with a transpiration coefficient of 750-1000, rise can growth only under a layer of 10-15 cm water, good oxygenated (Hagan R., 1968). Rise growing in Banat Plain have been realized in a surface irrigation system (Rogobete, 2006; Sandu, 1986).

Because soil and water contain soluble salts, such an irrigation system needs a permanently control in order to prevent degradation processes, like gleization - salinization - sodication (Oanea, 1977; Rogobete, 1993).

Must be also mentioned the scientific research of Oprea C. (1956; 1962; 1971) and Sandu Gh. (1984).

In the absence of a good management all the rice - fields (with one exception – Banloc) were abandoned because the level of rice production became non profitable. During of about 50 years of scientific research we have identified 11 abandoned rice-fields where the soils are strong degraded.

In the irrigation plot time of 125 years of Banloc a lot of properties are modified: the texture species become clayey, the bulk density is high even in the topsoil (1.33/1.68 $g.cm^{-3}$), infiltration rate is diminished from 38 $mm.h^{-1}$ to 5 $mm.h^{-1}$ at 50 cm depth, the level of calcium carbonate lowers from 40 cm to 150 cm depth.

MATERIALS AND METHODS

This scientific paper is the result of a prolonged researches in Banat territory, in the course of about 50th years. The pedological researches

have been made personal or most frequently within the Pedological and Agrochemical Studies Office Timișoara (OSPA) and in the Soil Science Laboratory of Politehnica University Timișoara. It has been identified in the Low Plaine between the rivers Mures – Timiș – Bârzava, 11 territories of abandoned rice – fields, but only one of them is in function, respectively Banloc.
In the case of the plot (53 ha) which has been reclaimed in the year 1988, we have recently analyzed some soil samples from a soil profile, determined as Anthrosols.

RESULTS AND DISCUSIONS

Humans began to affect the land in many of the ancient countries and the process knows a gradual intensification parallel with the population increasing. Our research has been focused of the Low Plaine situated between the rivers Mureş - Aranca - Timiș - Bega - Bârzava, in the western part of Romania.
The Low Plaine is covered with the Quaternary deposits which are formed by clay, loess, sands or gravels. Another characteristic is the high level of the groundwater and the dominance of values of 1.0-2.0 m isophreatic. The qualitative analyse of the water present in the water bearing horizons indicate a variable total soluble salts content of 0.9 g/l in sandy zones, 1.0-1.5 m in loamy zones and 3.0-5.0 g/l in clayey zones. The oscillations of the groundwater level in these zones are 2.0-2.5 m. Because the feeding aquifer is in a volcanic tuff or andesite zone, the content of groundwater in cations of Ca^{2+}, Mg^{2+} and Na^{+} is great.
In the condition, predominantly, in the Low Plaine of an exudative moisture regime of 4-5 months, the saline and sodic soils occupies a great area.
Because the phreatic water - bearing stratum is near by soil surface, the hydrostatic level is free and the feeding zone coincides with the extension of the stratum. The presence at surface of a rich parent material with soluble salts, like $CaHCO_3^-$, $MgHCO_3^-$ and Na_2HCO_3 is the main reason for the existence of the insular soil area with sodication phenomena.

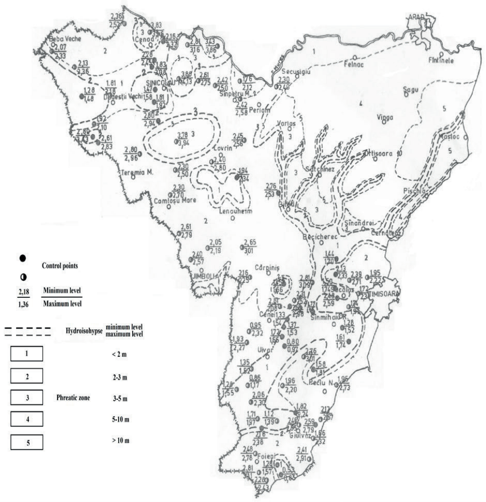

Figure 1. Hydrogeologic map

The recession of Pannonian Lake left behind an immense boggy area (Griselini, 1779) which is maintained pending to the 18th century on an area of about 877.000 ha (Historical Banat).
The land improvement arrangement initiated in the years 1716 have been radically modificated the aspect of Low Plaine (Rogobete, 1985). In fact, the land improvement works has been continued until the years 1970-2000 included in the seventh land improvement systems (Figure 2).

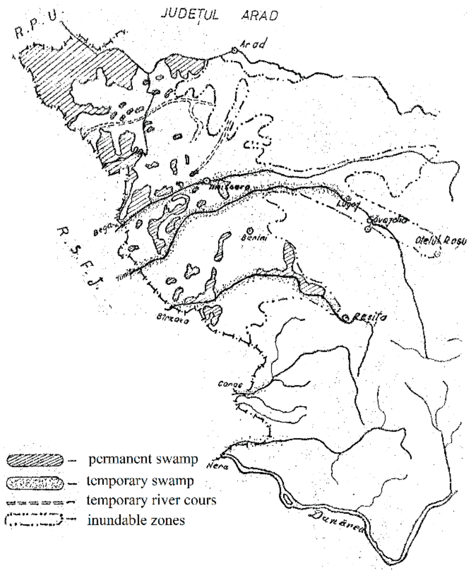

Figure 2. Map of Banat region, 17th century (after Grisselini)

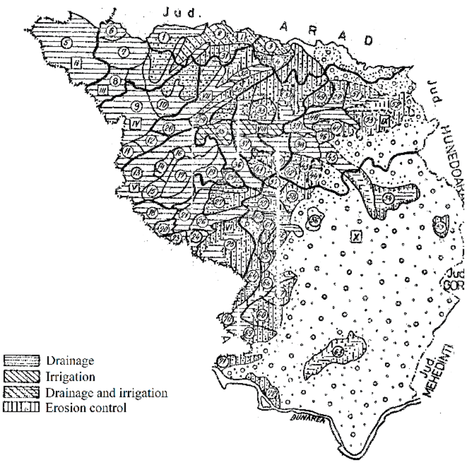

Figure 3. Land improvement map

Since the climatic conditions are favorable for rice cultivation in Banat region have been appeared a lot of rice fields arrangements.

Because of a bad management they have abandoned after a few years, with one exception Banloc (Figures 4, 5)

It will be presented the main rice fields from Banat:

1. Valcani

Hydrographical basin Aranca, cadastral territory 6198 ha

Table 1. Valcani Soil types, ha

AS	CZ	EC	VS	GS	SN	AT	Assoc
1044	912	87	1975	68	17	6	1661

Phreatic water excess - strong 4120 ha

Salinization - sodication - strong 719 ha

Soil profile from abandoned rice fields

Table 2. Vertosol salsodic (SRTS) - Pellic salic - sodic Vertisols (WRB), Analytical data Valcani

Depth, cm	11-27	27-58	58-78	78-108
Clay, %	66.5	63.4	62.5	42.0
Silt, %	14.3	17.4	16.8	23.8
BD, g/cm^3	1.51	1.49	1.49	1.40
pH_{H2O}	6.95	6.60	7.10	8.05
Humus, %	3.94	2.76		
CECs, me	45.43	49.28	48.70	40.0
Soluble salts, %			0.254	0.240
ESP			8.3	8.5

2. Sânpetru Mare

Feeding the two rice fields from river Aranca Cadastral territory 10.159 ha. There are two rice fields.

Table 3. Sânpetru Mare Soil types, ha

AS	CZ	VS	GS	SN	Association
1155	3678	2099	46	338	1860

Phreatic water, strong excess 339 ha

Salinization - sodication, strong excess 966 ha.

Table 4. Soloneț vertic - salic (SRTS) -Vertic -salic - Solonetz (WRB), Analytical data Sânpetru Mare

Depth, cm	3-16	16-30	30-50	75-95
Clay, %	36.7	41.1	40.3	38.0
Silt, %	27.2	27.2	26.4	30.5
BD, g/cm^3	1.43	1.46	1.42	1.44
pH_{H2O}	9.14	9.35	9.71	10.09
Humus, %	3.04	2.82	2.10	
CECs, me	27.85	36.58		
ESP	62.04	63.09		

Table 5. Vertosol gleyic - salic (SRTS) -Pellic - gleyic-salic (WRB), Analytical data Sânpetru Mare

Depth, cm	0-25	25-57	57-70	70-85
Clay, %	37.8	45.9	47.6	49.3
Silt, %	31.8	28.1	27.6	25.9
pH_{H2O}	6.50	7.57	8.33	8.94
Humus	3.53	3.42	2.53	
CECs, me	26.39	43.51	44.20	33.94
ESP			4.29	9.10
Soluble salt			86.9	147.0

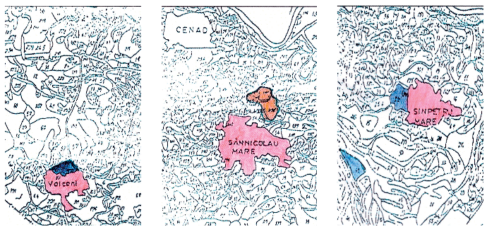

Figure 4. Rice fields Aranca hydrographical basin

3. Sânnicolau Mare

Hydrographical basin Mures-Aranca-Galatca Territory 13903 ha.

Table 6. Sânnicolau Mare Soil types, ha

AS	CZ	EC	VS	GS	SN	Association
1015	5329	2030	1016	634	127	2538

Strong phreatic excess 2470 ha

Salinization - sodication, strong 738 ha.

Table 7. Cernoziom gleic-sodic (SRTS) Gleyic-hyposodic Chernozems (WRB), Analytical data Sânnicolau Mare

Depth, cm	12-24	24-41	41-59	90-137	137-180
Clay, %	25.5	28.0	24.0	27.6	12.8
Silt, %	17.3	15.4	14.8	34.3	5.3
BD, g/cm^3	1.61	1.54	1.50		
pH_{H2O}	6.95	7.20	7.25	8.55	8.55
Humus, %	2.53	2.42			
CECs, me	15.87	17.23			
ESP				8.2	8.2

4. Otelec

Cadastral territory 8315 ha, the rice field was located on the left side of the Bega canal.

Table 8. Otelec Soil types, ha

AS	CZ	FZ	EC	VS	PE	GS	SN	Assoc
396	2432	2432	1038	1792	603	299	115	407

Strong phreatic water excess 1294 ha.
Salinization - sodication, strong 115 ha.

Table 9. Pelosol gleic salsodic (SRTS)-Gleyic-hyposalic-hyposodic Vertisols (WRB), Analytical data Otelec

Depth, cm	30-50	50-80	80-105	105-138
Clay, %	43.5	40.6	34.8	31.1
Silt, %	24.0	28.0	32.0	30.2
pH_{H2O}	8.30	8.30	8.90	8.80
Humus, %	3.28			
CECs, me	33.2	31.6	20.6	16.0
ESP	5.50	6.72	11.81	13.0
Soluble salts	56	56	116	130

5. Uivar

With the village Pustinis and Răuti, three rice-fields. The cadastral territory 11217 ha is traversed of the Bega channel with its affluents Beregsau and Timișat.

Table 10. Soil types, ha

AS	CZ	FZ	EC	VS	PE	GS	SN	Assoc
516	3162	864	1350	2147	785	389	131	895

Strong phreatic water excess 1474 ha.
Salinization - sodication, strong 135 ha.

Table 11. Cernoziom gleic-salinic (SRTS) Gleyic-hyposodic Chernozems (WRB), Analytical data Uivar

Depth, cm	20-35	35-50	50-65	65-100	100-130
Clay, %	31.4	32.2	32.3	32.1	29.0
Silt, %	23.9	24.8	22.8	19.8	20.9
BD, g/cm^3	1.51	1.47	1.62	1.60	
pH_{H2O}	8.33	8.45	8.49	8.53	8.68
Humus, %	2.73	1.79	0.94	0.58	
CECs, me	38.22	53.7	29.75	23.16	15.28
ESP, %		2.14	0.71	1.64	5.56
Soluble salts		140.5	186.1	186.1	180.1

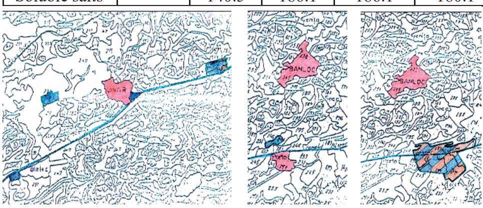

Figure 5. Rice fields Timiș-Bega-Bârzava hydrographical basin

6. Livezile

The cadastral territory is in Bârzava hydrographical basin, with an area of 5599 ha and traversed by the river Lanca and Birda, which are regularized and damming in.

Table 12. Soil types, ha

AS	CZ	FZ	EC	VS	PE	GS	SN
27	1133	821	2070	334	154	175	517

Strong phreatic water excess 1780 ha
Strong salinization - sodication 536 ha.

Table 13. Eutricambosol aluvic-gleic (SRTS)-Fluvi-gleyic eutric Cambisols (WRB), Analytical data Livezile

Depth, cm	15-38	38-55	55-85	85-125	125-180
Clay, %	39.8	37.7	38.4	39.4	42.6
Silt, %	31.5	30.7	30.9	32.6	24.4
pH_{H2O}	5.60	6.20	6.40	6.55	6.75
Humus, %	2.88	1.92			
CECs, me	31.00	30.24	30.46	32.58	32.39

7. Banloc

With the villages Partos, Ofsenita and Soca. The cadastral territory 11781 ha, placed in the Low Plaine Timiș-Bârzava, has the lowest altitude (75-85 m) from the Banat Plaine. The presence of ground water near the soil surface, with slow runoff determined the building three drainage systems in the zone, and two rice fields and a piscicultural arrangement with water from river Bârzava. The rice field from village Partos has been abandoned but the rice field from Banloc founded in the year 1786 is still in function.

Table 14. Banloc Soil types, ha

AS	CZ	EC	VS	PE	GS	SN	Assoc
437	720	2979	2850	358	274	28	2606

Strong phreatic water excess 2740 ha.
Strong salinization - sodication 65 ha.

Table 15. Eutricambosol gleic-sodic (SRTS)-Gleyic-sodic-eutric Cambisols (WRB), Analytical data Banloc

Depth, cm	0-28	28-44	59-78	78-93	93-112	112-125
Clay,<2μ	36.5	41.2	41.4	27.6	29.5	45.0
Silt, %	27.3	24.4	31.1	31.8	23.6	31.9
BD	1.50	1.56	1.54			
pH_{H2O}	6.80	6.70	7.10	8.10	8.10	8.25
Humus	2.73	2.42	1.63	1.43	0.86	0.53
CECs, me	31.24	30.19	31.75	29.13	29.8	38.40
ESP				22.6	18.1	28.3

8. Partos

Table 16. Pelosol gleic-sodic (SRTS) - Gleyic-sodic Vertisols (WRB), Analytical data Partos

Depth, cm	13-30	30-49	64-90	90-110
Clay, %	46.0	49.5	50.3	45.9
Silt, %	22.9	24.2	25.7	31.5
pH_{H2O}	6.95	7.25	7.90	8.30
Humus, %	3.16	3.06	2.66	
CECs, me	33.40	34.66	36.42	35.15
ESP		13.3	15.4	29.3

At these analyzed rice fields, can be added the rice fields from Timișoara, Beregsău, Gătaia and Denta which are also abandoned because due to the degradation processes become not profitable.

All the soil profiles, which have been used as rice fields and were abandoned, reveal strong degraded phenomena, especially phreatic water excess and gleization and also strong sodication processes.

All these phenomena are the result of two causes:

- a first cause is that the rice -fields are located on the soil with a low fertility because of a clayey texture or of a great ESP (15-30), for example on Solonetz;
- the second cause is a bad management in the time of rice field exploitation, which start up the degradation phenomena.

We consider that the bad management of the rice fields has a great contribution of the large spread in the Low Plain from Banat region of the soil type as Solonetz or sodic soils. For example, all the soil types like Chernozems, Cambisols, Fluvisols, Vertisols, are sodic. The degradation like sodication, take place not only in the rice field perimeter, but also in the limitrophe zones, and for that is imperative necessary as the drainage system to be in function, in order to control and maintain the level ground water at an optimum depth.

This supposes to realize maintenance works for the sustainable drainage systems.

Case study: Degraded soils improvement at the pig farm Beregsau - COMTIM.

Abandoned rice-field

The village Beregsau Mare and Mic is part of a great village Sacalaz, which has a territory of 11949 ha with 9432 ha arable.

Situated in the Low Plaine Timiș, 83-90m altitude, the territory is traversed by two rivers - Beregsau and Bega Veche. The level of ground water is oscillating between 0.5-3.0 m depth and with a medium mineralization-$Ca(HCO_3)_2$, $NaHCO_3$, $Mg(HCO_3)_2$.

In the 18th century, during the years 1765-1789, the village was colonized with german peoples - swabi. After the ample hydrotechnical and land improvement in the territory Sacalaz has been realized a piscicultural arrangement and a rice field. Because of wrong management, the rice field has been abandoned and the location remains with degraded soils which have salt and sodium in a high concentration.

Table 17. Soil cover of the arable territory (9438 ha)

Soil type	AS	CZ	FZ	EC	VS	PE	GS	SN
Area, ha	985	3719	617	2951	478	1320	249	302

All of the soil types are affected by degradation phenomena:

-water excess: at surface: moderate 254 ha, strong 930 ha;

-ground water: moderate 2223 ha, strong 1247 ha;

-salinization and sodication: moderate 2679 ha, strong 837 ha;

-compaction: 2073 ha;

The soil association, originated from the abandoned rice field which cover the plot of 53 ha was compound of the next soil types, in the year 1988:

1. Solonet gleic (SRTS) or Gleyic Solonetz (WRB) 45%.
2. Vertosol salinic (SRTS) or Pellic Hyposalic Vertisols (WRB) 30%.
3. Cernoziom salinic-sodic (SRTS) or Hyposalic - Hyposodic Chernozems (WRB) 25%.

Table 18. Solonet gleic (SRTS) – Gleyic Solonetz (WRB) Analytical data Beregsau 1988

Depth, cm	10-33	33-53	53-70	90-115
Clay, <2μ,%	46.2	50.70	51.8	36.9
Silt, %	21.6	16.1	22.0	25.8
Humus	2.88	1.88	1.66	1.87
pH_{H2O}	8.60	9.40	9.95	10.00
CECs, me	43.16	35.01	33.20	19.84
ESP	43.09	69.69	71.38	40.83

Table 19. Vertosol salinic (SRTS)-Pellic-hyposalic Vertisos (WRB) Analytical data Beregsau 1988

Depth, cm	0-25	41-61	61-76	76-105
Clay, <2μ,%	58.3	55.9	55.2	53.1
Silt, %	19.5	21.4	20.6	17.1
Humus, %	6.48	1.50	1.00	-
pH_{H2O}	7.40	8.30	8.40	8.41
CECs, me	48.50	44.00	42.00	40.00
ESP	3.98	4.68	4.14	
Cl^-, me		0.115	0.125	0.134
SO_4^{2-}, me		0.417	0.452	1.000
CO_3H^-, me		0.433	0.459	0.443

Table 20. Cernoziom salinic-sodic (SRTS)-Hyposalic-sodic Chernozems (WRB) Analytical data Beregsau 1988

Depth, cm	0-20	20-34	34-61	61-72	72-120
Clay, <2µ,%	30.1	34.8	41.3	35.0	20.2
Silt, %	19.8	22.5	17.8	18.0	16.2
Humus, %	3.35	3.28	2.93	1.05	
pH_{H2O}	6.67	8.30	9.19	9.40	8.89
CECs, me	31.20	30.45	32.20	28.32	21.76
ESP	6.80	10.08	13.29	8.85	8.50
Soluble salts	99.4	102.3	180.2	247.4	-

In August, 1988 year we sign a Protocol for research in order to improve the plot of 53 ha originated from the rice field. The improvement project relies on a detailed soil survey made of the author. The soil samples have been analyzed in the Soil Science Laboratory of Politehnica University Timişoara. The plot was included in the Drainage system Timişoara (Figure 6).

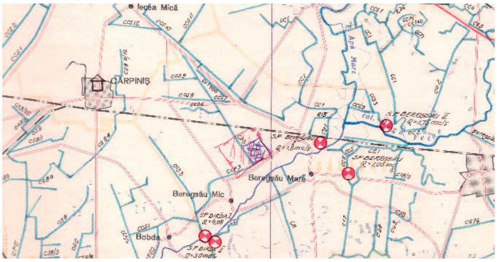

Figure 6. The improved plot of Beregsău

Soil improvement measures proposed and realized in about 6 months were:

1. leveling work for each plot with the slope orientated towards CA_3 or CCP_3 (supply or collector canals) corresponding the project;
2. tubular drainage at a medium depth of 1.00m with ballast filtering layer, which must be maintained at the surface of terrain after compaction; the distance between drains will be 10 m; the slope of the drains must be at least $1\ ^0/_{00}$; the rifled tube must be controlled, before laying the drain, the quality of the tubular drain.
3. it is necessary to retooling the existing collectors channels for a depth of 1.5 m;
4. mole drainage at 0.6 m depth, perpendicular on the tubular drainage chain, at 1.40m distance between the chain of mole drainages;
5. phosphogips amendment with 10 t/ha, incorporated by discing;
6. fertilization by natural organic manure obtained from the piggery, 80 t/ha incorporated by discing.
7. deep loosening at 0.5 m depth, with a simultaneous introduction of sand on the traces of the claws of the scarifier;
8. supply of earth material, fertile, in a layer of 20-30 cm depth, which must be maintained distinct towards the initial soil type, without mix and farming with a Cyzell or moldboardless plowing and seeded with perennial grass which has superficial roots.
9. leaching soluble salts by an over irrigation applied during autumn – beginning winter; the norm of leaching will be calculated after chemical analyses of the content of soluble salts from the soil and irrigation water and also of drained water.

The improvement measures have made by a specialized company IEELIF. Timişoara in the course of the year 1988.

In the following years, the reclaimed plot of 53 ha area became a new soil type and the level of production has known a significant rise.

Table 21. Antrosol aric-sodic (SRTS)-Aric-sodic Anthrosols (WRB) Analytical data Beregsau 2025

Depth, cm	20-30	40-60	60-80	80-100
Clay, <2µ,%	43.6	47.6	38.9	30.4
Silt, %	29.7	26.3	31.3	44.2
Humus, %	3.80	2.92	2.27	1.08
CECs, me	40.02	43.10	35.30	21.2
pH_{H2O}	8.18	8.27	8.46	8.52
ESP	5.73	11.65	36.20	22.54

Since the drainage system has been maintained only 10-15 years after the year 1989, the effect of improvement measures has been gradually mitigated. To make a parallel between the values of ESP from the Solonetz of the year 1988 with the ESP from the Anthrosols, respectively ESP=43.09-69.69 (1988) and 5.73-11.65 (2025), point out the radically reclamation the soil chemical conditions for plants cultivation.

CONCLUSIONS

Soil forming factors in the Banat Low Plaine are very favorable for the appearance of gleyzation, salinization and sodication processes.

The wide hydrotechnical works and land improvement measures have regularized the course of rivers Aranca, Timiș, Bega, Bârzava, and drying up the swamps. The agricultural land has strong increased to about 700000 ha in the region Banat and also the level of ground water has lowered in the Low Plaine at 3 – 5 m depth. The absence of the maintenance works in the drainage systems decreased the favorable effect regarding the soils fertility and the degraded phenomena turn up again.

The majority of soil types present a low salinization but the alkalization process is strong and the salsodic subtypes are dominantly. For example, there are Chernozems, Vertisols, Cambisols salsodic on about 30% from the total area.

The area in the Banat region with typically soil types with excessively clayey, gleysol and Solonetz is:

- Vertisols - 94883 ha (8.02%)
- Gleysols - 40764 ha (3.45%)
- Solonetz - 2106 ha (1.78%)

We consider that on this large area with gleyc and sodic soils has contributed, for certain, the wrong management practiced in the rice field.

Due to the negative soil profiles characteristics, were necessary a lot of kind meliorative measures, respectively hydro-pedo-agro-meliorative.

Between the eleven rice fields in which are dominantly soils as Vertisols, Gleysols, Solonetz and gleyic sodic, and salinic soils like Chernozems, Eutri-Cambisoils and Fluvisols, we have selected the abandoned rice field from COMTIM – Beregsău, because of the demand on research contract.

The improvement measures have been realized in a half year, but the cost was very high.

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THE TECHNOLOGIC PROCEDURES OF UTILIZING STRAW SURPLUSES AS FERTILIZER ON THE CHERNOZEMIC SOILS

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Abstract

Tillage and removal of agricultural production from fields leave the soil increasingly poor in organic matter. Currently, the reimbursement of organic fertilizers to the soil, with rising fuel prices, has become very expensive, exceeding €30/t within a three-km radius. In this context, straw surpluses from cereal crops are welcome to meet the soil's organic matter needs. They do not require loading, transportation and distribution costs, increasing the profitability of application. Experience has shown that on the chernozemic soils, straw incorporated during cereal harvesting does not affect the nitrogen supply of plants. This process makes it possible to fertilize with straw without supplementing with nitrogen, especially where nitrogen fertilizers are not available. It was shown that among the organic fertilizers, straw combined with nitrogen fertilizers has the highest humus restoration potential. The research show, that humus content increase between 150 and 270 kg/t of straw, humifying 14-30% of organic matter from straw.

Key words*: chernozemic soils, nitrogen fertilizers, organic matter, straw surpluses.*

INTRODUCTION

Agriculture in the Republic of Moldova is in a deep crisis regarding the replenishment and maintenance sources of the agricultural soil's fertility. The prices of industrial fertilizers have increased considerably, and the use of traditional organic fertilizers is limited by an increase in prices for services for their preparation and application, as well as a significant regression of livestock. The lack of financial resources for seeds, the procurement of fertilizers and chemical preparations for crop care indicate a major problem for farmers.

On the other hand, according to the agronomic law of Yu. Liebig (1842), in order to maintain the fertility of the soil, it is necessary to return to it that part of the lost nutrients that man took from it in the form of a harvest. The reserves of nutrients in the soil are limited, therefore we cannot permanently alienate from it. History has shown us that the prosperity and death of entire peoples and civilizations are linked to the way they treated the land (soil) on which they lived (Montgomery, 2013).

In this situation, straw surpluses remain a welcome opportunity to compensate the organic fertilizers deficit. Straw is formed on the lands where it must be applied, is evenly distributed on the soil surface, does not require loading and transportation costs. The purpose of this research was to investigate the straw - nitrogen relationship in several straw fertilization processes on the chernozemic soil.

MATERIALS AND METHODS

Planning and organization of the experiment

Since the straw from the spikes has an extended C:N ratio, it can cause microbiological immobilization of mineral nitrogen in the soil and suppress the provision of nitrogen to plants. In the specialized literature, it is recommended that the straw applied as fertilizer must be supplemented with nitrogen (Lixandru et al., 1990; Turcan et al., 1993; Rusu et al., 2005; Nicholson et al., 2007; Mineev et al., 2017; Tiantian et al., 2021; Bai-Jian Lin et al., 2024).

So, the chernozem soils have a high nitrification capacity, which can almost entirely meet the nitrogen needs of medium-sized crops. Long-term research has demonstrated that chernozemic soils without fertilization have the capacity to form up to 100 kg/ha of mineral nitrogen in a season in the 100 cm soil profile (Lungu et al., 2017; Andriesh, 2018).

In this context, we suspected that on chernozemic soils the nitrogen-fixing influence of straw is less pronounced than on poor soils with low nitrification capacity, if straw is applied early. To verify this hypothesis, a field experiment was set up to expand and streamline straw fertilization.

The experiment was founded in the summer of 2009 in a rotation with field crops at the Experimental Station of Erosion and Pedology of National Institute for Applied Research in Agriculture and Veterinary Medicine, located in the Ursoaia com., Cahul district, Republic of Moldova. The soil of the experimental field was identified as a slightly eroded ordinary loamy-clayey chernozem. The experiment scheme included ten variants (Table 1).

Table 1. Experience with the quantities of the main fertilizing elements fully incorporated with fertilizers in 2009, 2013 and 2017, kg/ha

Variant	C	N	P_2O_5	K_2O
1. Control, without fertilizers	0	0	0	0
2. Straw, 4 t/ha	4804	83	10	114
3. Straw, 4 t/ha + $N_{20}P_{20}$	4804	143	70	114
4. Straw, 8 t/ha + $N_{20}P_{20}$	9608	226	80	228
5. Straw, 8 t/ha	9608	166	20	228
6. $N_{20}P_{20}$	0	60	60	0
7. $N_{170}P_{180}$	0	510	540	0
8. Straw, 4 t/ha + $N_{140}P_{175}$	4804	509	539	114
9. Straw, 4 t/ha + Sheep manure, 16 t/ha	10463	509	430	895
10. Sheep manure, 20 t/ha	6820	514	479	931

The experience was based on four repetitions with a plot area of 120 m² (6 x 20 m). Straw and manure were applied three times by overlapping the materials, once every four years, in July 2009, 2013 and 2017. With the same periodicity of four years, chemical fertilizers in the form of amorphous and ammonium nitrate were also added.

In variants 7, 8, 9 and 10, equivalent amounts of nitrogen and phosphorus were added to the fertilizers with the intention of establishing more clearly the comparative role of straw organic matter and sheep manure in crop nutrition and yield.

In this context, both straw alone, without nitrogen supplementation, and straw supplemented with reduced doses of nitrogen were tested. The combination of straw with sheep manure, as well as with high doses of chemical fertilizers with nitrogen and phosphorus, was also tested. In order to more clearly elucidate the influence of straw as a fertilizer and amendment, they were applied in two doses: 4 t/ha and 8 t/ha.

When harvesting the spikelets, the straw and fertilizers weighed according to the planned doses were distributed uniformly by hand on the plots. After each fertilizer distribution, the experimental field was harrowed three times with a heavy disc harrow.

Weather conditions. As is known, the climate of the Republic of Moldova is moderately continental. Winter is short, mild and with little snow, and summer - hot, long-lasting and with insufficient amounts of precipitation. In the southern part of the country, these characteristics are more pronounced. Here the climate is considered the most arid and warm in the republic, temperatures being 2-3°C higher than in other regions (Sivun, 1982). In addition to the positive aspects of the climate, the abundance of sunlight and heat, there are also negative aspects, namely dry periods and large temperature variations.

In the southern part of the republic, temperatures above +10°C begin in the second decade of April and extend until the second decade of October. Their sum varies between 3200°C and 3400˚C. Under the influence of these temperatures, the region differs from the rest of the territory by a pronounced lack of moisture in the soil, frequent and extensive droughts.

The average annual amount of precipitation at the Cahul weather station is 451 mm, and about 65% of this amount falls during the warm period in the form of short-term showers. During this period of the year, an average of 291 mm of precipitation is deposited and about 3300˚C of active temperatures accumulate. An average of 795 mm/year can be consumed through evaporation in the area (Melniciuc, 2021).

Therefore, the water evaporation capacity is about twice as high as the volume of atmospheric deposition. The humidity coefficient is 0.57 (451/795). The optimal water demand for crops sown in spring in the Cahul station area is about 360 mm, and the amount of water evaporated from the soil from sowing to harvesting for this group of crops is on average 220 mm.

Therefore, the coverage of water needs for plants from precipitation is about 61%.
During the experiment, the agricultural years 2010 and 2013 stood out for their higher than the annual norm rainfall, when about 20% more precipitation than the annual norm (Table 2).

The lowest amounts of precipitation were noted in 2011, 2015, 2020 and 2021. Thus, the lowest harvest in the experiment was recorded in 2015 in the control variant - 1800 kg/ha cereal units. During the corn vegetation period, only 184 mm of precipitation was deposited, or 64 percent of the statistical norm.

Table 2. Crop succession and yield in the Control variant

Harvest year	Cultivated plant	Rainfall, mm		Harvest, kg/ha	
		per agricultural year	for 04 - 09 months	physical mass	cereal units (c.u.)
2010	Corn grains	535	428	4840	3870
2011	Spring barley	322	261	2240	2240
2012	Sunflower	388	268	1700	2500
2013	Winter barley	649	526	4930	4930
2014	Corn grains	441	361	5280	4220
2015	Corn grains	293	184	2250	1800
2016	Pea grains	419	304	2120	2540
2017	Winter barley	426	332	5520	5520
2018	Corn grains	305	170	5640	4510
2019	Sunflower	401	353	2050	3010
2020	Winter wheat	291	243	5080	5080
2021	Corn grains	393	315	7950	6360
Statistical average		*451*	*287*	-	*4658*

According to the data obtained, the highest yield, 6360 kg/ha of corn grains, was formed in 2021. This is explained by the fact that, although the total amount of precipitation in that year was 58 mm lower than the statistical norm, 80% of it was deposited during the vegetation period. This case clearly shows that providing plants with water depends not only on the total amount of precipitation, but also on the quality of its distribution during the vegetation period.

RESULTS AND DISCUSSIONS

Taking into account the fact that wheat straw has a C:N = 80:1 ratio, four times more extensive than the optimal one for the biochemical processes of providing plants with nitrogen, we set out to experiment with various models of combining straw with nitrogen. Nitrogen is contained in the soil in small quantities, which is why frequent fertilization of the soil and plants with this element is practiced. Monitoring the nutrition process is identified more correctly and precisely through chemical analysis of plants.

Description of the straw influence in the first year of action. Table 3 shows the results regarding the amount of nitrogen absorbed by corn from soil and fertilizers in 2010, a year with a good corn harvest. In the *Control* variant, the grain harvest was 4840 kg/ha. We owe this fact to favourable moisture conditions. In the agricultural year 2009-2010, 535 mm of precipitation was deposited and 80% of them watered the corn during the vegetation.
On this favourable moisture substrate, the tested fertilization procedures also manifested themselves appropriately. As expected, the highest yield in the experiment was formed in variant 10 (Sheep manure, 20 t/ha). Here, grain production increased, compared to the *Control* variant, by 39% (6740 x 100/4840). In clarifying the fertilizing influence of sheep manure, the fact that it has a higher concentration of nitrogen and other deficient elements was taken into account. In addition, before incorporation, it goes through several fermentation stages, through which the nutrients become accessible to plants. Straw, however, has a lower content of nutrients and in the experiment was applied in a fresh, unfermented state.

Table 3. Yield and nitrogen consumption from soil and fertilizers by corn crop in 2010

Variant	Grain			Secondary aerial production			Total N consumption kg/ha (***7)
	Yield, kg/ha (1)	N, % (2)	N assimilated kg/ha (*3)	Production kg/ha (4)	N % (5)	N assimilated, kg/ha (**6)	
1. Control, without fertilizers	4840	1.22	59	5664	0.86	49	108
2. Straw, 4 t/ha	4700	1.26	59	7293	0.85	62	121
3. Straw, 4 t/ha + $N_{20}P_{20}$	5240	1.40	73	7550	0.91	69	142
4. Straw, 8 t/ha + $N_{20}P_{20}$	5860	1.68	98	8036	0.97	78	176
5. Straw, 8 t/ha	5270	1.30	69	7728	0.91	70	139
6. $N_{20}P_{20}$	5100	1.41	72	6100	0.86	52	124
7. $N_{170}P_{180}$	5300	1.46	77	8183	0.91	74	152
8. Straw, 4 t/ha + $N_{140}P_{175}$	5540	1.51	84	9216	0.91	84	168
9. Straw, 4 t /ha + Sheep manure, 16 t/ha	6150	1.70	105	9600	0.93	89	194
10. Sheep manure, 20 t/ha	6740	1.59	107	11373	0.94	107	214
DL_{05}	*244*	*0.04*	*2.7*	*421*	*0.03*	*3.1*	*5.8*
Sx, %	*4,46*	*2.23*	*3.35*	*5,21*	*3.14*	*4.18*	*3.77*

Note: *3 = (1) x (2)/100; **6 = (4) x (5)/100; ***7 = (3)+ (6).

However, the straw incorporated separately demonstrated a fertilizing influence, even from the first year of action. In the variant 5 (Straw, 8 t/ha) the yield increased significantly compared to the *Control* by 430 kg/ha/year c.u. (cereal units) (5270-4840), or by 9%. In the variant (Straw, 4 t/ha) the yield was 3% lower than in the *Control* (4700 x 100/4840).

From a statistical point of view, this difference between the variants is considered irrelevant, that is, at the level of the reference variant. In other words, we can see that harvest mass was not negative, but at the level of the *Control*.

These results are somewhat contradictory to the data in the literature, mentioned above, where it is pointed out that straw applied separately decreases yields and must necessarily be supplemented with nitrogen-containing fertilizers. In the experimental case, when straw was applied in the dose of 4 t/ha, the yield was at the level of the *Control* variant, and when the dose of straw was doubled, a significant increase in yield was formed.

So, among the factors that conditioned the harvest formation in the variants treated only with straw, the accessible nitrogen was not the minimal factor that limited the yield formation. Indirectly, it is assumed that the soil compaction was the condition that limited the harvest level in this variant. Since, when incorporating 8 t/ha of straw, the yield increased suggestively compared to the variant treated with 4 t/ha of straw and to the *Control*.

The incorporated straw influences, first of all, the physical properties of the soil. With their very low density, of about 50 kg/m^3, they maintain the soil in a loose state. The quantity of 8 tons of freshly harvested straw occupies a volume of about 160 m^3 (8000/50). And when mixed with the ploughed soil layer, with an apparent density of about 1.20 t/m^3, it loosens its state up to 1.11 t/m^3 (2000 m^3 x 1.20 t/m^3 / 2000 m^3 + 160 m^3).

For loamy-clayey soils, which dominate in the Republic of Moldova, the calculated parameter means a very low apparent density, very high porosity and moderately loose soil (Canarache, 2000). Reducing the apparent density of the soil means increasing the permeability and water retention capacity. The value of 1.11 t/m^3 also highlights a decrease in resistance to root penetration, as well as to soil tillage mechanisms.

On the other hand, straw also demonstrated a direct biochemical fertilizing action. In variants 7 and 8, the same doses of 170 kg/ha of nitrogen were applied. The only difference is that in variant 8 (Straw, 4 t + $N_{140}P_{175}$, 30 kg nitrogen per dose) the amount contained in the straw was applied. Compared to variant 7, a suggestive increase of 240 kg/ha c.u. (5540-5300) was formed. The corn plants in variant 8 were more fully provided with nitrogen. If in variant 7 the plants assimilated a total of 152 kg for the formation of the harvest, then in variant 8, significantly more - 168 kg/ha of nitrogen.

But the results are even more striking if we add to the comparison variant 9 (Straw, 4 t + Sheep manure, 16 t/ha). Here the same amount of nitrogen was applied, 170 kg/ha, but completely in the form of organic matter. The increase in grain yield in this variant was 1310 kg/ha (6150-4840) or 27% compared to the *Control* variant (1310 x 100 /4840). We assume that this phenomenon is explained by the fact that the plants were more fully provided with other nutritional elements from the sheep manure, which they did not find in the soil of other variants.

Variant 10 (Sheep manure, 20 t/ha), similarly, with the amount of 170 kg/ha nitrogen was placed for comparison with the other variants in the scheme. It is known that sheep manure is the most complex, widespread and common fertilizer with which other forms of organic fertilizers are compared (Țurcan, Sergentu, Banaru et al., 1993). In experience, the variant 10 served as a reference, as a measure of comparison of the tested procedures. Here the indicators taken in the research were the highest. The yield index increased by 1900 kg/ha of cereal grains (6740-4840) or by 39% (6740 x 100/4840). Nitrogen consumption by corn doubled, compared to the reference variant (214/108).

Evolution of harvests over time in the experiment. For a more clarification of the fertilizing influence of straw, the results on aboveground plant production and nitrogen used by plants were monitored over 12 years. The given period was divided into three quadrennial time intervals, according to the application of fertilizers in the experiment (Table 4). Fertilizers, according to the scheme, including chemical ones, were administered quadrennial three times in 2009, 2013 and 2017.

Table 4. Yield and nitrogen consumption in the experiment by fertilization stages, kg/ha

Variant	Average annual saleable harvest in grain units by years				The amount of nitrogen assimilated seasonally by plants			
	2010-2013	2014-2017	2018-2021	Average	2010-2013	2014-2017	2018-2021	Average
1. Control, without fertilizers	3385	3523	4738	3882	83	112	111	102
2. Straw, 4 t/ha	3447	3691	4567	3902	86	114	110	103
3. Straw, 4 t/ha + $N_{20}P_{20}$	3909	3771	5422	4367	119	118	132	123
4. Straw, 8 t + $N_{20}P_{20}$	4069	4102	5539	4570	134	139	139	137
5. Straw, 8 t/ha	3846	3611	4928	4128	114	111	118	114
6. $N_{20}P_{20}$	3537	3705	4715	3985	96	117	112	108
7. $N_{170}P_{180}$	3851	3658	5380	4296	116	119	136	124
8. Straw, 4 t /ha + $N_{140}P_{175}$	4082	4352	5680	4705	126	129	137	130
9. Straw, 4 t/ha + Sheep manure, 16 t/ha	4195	4546	5577	4773	111	145	141	133
10. Sheep manure, 20 t/ha	4492	4278	5297	4689	124	136	133	131
Average of variants	*3881*	*3924*	*5184*	*4330*	*111*	*124*	*127*	*121*

An overview of the presented results indicates an increase in plant productivity at the fertilization stages. The phenomenon was demonstrated in all variants of the experiment both in terms of plant productivity indices and nitrogen consumption values. In variant 2 (Straw, 4 t/ha) the average annual production value in the period 2010-2013 was 3447 kg/ha c.u. In the period 2014-2017 the average annual production increased by 244 kg/year, reaching 3691 kg/ha. And, in the period 2018-2021 wheat production increased, compared to the previous four-year period, by 876 kg/year (4567-3691).

More relevant was the increase in marketable production according to the average values of all variants. In the first period (2010-2013) the average production per experience was 3881 kg/ha c.u. In the second period (2014-2017) - 3924 kg/ha, with an increase of 43 kg/year (3924-3881). And, in the next period of years, 2018-2021, the average annual harvest per experience evolved to 5184 kg/ha, forming a difference of 1260 kg/year, compared to the previous period.

The same evolution was also noted according to the indices of nitrogen consumption from the soil and fertilizers by plants in the experimental variants. For example, on the plots of variant 8 (Straw, 4 t/ha + $N_{140}P_{175}$) the amount of nitrogen concentrated in the aboveground part of the plants constituted 126 kg/ha/year in the first observation stage. In the second - 129 kg and in the third - 136 kg/ha/year nitrogen. Similar increases in the amount of nitrogen assimilated by plants were also observed according to the average annual indices of nitrogen absorption by plants in the experiment.

We assume that the increase in productivity and nitrogen assimilation by crops, across the entire experimental field over time, is due to the increasingly efficient agrotechnique implemented at the station. The average productivity per stage of plants grown on the experimental soil increased, compared to the stage of 2010-2013, by about 12% (4330 x 100/3881). Amount of nitrogen absorbed annually increased by 9 % (121 x 100/111).

The significance of the combined application of straw with other fertilizers. In addition to the beneficial changes in yields and nitrogen assimilation over time, the experience also noted advantageous transformations from combining straw with other fertilizers. For example, in variant 5 (Straw, 8 t/ha), the production increased compared to the *Control* by 461 kg in the first stage of fertilization (3846-3385). In the second stage - by 88 kg and in the third - by 190 kg/ha/year grain units. The average value of the yield supplement in this variant on all three stages of fertilization was 246 kg/ha/year grain units (4128-3882).

When combining straw with sheep manure or chemical fertilizers, the yields increased more clearly and suggestively. Even in tiny doses, of 20 kg/ha of active substance and applied at a considerable periodicity, of four years, ammonium nitrate and amorphous essentially stimulated the fertilizing potential of straw. If in the years 2010-2013 in variant 2 (Straw, 4 t/ha) the average annual yield increased compared to the *Control* by 62 kg (3447-3385), then in variant 3 (Straw, 4 t/ha + $N_{20}P_{20}$) the annual yield increases advanced more than 8 times, making up 524 kg/ha (3909-3385).

Similar differences in the mentioned variants were also noted in the second period of fertilizer application from 2014 to 2017. However, here the amplitudes of the differences compared to the *Control* were shorter, amounting to 168 kg (3697-3523) and, respectively, 248 kg/ha c.u. (3771-3523). Such stimulating influences on plant productivity were also observed at other doses of straw and fertilizer incorporation.

When straw was applied together with chemical fertilizers, a very advantageous synergistic phenomenon was also detected. The joint action of straw with chemical fertilizers formed a production increase greater than the sum of the increases from straw and fertilizers applied separately. For example, according to the average indices from all three stages of experimentation, in variant 3 (Straw, 4 t + $N_{20}P_{20}$) the increase was 485 kg/ha/year c.u. (4367-3882). In variant 2 (Straw, 4 t/ha) the increase was 20 kg (3902-3882), and in variant 6 ($N_{20}P_{20}$) – 103 kg (3985-3882). The sum of the yields increases from the separate application of fertilizers amounted to 123 kg/ha (20 + 103), being 3.9 times lower than the increase obtained from the combined incorporation of straw with chemical fertilizers (485/123).

Such significant synergistic increases in size were also observed when increasing the dose of straw or incorporated chemical fertilizers. The average value of the yield increase in variant 4 (Straw, 8 t/ha + $N_{20}P_{20}$) was 688 kg/ha/year c.u. (4570-3882). In variant 5 (Straw, 8 t/ha) an addition of 246 kg (4128-3882) was formed and in variant 6 ($N_{20}P_{20}$) - 103 kg/ha c.u. (3985-3882). Therefore, the sum of the production increases from the separate application of straw and chemical fertilizers amounted to 349 kg/ha (246 + 103). The sum being about two times lower than the increase obtained by applying these two fertilizers together (688/349).

Since the association of straw with chemical fertilizers, the provision of nitrogen to plants has improved. According to the seasonal average values, it was seen that in variant 2 (Straw, 4 t/ha) plants used an average of 1 kg/ha/year more nitrogen than in the *Control* (103-102). In variant 6 ($N_{20}P_{20}$), plants assimilated an average of 6 kg/ha/year more nitrogen (108-102). The nitrogen supplement from these two variants, with separate application of fertilizers, amounted to 7 kg/ha. And, applied together, these two fertilizers multiplied the amount of nitrogen absorbed three times, reaching 21 kg/ha/year (123-102).

These indicators were characterized with even more relevant parameters when the straw dose was increased. According to the average results by stages, the annual amount of nitrogen assimilated by plants in variant 5 (Straw, 8 t/ha) increased, compared to the *Control*, by 12 kg (114-102). In variant 6 ($N_{20}P_{20}$) 6 kg/ha more nitrogen was used. The sum of the nitrogen increase compared to the *Control* from the separate application of fertilizers was 18 kg (12 + 6). And, from the associated incorporation of these two fertilizers - 35 kg/ha/year nitrogen (137-102). The synergistic effect from the joint action of fertilizers was 17 kg/ha nitrogen (35-18). In relative units, the synergistic increase was 48% (17 x 100/35). In fact, half of the assimilated nitrogen was made available to the plants for free, through synergy, only through the process of applying fertilizers together.

After carbon, nitrogen is considered the second chemical element that ensures life and is indispensable for living beings. It enters the structure of proteins, nucleic acids and other organic compounds, which constitute the physical body of living beings. It is the element of greatest importance in plant nutrition and the decisive factor in the growth of agricultural crops. However, in soils it is found in small quantities, frequently limiting the development of plants.

Analysis of the phenomenon of synergy on the yield and provision of plants with nitrogen when straw is applied together with chemical fertilizers allows us to certify that not only chemical fertilizers increase the fertilizing potential of straw, but also straw increases the yield of chemical fertilizers.

CONCLUSIONS

1. Soils in the agricultural circuit need to be supplemented with organic matter and primary elements alienated as a result of long-term plant cultivation. However, organic fertilizers accumulate too little and are very expensive to use. Chemical fertilizers, and to this day, are made at high prices. In this context, straw surpluses are appreciated as an accessible and considerable resource of organic matter for agricultural soils.

2. On chernozem soil, where the experiment was carried out, the phenomenon of a decrease in plant production from straw incorporation was not confirmed. This is due to the ability of chernozemic soils to form, through humus mineralization, sufficient nitrogen necessary for the microbiological processing of the applied straw. Therefore, in the pedoclimatic conditions of the Republic of Moldova, straw surpluses can be incorporated as fertilizer in advance and without being supplemented with nitrogen-containing fertilizers.

3. Of course, where available, nitrogen fertilizers should be associated with straw. By combining straw with nitrogen fertilizers, the fertilizing effect of both fertilizers is increased. A synergistic crop yield is formed that is more than twice as high as the sum of the increases from both fertilizers applied separately. The higher the dose of straw or chemical fertilizers in the mixture, the more impressive the synergistic increase becomes.

4. The most efficient method of applying straw as fertilizer has proven to be the method consisting of 4 t/ha of chopped straw and distributed at harvest in the stubble. Over which chemical fertilizers were distributed in doses of $N_{20}P_{20}$. In physical quantities for one hectare, these fertilizers can be represented by 40 kg of ammophos and 44 kg of ammonium nitrate. Both types of fertilizers are incorporated once for four years. Wheat also returns to field rotations with about the same periodicity. This method ensures an average annual increase of 480 kg/ha of conventional grain wheat.

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Zea mays L.: REVIEW OF MORPHOLOGY OF TASSEL COMPONENTS THAT CAN INFLUENCE THE AMOUNT OF POLLEN

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Abstract

Pollination is essential for hybrid seed production in maize, relying heavily on the pollen output of inbred male plants. Effective pollination ensures kernel development, genetic purity, and high-quality hybrid seeds. Tassel morphology—including traits like weight, branch count, and Tassel Area Index - plays a key role in pollen production. Breeding programs must optimize these traits to balance pollen availability with minimal negative effects, such as leaf shading. Integrating genetic and phenotypic assessments can enhance pollination efficiency and hybrid reliability. This approach supports yield stability and resilience under diverse environmental conditions, contributing to sustainable maize production.

Key words*: pollen. Zea mays L., tassel morphology, pollination.*

INTRODUCTION

Maize (*Zea mays* L.) is recognized as the most widely cultivated cereal globally, with the United States of America, China, and Brazil leading production rankings (FAO, 2023). In 2023, the global area dedicated to maize cultivation was estimated to be between 200 and 210 million hectares. This crop's widespread cultivation is attributed to its remarkable productivity and versatility. For example, maize serves as a critical component in diverse industries, including animal feed, staple foods such as flours, hominy, oil, and bread, and even in specialized sectors like brewing, pharmaceuticals, and mining (Strazzi, 2015). In Europe, approximately 16 to 18 million hectares were sown with maize during the 2023 season. This figure varied slightly due to factors such as weather conditions, market dynamics, and crop rotation practices. Among the leading European maize producers are France, Ukraine, Romania, and Hungary, with Romania maintaining a dominant position in maize cultivation. Recent data highlights that Romania consistently cultivates around 2.5 million hectares annually, making it the European Union's largest producer in terms of cultivated area (FAO, 2023). This leadership can be attributed to Romania's favorable climatic and soil conditions, particularly in its southern and eastern regions. From a personal perspective, Romania's strategic emphasis on maize cultivation reflects its broader agricultural strengths and adaptability. These favorable conditions could position the country as a potential hub for innovation in maize-based product development, which could enhance its competitiveness in the global market. The multiple applications of maize in food production, industrial uses, and beyond further underscore its significance in ensuring both economic stability and food security.

Maize holds substantial economic significance, and breeding programs are vital for developing genotypes with agronomic traits that align with producer needs and consumer market expectations. Modern maize genotypes have been optimized for traits such as reduced plant and ear heights, more upright leaf angles, smaller tassels (fewer branches and lower mass), shorter intervals between tasselling and silking, decreased grain protein content, and increased yield potential (Duvick, 2005).

One of the most critical stages in maize development is flowering, which directly impacts yield. This phase typically spans 5 to 14 days (Ogden et al., 1969) and begins with pollen release from the tassel, followed shortly by silk emergence. Remarkably, each maize plant produces approximately 25 million pollen grains, ensuring a robust reproductive capacity with around 25,000 pollen grains available per silk (Kiesselbach et al., 1949). This interplay between tassel and silk dynamics underscores the complexity of maize reproductive biology.

Tassel size plays a pivotal role in yield determination. Larger tassels can reduce grain yield by shading upper leaves, thereby diminishing photosynthetic efficiency, and by acting as sinks for photo assimilates (Edwards, 2011; Souza et al., 2015). However, in environments with stress factors such as limited water availability, larger tassels may become advantageous by ensuring sufficient pollen production for fertilization (Parvez, 2007). Breeding programs have thus focused on selecting smaller tassels to reduce shading effects, enhance photosynthetic performance, and allow higher planting densities. While this strategy improves yield potential, it has inadvertently reduced pollen production (Duvick & Cassmann, 1999). Consequently, understanding factors influencing pollen production and germination has become a priority to support breeding programs and hybrid seed production. Tassel size, defined by traits such as branch number, branch length, spikelet count, and tassel area index, exhibits a complex relationship with yield. Specifically, a negative correlation with grain yield is often observed, alongside a positive correlation with pollen production (Fonseca et al., 2003). This duality poses challenges in breeding programs aimed at optimizing tassel traits.

The reduction of tassel size has been instrumental in improving planting efficiency and yield under optimal conditions, there is a growing need to develop adaptive strategies for stress-prone environments. Incorporating genetic diversity and exploring innovative breeding techniques could balance the trade-offs between pollen production and yield. This balance is critical to sustaining productivity as environmental conditions continue to fluctuate due to climate change.

TASSEL CHARACTERISTICS AND THEIR ROLE IN MAIZE POLLEN PRODUCTION

This scientific publication provides an in-depth analysis of tassel characteristics—such as tassel weight, stalk diameter, number of primary branches, total branch length, tassel length from lower and upper branches, and Tassel Area Index - and their influence on pollen production in maize inbred plants. The study examines the morphology of tassel components under conditions of heat stress and evaluates how these changes impact pollen production. Pollination is a critical process in the growth and development of higher plants and is particularly sensitive to unfavourable environmental conditions (Ali et al., 2018). Among these, heat stress is a prominent environmental challenge that significantly affects the physiological and phenotypic traits of reproductive organs, as well as the pollination process (Hatfield and Prueger, 2015; Gabaldón-Leal et al., 2016). Even short episodes of heat stress during the crucial flowering stage can result in substantial yield losses (Tian et al., 2018; Wang et al., 2022).

This research underscores the importance of understanding the intricate relationships between tassel morphology and pollen production under stress conditions. By identifying key traits associated with resilience, the study aims to contribute to breeding strategies that enhance maize productivity in the face of climate variability.

GENETIC AND MORPHOLOGICAL INTERACTIONS OF TASSEL TRAITS: IMPLICATIONS FOR POLLEN PRODUCTION AND YIELD IN MAIZE

In maize breeding, increasing emphasis is placed on selecting traits that maximize yield by optimizing energy conversion processes. Key traits include plant height, ear height, leaf number, and leaf area. Additionally, tassel characteristics play a significant role in influencing plant performance and productivity (Body et al., 2008).

During the development of inbred lines, tassels often display reduced development, diminished pollen production, and, in extreme cases, male sterility. These effects, attributed to inbreeding, lead to significant variability in pollen competitiveness among inbred lines (Sari-Gorla et al., 1975) and F_1 hybrids (Pfahler, 1967). Excessive selection pressure, compounded by the genetic background of the lines, can detrimentally impact both male and female reproductive traits in maize. This highlights a critical challenge in breeding programs: maintaining a balance between inbreeding to fix desirable traits and avoiding the associated negative effects on fertility. From a practical standpoint, addressing this issue requires a multifaceted approach. Breeding strategies must incorporate a deeper understanding of genetic interactions that drive these reproductive changes. The focus should shift from solely maximizing yield potential to maintaining a robust reproductive framework, particularly under the stress conditions often encountered in nurseries and seed production environments. This balance is essential not only for the development of high-performing hybrids but also for ensuring the sustainability of breeding programs over time (Vidal-Martínez et al., 2001). Therefore, integrating genomic tools and phenotypic assessments could help breeders identify genotypes with improved resilience to inbreeding depression. Additionally, maintaining genetic diversity within breeding populations could serve as a buffer against the adverse effects of excessive inbreeding, ensuring that the development of new inbred lines supports both productivity and reproductive success.

The tassel, the male inflorescence of maize, is a commonly studied aspect of the plant. Maize, being monoecious and heteroecious, depends significantly on the development of its male flowers for reproductive success (Bocz, 1992). The morphology of the tassel, especially traits affecting pollen production, plays a vital role in seed production and the selection of hybrids. Numerous studies have investigated the connection between pollen production and tassel characteristics (Vidal-Martínez et al., 2001; 2004; Fonseca et al., 2003; Rácz et al., 2006). Additionally, the genetic basis of tassel traits has been explored. Mock and Schuetz (1974) found that the number of tassel branches is a quantitative trait with high heritability. Geraldi et al. (1978) also provided heritability estimates, reporting 86.1% for tassel weight, 45.8% for tassel branch number, and 28.8% for tassel length. However, the inheritance mechanisms of these traits remain incompletely understood (Berke and Rocheford, 1999).

Research has consistently shown that an increase in the number of tassel branches negatively impacts grain yield (Geraldi et al., 1978; 1985; Vidal-Martínez et al., 2001; Gyenesné Hegyi et al., 2001; Hegyi, 2003). This is likely because the plant's resources are diverted toward tassel development, reducing the energy available for grain formation. Similarly, tassel weight is negatively correlated with grain yield but positively correlated with the number of branches (Geraldi et al., 1985). These findings suggest that selecting for maize genetics with fewer tassel branches and smaller tassels could indirectly improve kernel yield. This improvement would result from reduced energy expenditure on tassel development and minimized shading effects on the upper leaves, which are critical for photosynthesis (Lambert and Johnson, 1977). However, it is important to note that smaller tassels in male parental inbred lines may create challenges for F_1 seed production and the maintenance of male lines. This is because smaller tassels produce less pollen and have reduced dispersal efficiency (Wych, 1988). This dilemma highlights the importance of finding a balance between tassel size and pollination efficiency in breeding programs.

The number of tassel branches is a key factor in pollen production (Vidal-Martínez et al., 2001a), and a higher number of branches tends to dominate over fewer branches (Mock and Schuetz, 1974; Hegyi, 2003). Hegyi (2003) observed an inverse relationship between the productivity of individual lines and the number of tassel branches. This suggests that while a higher number of branches may increase pollen production, it can simultaneously reduce grain yield. Analyses using Pearson's correlation coefficient to examine the relationships between tassel components and quantitative traits indicate that these traits interact both directly and indirectly (Body et al., 2008). The presence of moderate to strong correlations, supported by high reliability, underscores the potential for these traits to influence one another indirectly. These observations provide valuable insights for breeding programs, suggesting that optimizing tassel traits could have beneficial effects on other plant characteristics.

From my perspective, these findings should encourage researchers and plant breeders to adopt a more holistic approach to maize improvement. For example, strategies could be explored to minimize the negative effects of large tassels, such as selecting for more compact tassels or optimizing their positioning on the plant. At the same time, it is essential to maintain an adequate level of pollen production to ensure successful pollination and seed formation. Additionally, I believe that integrating modern technologies, such as imaging analysis and 3D modelling of tassels, could provide a deeper understanding of the relationships between tassel morphology and crop performance. These tools could facilitate the identification of innovative solutions for tassel optimization, contributing to improved yield and sustainability in maize cultivation.

Morphological traits and grain yield components develop sequentially during plant growth. These traits are quantitative, governed by multiple genes (Ledent, 1984), and often show a genetic correlation with grain yield, exhibiting high heritability (Gallais, 1984).

CONCLUSIONS

Tassel stalk diameter displayed a moderate positive correlation with tassel length measured from the lower side branch, while its correlation with tassel length from the upper side branch was weak but statistically significant (P=0.01%). However, its associations with other traits were negligible. Unlike tassel stalk diameter, most tassel characteristics exhibited medium to high heritability values, as noted by Upadyayula et al. (2006).

The Tassel Area Index (TAI) exhibited a significant negative correlation with plant height, crop yield, and thousand kernel weight (P=0.01%). A moderate negative correlation was also noted with ear height and leaf area index (Body et al., 2008). On the other hand, multiple studies have identified strong positive correlations between leaf area index, plant height, and crop yield (Zsubori et al., 2002; El Hallof and Sárvári, 2006; Jakab, 2003). These opposing relationships underscore the dual influence of tassel morphology, as its shading effect on leaf area contributes to the negative associations with yield-related traits. These findings are consistent with earlier research by Lambert and Johnson (1977) and further validate the relevance of TAI in plant breeding initiatives.

In my view, TAI serves as a critical indicator for optimizing maize breeding strategies. As Fonseca et al. (2003) suggested, TAI can predict the pollen-producing potential of maize genotypes, underscoring its relevance in parent selection for seed production. However, our findings show a negative correlation between TAI and yield-related traits, indicating that a balance must be struck between sufficient pollen production and minimizing the shading effects of larger tassels. Interestingly, a weak but positive correlation was observed between TAI and test weight, suggesting that further exploration of this relationship could refine selection criteria for high-performing genotypes.

Thousand kernel weight showed a moderate negative correlation with test weight at a high reliability level (Body et al., 2008). This result diverges from the findings of Li et al. (2007), who reported significant positive correlations, and from earlier studies by Pomeranz et al. (1986) and Dorsey-Reading et al. (1991), which found weak or no significant correlations between these parameters. These discrepancies underline the complexity of genetic and environmental factors influencing grain traits and emphasize the need for further investigations into these relationships.

The importance of tassel components in maize breeding is expected to increase significantly in the near future. Analysing correlations among tassel traits offers crucial information that can be effectively utilized in selection strategies. Notably, the tassel area index (TAI), represents the combined influence of various tassel components, presents a valuable approach for targeting traits with relatively low heritability.

From a practical perspective, more emphasis should be placed on research into tassel morphology, not only in seed multiplication programs but also in conventional maize production systems. Such efforts could enhance efficiency and yield outcomes by enabling breeders to identify and select genotypes with optimal tassel characteristics for different production environments. The integration of these findings into modern breeding programs could lead to more resilient and productive maize hybrids, ensuring greater adaptability to changing climatic and agricultural conditions.

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INFLUENCE OF CHANGING CLIMATIC CONDITIONS ON THE QUALITY TRAITS OF COMMON WINTER WHEAT GROWN IN THE PAZARDZHIK REGION

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Abstract

The trial was conducted for a 5-year period (2019-2023) on the experimental field of irrigated agriculture experimental station Pazardzhik. In the period 2019-2023 in the field of irrigated agriculture experimental station Pazardzhik on cinnamon forest soil at humus 1.2-1.5% and pH 5.5-6 is set trial, which includes 9 varieties of common winter wheat selection of IPGR Sadovo. It was laid out using the long strip method in four replications with a harvest plot size of 10 m2. The sowing rate is 550 gs/m2. Fertilization was with phosphorus (20 kg/da), applied as triple superphosphate pre-sowing. The entire nitrogen fertilizer rate was applied as N12 (applied as ammonium nitrate). The quality parameters sedimentation value; grain vitreousness; fermentation number; crude protein; wet gluten content; gluten release, dry gluten; bread making strong index were monitored in the grain quality laboratory at IPGR, Sadovo. Gluten relaxation, baking stomg index and dry gluten were determined. ANOVA, Duncan test, cluster and correlation analysis were used for mathematical treatment of data.

***Key words**: common winter wheat, grain quality, changing climatic conditions.*

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the major crops closely linked to the nourishment and development of modern society. It is of strategic importance in solving food security problems, being used as a raw material for the production of various food products in many industries (Ekinci & Gökbulut, 2020; Belkina et al., 2018; Manukyan et al., 2023; Nosova et al., 2021). With population growth and urbanization, wheat grain consumption is also increasing (Hongjie et al., 2019; Li et al., 2019; Robles-Zazueta et al., 2024). The way to developing wheat varieties with maximum yield of quality wheat is extremely difficult. Wheat breeders are constantly challenged to develop new genotypes that respond to changing environmental conditions with high ecological plasticity (Dowla, et al., 2018; Gungor & Dumlupinar, 2019; Aydogan et al., 2020; Robles-Zazueta et al., 2024).

It has been suggested that changes in climatic conditions over time will cause further abiotic and biotic changes in grain production that are likely to lead to significant changes in wheat yield and quality (Fradgley et al., 2023).

The characteristics that affect the quality of wheat can be broadly grouped into two main groups. The first group of traits are heritable, those under genetic control. The second group includes properties that are influenced by environmental conditions and the specific factors of the growing area. Common winter wheat is a microclimate crop. It influences the expression of the genetic traits of a given genotype related to yield and grain quality. Intensely changing biotic and abiotic factors strongly influence a given variety that interacts with the environment in which it is grown (Moskalets & Rybalchenko, 2015; Harkness et al., 2020; Dennis et al., 2021). The quality of the harvested grain determines its nutritional value. It includes a complex of indicators determining its technological characteristic (Fradgley et al., 2023). The values of these indicators in each individual variety are genetically determined, but are also influenced by the agroecological conditions of a given growing region, climatic factors during the vegetation of the plants, the applied agrotechniques (Ivanova & Tsenov, 2009; Cifci & Yağdi, 2010; Delibaltova et al., 2014).

Therefore, research that is related to the cultivation of common winter wheat varieties in different regions has a certain scientific and practical importance. A number of our scientists are investigating the changing environmental conditions in different regions of Bulgaria and their impact on wheat productivity and quality (Tanchev, 2008; Stoeva & Ivanova, 2009; Ilieva, 2011; Kirchev & Delibaltova, 2016; Uhr & Samodova, 2020; Dragov & Samodova, 2020; Gubatov & Delibaltova, 2020; Tsenov et al., 2021; Angelova et al., 2023).

The varieties bred at IPGR Sadovo enable us to cover the wide range of quality requirements for different types of pasta production. Many years of research have shown that the varieties selected by IPGR have good ecological plasticity.

They retain good quality and quantity parameters regardless of the conditions of the growing region. The breeding of the Sadovo Institute has created some of the best quality wheat. The Pobeda variety is a national quality standard, Sashez is a new achievement in grain quality breeding, they are strong wheats with excellent dairy and baking qualities of the grain (gr. A - Executive agency for variety testing, approbation and seed control), which can serve as flour improvers.

A way of establishing the ability of varieties to resist various abiotic and biotic stresses is to test them in a variety of ecological zones. This enables us to select materials that efficiently use the resources of the surrounding environment and its features, which in turn enables the creation of ecologically plastic, high-yielding and high quality parameter varieties of common winter wheat (Kirchev & Delibaltova, 2016; Andrusevich et al., 2018; Öztürk et al., 2019; Uhr & Samodova, 2020; Tsenov et al., 2021; Güngör et al., 2022; Angelova et al., 2023).

The objective of the present study is to establish and monitor the grain quality of common winter wheat varieties, breeding by IRGR Sadovo, grown in the area of the Experimental Station of Irrigated Agriculture Pazardzhik.

MATERIALS AND METHODS

In the period 2019-2023 in the field of the Experimental Station of Irrigated Agriculture Pazardzhik on pseudopodzolic Cinnamon soil at humus 1.2-1.5% and pH 5.5-6 was carried out trial, which includes 9 varieties of common winter wheat selection of IPGR Sadovo. Plotted using the long strip method in four replications with a harvest plot size of 10 m^2. Sowing is with 550 germinating seeds/m^2. Fertilization with phosphorus (20 kg/da) was given pre-sowing in the form of triple superphosphate. The entire nitrogen fertilizer rate was applied as N_{12} (in the form of ammonium nitrate).

The laboratory tests for grain quality were carried out in the technological laboratory of IPGR - Sadovo. The following were analysed: VG - Grain vitreousness; SdV -Sedimentation value; FN-Fermentation number; Wet gluten content - WGC; Gluten release - RG; Dry gluten – DG; bread making strong index – BMSI.

Technological assessment for grain and bread-making quality

Vitreousness (VG), particularly for grain quality in durum wheat was determined according to BSS 13378:1976, BSS EN 15 585:2008 (https://bdsbg.org/bg/project/show/bds:proj:17689).

The Sedimentation Value (SdV) (Iced Acetic Acid Test – 2%, (Pumpyanskiy, 1971) test was used to provide information on the protein quality and baking properties of the wheat (Angelova et al., 2020; Galushko & Sokolenko, 2021; Uhr et al., 2023).

The Fermentation Number (FN) – Pelschenke test was used to assess the fermentation capacity of the wheat which is important for bread-making quality. The test is based on the retention of CO_2 gases released during dough fermentation. A 10 g sample of grain meal is mixed with a yeast solution (a biological product, representing a concentrated mass of yeast of the Saccharomyces cerevisiae species) in two replicates (Pelshenke et al., 1953). The experiment was carried out under controlled conditions (30^{o}C - water thermostat). The longer the retention time of the sample on the water surface, the better the quality of the gluten. (Angelova et al., 2020; Galushko and Sokolenko, 2021; Uhr et al., 2023).

Gluten Content and Quality (WGC) (BSS EN ISO 21 415-2:2008, BSS EN ISO 21415-1:2007) test was applied to determine gluten characteristics, which are critical for the end-use quality of wheat, gluten relaxation, mm (BDS 13375:1990/Amendment 1: 1993), (https://bds-bg.org/bg/project/show/bds:proj:17686).

Dry Gluten (DG) was done according to the ISO 21415-3:2006, BSS EN ISO 21415-4:2007 standards to further understand the protein quality of wheat samples (https://www.iso.org/standard/35864.html).

The mathematical processing of the data was performed by applying ANOVA, Duncan test, cluster and correlation analysis. SPSS 19 and Microsoft excel for Windows were used.

RESULTS AND DISCUSSIONS

The research was conducted in the experimental station of irrigated agriculture with Ivaylo, Pazardzhik on Pseudopodzolic Cinnamon soil. It belongs to the European-continental area and is referred to the transitional-continental sub-region in the region of the Upper Thracian Lowland. The field is situated on an old well-bedded terrace of the river Topolnitsa (Teoharov, 2019).

Field studies

According to literature data, the soil distinction is referred to as Izluzhena Cinnamon Forest soil (Penkov et al., 1992; Ivanov, 2019; Kirilov, 2022).

Ivanov (2019) made complete and detailed field investigations of the experimental station and found: that the mechanical composition is highly differentiated layer by layer. The humus horizon A1l (0-34 cm) has a light sandy-clay mechanical composition, is compacted with a sputtered structure and is poor in silt particles. A hard soil crust forms after more intense rain. In the humic podzolic horizon the silt content (<0.001) is 16.5% and physical clay content (<0.01) is 27.65%, respectively, while in the adjacent horizon 34-56 cm this content is 28.9% and 40.8%, respectively, i.e. about 2 times higher, this textural differentiation is characteristic of this soil differentiation (Dimitrov et al., 2016).

Teoharov (2019) reported that these soils are very poor in organic matter. The cultivated areas contain about 1-1.5% humus, mostly concentrated in the ploughsoil, which is mostly represented by the alluvial horizon. The composition of humus is dominated by fulvic acids. The amount of total nitrogen is usually very low and does not exceed 0.10-0.12%. These results are confirmed by Ivanov (2019), as he found a satisfactory content of total phosphorus, from 0.105 to 0.128%. As well as slightly acidic reaction pH 5.2 - 5.3 of the soil solution. No carbonates were detected on the profile. The humus content is low - from 1.09 to 2.13%.

The soil has a satisfactory content of absorbable forms of the main nutrient elements - nitrogen, phosphorus and potassium, which is the result of the use in previous years of the experimental area for breeding trials. The results obtained were confirmed by Kirilov (2022).

Meteriological studies

The Pazardzhik field belongs to the Transitional-Continental climatic sub-region in the Upper Thracian Lowland. The climate is temperate-continental, but is influenced by currents along the Topolnitsa and Maritsa rivers (Ivanov, 2019).

Climatically, the study years are very diverse.

Rainfall is unevenly distributed over time. Very often there is a strong lack of water in the wetter months, and in other cases there is waterlogging, reaching swamping and flooding. Temperatures below the multi-year values are recorded for 2019, 2020 and 2023, and for the other two years the values are aligned with the multi-year values.

The most important months for the formation of the quality of wheat are May and June, when we have the grain filling, its waxy and full maturity. Grain filling starts at the beginning of the milky stage and continues until the beginning of waxy maturity. Maturation is characterised by the complete anatomical separation of the grain from the mother plant and the cessation of the accumulation of reserve nutrients and enzymes. Full maturity occurs at the end of June.

In dry and warm climates, protein accumulation in grains is enhanced. Such conditions shorten grain formation and filling periods, accelerate ripening and consequently increase the proportional protein content of grain composition (Moayedi et al., 2021; Clauw et al., 2024; Chețan et al., 2024)

For the month of May, temperatures are reported to be 0.8 below multi-year values for 2022 to 4.1 for 2023. Reported precipitation for the period is well below normal with 17.4 for 2021 to 48.6 for 2022 unevenly distributed throughout the month. The month of June in terms of temperature is warmer than the perennial values highest temperature was recorded in 2019 at 22.4 and lowest in 2020 at 20.9. Precipitation below normal was recorded for 2020, 2022 and 2023 and above normal for 2019 and 2021. In all years they are unevenly distributed over the month (Table 1).

Table 1. Average monthly temperatures and rainfall totals for the months of March to June for the years 2019 to 2023

Months/Year	III	IV	V	VI
Average monthly temperatures in ^{0}C				
2019	9.8	11.7	17.2	22.4
2020	8.3	11.1	17.0	20.9
2021	5.5	12.1	16.9	21.1
2022	4.8	12.0	18.0	22.3
2023	7.9	11.3	14.7	21.4
for 70 years	6.2	12.0	18.8	20.6
Raifall, mm				
2019	3.5	59.8	33.6	128.9
2020	113.9	80.0	30.7	34.3
2021	35.6	75.2	45.3	74.0
2022	20.9	50.0	14.1	50.7
2023	33.4	55.3	43.1	41.9
for 70 years	37.9	45.2	62.7	56.4

The physical traits and chemical composition of wheat change under the influence of soil and climatic conditions (Delibaltova et al., 2014; Nekrasov et al., 2021; Uhr et al., 2022; Angelova et al., 2023).

The most important grain quality traits by which varieties are evaluated for quality are vitreousness, gluten quantity and quality, and sedimentation value.

Table 2 presents the mean values of the studied traits and their corresponding evidences according to Duncan's multiple comparison test between genotypes.

Vitreousness is very important in determining the quality of the grain, it is a varietal characteristic. This indicator reflects the structural features of the grain endosperm. It is closely related to the protein content and technological properties of the wheat. There is information that vitreous grain justifies higher milling quality (Egorov, 2002; Filipov, 2004; Ionova et al., 2017; Galushko et al., 2019). Galushko et al. (2021) found a positive relationship between grain virtuousness and bread volume in winter wheat (r = 0.57).

The results of the study showed that the mean values over the period by genotypes ranged from 49.200 Fermer to 64.200 for Pobeda (Table 2). The highest values for the indicator were recorded in the 2022 crop year, the environmental conditions were favorable and the varieties formed trait values above Executive agency for variety testing, approbation and seed control requirements (50%) for quality group A. The coefficient of variation was 12.62% (average) (Dimova & Marinkov, 1999).

While the physical parameters of the grain are primarily important for the milling quality of the wheat and for the potential flour yield, its chemical composition and mainly the gluten complex are crucial for the baking characteristics of the varieties.

Table 2. Average values of quality traits in winter wheat varieties for the period 2019-2023

Variety	Grain vitreousness	Sedimantation value	Fermentation number	Wet gluten yield	Gluten release	Bread making strong index	Dry gluten
Sadovo 1	55.400 ab	52.600 ab	59.800 a	29.432 ae	13.100 a	50.000 a	9.538 de
Pobeda	64.200 b	62.000 cd	100.400 cd	31.656 abc	12.300 a	52.200 a	10.400 abc
Boryana	54.000 ab	58.000 abcd	67.800 ab	30.020 ab	11.600 a	50.800 a	9.736 ad
Nadita	56.400 ab	60.600 bcd	71.400 ab	32.676 bc	12.700 a	50.800 a	10.554 bc
Gizda	56.400 ab	55.600 abcd	72.000 ab	32.256 abc	11.700 a	51.800 a	10.444 abc
Geya1	39.000 c	42.600 e	36.000 e	25.816 d	11.400 a	45.600 b	8.068 f
Nikibo	52.800 ab	49.400 ab	60.000 a	26.724 de	10.800 a	48.600 ab	8.850 e
Fermer	49.200 ac	54.400 abc	88.400 bc	31.824 abc	12.200 a	51.000 a	10.040 abc
Sashets	53.800 ab	64.000 d	111.600 d	34.608 c	13.300 a	50.400 a	11.182 c
Mean	53.47	55.47	74.16	30.56	12.12	50.13	9.87
Minimum	39.00	42.60	36.00	25.82	10.80	45.60	8.07
Maximum	64.20	64.00	111.60	34.61	13.30	52.20	11.18
Std. Error	2.25	2.24	7.65	0.95	0.27	0.66	0.32
Std. Dev.	6.75	6.71	22.94	2.86	0.82	1.99	0.95
CV, %	12.62	12.09	30.93	9.35	6.80	3.97	9.64

The sedimentation value (cm^3) is a method of examining the settling of flour in 2% acetic acid, which accurately indicates the quality of gluten and dough.

Sedimentation value has been found to correlate with gluten content, gluten quality, and bread volume as a function of protein content (Boyadjieva & Mangova, 2007; Pshenichnaya & Dorokhov, 2017; Galushko et al., 2021).

Therefore, measuring this value is extremely useful for grain quality. Sediment value has also been found to be influenced by heritability (58.6%) and is mostly determined by genotype, but environmental factors (24.9%) are also significant (Lorenzo & Kronstad, 1987; Grausgruber et al., 2000; Hruskova and Famera, 2003; Kibkalo, 2022).

According to the sedimentation number, the varieties Pobeda, Sashets, Nadita, for the whole period of the study show values above the requirements for group A (50cm^3), in the case of the rest of the studied materials we observe a slight decrease of the indicator over the years depending on the conditions of the year. The reported average value for the period is 55.47 (above the requirements for group A). The coefficient of variation of the attribute is average (12.09%).

The joint consideration of the Pelshenke fermentation number (min) trait (Pelshenke et al., 1953) together with the sedimentation number allows us to assess the interdependence between the quality and quantity of gluten by the magnitude of their values (Hermuth, 2019; Angelova et al., 2020; Galushko & Sokolenko, 2021; Uhr et al., 2023).

The results obtained in the present study show high values of this trait in the first (2019) and third (2021) years in the high quality wheats Pobeda and Sashez. For the remaining varieties, the values are below 100 minutes. On average for the study period for the trait lower results were obtained (74.16 min) The coefficient of variation was high 30.93. Our previous studies confirm the high variation of the trait (Angelova et al., 2023).

Asseng et al. (2019) assessed the impact of climate change on wheat protein from a global perspective, concluding that climate change may affect wheat quality by impacting wheat protein synthesis and accumulation (Yuan et al., 2024).

The yield of wet gluten in the grain ensures the appropriate amount of gluten in the flours and gives us an idea of the protein content and nutritional value. Gluten is highly dependent on wheat type and variety, soil and climatic conditions, fertilization, etc. The baking strength number provides the level of bread quality - volumetric yield of bread with good formability (Abduazimov, 2018; Nekrasov et al., 2021; Juraev et al., 2023).

Wet gluten is an extremely important trait for grain processing products- flour, dough, bread, etc. (Dimitrova-Doneva et al., 2002; Dochev 2011). The unique ability of gluten proteins to form a complex called gluten predetermines the leading role of wheat among all cereals (Podgorny et al., 2020). Research by Popa et al.

(2014) proves that the best predictor of bread quality is not the gluten index parameter as such, but the amount of wet gluten at the time of determining this parameter. It also correlated significantly with bread volume (r = 0.79***) and h/d ratio (r = 0.73 ***).

Our results show that the varieties grown in the soil-climatic conditions of the Ivaylo Experimental Station area showed, on average, a high retention of wet gluten content (30.56%) over the study period. The average values for the varieties, with the exception of variety Geya 1 (25.8%) and variety Nikibo (26.7%), were above the quality requirements for group A. The best results were obtained in the 2022 crop year, during which the indicator values were above IACAS requirements (28%). Reported wet gluten yields ranged from 30.64% for Sadovo 1 to 40.06% for Sashez (data not tabulated). The coefficient of variation is low (CV, 9.35%).

For gluten relaxation, we observed values ranging from 8 to 13.5 mm during the first three years of the study (2019-2021). There is an increase in the trait values in the following two years from 11.5 mm to 18 mm. On average over the five year period the result is 12.12 mm, with the lowest being in Nikibo 10.8 mm, and the highest in Sashez 13.3 mm. The coefficient of variation for this trait is low (CV, 6.8%). For the BMSI, the variation is low (CV, 3.97%), and the values obtained for the trait depend on gluten relaxation. The highest values are in 2019 and 2021, during which a lower gluten relaxation is reported. For dry gluten, the average values for the period ranged from 8.07% for variety Geya 1 to 11.18 for Sashez. The average for the period was 9.87%. The variation for the trait is low (CV, 9.64%).

Microclimate has a strong influence on the expression of genetic traits of the varieties related to productivity and grain quality.

The variety, as a genotype or a combination of close genotypes, exists and interacts with the given environment, encountering biotic and abiotic factors that change intensively, indicating its strong influence (Uhr & Samodova, 2020; Dennis et al., 2021; Uhr et al., 2022).

The varieties monitored in the study cover the three wheat quality groups Group A - "strong wheat" - Pobeda and Sashets.

Group B - "medium wheats with increased strength"- Sadovo 1, Nikibo, Boriana and Farmer.

Group W - "medium strength wheats"- Geya-1, Gizda and Nadita.

Knowledge of the influence of G × E (genotype × environment) can be used to adapt crops to specific environmental conditions or to select broadly adaptive varieties that are resistant to variable environments. The environment is rarely fully conducive to expressing the full potential of the varieties sown. Grain quality traits expressed by the studied parameters are largely under genetic control, but are also influenced by growing environment conditions (Atanasova et al., 2010; Eagles et al., 2002; Laidig et al., 2017; Podgorny et al., 2020).

The ANOVA results (Table 3) show that both location and cultivar and the interaction between these two factors have the largest contribution to the strength of factor influence on the quality parameters studied. The influence of year and environmental conditions had the greatest contribution on quality parameters such as grain vitreousness (63.9%), gluten relaxation (59.7%), sedimentation value (58.6%) and wet gluten content (43.4%). Results close to ours were obtained by Szafranska et al. (2024).

Genotype had the strongest influence in dry gluten yield (46.2%) and FN (37.3%) although, in FN, the year of cultivation also had an influence (31.6%).

The influence of G*E was strong in BMSI (46.9%), RG (31.4%) and FN (30.9%). When studying the genotype*environment interaction in common winter wheat cultivars and its quality Tsenov et al. 2004 obtained results similar to ours. The values obtained by Stoeva & Penchev (1999, 2005) are largely analogous, demonstrating the determining role of year in expressing wheat quality potential.

Table 3. Analysis of variance for the studied traits ANOVA, determination of influence of the factor strength η, %

Grain vitreousness	SS	DF	MS	F	P	η, %	SIG
YEAR	21427.6	4	5356.9	6343.7	0.00	63.9	***
GENOTYPE	5467.2	8	683.4	809.3	0.00	16.3	***
GxE	6546.8	32	204.6	242.3	0.00	19.5	***
Error	76.0	90	0.8			0.2	
						100	
Fermentation number	SS	DF	MS	F	P	η,%	SIG
YEAR	53415.1	4	13353.8	3196.4	0.00	31.6	***
GENOTYPE	63130.5	8	7891.3	1888.9	0.00	37.3	***
GxE	52216.1	32	1631.8	390.6	0.00	30.9	***
Error	376.0	90	4.2			0.2	
						100	
Gluten release	SS	DF	MS	F	P	η, %	SIG
YEAR	732.23	4	183.06	578.08	0.00	59.7	***
GENOTYPE	81.53	8	10.19	32.18	0.00	6.6	***
GxE	384.97	32	12.03	37.99	0.00	31.4	***
Error	28.50	90	0.32			2.3	
						100	
Sedimantation value	SS	DF	MS	F	P	η, %	SIG
YEAR	12720.9	4	3180.2	3577.8	0.00	58.6	***
GENOTYPE	5400.0	8	675.0	759.4	0.00	24.9	***
GxE	3518.7	32	110.0	123.7	0.00	16.2	***
Error	80.0	90	0.9			0.4	
						100	
Wet gluten content	SS	DF	MS	F	P	η,%	SIG
YEAR	1256.6	4	314.2	32484	0.00	43.4	***
GENOTYPE	979.9	8	122.5	12665	0.00	33.8	***
GxE	660.9	32	20.7	2136	0.00	22.8	***
Error	0.9	90	0.0			0.0	
						100	
Bread making strong	SS	DF	MS	F	P	η, %	SIG
YEAR	1113.6	4	278.4	348.0	0.00	35.6	***
GENOTYPE	475.2	8	59.4	74.2	0.00	15.2	***
GxE	1468.8	32	45.9	57.4	0.00	46.9	***
Error	72.0	90	0.8			2.3	
						100	
Dry gluten	SS	DF	MS	F	P	η,%	SIG
YEAR	67.04	4	16.76	2423	0.00	28.5	***
GENOTYPE	108.66	8	13.58	1964	0.00	46.2	***
GxE	59.04	32	1.85	267	0.00	25.1	***
Error	0.62	90	0.01			0.3	
						100	

Correlation analysis (Table 4) was performed to assess the relationships among the qualitative indices under study, and the results showed that there was a strong positive correlation of DG with SdV (0. 941**), FN (0.866**), WGC (0.985**) and BMSI (0.838**) at 0.01 level of evidence and significant at 0.05 level of evidence with VG (0.684*) and GR (0.669*).

Analysis of our data shows that there is positive correlation in SdV with FN (0.864**), WGC (0.897**), BMSI (0.826**), DG (0.941**) at 0.01 level of evidence. It is also significant for FN and SdV (0.864**). A strong positive correlation was also observed for the WGC parameters with SdV (0.897**) and FN (0.846**). A strong correlation was also found for BMSI with VG (0.857**), SdV (0.826**). Moderate correlation relationships between VG, SdV and WGC were obtained close to our results in Grain Quality study of new winter wheat genotypes (Galushko et al., 2021). A positively significant correlation over the years of study between sedimentation value and wet gluten content (r = 0.63) was also obtained by Sokolenko et al., 2021. Correlation analysis performed by Podgorny et al. 2020 revealed a relationship between yields and quality traits. As in our previous studies (Table 4).

Table 4. Correlations between the qualitative traits studied

Correlations							
	Grain vitreousness	Sedimantation value	Fermentation number	Wet gluten yield	Gluten release	Bread making strong index	Dry gluten
Grain vitreousness	1						
Sedimantation value	0.770*	1					
Fermentation number	0.605	0.864**	1				
Wet gluten yield	0.568	0.897**	0.846**	1			
Gluten release	0.345	0.615	0.56	0.703*	1		
Bread making strong	0.857**	0.826**	0.722*	0.788*	0.4	1	
Dry gluten	0.684*	0.941**	0.866**	0.985**	0.669*	0.838**	1
*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed)							

Cluster analysis was performed to determine the genetic similarity among the varieties studied. The results are presented in a dendrogram (Figure 1) The figure shows that according to the quality traits studied, the varieties are divided into three main cluster groups. Genetically the most similar varieties are Nadita and Gizda (corresponding to quality group W), followed by Sadovo 1 and Nikibbo (corresponding to quality group B) they form the first cluster group with two subclusters. The second cluster is formed by the high quality Group A wheats. Genetically the most distant in quality are the varieties Geya 1 and Sashez, followed by Geya 1 and Pobeda. This analysis gives us a good idea of the genetic closeness and remoteness of the varieties depending on their quality parameters.

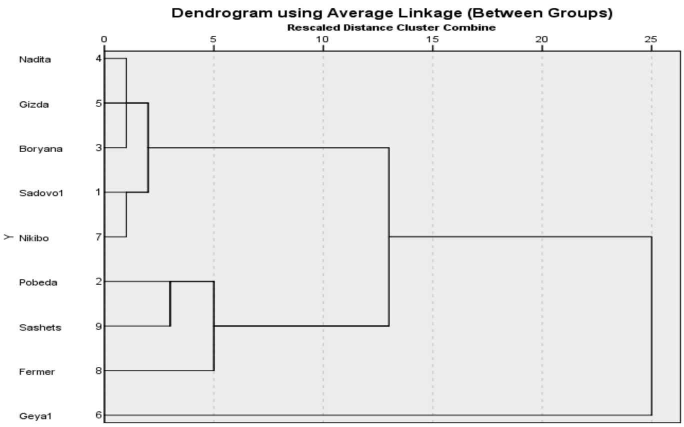

Figure 1. Dendrogram of cluster analysis

CONCLUSIONS

Each of the quality traits examined provides information on part of the "grain quality" complex. It is therefore important to follow the level of varieties as a whole, as well as their performance under environmental variables. The varieties Pobeda and Sashez are high quality varieties in terms of the indices which are tracked correspond to the quality of wheat Group A - "strong wheat".

The varieties Sadovo 1, Boriana and Fermer meet and exceed the quality requirements of Group B - "medium wheat with increased strength". Only in the case of Nikibo, there is an underestimation of the values of individual indicators in individual years. The conditions in the growing area have affected the outbred genotypes differently.

In the case of Group W wheats - 'medium strength wheats' - Gizda and Nadita have shown excellent results over the five-year period,

exceeding the requirements for Group W. In the case of variety Geya-1, lower results were observed, but they covered the requirements for the group in which the variety was classified.
The conditions in the Pazardzhik area are suitable for growing quality grain.

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PEA PROTEINS AS AN ALTERNATIVE TO PROTEINS OF ANIMAL ORIGIN FOR WINE CLARIFICATION - A MINIREVIEW

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Abstract

Pea (Pisum sativum) is a leguminose crop cultivated worldwide for its high protein content. Pea protein is already used as a nutraceutical or food ingredient due to its low allergenic effects. In recent years it started to be applied in wine clarification as an alternative to the use of proteins of animal origin (casein, ovalbumin, gelatin), which are not suitable for vegetarians and can also cause allergic reactions in some people. As an adjuvant for wine fining, the pea protein removes some of the undesirable oxidisable polyphenols in wines and some other compounds, with good effect on the colour and taste. This paper discusses the types of pea extracts and their advantages and limitations as replacements of animal proteins in winemaking. Mechanisms of molecular interactions with the wine compounds and effects are presented in comparison with those produced by fining with traditionally used agents.

Key words*: pea proteins, pea extracts, wine, fining agents.*

INTRODUCTION

Pea (*Pisum sativum*) is a leguminous plant belonging to the *Fabaceae* family, cultivated worldwide as commercial crop, forage, rotational or cover crop (Pavek, 2012), due to its high protein content, reaching 20-25% (Shanthakumar et al., 2022).

Other compounds found in pea are fibres, starch, trace elements and some phytochemical substances which may also be important for antimicrobial, anti-inflammatory, antioxidant and even anticancer properties (Rungruangmaitree and Jiraungkoorskul, 2017).

Pea is relatively easy to grow, does not necessarily need fertilizers as it is can fix atmospheric nitrogen (Wang et al., 2020), is drought tolerant because has a deep root system (Meena et al., 2018) and has a low carbon dioxide emission 0.98 CO_2 equivalents per kg of peas, significantly lower than 1.79 for beans or 99.48 for beef, 23.88 for cheese and 12.31 for pork (Ritchie et al., 2022).

As more and more people shift to vegetarian or vegan products for diet or health reasons, pea protein emerged as a very good alternative to replace ingredients of animal origin. Egg and dairy ingredients replacement with pea-based ingredients are very often researched (Hedayati et al., 2022; Wu, 2022).

Pea protein extracts became more popular as food supplements or for many applications in food industry, due to its affordability, availability, nutritional value and potential health benefits (Boye et al., 2010; Lam et al., 2016; Lu et al., 2020).

MATERIALS AND METHODS

Some of the most important scientific databases of references on life sciences were systematically searched using the terms “pea”, “pea protein”, “pea isolate”, “pea hydrolysate”, “vegetal protein” coupled with “wine”, “fining”, “fining agents”. The search was performed in ScienceDirect, Scopus, Elsevier and PubMed up to January 2025.

A separate literature search was performed using the terms “animal protein” or "vegetal protein” and “wine" in combination with "alternative", “allergenicity” to document the reasons for replacing animal protein fining in wines.

Papers and some reviews specifically addressing the topic of pea protein as a fining agent for wines were favoured, but other papers related with the pea protein uses and reaction mechanisms were also included.

Pea protein research developed very much in the past years, but the research on fining wines with pea protein is still limited. For example, on Science Direct Freedom Collection, a search with the term "pea protein" rendered 22 results for 2001, growing slowly to 111 in 2015 and then increasing abruptly and reaching 1554 in 2024 (Figure 1 a). In the same time, the search with "pea protein" coupled with "wine finding" rendered very few results (Figure 1 b), especially in the last years, showing that this new application in wine clarification is an emerging research topic.

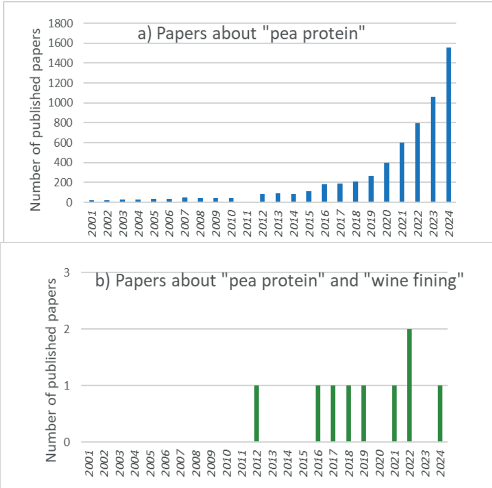

Figure 1. Evolution of published papers from 2001 to 2024 related to pea protein in general (a) and application of pea protein in wine fining (b)

RESULTS AND DISCUSSIONS

Pea protein as food

Plants have been used for centuries as part of human diet, providing energy and important nutritional compounds. Among plants, pulses include the nutritionally-dense edible seeds of legumes, such as beans, peas, chickpeas and lentils. Like any pulse, pea is composed mainly of starch embedded in proteins, fibres and lipids (Pelgrom et al., 2015).

Based on several studies included in a comprehensive review (Wu et al., 2023), the approximate composition of pea consists of 59.32–69.59% carbohydrates (more specifically 39.44% to 46.23% starch and 23.23% to 30.72% dietary fibres, of which 3.91-8.01% of soluble fibre and 19.32-23.1% of insoluble fibre). It also contains 20-25% proteins and 3.06-7.3% lipids.

However, when it comes to plant proteins, it is also known that they are not as well balanced as the animal proteins, regarding the content of essential and non-essential amino acids. Plant proteins may be deficient in several essential amino acids (Berrazaga et al., 2019) and pea is no exception, cysteine and methionine being the limiting amino acids in pea protein, as well as tryptophan (FAO, 2025). Lysin is, however, well represented in pea proteins (Shi et al., 2018), as well as arginine (Robinson and Domoney, 2021). Plant proteins are also less digestible than the animal ones (Kaur et al., 2022), due to the presence of fibres which inhibit the proteolytic enzymes (Doudu et al., 2003), the digestibility rate of cooked pea proteins being 73-94% (Khattab, S and Nyachoti, 2009).

Moreover, structural differences are also found, plant proteins having in their structures fewer α-helixes and more β-sheets than animal proteins, which increase with heating and adversely affects their digestibility (Carbonaro et al., 2012).

To improve the nutritional value and to better valorise peas, protein is often extracted for various applications.

Pea protein extraction

Plant proteins, including pea's, are classified in accordance to their solubility in four major classes: globulin, albumin, prolamin, and glutelin (Markgren and Johansson, 2020), most of them having storage functions for the plant, especially globulin. Globulin (legumin and vicilin) is the fraction soluble in solutions of salt, representing 55-65% of pea protein (Lu et al., 2020).

Pea protein is obtained by removing the outer shell of the peas and milling the rest into flour. The fibres and starch are subsequently removed from the flour. Pea protein concentrate is the least processed and contains also carbohydrates and lipids, while pea protein isolates and hydrolysed pea protein are higher in protein.

To extract the pea protein several methods can be applied to obtain enriched protein fractions or isolates. The most used methods are wet fractionation, which is based on the solubilization of the starch and protein in water at different conditions, and dry fractionation, which is based on separation by density and particle size (Klupšaitė & Juodeikienė, 2015).

The wet fractionation method leads to higher protein concentration (up to 94%), but is very demanding in time and energy, while the dry fractionation reaches a concentration of up to 75%, but is more sustainable (Allotey et al., 2022) and preserves better the natural structure and function of the proteins (Pelgrom et al., 2015; Pelgrom et al., 2013).

More sophisticated extraction methods include the use of enzymes, ultrasounds, radio-frequency, microwaves, high pressure, pulsed electric fields, intense pulsed light and so on (Rajpurohit and Li, 2023).

These extracts have may uses and certain reviews are available to point out this diversity from food applications (Shanthakumar et al., 2022) to food supplements and pharmaceutical products, edible coatings for fruits and vegetables, emulsifiers, drug delivery and non-edible applications (Kumar et al., 2022).

These proteins can be used as such or can be modified by various treatments such as heat, pressure, extrusion, plasma, ultrasounds, chemical modifications, fermentation, enzymatic transformations and so on, in order to improve their functional properties (Shanthakumar et al., 2022).

Wine fining with proteins

Of all the applications of the pea protein isolates, the use in wine is only recent and is mainly based on their capacity to bind with the tannins, a property discussed in the subchapter which follows.

Pea protein is applied in wine as an adjuvant and the technological process for which is used is called fining. This is a practice based on using some substances, called fining agents, to clarify and improve the filterability of a liquid product, such as wine, juice, beer - thus preventing unwanted sensory effects and the forming of sediments after bottling.

Generally, the fining agents are selected to be able to remove undesirable particles, haze and, in some beverages, yeasts after fermentation.

In wine, fining is applied to modulate the organoleptic properties, including, but not limiting to the visual ones. Thus, the expected effects of wine fining are as follows:

- Reduce turbidity for a better visual effect, but also as a means for reducing unwanted compounds. Turbidity is reduced through adsorption on the fining agent molecules, either in gravitational sedimentation or in flotation.
- Remove C6-aldehydes and C6-alcohols inducing herbaceous notes.
- Remove enzymes such as polyphenol-oxidases which lead to wine browning or pinking, and esterases, which promote the loss of aroma, and elemental sulphur, which could lead to reductive aroma.
- Remove phenolic compounds to reduce white wine oxidability and avoid browning and pinking in the presence of oxygen and oxidoreductase enzymes.
- Remove phenolic compounds to avoid bitterness, which is mainly imputable to flavan-3-ols, but may also be caused by some flavanols and derivatives of benzoic and hydroxycinnamic acids (Ferrer-Gallego et al., 2014; Ferrer-Gallego et al., 2016).
- Remove phenolic compounds to avoid the astringency determined by flavanols and their polymers with low molecular weight from the classes of procyanidins/ prodelphinidins sub-class of condensed (or proanthocyanic) tannins (OIV Resolution Oenological Tannins OIV-OENO 675A-2022; Vignault, 2019). These compounds are present especially in red wines and they naturally decrease by polymerization and precipitation during wine maturation and aging (Quijada-Morín et al, 2014; Ramos-Pineda et al., 2017), but their removal by fining speeds up the process of taming an unbalanced astringency and is especially useful for wines, white or red, which are not maturated or aged for long times or at all.

Protein based fining agents, pea protein isolates included, cannot induce all the effects underlined above, but they have important and various effects at the polyphenol compounds level, due to their ability to interact with several phenolic classes in selective ways in accordance to their composition (Río Segade et al., 2020).

Animal protein fining agents, their allergenic potential and vegetal alternatives for wine fining

Proteins of animal origin, such as gelatine, ovalbumin and caseins, are the most regularly used protein-based fining agents for wine, an overview of these agents being synthetically presented by Obreque-Slier et al. in support of their applicative research done in 2023 (Obreque-Slier et al., 2023). Gelatine of porcine origin is frequently used as it is rich in proline and selectively removes tannins with high molecular weight, thus removing up to 20% of the initial tannin (Maury et al., 2001).

Gelatine is most effective in reducing the bitter aftertaste, making wines softer or thinner; casein prevents oxidation in both white and red wines; and albumin is a very good fining agent for tannic red wines (AWRI, 2024; Braga, et al., 2007). Isinglass, protein originating in fish, is also used in white wines to intensify yellow colour (AWRI, 2024).

Despite their long-time application and demonstrated effectiveness, animal-based fining agents could present a risk for individuals with allergies or food intolerances (Peñas et al., 2015) and legislative decisions have been made for their regulation or labelling (Regulation (EU) No 1169/2011; Regulation (EU) 2019/33).

As fining agents are defined as processing aids (Resolution OIV-OENO 567A-2016) and are eliminated themselves through subsequent finings, decanting and filtration operations before wine is bottled, there is a reasonable concern that some small residues may still remain in the wine.

A team of researchers determined in model wines that 24-58% of initial proteins remained after fining, the values varying in accordance with the quantification method used (Maury et al., 2019). But these values were obtained in model wines, not in actual wines, which have a more complex composition and in which many technological operations are applied (fining with bentonite to remove proteins, decanting and filtering). Peñas et al. (2015) showed in their review with data obtained from real wines that all studies used for the review indicated that most wines at bottling time were free from allergenic proteins as residues of allergenic fining agents, but they also showed that in some cases relatively high quantities of especially egg white proteins, as well as some amounts of milk proteins, were still present. Casein is less likely to be found in wines fined with this agent (Restani et al., 2012).

According to Article 51 of Regulation (EU) No. 579/2012 allergenic products which were used for wine treatments, including milk-based and eggs-based products, have to be declared on the label (Commission Implementing Regulation (EU) No 579/2012) if they are detected in the final wine by using the OIV ELISA method OIV-MA-AS315-23 for the quantification of potentially allergenic residues of fining agent proteins (Resolution OIV-Oeno 427-2010 modified by OIV-COMEX 502-2012; Weber et al., 2007). Other methods for simultaneous quantitative detection of protein residues by High-Resolution Mass Spectrometry (Monaci et al., 2013) UPLC-MS/MS (various caseins, α-lactalbumin, β-lactoglobulin, lysozyme, ovalbumin and ovotransferrin) have also been proposed (Yang et al., 2021).

In the light of these drawbacks, alternatives from vegetal sources are actively sought and new products started to be commercially proposed, to be used in various winemaking stages, in doses between 10 and 30 g/hl.

A few licence-protected plant-based products are available for wine fining or wine clarification through flotation, but they are not usually composed only of proteins, although some contain mostly proteins, such as:

- pea proteins: Plantis L (Enartis, Trecate, Italy), Proveget Premium and Proveget 100 (Agrovin, Ciudad Real, Spain), GreenFine® Mix/Rosé, Nature, Must, X-Press, Must-L, Intense etc. (Lamothe Abiet Canéjan, France); Fitoproteina P (Enologica Vason, Verona, Italy), Protein Clair Liquid and Special (LaFood, Fasano, Italy);
- potato proteins/patatins: Plantis® PQ (Enartis, Trecate, Italy), Proveget Fine (Agrovin, Cidad Real, Spain), Vegefine™, Vegeflot™, Oenofine™ Pink, Nature and RedY etc. (Laffort, Floirac, France), Fitoproteina XP (Enologica Vason, Verona, Italy), Protein Clair VP, VP Special, PP (LaFood, Fasano, Italy).

Products containing combinations of several proteins were classified according to the main protein in their composition. The list provided is not exhaustive and is only showing the present state, as products may be discontinued in the future and replaced by other new ones.

However, plant proteins may also generate immune responses in humans, as it is the case of gluten, which has negative effects on people prone to IgE-mediated allergic reactions (Simonato et al., 2001) or suffering from celiac disease (Cohen et al., 2019). For this reason, in spite of some studies showing their technological efficacy, glutens or other wheat proteins were removed by the OIV in 2024 from the list of fining agents approved for wine (Antoce, 2025). Pea protein is approved since 2004 (resolution OIV-OENO 28/2004) as part of the same resolution in which wheat protein was also approved, but later on removed (Resolution OIV-OENO 723-2024).

In compensation, patatin from potato was included since 2013 (resolution OENO 495 - 2013), along with the pea protein, as both have a very low allergenic potential, with very few cases reported. From pea, for example, the protein 7S globulin Pis s1 was found to be potentially allergic for some children (Popp et al., 2020), but not for adults.

Potato and pea proteins rarely induce allergies by themselves, but, because of common IgE epitopes, a cross-reaction cannot be excluded in the case of people sensitized to latex (Schmidt et al., 2002) or legumes (Robinson et al., 2022), such as peanut, soy or lupine.

Proteins from maize, rice, other legumes such as lentil, soybean or faba bean are not commercially available, even though some research shows that some of them have good prospects, with an efficiency comparable with that of the gelatine (Marangon et al., 2019).

Grape seed protein extract (GSPE) with a minimum 40% protein is also under evaluation (Gazzola et al., 2017.). Yeast protein extract is also considered a protein of vegetal origin and is present in various composed fining agents (Gaspar et al., 2019).

Pea protein for wine fining – properties, traits and reaction mechanisms

For the use of pea protein in food there is no quantitative limit, and this is also recognized by its inclusion the FDA's GRAS (Generally Recognized as Safe) database (GRAS Notice No. GRN 000182, 2025).

As a processing aid in wine there are, however, limits set. The OIV recommends that the maximum usage dose to be used for fining be less than 50 g/hl and should be established based on laboratory trials (OIV-International Code of Oenological Practices, 2025). Same dose is also allowed in the USA since 2022, when permission was given for continuing the use of pea protein in wine and grape juice (Alcohol and Tobacco Tax and Trade Bureau, 2025).

The effectiveness of the pea protein depends on the type, but also on the composition of the wine which is fined with it, especially on the polyphenol classes (Segrade et al., 2019).

A drawback of wine fining is the loss of some colour pigments. Proteins interact differently with anthocyanins or with colourless phenolics, plant proteins being more protective of the red wine colour (Gordillo et al., 2021). However, some degree of colour loss is inherently reported when using plant proteins, too. A pea-based protein used in high dose in Primitivo and Montepulciano red wines fining decreased the anthocyanin levels by 7.7% and 3.5%, respectively (Segade et al., 2019). The same study also showed that the colour intensity is affected differently depending on the pea protein type, so that some proteins may not lower visibly the colour intensity, while others can remove also flavonols, beside anthocyanins, contributing further to the loss of colour. The initial quantity and types of anthocyanins and their stability is also a factor in resistance to colour loss due to fining, which makes Syrah wines more resistant to visible colour loss, while Nebiollo wine colour was strongly affected.

Similarly with egg protein, pea protein is able to decrease the Syrah wine colour intensity by 5%, increase lightness by 5%, without significantly affecting the hue measured by CIELab method, indicating that it reduces better the content of copigments rather than other colour components (Gordillo et al., 2021). Other studies showed that while gelatine can remove monomeric anthocyanins and anthocyanin-flavanol copolymers, the pea protein does not do this to a significant degree. Of all monomeric anthocyanins, pea protein influenced most the cyanidin-3-glucoside, but not the other glycosides (Granato et al., 2018)

This can explain also why greater colour loss is observed in varieties richer in cyanidin-3-glucoside, such as Nebiollo and Primitivo (Segrade et al., 2019). Pea protein is selective in removing anthocyanidins. In the young wines pea protein removed about 6% of malvidin derivatives, such as Mv-3-glc (from 51.95 ± 0.20 to 49.18±0.54), Mv-3-acetylglc (from 14.22 ± 0.31 to 13.07 ± 0.14), Mv-3-p-coumglc (from 8.05 ± 0.08 to 7.35 ± 0.18), as well as Pt-3-glc (from 11.16 ± 0.18 to 10.18 ± 0.24), which is similar to the effect of egg albumin on these compounds. But, unlike egg albumin, pea protein also removed as well Dp-3-glc (from 9.88 ± 0.08 to 9.06 ± 0.20) and Cy-3-glc (from 1.32 ± 0.02 to 1.23 ± 0.01) (Gordillo et al., 2021)

The mechanisms involved in these protein-polyphenol binding are determined by the formation of hydrogen bonds and hydrophobic interactions. If the protein concentration is small, the polyphenols cover their surface, lowering their hydrophilic character, which leads to flocculation and precipitation. Conversely, if the protein concentration is high, the protein is covered by phenolic compounds which also lead to precipitation (Ribéreau-Gayon et al., 2021). As compared to plant proteins, gelatine is forming more hydrogen bonds (Zoecklein et al., 1999). Plant proteins have their affinity for combining with polyphenols explained by their high proline content. The proline residues force the protein into a more irregular structure which provides higher accessibility of binding sites which interact with phenolic compounds (Kieserling et al., 2024).

The study made by Granato and their team in 2018 shows that the natural pea protein is not biding very well with proanthocyanidins (tannins) in a red wine, irrespective if the wine is young or aged. This behaviour of the pea protein also correlates with the observations that the hue of the wine colour is not affected, which can be a very good effect sometimes, but which also means that is less effective in removing browning in white wines (Cosme et al., 2012). However, the affinity can be increased if the pea protein is enzymatically hydrolysed to reduce its size, while keeping the hydrophobic binding sites (Granato et al., 2018). Pea protein was similarly effective in removing monomeric and dimeric flavonols, as much as the other commercial fining agents tested, but not as effective as lentil proteins (Granato et al., 2014).

By binding and precipitating polyphenols proteins reduce not only colour, but also the astringency and bitterness induced by certain classes of polyphenols. The perceptions of astringency and bitterness are produced by molecules in the class of flavanols, a group of polyphenols including various compounds, from the monomeric flavan-3-ols to oligomeric flavanols, and to polymeric procyanidins also called condensed tannins.

As pea protein has a limited affinity for tannins, its efficiency is lower than that of the gelatine or potato protein when it comes to removing astringency, but the reduction of astringency is demonstrated to be similar to the one produced by polyvinylpolypyrrolidone (PVPP), a synthetic molecule very efficient for wine fining (Kang et al., 2018). Some studies show that, for removing astringency, potato protein could be a better choice (Gambuti et al., 2012; Gambuti et al., 2016).

For the removing of bitterness, pea protein can be a very good alternative. Segade et al. (2019) showed that pea protein is very effective in reducing polymeric and oligomeric flavanols by 7.1% and 11.1%, respectively, being better for this effect than other fining agents tested. These oligomeric flavanols are highly correlated with the bitterness of the wine (Griffin et al., 2020).

The degree of flavanol polymerization is correlated to the intensity of bitterness and astringency, thus the longer the molecular chain, the less bitterness and the highest the perceived intensity of astringency (Sun et al., 2013).

The effect of pea protein on flavanols depends also on the dose, as well as on the phenolic matrix of the treated wine. In Montepulciano wines Segade et al. (2019) observed a reduction of 7.0% of oligomeric flavanols for a low dose of pea protein used, and a 10.8% reduction in case of a high dose, but no significant reduction of polymeric flavanols, irrespective of the dose. In Syrah, one of the pea proteins tested had a significant effect only on polymeric flavanols, irrespective of the dose, while in Nebiollo another pea protein removed equally oligomeric and polymeric flavanols (Segade et al., 2019). The flavanols composition may also play a role in their interaction with the pea protein, galloylation percentage being cited as a factor of increased biding with proteins (de Freitas, 2012).

The use of pea protein as a fining agent in wine can also have an impact on the flavour, either by removing some of the volatile compounds, but also by contributing some. Pulses and their proteins are known to have a specific flavour, which is not always appreciated by the consumers, and there is a concern that certain flavours can be transferred in the wine during the process of fining. Such compounds identified in the flavour of pulses are the 2-penten-1-ol and 2-octenal (Bi et al., 2020), hexanal and 3-*cis*-hexenal, aldehydes which have a beany, grassy and green-leaf aroma and result from the oxidation of lipids catalysed by the enzyme lipoxygenase (LOX) (Bi et al., 2022; Roland, et al., 2017), as well as 3,5-octadien-2-one, nonanol (Trikusuma et al., 2020). In addition, the presence of some pyrazines in higher content, such 2-methoxy-3-isopropyl-(5 or 6)-methyl pyrazine (Zhang et al., 2020; Trikusuma et al., 2020) lead to pea off-flavours of earthy type. Many other substances are cited in the literature as participating to the formation of the typical off-flavour of pea (Karolkowski, et al., 2021; Murray et al., 1976; Murray et al., 1970), but all these substances are in small quantities and were not reported so far to determine an off flavour in the wines treated with pea protein or hydrolysates, although this possibility cannot be excluded. As well, there may also be some non-volatile compounds that could be transferred to wine, for example caffeic acid or saponins, which are known to be present in the pea flour or extracts and confer a bitter taste (Curl et al., 1985).

Conversely, pea protein added in must or wine may selectively remove volatile compounds, by reversible or non-reversible mechanisms (Bi et al., 2022), with various effects. Low hydrophobicity of certain molecules correlates with low retention by pea protein. While fining agents such as PVPP especially reduce the volatile ethyl esters, pea protein was found to especially reduce the amount of terpenes (Antoce and Cojocaru, 2024), a fact that may affect the aromatic profile of some muscat type-aroma. A study performed on a terpenic variety of grapevine called Tamâioasa românească shows that, when it is applied in must, before fermentation, the effects on volatile profile of pea protein treatments in dose of 20 g/hL are not statistically significant compared to the PVPP, thus making it a good alternative to PVPP in winemaking (Antoce and Cojocaru, 2024). Further research is going to be necessary to prove that the application of pea protein for wine fining does not impart unwanted flavours and/or that does not remove key aroma compounds, but the results so far are encouraging. In a study comparing PVVP with pea protein and K-caseinate fining agents it was showed that, from the viewpoint of the sensory effect, there were no significant differences (Cosme, 2012).

Other properties of pea protein and hydrolysates may also be of interest for the application in wine fining, such as their solubility or foaming ability. As it is the case with all proteins, the solubility depends on the pH of the media, being the least soluble at the isoelectric points, which for pulse proteins are between pH 4 and 6 (Ma et al., 2022), values which are, in general, above those found in wines. Granato (2014) experimented with the use of insoluble protein isolates from pea for fining white wines, as insolubility is an important trait of fining agents which must be separated from the wine after the treatment, by decanting or filtration. Foaming properties were mostly studied in connection with the preparation of other foods (Ma et al., 2022), but for the compounds based on pea protein destined to be used in the technique of wine flotation, foaming could also be relevant.

Other issues which have not yet been addressed in the scientific literature are the influence of fining must or wine with pea protein on the microorganisms (some of which are useful in alcoholic or malolactic fermentation, or undesirable such as spoilage lactic or acetic bacteria or Brettanomyces yeasts). Also, it may be of interest to determine if soluble peptides could be formed through protein hydrolysis, which might remain in wine after fining.

While the over-fining phenomenon, when too much fining agent remaining in the final product also creates turbidity, is not reported to be associated with the use of pea protein, it should also be of interest to check the compactness of the formed deposit, so that not too much wine is caught in the lees.

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EFFECTS OF TILLAGE AND NITROGEN RATE ON SUNFLOWER IN THE SPECIFIC CONDITIONS FROM DANUBE MEADOW

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Abstract

Within sunflower crop technology, tillage and nitrogen rate are among the most important factors significantly affecting the grain yield and yielding elements. Therefore, this paper aims to present the results of the performed research regarding the effects of different tillage and nitrogen rates on sunflower grain yield and yielding elements in the specific growing conditions of Danube Meadow from South Romania. In this respect, the research was performed in the years 2023 and 2024, in field experiments under rainfed conditions located in the Danube Meadow from South Romania. The experimental factors were Tillage methods, with 5 variants (a1. Plowing performed with a 4-furrow reversible plough at depth of 25 cm + 2 passes with a disc harrow; a2. Scarifying at a depth of 35 cm + 2 passes with a disc harrow; a3. Cultivation with Gruber Tiger cultivator at a depth of 25 cm; a4. Cultivation with Gruber Tiger cultivator at a depth of 15 cm; a5. Disc harrow at a depth of 15 cm - 2 passes), and Nitrogen rate, with 4 variants (b1. N0 - unfertilized; b2. 60 kg/ha; b3. 90 kg/ha; b4. 120 kg/ha). The obtained results highlighted the better values of the grain yield and yielding elements at deep and reduced tillage (scarifying at 35 cm + 2 disc harrows and Gruber Tiger at 25 cm) in favourable climatic conditions, while in the context of less favourable climatic conditions the best results were obtained in the context of shallow and reduced tillage of Gruber Tiger at 15 cm (2 passes). The obtained results showed that the values for all the analysed yield elements and the grain yield constantly increased with increasing the nitrogen rate from 0 to 60, respectively to 90 and 120 kg/ha, regardless of tillage method and the climatic conditions of the year.

Key words: *sunflower, tillage, nitrogen rate, grains yield, yielding elements.*

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is the most important oil crop, with more than 1 million ha cultivated annually. Sunflower is a versatile crop, this characteristic being given both by the multiple uses of the crop and by the possibility of cultivating it in different environmental and technological conditions (Ion, 2021).

Considerable research has been performed on different tillage systems in agricultural production methods and tillage systems in sunflower and other crop (Sessiz et al., 2008). Sunflower cultivation efficiency is determined by a correct selection of agrotechnical measures applied from soil tillage after previous crop to sunflower sowing (Aksyonov, 2010). Information on soil tillage system is necessary in sunflower cropping for maximize and improve the quality and the level of yield (Petcu et al., 2020).

Within the framework of crop technologies, soil tillage represents an important link and therefore it is necessary to be carried out in the best conditions (Khan et al., 2021). Thus, the farmer must know some particularities and type of soil, the presence of problem weeds, as well as some characteristics of the crops, in order to develop tillage methods, the necessary equipment and execution indices (Carr et al., 2013).

In Europe, there has been a significant change in the way tillage is approached in recent years, this change being due to a growing awareness among farmers, politicians and society as a whole that soil is not a renewable resource in itself (Sojnóczki et al., 2023).

Depending on the crops and local conditions, several tillages can be performed. In addition to the classic tillage system based on ploughing, in order to save energy embedded in field crop technology, to better water conservation in the soil, and to avoid repeated passes with tractors and agricultural machinery, with negative effects on the soil, more and more farmers are accustomed to use a minimum tillage system.

Compared to conventional tillage, zero or reduced or minimum tillage facilitates timely sowing, may increase yield, and reduce production costs and boosts farm income, but on

the other hand, weeds are a major constraint in maize production in reduced tillage practices (Samrat et al., 2021).
Conservation tillage has attracted increased attention in recent years due urgent needs for erosion control and water conservation in various geographic regions of the world (Deng et al., 2022). Soil and water conservation-oriented tillage methods include conservation tillage, strip tillage, and mulch tillage (Galzki et al., 2011).
In many countries all over the world, the increase in fertilizer price in combination with the increasing cost of fossil fuels has caused producers to consider conservation agriculture, which includes lower impact cultivation practices such as no tillage cultivation, permanent crop residues on the surface, and crop rotation (Blanco-Sepúlveda et al., 2021; Wolschick et al., 2021). Conservation tillage is able to significantly improve soil properties (physical, biological, and chemical) and other biotic factors, reduce soil erosion, improve the water infiltration, and help in the reduction of the production costs (Pittelkow et al., 2015; Giller et al., 2015; Tarolli et al., 2019).
Although it is a large consumer of nutrients, sunflower utilizes fertilizers less well than wheat or other plants, which is largely due to the high capacity of its root system to extract the necessary nutrients, even those that are poorly soluble, from a deep soil profile (Vrânceanu, 2000). The absorption of nutrients is rapid in sunflower, in relation to the rate of dry matter production during the early stages of development (Bîrnaure, 1991). Sunflower is sensitive to both nitrogen deficiency and excess, especially in the early stages, which will have negative repercussions on the development and growth processes, and of course, on grain yield.
On a global scale, nitrogen is the most used fertilizer nutrient in agriculture. Studies have shown that cultivated plant species use only about 50% of applied N effectively, while the rest is lost through various pathways to the environment (Govindasamy et al., 2023).
Nitrogen is the major nutrient required by sunflowers, and has the greatest impact on seed size, leaf size and number of leaves, test weight and yield (Toosi & Azizi, 2014). The large variation in the response of sunflower to nitrogen fertilization indicates the need for studies to better adjust the optimum levels of this nutrient for production conditions (Coêlho et al., 2022).
This paper aims to present the results of the performed research regarding the effects of different tillage and nitrogen rates on sunflower grain yield and yielding elements in the specific growing conditions of Danube Meadow from South Romania.

MATERIALS AND METHODS

Research was performed in field experiments under rainfed conditions in years 2023 and 2024. The field experiment was located in the Danube Meadow from South Romania, respectively near Oltenița city from Călărași County.
The relief in the area of research bears the imprint of meadow characteristics. This is characterized by a horizontal plane, with a land slope between 0.5 and 2%.
The soil is of alluvial type, and has a medium to heavy texture, an upper horizon of 20-35 cm thick, a dark brown - yellowish colour, and a granular structure.
The temperature values recorded both in 2023 and 2024 are notable for exceeding values in summer time by over 2-3ºC compared to the average for the area (27ºC) (Figure 1). The maximum recorded value was 42.2ºC on August 2024, and the minimum recorded value was -2.9ºC on February 17, 2023. The year 2024 was warmer than the year 2023, especially in the summer and winter seasons.

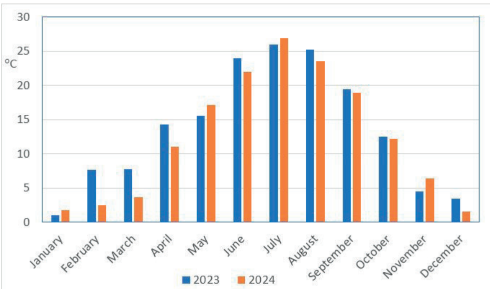

Figure 1. Temperatures in experimental field (2023-2024)

Regarding rainfall, there was a major water deficit in February, but a rainy period in April, while the summer months had small rainfall (Figure 2). From May to July, when sunflower plants are in the most sensitive period to water stress, the year 2024 was droughter than 2023.

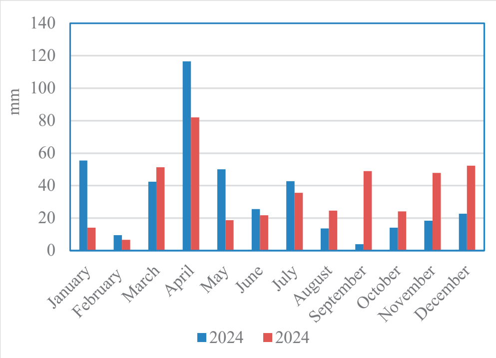

Figure 2. Rainfall in experimental field (2023-2024)

The field experiment was based on the method of subdivided plots into 3 replications, having two experimental factors, respectively:

- Factor A: Tillage method, with 5 variants:
  - a1. Plowing performed with a 4-furrow reversible plough at depth of 25 cm + 2 passes with a disc harrow (Control variant);
  - a2. Scarifying with Artiglio Scarifier at a depth of 35 cm + 2 passes with a disc harrow;
  - a3. Cultivation with Gruber Tiger cultivator at a depth of 25 cm;
  - a4. Cultivation with Gruber Tiger cultivator at a depth of 15 cm;
  - a5. Disc harrow with Horsh Joker Disc at a depth of 15 cm (2 passes).
- Factor B: Nitrogen rate, with 4 variants:
  - b1. N0 - unfertilized (Control variant);
  - b2. 60 kg/ha;
  - b3. 90 kg/ha;
  - b4. 120 kg/ha.

Each experimental variant had 120 m^2, resulting from 20 m length and 6 m width.

The tillage according to the classic system (plowing and scarifying) took place in the previous fall, and the minimum tillage (Gruber Tiger 15 cm, Gruber Tiger 25 cm, Disc harrow (2 passed) were performed in the spring.

In all experimental variants, except for Control variant, before seedbed preparation there was applied the complex fertilizer 16:16:16 in a rate of 250 kg/ha, assuring 40 kg/ha as active substance of N, P_2O_5 and K_2O. In May, the nitrogen rate according to the experimental variant was assured by the second fertilization applying the liquid fertilizer UAN (Urea Ammonium Nitrate Solution) containing 32% nitrogen. Thus, in 2023, on 19th of May, and two weeks early in 2024, on 5th of May, there was applied 62,5 l/ha of UAN for the variant b2, 156 l/ha of UAN for the variant b3, and 250 l/ha of UAN for the variant b4.

The sunflower hybrid was Sumiko (resistant to Express herbicide) and the previous plant was maize.

The sowing was carried out on 3rd of May in 2023 and 2 weeks early in 2024, respectively on 20th of April. The sowing was carried out with a John Deer tractor and a Gaspardo Maestro seeder with 8 rows, at a depth of 5-6 cm, a distance between rows of 70 cm and a sowing density of 65,000 germinating grains/ha.

The control of annual and perennial dicotyledonous weeds (including *Xanthium* spp., *Cirsium arvense, Datura stramonium, Solanum nigrum*) was performed by applying the herbicide Express 50 SG (Tribenuron-methyl 500 g/kg) in a rate of 30 g/ha + 250 ml/ha of Trend 90 adhesive. The herbicide was applied in the sunflower plant growing stage of 4-6 leaves (BBCH 14-16), when the weeds were in the 2-4 leaf phase, and the *Cirsium arvense* had maximum 10 cm high.

Against *Sorghum halepense* and other annual monocotyledonous weeds (*Setaria* spp., *Echinochloa crus-galli, Digitaria sanguinalis*), there was used the herbicide Pantera (Quizalofop-P-tefuryl 40 g/l) in a rate of 1 l/ha, applied in the weed stage of 1-3 leaves.

Against pathogens (*Alternariaster helianthi*, *Diaporthe helianthi*, *Sclerotinia sclerotiorum, Botrytis cinerea, Plenodomus lindquistii*), Pictor fungicide (Boscalid 200 g/l + Dimoxystrobin 200 g/l) was used in a rate of 0.5 l/ha in the sunflower plant stage of 6-8 leaves (BBCH 16-18). The treatment was repeated in the flower bud phase.

Harvesting was carried out with the New Holland CR9090 combine on 10th-20th of August (2023-2024).

For each experimental variant, the following determinations were performed at harvest: plant density; the height of plants; the head diameter (cm); the number of grains per head; the grain weight per head (g); Thousand Grains Weight - TWG (g); grain yield (kg/ha).

RESULTS AND DISCUSSIONS

Tillage effects on sunflower grain yield and yielding elements

In the case of the tillage influence on the plant density at harvest, it can be seen from Table 1 that in the case of variants with ploughing, Gruber Tiger (25 cm) and scarifying, there were registered the best plant density in the year 2023, with over 59,000 plants/ha. In the year 2024, the highest plant density was recorded at variant with Gruber Tiger (15 cm), with over 60,000 plants/ha, with close values for the variant with disc harrow. So, in the more favourable climatic conditions of the year 2023, the highest plant densities were registered in the case of deep tillage, while in the less favourable climatic conditions of the year 2024, the highest plant densities were registered in the case of shallow tillage. Plowing was to extremes, with highest plant density in the year 2023, but with the smallest plant density in 2024.

Table 1. Soil tillage effect on sunflower plant density at harvest

Tillage	Plant density (no/ha)		
	2023	2024	*Average*
Plowing (25 cm + 2 passes with disc harrow)	59422	52025	*55724*
Scarifying (35 cm + 2 passes with disc harrow)	59136	53589	*56363*
Gruber Tiger (25 cm)	59375	55276	*57326*
Gruber Tiger (15 cm)	55643	60243	*57943*
Disc harrow (15 cm x 2 passes)	55541	58742	*57142*
Average	*57823*	*55975*	*56900*

As in the case of the plant density, the plant height registered the highest values in 2023 at the variants with ploughing, Gruber Tiger (25 cm) and scarifying, with values over 170 cm (Table 2). In 2024, the highest values were registered at the variants with Gruber Tiger at 25 and 15 cm. Again, in the more favourable climatic conditions of the year 2023, the highest plant heights were registered in the case of deep tillage, while in the less favourable climatic conditions of the year 2024, the highest plant heights were registered in the case of shallow tillage. Plowing was giving the smallest value in the case of the year 2024.

Table 2. Soil tillage effect on sunflower plant height

Tillage	Plant height (cm)		
	2023	2024	*Average*
Plowing (25 cm + 2 passes with disc harrow)	170.9	156.7	*163.8*
Scarifying (35 cm + 2 passes with disc harrow)	174.3	162.6	*168.5*
Gruber Tiger (25 cm)	173.2	165.9	*169.5*
Gruber Tiger (15 cm)	164.4	164.7	*164.5*
Disc harrow (15 cm x 2 passes)	168.3	162.8	*165.5*
Average	*170.2*	*162.5*	*166.4*

The head diameter registered values over 17 cm to all experimental variants in the year 2023, except for the variant with disc harrow, where the hear diameter was of 16.6 cm (Table 3). In the year 2024, only variant with Gruber Tiger at 15 cm gave a head diameter over 16 cm (16.8 cm). As average values, the highest value of the head diameter was registered at the variant with Gruber Tiger at 15 cm (17.1 cm), while the smallest value was registered at the variant with disc harrow (15.9 cm).

Table 3. Soil tillage effect on sunflower head diameter

Tillage	Head diameter (cm)		
	2023	2024	*Average*
Plowing (25 cm + 2 passes with disc harrow)	17.0	15.2	*16.1*
Scarifying (35 cm + 2 passes with disc harrow)	17.3	15.2	*16.2*
Gruber Tiger (25 cm)	17.5	15.1	*16.3*
Gruber Tiger (15 cm)	17.3	16.8	*17.1*
Disc harrow (15 cm x 2 passes)	16.6	15.3	*15.9*
Average	*17.1*	*15.5*	*16.3*

The number of grains per head registered the highest values in the year 2023 in the case of tillage reduced and deep variant (scarifying and Gruber Tiger at 25 cm), while in the year 2024 the tillage variant with Gruber Tiger at 15 cm gave the highest number of grains per head (Table 4). The smallest value of the number of grains per head were registered in the year 2023 for the tillage variant with disk harrow (1305 grains per head), while in the year 2024 it was obtained in the case of variant with plowing (1034 grains per head).

Table 4. Soil tillage effect on number of grains per head

Tillage	Number of grains per head		
	2023	2024	*Average*
Plowing (25 cm + 2 passes with disc harrow)	1387	1034	*1211*
Scarifying (35 cm + 2 passes with disc harrow)	1419	1079	*1249*
Gruber Tiger (25 cm)	1401	1057	*1229*
Gruber Tiger (15 cm)	1368	1135	*1252*
Disc harrow (15 cm x 2 passes)	1305	1082	*1194*
Average	*1376*	*1077*	*1227*

The grain weight per head registered the highest value in the year 2023 in the case of tillage variant with scarifying (60.67 g), while in the year 2024 it was obtained at the tillage variant with Gruber Tiger at 15 cm (47.25 g), this variant standing out with a good value also in the year 2023 (Table 5). As in the case of the number of grains per head, the smallest value of the grain weight per head were registered in the year 2023 for the tillage variant with disk harrow (53.77 g), while in the year 2024 it was obtained in the case of variant with plowing (37.65 g).

Table 5. Soil tillage effect on grain weight per head

Tillage	Grain weight per head (g)		
	2023	2024	*Average*
Plowing (25 cm + 2 passes with disc harrow)	56.77	37.65	*47.21*
Scarifying (35 cm + 2 passes with disc harrow)	60.67	38.42	*49.55*
Gruber Tiger (25 cm)	58.15	39.32	*48.74*
Gruber Tiger (15 cm)	59.17	47.25	*53.21*
Disc harrow (15 cm x 2 passes)	53.77	40.11	*46.94*
Average	*57.71*	*40.55*	*49.13*

TGW registered values above 40 g in the year 2024 for all the tillage variants expect for the variant with disk harrow with 39.61 g (Table 6). In the year 2024, the highest value of TGW was registered in the case of variant with Gruber Tiger at 15 cm, this being the only variant with more than 40 g (42.46 g). In the year 2024, the smallest value of TGW was registered in the case of variant with plowing (36.06 g).
The grain yield registered the highest values in the year 2023 in the case of reduced and deep tillage variant (scarifying with 3616 kg/ha and Gruber Tiger at 25 cm with 3492 kg/ha), while in the year 2024 the tillage variant with Gruber Tiger at 15 cm gave the highest grain yield (2921 kg/ha) (Table 7). The smallest value of the grain yield was registered in the year 2023 for the tillage variant with disk harrow (3177 kg/ha), while in the year 2024 it was obtained in the case of variant with plowing (1939 kg/ha, this tillage variant being the only one with less than 2000 kg/ha). It has to be underline that in the favourable climatic conditions of the year 2023, all the tillage variants gave grain yield above 3000 kg/ha.
The less favourable climatic conditions of the year 2024 gave an average grain yield with about 1000 kg/ha compared to the year 2023.

Table 6. Soil tillage effect on TGW

Tillage	TGW (g)		
	2023	2024	*Average*
Plowing (25 cm + 2 passes with disc harrow)	40.77	36.06	*38.42*
Scarifying (35 cm + 2 passes with disc harrow)	42.61	36.27	*39.44*
Gruber Tiger (25 cm)	41.18	37.43	*39.31*
Gruber Tiger (15 cm)	40.30	42.46	*41.38*
Disc harrow (15 cm x 2 passes)	39.61	38.15	*38.88*
Average	*40.89*	*38.07*	*39.49*

Table 7. Soil tillage effect on sunflower grain yield

Tillage	Sunflower grain yield (kg/ha)		
	2023	2024	*Average*
Plowing (25 cm + 2 passes with disc harrow)	3388	1938	*2663*
Scarifying (35 cm + 2 passes with disc harrow)	3616	2098	*2857*
Gruber Tiger (25 cm)	3492	2191	*2842*
Gruber Tiger (15 cm)	3312	2921	*3117*
Disc harrow (15 cm x 2 passes)	3177	2469	*2823*
Average	*3397*	*2323*	*2860*

Nitrogen rate effects on sunflower grain yield and yielding elements
The obtained results showed that the values for all the analysed yield elements and the grain yield constantly increased with increasing the nitrogen rate from 0 to 60, respectively to 90 and 120 kg/ha, regardless of the climatic conditions of the year (Tables 8-14).

Table 8. Nitrogen rate effect on sunflower plant density at harvest

Nitrogen rate (kg/ha)	Plant density (no/ha)		
	2023	2024	*Average*
N0	54228	53639	*53933*
N60	57071	55828	*56450*
N90	59400	57343	*58372*
N120	60595	57090	*58843*
Average	*57823*	*55975*	*56900*

Table 9. Nitrogen rate effect on sunflower plant height

Nitrogen rate (kg/ha)	Plant height (cm)		
	2023	2024	*Average*
N0	157.0	152.0	*154.5*
N60	164.9	159.9	*162.4*
N90	170.9	167.1	*169.0*
N120	188.1	171.1	*179.6*
Average	*170.2*	*162.5*	*166.4*

Table 10. Nitrogen rate effect on sunflower head diameter

Nitrogen rate (kg/ha)	Head diameter (cm)		
	2023	2024	*Average*
N0	15.9	14.4	*15.2*
N60	17.3	15.4	*16.3*
N90	17.4	15.8	*16.6*
N120	17.9	16.5	*17.2*
Average	*17.1*	*15.5*	*16.3*

Table 11. Nitrogen rate effect on number of grains per head

Nitrogen rate (kg/ha)	Number of grains per head		
	2023	2024	*Average*
N0	1144	941	*1043*
N60	1370	1050	*1210*
N90	1474	1093	*1284*
N120	1516	1226	*1371*
Average	*1376*	*1077*	*1227*

Table 12. Nitrogen rate effect on grain weight per head

Nitrogen rate (kg/ha)	Grain weight per head (g)		
	2023	2024	*Average*
N0	42.51	36.61	*39.56*
N60	55.54	39.14	*47.34*
N90	61.64	40.57	*51.11*
N120	71.14	45.87	*58.51*
Average	*57.71*	*40.55*	*49.13*

Table 13. Nitrogen rate effect on TGW

Nitrogen rate (kg/ha)	TGW (g)		
	2023	2024	*Average*
N0	37.27	37.84	*37.56*
N60	40.41	38.02	*39.22*
N90	41.44	37.26	*39.35*
N120	44.45	39.16	*41.81*
Average	*40.89*	*38.07*	*39.49*

Table 14. Nitrogen rate effect on sunflower grain yield

Nitrogen rate (kg/ha)	Grain weight (kg/ha)		
	2023	2024	*Average*
N0	2347	1919	*2133*
N60	3333	2230	*2782*
N90	3592	2357	*2975*
N120	4315	2787	*3551*
Average	*3397*	*2323*	*2860*

Tillage and nitrogen rate effects on sunflower grain yield and yielding elements

In the year 2023, tillage methods combined with nitrogen rate led to a variation of the plant density at harvest from 51237 to 61904 plants/ha, while in the year 2024 this variation was between 50000 to 62357 plants/ha (Table 15). In the year 2023, regardless of tillage variant the plant density increased with nitrogen rate increase, but in the less favourable climatic conditions of the year 2024 this increase was just up to the nitrogen rate of 60 kg/ha, expect for the tillage variants with Grube Tiger at 25 cm and disc harrow.

Table 15. Soil tillage and nitrogen rate effect on sunflower plant density at harvest

Tillage methods	Nitrogen rate	Plant density (no of plants/ha)		
		2023	2024	*Average*
Plowing (25 cm + 2 passes with disc harrow)	N0	57737	50531	*54134*
	N60	58309	54523	*56416*
	N90	59737	53046	*56392*
	N120	61904	50000	*55952*
	Average	*59422*	*52025*	*55724*
Scarifying (35 cm + 2 passes with disc harrow)	N0	54047	53643	*53845*
	N60	59523	54404	*56964*
	N90	61309	54071	*57690*
	N120	61666	52237	*56952*
	Average	*59136*	*53589*	*56363*
Gruber Tiger (25 cm)	N0	54047	50777	*52412*
	N60	60119	51762	*55941*
	N90	61429	58329	*59879*
	N120	61904	60237	*61071*
	Average	*59375*	*55276*	*57326*
Gruber Tiger (15 cm)	N0	54071	58071	*56071*
	N60	55357	62357	*58857*
	N90	56071	59471	*57771*
	N120	57071	61071	*59071*
	Average	*55643*	*60243*	*57943*
Disc harrow (15 cm x 2 passes)	N0	51237	55171	*53204*
	N60	52047	56095	*54071*
	N90	58452	61799	*60126*
	N120	60429	61904	*61167*
	Average	*55541*	58742	*57142*

In the year 2023, tillage methods combined with nitrogen rate led to a variation of the plant height from 151.3 to 205 cm, while in the year 2024 this variation was between 145 to 176 cm

(Table 16). Generally, regardless of tillage variant and climatic conditions of the year, the plant height increased with nitrogen rate increase.

Table 16. Soil tillage and nitrogen rate effect on sunflower plant height

Tillage methods	Nitrogen rate	Plant height (cm)		
		2023	2024	*Average*
Plowing (25 cm + 2 passes with disc harrow)	N0	165.0	145.0	*155.0*
	N60	164.3	154.3	*159.3*
	N90	181.7	161.7	*171.7*
	N120	172.7	165.7	*169.2*
	Average	*170.9*	*156.7*	*163.8*
Scarifying (35 cm + 2 passes with disc harrow)	N0	160.0	153.0	*156.5*
	N60	168.3	158.3	*163.3*
	N90	169.0	169.0	*169.0*
	N120	200.0	170.0	*185.0*
	Average	*174.3*	*162.6*	*168.5*
Gruber Tiger (25 cm)	N0	155.0	155.0	*155.0*
	N60	164.3	164.3	*164.3*
	N90	168.3	168.3	*168.3*
	N120	205.0	176.0	*190.5*
	Average	*173.2*	*165.9*	*169.5*
Gruber Tiger (15 cm)	N0	153.7	155.7	*154.7*
	N60	160.7	161.7	*161.2*
	N90	160.0	168.0	*164.0*
	N120	183.3	173.3	*178.3*
	Average	*164.4*	*164.7*	*164.5*
Disc harrow (15 cm x 2 passes)	N0	151.3	151.3	*151.3*
	N60	166.7	160.7	*163.7*
	N90	175.7	168.7	*172.2*
	N120	179.3	170.3	*174.8*
	Average	*168.3*	*162.8*	*165.5*

In the year 2023, tillage methods combined with nitrogen rate led to a variation of the head diameter from 15.2 to 18.6 cm, while in the year 2024 this variation was between 13.7 to 17.8 cm (Table 17). Generally, regardless of tillage variant and climatic conditions of the year, the head diameter increased with nitrogen rate increase.

In the year 2023, tillage methods combined with nitrogen rate led to a variation of the number of grains per head from 974 to 1591, while in the year 2024 this variation was between 828 to 1362 (Table 18). Generally, regardless of tillage variant and climatic conditions of the year, the number of grains per head increased with nitrogen rate increase.

In the year 2023, tillage methods combined with nitrogen rate led to a variation of the grain weight per head from 36.65 to 77.61 g, while in the year 2024 this variation was between 32.65 to 62.28 g (Table 19). Generally, regardless of tillage variant and climatic conditions of the year, the grain weight per head increased with nitrogen rate increase.

Table 17. Soil tillage and nitrogen rate effect on sunflower head diameter

Tillage methods	Nitrogen rate	Head diameter (cm)		
		2023	2024	*Average*
Plowing (25 cm + 2 passes with disc harrow)	N0	16.1	14.4	*15.3*
	N60	17.2	15.0	*16.1*
	N90	17.0	15.3	*16.1*
	N120	17.7	16.0	*16.8*
	Average	*17.0*	*15.2*	*16.1*
Scarifying (35 cm + 2 passes with disc harrow)	N0	16.7	14.7	*15.7*
	N60	16.9	14.9	*15.9*
	N90	17.5	15.2	*16.4*
	N120	18.1	16.1	*17.1*
	Average	*17.3*	*15.2*	*16.2*
Gruber Tiger (25 cm)	N0	15.9	14.2	*15.0*
	N60	17.5	14.8	*16.1*
	N90	17.9	15.3	*16.6*
	N120	18.6	16.3	*17.4*
	Average	*17.5*	*15.1*	*16.3*
Gruber Tiger (15 cm)	N0	15.2	15.2	*15.2*
	N60	17.8	16.8	*17.3*
	N90	17.9	17.5	*17.7*
	N120	18.3	17.8	*18.1*
	Average	*17.3*	*16.8*	*17.1*
Disc harrow (15 cm x 2 passes)	N0	15.4	13.7	*14.6*
	N60	17.3	15.3	*16.3*
	N90	16.9	15.9	*16.4*
	N120	16.9	16.1	*16.5*
	Average	*16.6*	*15.3*	*15.9*

Table 18. Soil tillage and nitrogen rate effect on number on grains per head

Tillage methods	Nitrogen rate	Number of grains/head		
		2023	2024	*Average*
Plowing (25 cm + 2 passes with disc harrow)	N0	1195	988	*1092*
	N60	1362	995	*1179*
	N90	1494	1023	*1259*
	N120	1496	1129	*1313*
	Average	*1387*	*1034*	*1211*
Scarifying (35 cm + 2 passes with disc harrow)	N0	1360	926	*1143*
	N60	1312	1045	*1179*
	N90	1474	1127	*1301*
	N120	1528	1219	*1374*
	Average	*1419*	*1079*	*1249*
Gruber Tiger (25 cm)	N0	1086	939	*1013*
	N60	1399	1132	*1266*
	N90	1528	1028	*1278*
	N120	1591	1129	*1360*
	Average	*1401*	*1057*	*1229*
Gruber Tiger (15 cm)	N0	1104	1024	*1064*
	N60	1397	1030	*1214*
	N90	1409	1124	*1267*
	N120	1562	1362	*1462*
	Average	*1368*	*1135*	*1252*
Disc harrow (15 cm x 2 passes)	N0	974	828	*901*
	N60	1380	1046	*1213*
	N90	1463	1163	*1313*
	N120	1402	1292	*1347*
	Average	*1305*	*1082*	*1194*

Table 19. Soil tillage and nitrogen rate effect on grain weight per head

Tillage methods	Nitrogen rate	Grain weight per head (g)		
		2023	2024	*Average*
Plowing (25 cm + 2 passes with disc harrow)	N0	45.01	38.85	*41.93*
	N60	54.74	41.08	*47.91*
	N90	60.96	34.30	*47.63*
	N120	66.37	36.37	*51.37*
	Average	*56.77*	*37.65*	*47.21*
Scarifying (35 cm + 2 passes with disc harrow)	N0	50.98	32.65	*41.82*
	N60	53.38	36.71	*45.05*
	N90	63.13	40.13	*51.63*
	N120	75.17	44.17	*59.67*
	Average	*60.67*	*38.42*	*49.55*
Gruber Tiger (25 cm)	N0	39.92	35.92	*37.92*
	N60	57.55	39.22	*48.39*
	N90	63.28	39.62	*51.45*
	N120	71.85	42.52	*57.19*
	Average	*58.15*	*39.32*	*48.74*
Gruber Tiger (15 cm)	N0	39.99	38.99	*39.49*
	N60	58.10	41.43	*49.77*
	N90	60.96	46.29	*53.63*
	N120	77.61	62.28	*69.95*
	Average	*59.17*	*47.25*	*53.21*
Disc harrow (15 cm x 2 passes)	N0	36.65	36.65	*36.65*
	N60	53.91	37.24	*45.58*
	N90	59.85	42.52	*51.19*
	N120	64.68	44.01	*54.35*
	Average	*53.77*	*40.11*	*46.94*

In the year 2023, tillage methods combined with nitrogen rate led to a variation of the TGW from 36.38 to 49.27 g, while in the year 2024 this variation was between 34.45 to 45.83 g (Table 20).

Table 20. Soil tillage and nitrogen rate effect on TGW

Tillage methods	Nitrogen rate	TGW (g)		
		2023	2024	*Average*
Plowing (25 cm + 2 passes with disc harrow)	N0	37.83	36.31	*37.07*
	N60	40.28	36.32	*38.30*
	N90	40.83	34.45	*37.64*
	N120	44.15	37.15	*40.65*
	Average	*40.77*	*36.06*	*38.42*
Scarifying (35 cm + 2 passes with disc harrow)	N0	37.57	35.32	*36.45*
	N60	40.73	37.18	*38.96*
	N90	42.87	35.65	*39.26*
	N120	49.27	36.91	*43.09*
	Average	*42.61*	*36.27*	*39.44*
Gruber Tiger (25 cm)	N0	36.86	37.93	*37.40*
	N60	41.22	36.65	*38.94*
	N90	41.39	37.39	*39.39*
	N120	45.25	37.73	*41.49*
	Average	*41.18*	*37.43*	*39.31*
Gruber Tiger (15 cm)	N0	36.38	42.4	*39.39*
	N60	40.54	40.38	*40.46*
	N90	41.8	41.23	*41.52*
	N120	42.46	45.83	*44.15*
	Average	*40.30*	*42.46*	*41.38*
Disc harrow (15 cm x 2 passes)	N0	37.72	37.24	*37.48*
	N60	39.29	39.58	*39.44*
	N90	40.3	37.6	*38.95*
	N120	41.12	38.19	*39.66*
	Average	*39.61*	*38.15*	*38.88*

Generally, regardless of tillage variant and climatic conditions of the year, the TGW increased with nitrogen rate increase.

In the year 2023, tillage methods combined with nitrogen rate led to a variation of the grain yield from 1988 kg/ha (for the variant combining disc harrow with 0 kg/ha of nitrogen) to 4660 kg/ha (for the variant combining scarifying with 120 kg/ha of nitrogen), while in the year 2024 this variation was between 1701 kg/ha (for the variant combining disc harrow with 0 kg/ha of nitrogen) to 3813 kg/ha (for the variant combining Gruber Tiger at 15 cm with 120 kg/ha of nitrogen) (Table 21). Generally, regardless of tillage variant and climatic conditions of the year, the grain yield increased with nitrogen rate increase.

Table 21. Soil tillage and nitrogen rate effect on sunflower grain yield

Tillage methods	Nitrogen rate	Grain yield (kg/ha)		
		2023	2024	*Average*
Plowing (25 cm + 2 passes with disc harrow)	N0	2448	1813	*2131*
	N60	3280	1971	*2626*
	N90	3738	1869	*2804*
	N120	4087	2098	*3093*
	Average	*3388*	*1938*	*2663*
Scarifying (35 cm + 2 passes with disc harrow)	N0	2881	1755	*2318*
	N60	3280	2114	*2697*
	N90	3644	2172	*2908*
	N120	4660	2351	*3506*
	Average	*3616*	*2098*	*2857*
Gruber Tiger (25 cm)	N0	2165	1808	*1987*
	N60	3545	2148	*2847*
	N90	3801	2243	*3022*
	N120	4456	2567	*3512*
	Average	*3492*	*2191*	*2842*
Gruber Tiger (15 cm)	N0	2251	2520	*2386*
	N60	3215	2595	*2905*
	N90	3433	2756	*3095*
	N120	4348	3813	*4081*
	Average	*3312*	*2921*	*3117*
Disc harrow (15 cm x 2 passes)	N0	1988	1701	*1845*
	N60	3346	2323	*2835*
	N90	3346	2746	*3046*
	N120	4026	3104	*3565*
	Average	*3177*	*2469*	*2823*

CONCLUSIONS

The obtained results highlighted the better values of the grain yield and yielding elements at deep and reduced tillage (scarifying at 35 cm + 2 disc harrows and Gruber Tiger at 25 cm) in favourable climatic conditions, while in the context of less favourable climatic conditions the best results were obtained in the context of shallow and reduced tillage of Gruber Tiger at 15 cm (2 passes).

The worst results were obtained in the case of tillage with disc harrow at 15 cm (2 passes) in favourable climatic conditions, and in the case of tillage with plowing at 25 cm + disc harrow (2 passes) less favourable climatic conditions.

The obtained results showed that the values for all the analysed yield elements and the grain yield constantly increased with increasing the nitrogen rate from 0 to 60, respectively to 90 and 120 kg/ha, regardless of tillage method and the climatic conditions of the year.

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AFTER EFFECT OF THE HERBICIDE ENVOKE ON THE ROOT WEIGHT AND THE SPROUT WEIGHT OF COTTON SEEDS (*Gossypium hirsutum* L.)

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Abstract

The after effect of the herbicide Envoke on the root weight and the sprout weight of cotton seeds was studied in two cultivars - Chirpan-539 and Helius (Gossypium hirsutum L.). The herbicide was applied during the crop's vegetation, once during the 4-5 leaf stage and twice during the 4-5 leaf and budding stages of the cotton. It was tested at the doses - 10 g.ha^{-1}, 15 g.ha^{-1} and 20 g.ha^{-1}. Seed germination samples taken from cotton plants treated with the herbicide Envoke during the growing season were placed. The root weight and the sprout weight were recorded on the 7th day after the samples were planted. The results obtained from each herbicide variant were compared with those of the two controls - the untreated control and the economic control. The herbicide Envoke, applied during the growing season of the two cotton cultivars - Chirpan-539 and Helius, did not have aftereffect the root weight of the cotton seeds. The herbicide had not negative affect on the sprout weight of the cultivar Helius, but did effect on this indicator of the cultivar Chirpan-539.

Key words*: cotton, herbicides, foliar treatment, cultivars, root weight, sprout weight.*

INTRODUCTION

The Cotton is a crop with a long growing season. Due to its weak competitive ability, it is sensitive to weeding in the early stages of its development (Berger et al., 2015). Then even a lower degree of weeding can affect the growth, the development, the yield, the seed germination properties and the fiber quality (Dogan et al., 2015; Charles & York, 2019). Weed control during the cotton growing season is an important part of cotton production technology (Jabran, 2016).

The problem of primary weed infestation with broadleaf and weeds of the *Poaceae* family, as well as weed infestation with weeds of the *Poaceae* family during the cotton growing season, has been largely solved (Chachalis & Galanis, 2007; Kahramanoglu & Uygur, 2010; Singh et al., 2016; Tariq et al., 2018a,b). Today, in the conventional crop cultivation technology, are a problem the broadleaf weeds during the growing season and the lack of the effective and the selective herbicides to control them. (Stoychev, 2013; Barakova, 2017). A large part of the research conducted with foliar herbicides shows that they exhibit phytotoxic effects on the crop (Montazeri, 2009; Barakova et al., 2018; 2021; Barakova, 2024; 2025). To date, there is little data on whether the herbicides applied during the growing season have an impact on the sowing properties of the cotton seeds. From what has been stated so far, it follows that it is necessary to search for not only efficacy, but also selective vegetation herbicides for the this crop.

The aim of the study is to investigate the aftereffect of the herbicide Envoke on the root weight and sprout weight of cotton seeds in the cultivars Chirpan-539 and Helius (*Gossypium hirsutum* L.).

MATERIALS AND METHODS

Research was conducted during the period 2022-2024. At the Field Crops Institute in Chirpan, under laboratory conditions, the aftereffect of the vegetation herbicide Envoke (trifloxysulforon-sodium) was studied in the cotton cultivars (*Gossypium hirsutum* L.) - Chirpan-539 and Helius.

The herbicide was applied once in the 4-5 leaf stage and twice during the 4-5 leaf and cotton budding stages. The doses in which the herbicide preparation was tested were 10 g.ha^{-1}, 15 g.ha^{-1} and 20 g.ha^{-1}. The variants of the study are listed in Table 1. The herbicide Envoke was applied with the adjuvant

Supersonic - 500 $ml.ha^{-1}$. All variants of the herbicide were applied with a backpack sprayer during the crop growth period – with working solution 300 $l.ha^{-1}$.

Table 1. Variants of the study

Cultivars	Cultivar	Herbicide	Phenological stages during treatment	Tested doses
Cultivars	Chirpan-539	Untreated control		
		Economic control		
		Herbicide	Phenological stages during treatment	Tested doses
		Envoke	4-5 leaf	10 $g.ha^{-1}$
				15 $g.ha^{-1}$
				20 $g.ha^{-1}$
			budding stage	10 $g.ha^{-1}$
				15 $g.ha^{-1}$
				20 $g.ha^{-1}$
			4-5 leaf and budding stages	10 $g.ha^{-1}$ + 10 $g.ha^{-1}$
				15 $g.ha^{-1}$ + 15 $g.ha^{-1}$
				20 $g.ha^{-1}$ + 20 $g.ha^{-1}$
	Helius	Untreated control		
		Economic control		
		Herbicide	Phenological stages during treatment	Tested doses
		Envoke	4-5 leaf	10 $g.ha^{-1}$
				15 $g.ha^{-1}$
				20 $g.ha^{-1}$
			budding stage	10 $g.ha^{-1}$
				15 $g.ha^{-1}$
				20 $g.ha^{-1}$
			4-5 leaf and budding stages	10 $g.ha^{-1}$ + 10 $g.ha^{-1}$
				15 $g.ha^{-1}$ + 15 $g.ha^{-1}$
				20 $g.ha^{-1}$ + 20 $g.ha^{-1}$

The herbicide Envoke was applied against the background of the herbicide combination Dual Gold 960 EC (S-metolachlor) – 1.2 $l.ha^{-1}$ + Smerch 24 EC (oxyfluorfen) – 1.0 $l.ha^{-1}$. It was applied before cotton emergence with a working solution of 400 $l.ha^{-1}$, in order to combat primary proliferation of weeds of the *Poaceae* family and broadleaf weeds.

The untreated control was left without hoeing and without herbicide treatment. The economic control was untreated and the weeds there were removed by three hoeings during the vegetation of the crop.

The root weight and the weight of the sprout of the cotton seeds were studied. The indicators were reported in grams (g). Germination samples of 100 pieces per variant (twenty five samples in one repetition) were set for both cotton cultivars. The seeds were from plants that were treated with the tested doses of the herbicide during the growing season.The root and sprout weights were recorded on the seventh day after the samples had been planted.

The values obtained from the herbicide variants were compared with those of the two controls.

A statistical assessment was made to characterize the representativeness and reliability of the influence of the studied indicators through variance analysis and Fisher's parametric F criterion (Shanin, 1977; Barov, 1982). The ANOVA123 program (Lidanski, 1988) was used when analyzing variance.

RESULTS AND DISCUSSIONS

In the cotton cultivar Chirpan-539, the average root weight for the study period varied from 2.1 g to 2.8 g (Table 2). The reported root weight value for both controls was 2.8 g. The values of the indicator for all variants of the Envoke herbicide are approximately equal to that of the commercial control, and the differences between them are mathematically unproven.

The root weight for the cultivar Helius varies within wider limits – from 2.1 g to 3.3 g. The lowest value of the indicator was measured for the untreated control – 2.1 g. The root weight for the commercial control is 2.5 g. The values of the indicator for the herbicide variants are equal to or exceed the value of the commercial control.

On average, during the study period, the vegetation treatment of the two cotton cultivars with the herbicide Envoke, at the tested doses and different stages of the crop development, did not have aftereffect on the root weight of the cotton seeds.

Table 2. Aftereffect of the herbicide Envoke on the root weight of cotton seeds (2022-2024)

Factor A		Variants of the study			Root weight of cotton seeds, g 2022	2023	2024	Mean
Cultivars	Chirpan-539	Untreated control			2.3	3.2	3.0	2.8
Cultivars	Chirpan-539	Economic control			2.0	3.5	2.8	2.8
Cultivars	Chirpan-539	Factor B						
Cultivars	Chirpan-539	Envoke	4-5 leaf	10 $g.ha^{-1}$	2.2	2.0	2.0	2.1
Cultivars	Chirpan-539	Envoke	4-5 leaf	15 $g.ha^{-1}$	2.1	3.0	2.6	2.6
Cultivars	Chirpan-539	Envoke	4-5 leaf	20 $g.ha^{-1}$	1.7	2.9	2.5	2.4
Cultivars	Chirpan-539	Envoke	budding stage	10 $g.ha^{-1}$	2.7	3.0	2.8	2.8
Cultivars	Chirpan-539	Envoke	budding stage	15 $g.ha^{-1}$	2.6	3.0	2.6	2.7
Cultivars	Chirpan-539	Envoke	budding stage	20 $g.ha^{-1}$	2.7	2.6	2.8	2.7
Cultivars	Chirpan-539	Envoke	4-5 leaf and budding stages	10 $g.ha^{-1}$ + 10 $g.ha^{-1}$	2.4	3.0	2.6	2.7
Cultivars	Chirpan-539	Envoke	4-5 leaf and budding stages	15 $g.ha^{-1}$ + 15 $g.ha^{-1}$	2.2	2.7	2.6	2.5
Cultivars	Chirpan-539	Envoke	4-5 leaf and budding stages	20 $g.ha^{-1}$ + 20 $g.ha^{-1}$	2.5	3.0	2.7	2.7
Cultivars	Helius	Untreated control			2.0	2.2	2.0	2.1
Cultivars	Helius	Economic control			2.9	2.0	2.5	2.5
Cultivars	Helius	Factor B						
Cultivars	Helius	Envoke	4-5 leaf	10 $g.ha^{-1}$	2.2	3.6	3.0	2.9
Cultivars	Helius	Envoke	4-5 leaf	15 $g.ha^{-1}$	2.7	3.8	3.4	3.3
Cultivars	Helius	Envoke	4-5 leaf	20 $g.ha^{-1}$	2.7	3.0	3.2	3.0
Cultivars	Helius	Envoke	budding stage	10 $g.ha^{-1}$	2.3	3.0	2.7	2.7
Cultivars	Helius	Envoke	budding stage	15 $g.ha^{-1}$	2.5	2.4	2.6	2.5
Cultivars	Helius	Envoke	budding stage	20 $g.ha^{-1}$	1.7	3.0	2.4	2.4
Cultivars	Helius	Envoke	4-5 leaf and budding stages	10 $g.ha^{-1}$ + 10 $g.ha^{-1}$	2.4	2.8	2.6	2.6
Cultivars	Helius	Envoke	4-5 leaf and budding stages	15 $g.ha^{-1}$ + 15 $g.ha^{-1}$	2.6	3.0	2.8	2.8
Cultivars	Helius	Envoke	4-5 leaf and budding stages	20 $g.ha^{-1}$ + 20 $g.ha^{-1}$	2.3	3.9	3.8	3.3

LSD g:
F.A p≤5%=0.1 p≤1%=0.2 p≤0.1%=0.3
F.B p≤5%=0.4 p≤1%=0.5 p≤0.1%=0.7
AxB p≤5%=0.5 p≤1%=0.7 p≤0.1%=0.9

Regarding the root weight, an analysis of variance was performed (Table 3). It was found that the influence of the variants of the study was 42.9%. It was proven at p≤1%. This on the years of the study was 26.3%, which was proven at p≤0.1%.

The influence of the cultivars, which was 1.4%, and of the variants with the herbicide – 13.2%, was not proven. The interaction of the cultivars with the variants of the herbicide (A×B) was proven – 28.3%, at p≤1%.

Table 3. Analysis of variance for the root weight of cotton seeds

Source of variation	Degrees of freedom	Sum of squares	Influence of factor, %	Mean square	Fisher's criteria	Probability level
Total	65	14.7	100	-	-	-
Years	2	3.8	26.3	1.9	17.9	***
Variants	21	6.3	42.9	0.3	2.7	**
Factor A - Cultivars	1	0.2	1.4	0.2	1.9	ns
Factor B - Variants of the herbicide	10	1.9	13.2	0.1	1.8	ns
AxB	10	4.1	28.3	0.4	3.8	**
Pooled error	42	4.5	30.8	0.1	-	-

*p≤5% **p≤1% ***p≤0.1%

In the cotton cultivar Chirpan-539, the average sprout weight for the study period varied from 13.7 g to 18.0 g (Table 4). The reported sprout weight value in the weeded (i.e. untreated) control was 16.9 g. The obtained value of the indicator from the farm control was 18.0 g. In all variants with the herbicide Envoke, values lower than those of the farm control were reported. This was also established in the individual years of the study.
The sprout weight in the cultivar Helius varied within narrower limits from 13.4 g to 17.2 g. In the untreated control, the measured value of the indicator was 13.4 g. In the economic control, the root weight was 14.3 g. The values of the indicator in the herbicide variants exceeded the value of the economic control.
The application of the herbicide Envoke, during the indicated stages of the cotton development in doses of 10 $g.ha^{-1}$, 15 $g.ha^{-1}$ and 20 $g.ha^{-1}$, had an affect on the sprout weight of the seeds in the cultivar Chirpan-539. The herbicide did not aftereffect on the sprout weight in the cultivar Helius.

Table 4. Aftereffect of the herbicide Envoke on the sprout weight of cotton seeds (2022-2024)

Factor A		Variants of the study			Sprout weight of cotton seeds, g			
					2022	2023	2024	Mean
Cultivars	Chirpan-539	Untreated control			17.6	16.3	16.8	16.9
		Economic control			18.3	17.7	18.0	18.0
		Factor B						
		Envoke	4-5 leaf	10 $g.ha^{-1}$	16.4	18.6	13.2	15.4
				15 $g.ha^{-1}$	16.4	15.6	15.8	15.9
				20 $g.ha^{-1}$	13.1	14.4	13.7	13.7
		Envoke	budding stage	10 $g.ha^{-1}$	15.3	15.8	14.6	15.2
				15 $g.ha^{-1}$	16.6	15.1	16.2	16.0
				20 $g.ha^{-1}$	17.0	15.0	15.2	15.7
		Envoke	4-5 leaf and budding stages	10 $g.ha^{-1}$ + 10 $g.ha^{-1}$	16.3	14.7	15.6	15.2
				15 $g.ha^{-1}$ + 15 $g.ha^{-1}$	16.1	14.4	14.3	14.9
				20 $g.ha^{-1}$ + 20 $g.ha^{-1}$	17.1	13.7	15.4	15.4
	Helius	Untreated control			15.3	11.4	13.5	13.4
		Economic control			19.4	9.3	14.3	14.3
		Factor B						
		Envoke	4-5 leaf	10 $g.ha^{-1}$	17.5	14.8	15.8	16.0
				15 $g.ha^{-1}$	17.1	17.0	17.4	17.2
				20 $g.ha^{-1}$	15.7	18.0	15.4	16.7
		Envoke	budding stage	10 $g.ha^{-1}$	15.7	16.8	16.6	16.4
				15 $g.ha^{-1}$	15.8	14.9	13.8	14.8
				20 $g.ha^{-1}$	15.0	14.8	15.2	15.0
		Envoke	4-5 leaf and budding stages	10 $g.ha^{-1}$ + 10 $g.ha^{-1}$	16.8	14.2	14.6	15.2
				15 $g.ha^{-1}$ + 15 $g.ha^{-1}$	15.1	15.0	14.8	15.0
				20 $g.ha^{-1}$ + 20 $g.ha^{-1}$	18.3	15.0	16.8	16.7

LSD g:
F.A $p \leq 5\% = 0.5$ $p \leq 1\% = 0.6$ $p \leq 0.1\% = 0.8$
F.B $p \leq 5\% = 1.1$ $p \leq 1\% = 1.5$ $p \leq 0.1\% = 1.9$
AxB $p \leq 5\% = 1.6$ $p \leq 1\% = 2.1$ $p \leq 0.1\% = 2.7$

Regarding the sprout weight, from the analysis of variance (Table 5) it was found that the influence of the study variants was the greatest – 59.5%. It was proven at $p \leq 0.1\%$. The years of the study had an influence 10.4%, proven at $p \leq 1\%$. The cultivars influence by 0.6%, but this had not been proven. The influence of the variants with the herbicide was 9.8%, but it had also not been proven. With 49.1%, with a reliability of $p \leq 0.1\%$ of the results obtained, the interaction of the cotton cultivars with the tested variants of the herbicide (A×B) had been proven.

Table 5. Analysis of variance for the sprout weight of cotton seeds

Source of variation	Degrees of freedom	Sum of squares	Influence of factor, %	Mean square	Fisher's criteria	Probability level
Total	65	125.2	100	-	-	-
Years	2	12.9	10.4	6.4	7.2	**
Variants	21	74.5	59.5	3.5	3.9	***
Factor A - Cultivars	1	0.7	0.6	0.7	0.8	ns
Factor B - Variants of the herbicide	10	12.5	9.8	1.2	1.3	ns
AxB	10	61.4	49.1	6.1	5.8	***
Pooled error	42	37.6	30.1	0.8	-	-

*p≤5% **p≤1% ***p≤0.1%

CONCLUSIONS

The vegetation treatment of the cotton cultivars Chirpan-539 and Helius with the herbicide Envoke in doses of 10 $g.ha^{-1}$, 15 $g.ha^{-1}$ and 20 $g.ha^{-1}$ and at different stages of the crop development had no affect on the root weight of the cotton seeds.

An aftereffect of the herbicide Envoke on the sprout weight in the cultivar Chirpan-539 was established.

In cultivar Helius, the tested herbicide did no effect on the sprout weight of the cotton seeds.

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RESEARCH REGARDING THE AMOUNT OF FLOWERS AND AERIAL PARTS OF LAVENDER ACCORDING TO PEDOCLIMATIC CONDITIONS

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Abstract

The present study refers to the amount of fresh flowers (inflorescence) and fresh aerial parts of lavender (herba), depending on the year of cultivation and pedoclimatic conditions. The study was carried out in 3 locations: Deta City (Timiş County), Mailat locality (Arad County) and Vinga locality (Arad County), on different soils. The amount of fresh flowers per plant and per hectare ranged from 70 grams/plant to 1322 kg/hectare at Deta and between 82 grams/plant and 1476 kg/hectare at Vinga, in the second year and between 214 grams/plant and 3638 kg/hectare at Deta and between 245 grams/plant and 4375 kg/hectare at Mailat in the third year. Regarding the amount of fresh herba per plant and per hectare, they ranged from 140 grams/plant to 2380 kg/hectare at Deta and between 162 grams/plant and 2916 kg/hectare at Vinga, in the second year and between 411 grams/plant and 6987 kg/hectare at Deta and between 487 grams/plant and 4696 kg/hectare at Mailat, in the third year.

Key words: *aromatic plant, crop yield, Lavandula sp.*

INTRODUCTION

Lavender is an aromatic shrub of Mediterranean origin, used for therapeutic, ornamental or food purposes, as a spice, but consecrated through its use in the cosmetics and perfume industry, thanks to its high content of essential oils and their quality (Kara & Baydar, 2013; Imbrea et al., 2016; Sönmez et al., 2018; Giray, 2018; Mac & Harris, 2002; Butta et al., 2023).

Lavender flowers are the main source of essential oils, which are rich in compounds such as linalool and linalyl acetate (Zuzarte et al., 2013; Guriță et al., 2019). According to a recent study, these compounds are responsible for lavender's aromatic properties and its therapeutic effects, including anxiolytic, sedative, and antimicrobial activities (Rus, et al., 2016). The flowers are harvested at flowering when the concentration of these volatile compounds reaches its peak, optimizing the yield and quality of the oil (Mason 2014; Lucean et al., 2018).

In addition to oil production, lavender flowers play an essential role in pollination and biodiversity. Recent studies have demonstrated that lavender flowers attract a variety of pollinators, including bees and butterflies, improving the health of the local ecosystem (Espinosa & Bieski, 2014; Passalacqua et al., 2017; Beicu et al., 2023*)*. Additionally, lavender's floral morphology has been linked to drought tolerance, allowing it to thrive in arid climates, as noted (Kazan & Manners, 2011; Hawke, 2017).

Lavender herb, which includes stems, leaves, and flowers, is valued not only for its essential oil but also for its bioactive compounds. A review by Cavanagh and Wilkinson (2002) in Phytotherapy Research highlights the antioxidant, anti-inflammatory, and antifungal properties of the plant, which contribute to its use in traditional medicine to treat conditions such as insomnia, headaches, and skin infections (Upson, 2002; Upson & Andrews, 2004).

From an agronomic point of view, the yield of lavender biomass is crucial for both the production of essential oils and the dry herb market. Other studies indicates that plant yields are influenced by environmental factors such as soil type, irrigation, and climatic conditions, with optimal yields occurring in the second or third year of cultivation (Andrade et al., 1999; Gonciariuc et al., 2019).

MATERIALS AND METHODS

The study was carried out in three locations, namely Deta (Timiș County), Vinga and Mailat (Arad County). Biological material - the general objective of the research aims at the behavior of a variety of *Lavandula angustifolia* in conventional and organic cultivation in terms of the level of flower and herb production/individual obtained in different years. The year 2023 represents the first year of study and is the 2nd year after planting, and the year 2024 represents the 2nd year of study, namely the 3rd year after planting. In the first year after planting, no flowers were harvested from lavender plants to allow the development of the root system of the plant.

To determine the amount of lavender and herb/individual flowers, 25 individuals from each site were sampled and the stems were cut about 5-10 cm above the base of the plant**.** To obtain only the flowers, cut only the tips of the stem 3-5 cm below the inflorescence. The amount of flowers/individuals and the of herba/individuals were weighed, I mention that in 2024 as the plants were mature, the woody stems were removed before weighing. To determine the number of flowers and herba/plant, the number of cuttings per ha was taken into account and the average quantity resulting from the determinations of the 25 individuals was multiplied by the number of cuttings.

The ideal soil for a lavender plantation must be well drained and with a clayey or sandy structure – thus the plantation in Deta, Timiș county is located on a chernozem-type soil, the one in Mailat locality in Arad County on a reddish-brown forest soil and the one in Vinga locality in Arad County on a brown forest soil.

Cultivation technology: at the establishment of the plantations, the soil tillage consisted of an autumn ploughing (basic soil tillage) at a depth of 30 cm, then shredding and leveling works were carried out in order to keep the surface clean of weeds. Before the actual establishment of the plantation (planting), a superficial preparation of the land was carried out with the combiner. Before planting, the land was marked and staked according to the planting scheme established with a planting norm that varied between 17000 and 18000 cuttings per ha. For the establishment of the plantations, the use of cuttings was chosen, knowing that this option ensures a better stability of the crop both in terms of growth and development, as well as its productivity. The planting was carried out manually with cuttings of *Lavandula angustifolia* Vera in the autumn of 2021 in pits with a depth of 15 cm, using trimming as a cleaning process (cutting the tips of the shoots and roots) followed by sludge and then planting. For the maintenance of the crop, between 8 and 10 mechanical pruning between the rows and manual pruning between plants were carried out starting with April of each year.

The harvest was carried out at full flowering when at least 75% of the information was opened - technical ripening in the second half of June, since in this phenophase the volatile oil content is at the highest level. It should be noted that the harvest was carried out in the morning, between 7 and 11 a.m., in sunny weather conditions, without wind, dew or fog, in order to preserve and ensure in optimal parameters, the quantity and quality of the volatile oil in the inflorescences.

RESULTS AND DISCUSSIONS

The results of the research on the number of flowers/plant according to the experimental year are presented in Table 1.

The results of the research on the amount of Flowers/Plant in lavender according to the crop year (Table 1), show a significant increase in the amount of flowers per plant between the crop years 2023 and 2024: the year 2023 - 77g flowers/plant, the year 2024 - 230 g flowers/plant, the difference of 153 g flowers/plant, is distinctly significant.

This significant increase ($p < 0.01$) can be attributed to several factors. First of all, lavender, as a perennial, shows a progressive increase in biomass and flowering capacity as the root system develops and the plant adapts to pedoclimatic conditions. Thus, in the second year of study (2024) year - 3 after planting, the plants benefit from a better developed root system, which allows them a more efficient absorption of nutrients and water, which translates into more abundant flowering.

It is also possible that inter-annual climate variations contributed to this significant difference. Favorable weather conditions in

2024, such as a higher average temperature during the growing season and an optimal rainfall regime, could explain this substantial increase in the amount of flowers.

Statistical tests confirm the robustness of the difference: the value of the significant difference at 5% (LD5% = 30.53) is far exceeded by the observed difference (153 g flowers/plant), emphasizing the importance of the crop year as a determining factor.

Table 1. Amount of flowers/plant depending on the cultivation location

Year of Culture	Flowers/ plant (g)	%	Difference	Significance
A1 – 2023	77	100		
A2 - 2024	230	299	153	**
Dl 5%= 30.53, Dl 1%= 70.41, Dl01%= 224.12				

The amount of flowers/plant depending on the cultivation location is shown in Table 2. The comparative analysis of the three crop localities - Deta, Vinga, and Mailat - showed the following results: Deta (b1): 162 g flowers/plant; Vinga (b2): 156 g flowers/plant; Mailat (b3): 142 g flowers/plant.

Table 2. Amount of flowers/plant depending on the cultivation location

Locality	Flowers/ plant (g)	%	Difference	Meaning
b1 - Deta	142	100		
b2 - Vinga	156	110	14	Ns
b3- Mailat	162	114	20	Ns
Dl 5%= 37.39, Dl 1%= 86.24, Dl01%= 274.49				

TEST DUNCAN - factor B, DL5% = 37.39

```
Date originale                Date sorate
 Mean   1 =   142.0  A   Mean   3 =   162.0  A
 Mean   2 =   156.0  A   Mean   2 =   156.0  A
 Mean   3 =   162.0  A   Mean   1 =   142.0  A
```

Mean 1 – b1 – Deta
Mean 2 – b2 – Vinga
Mean 3 – b3 – Mailat

Although there are numerical variations between localities, they are not statistically significant, as indicated by the Duncan test, where all averages are classified in the same statistical group (A). The differences recorded are below the limit of the significant difference at 5% (LD5% = 37.39), which suggests a homogeneity of edaphic and climatic conditions between the three locations.

This homogeneity is explained by the similarity of the pedological characteristics (soil texture, pH, organic matter content) and climatic characteristics (average temperature, precipitation) of the three localities. At the same time, the applied culture technology was uniform in all three locations, which contributed to reducing the variability between locations.

The contribution of factors A [crop year] and B [crop locality] on the amount of flowers/plant in lavender is shown in Figure 1. The graph of the contribution of the factors reveals the contribution of each factor analyzed to the total variability of the quantity of flowers/plant: Crop year (Factor A): 96.44%; Locality (Factor B): 1.13%; Experimental error: 0.42%.

These results show that the year of cultivation is the main determining factor in the variation of the amount of flowers/plant. Its contribution of 96.44% indicates an overwhelming influence, which is typical for perennial crops in which plant age and annual climatic conditions play a key role.

The locality, with a contribution of only 1.13%, does not exert a significant influence on flower production, which can be interpreted as evidence of lavender's adaptability to a wide range of pedoclimatic conditions or as a consequence of similar soil and climate conditions in the analyzed locations.

The low experimental error value (0.42%) reflects a rigorous experimental design and proper management of variables, ensuring high reliability of the results obtained.

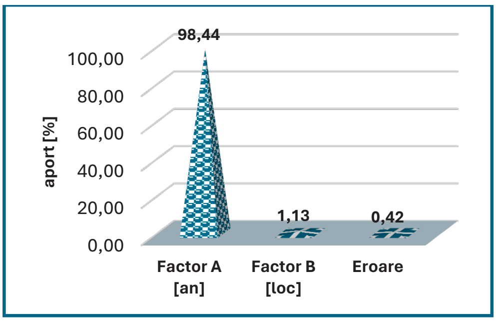

Figure 1. Contribution of factors A [crop year] and B [crop locality] on the amount of flowers/plant in lavender

The results of the research on the amount of herb/plant according to the experimental year are presented in Table 3. The year 2023 represents the first year of study and is the 2nd year after planting, and the year 2024 represents the 2nd year of study, namely the

3rd year after planting. The data indicate a significant increase in the amount of herb harvested per plant between crop years 2023 and 2024: 2023 (a1): 152 g/plant; 2024 (a2): 449 g/plant, the difference of 297 g/plant in the second year of study compared to the first year is statistically ensured as distinctly significant, indicating that the results are not due to chance, but real and measurable factors. The almost threefold increase in the amount of herb/plant between 2023 and 2024 reflects both the positive effect of plant maturation and the favorability of pedoclimatic conditions. Lavender, as a perennial species, presents a natural growth dynamic in which the first 1-2 seasons are intended for stabilization and development of the root system. After this period, the plants reach a maximum production potential.

Table 3. Amount of herb/plant by year of cultivation

Year of Culture	Herb/Plant (g)	%	Difference (g)	Significance
A1 - 2023	152	100		
A2 - 2024	449	295	297	**
Dl 5%= 79.96, Dl 1%= 184.44, Dl01%= 587.04				

In addition to the effect of plant age, the significant increase in biomass in 2024 can be attributed to agronomic and climatic factors such as: favorable weather conditions: a warmer and wetter spring that favored vegetative growth; adaptation of plants to the growing environment: as the plants acclimatize, the yield increases.

The results of the research on the amount of herb/plant according to the cultivation location are presented in Table 4.

Table 4. Amount of herb/plant depending on the cultivation location

Locality	Herb/ Plant (g)	%	Difference (g)	Meaning
b1 – Deta	275.5	100		
b2 - Vinga	305.5	111	30	Ns
b3 – Mailat	320.5	116	45	Ns
Dl 5%= 97.33, Dl 1%= 225.89, Dl01%= 718.98				

The comparative analysis of the three locations (Deta, Vinga, and Mailat) showed the following results: Deta (b1): 275.5 g/plant; Vinga (b2): 305.5 g/plant and Mailat (b3): 320.5 g/plant. The differences observed between localities (30 g between Deta and Vinga and 45 g between Deta and Mailat) are not statistically significant (ns).

Although numerically Mailat recorded the highest production of herb/plant, followed by Vinga and Deta, these differences are statistically insignificant. This suggests a homogeneity of edaphic and climatic conditions between the three locations, and a high adaptability of lavender to various growing environments. Specifically, soil conditions (pH, texture, organic matter content) and climate (average temperature, rainfall regime) are similar between locations. The cultivation technology applied uniformly in all locations (fertilization, irrigation, phytosanitary management) has reduced the variability of production. Lavender exhibits high resilience to minor variations in environmental conditions.

The value of the significant difference at 5% for the locality factor (LD5% = 97.33 g) is considerably higher than the differences recorded between locations (30-45 g). This fact indicates a minor influence of the crop locality on the amount of herb/plant, emphasizing instead the determining role of the crop year and the conditions associated with it.

The results also suggest that the choice of locality has little impact on herba production, meaning that lavender can be successfully grown in a variety of locations, as long as the minimum soil and climate requirements are met.

Given the major influence of the crop year, farmers should focus their efforts on optimizing agrotechnical practices (fertilization, irrigation, disease and pest management) and monitoring climatic conditions to anticipate and adjust technological strategies.

The results of the research demonstrate that the crop year has a significant impact on the production of lavender herb, with a spectacular increase in biomass in the second year of cultivation. The locality of cultivation has a low, statistically insignificant influence, emphasizing the ability of lavender to adapt to a variety of edaphic and climatic conditions.

These findings can guide farm management decisions and help optimize yields in lavender crops, with a focus on adapting crop technologies to climatic conditions and maximizing the potential of plants in the long term.

DUNCAN TEST - factor B, LD5% = 97.93

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Original dates
 Mean 1 = 275.5 A Mean 3 = 320.5 A
 Mean 2 = 305.5 A Mean 2 = 305.5 A
 Mean 3 = 320.5 A Mean 1 = 275.5 A
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Mean 1 – b1 – Deta
Mean 2 – b2 – Vinga
Mean 3 – b3 – Mailat

As a result of the 3 comparisons [C_3^2], only one class A was obtained.

The DUNCAN test is used to compare the means of several groups and to identify significant differences between them. In this case, the analysis compares the amount of herb/plant depending on the locality of cultivation: b1 – Deta: 275.5 g/plant; b2 – Vinga: 305.5 g/plant and b3 – Mailat: 320.5 g/plant. All averages are accompanied by the same statistical letter (A), which indicates that the differences between the locations are not significant at the 5% significance level.

Although numerically there is a slight increase in the amount of herb/plant from Deta to Mailat, this difference is not large enough to be considered statistically significant. The lack of statistical significance indicates that the observed variations can be attributed to natural variation or other uncontrolled factors, rather than to actual differences between locations.

In Deta (275.5 g/plant) it has a slightly lower production compared to Vinga (305.5 g/plant) and Mailat (320.5 g/plant), but the differences are minor and do not reflect a clear agronomic advantage for a certain location. This homogeneity of the results can be interpreted as a high resilience of lavender to minor edaphic and climatic variations between these locations. This result can encourage farmers to adopt sustainable practices without significantly compromising production.

As variations between locations are insignificant, in order to increase productivity, the focus should be on optimising environmental and agrotechnical factors: controlled irrigation and efficient management of water resources; fertilization adapted to the specific needs of lavender and weed control and pest control

The results of the DUNCAN test indicate that the locality of cultivation does not have a significant impact on the amount of herba in lavender, which confirms the ability of this plant to adapt to varied conditions. This suggests that agronomic management strategies and annual (climatic) conditions are the key factors for optimising production.

Based on these results, farmers can consider lavender a flexible and resilient crop with the potential to grow in various environments without major differences in productivity.

The contribution of factors A [crop year], B [crop locality] on the amount of herb/plant is shown in Figure 2.

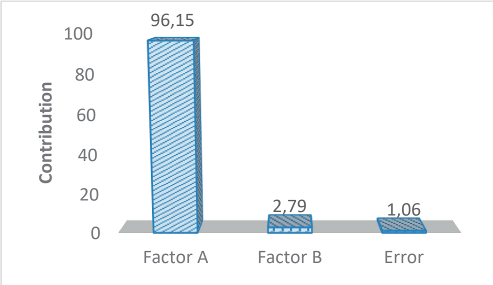

Figure 2. Contribution of factors A [crop year], B [crop locality] on the amount of herb/plant

Figure 2 shows the percentage contribution of the analyzed factors - the year of cultivation (Factor A) and the locality of cultivation (Factor B) - on the variation of the amount of herb/plant. In addition, the experimental error is also evaluated, which reflects the variability not explained by these factors.

The year of cultivation is the determining factor in the production of herba in lavender, explaining 97.68% of the total variation. This result emphasizes the overwhelming influence of climatic conditions and plant age on vegetative development.

The significant increase in production in 2024, compared to 2023, is the result of several factors, such as plant maturation and climatic conditions.

Lavender, being a perennial plant, registers a progressive increase in biomass in the first years of cultivation. In the second year of vegetation, the root system is better developed, which facilitates the absorption of nutrients and water, leading to a substantial increase in the amount of herb.

Differences in temperature, rainfall and brightness between growing years can significantly influence the growth and development of lavender. This result indicates

that optimizing annual management practices is essential to maximize production. The locality of cultivation contributes to a very small extent to the variation in the amount of herb/plant, explaining only 1.55% of the variability. This low influence can be explained by lavender's ability to adapt to various pedoclimatic conditions. Lavender is recognized for its tolerance to varied soils and drought resistance, which gives it high flexibility in terms of growing location. It follows that the locality of cultivation is not a critical factor in determining the production of herb, which gives farmers great flexibility in choosing land. This suggests that lavender can be successfully grown in various regions without the risk of significant drops in yield.
The experimental error of 0.76% is extremely low, which reflects a solid experimental design and high data consistency. A small error indicates that the measurements are accurate and reproducible, and the influence of uncontrolled factors is minimal. This low level of error suggests that cropping practices were uniformly applied and that uncontrolled agronomic factors (such as pest infestation or unforeseen soil variations) had an insignificant impact on production.
Table 5 shows the results regarding the quantity of flowers/ha depending on the crop year. In the first year of study 2023 (a1) - year 2 from planting, 1353 kg of flowers/ha were obtained, considered the reference year (Mt), and in 2024 (a2) - year 3 from planting, 4111 kg of flowers/ha, representing an increase of 304% compared to 2023.

Table 5. Quantity of flowers/ha according to the year of cultivation

Year of Culture	Flowers/ha (kg)	%	Difference	Significance
A1 - 2023	1353	100		
A2 - 2024	4111	304	2758	**
Dl 5%= 689.64, Dl 1%= 1590.69, Dl01%= 5062.95				

DUNCAN TEST - factor B, LD5% = 844.6

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Original dates
 Mean 1 = 2414.  A Mean 2 = 2898.  A
 Mean 2 = 2898.  A Mean 3 = 2884.  A
 Mean 3 = 2884.  A Mean 1 = 2414.  A
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Mean 1 – b1 – Deta
Mean 2 – b2 – Vinga
Mean 3 – b3 – Mailat

The absolute difference is 2758 kg of flowers/ha, the difference between the two years is marked with **, which indicates a distinctly statistically significant difference at a significance level of 5% (DL 5% = 689.64).
This significant increase is due to the maturity of the plants and the climatic conditions. Also, applying more efficient care techniques or adapting fertilization and irrigation can help increase yield.
All media are classified in the same significance group: "A", which indicates that there are no statistically significant differences between locations. Interpreting the differences between locations:
- Vinga vs. Deta: Difference = 2898 - 2414 = 484 kg flowers/ha. This difference is smaller than the LD5% (844.6), so it is not statistically significant.
- Mailat vs. Deta: Difference = 2884 - 2414 = 470 kg flowers/ha. The difference is also smaller than the LD5%, so not statistically significant.
-Vinga vs. Mailat: The difference = 2898 - 2884 = 14 kg flowers/ha, This difference is negligible and well below the significance threshold.
All three locations (Deta, Vinga and Mailat) show statistically similar yields. Even though Vinga and Mailat had slightly higher productions than Deta, the differences are not enough to be considered statistically relevant. The choice of location between the three tested will not have a significant impact on the production of lavender flowers. Thus, farmers can decide to place crops based on other factors, such as land accessibility, costs or infrastructure, without compromising yield. The soils and climatic conditions in the three locations can be similar enough that they do not generate significant differences in production. The application of standardized agricultural techniques can contribute to yield uniformity
Figure 3 shows the relative contribution of two main factors - Crop year (Factor A) and Crop locality (Factor B) - on the number of flowers/ha, as well as the contribution of the experimental error.
The year of cultivation contributes 96.77% to the variation in the amount of flowers/ha. This is an extremely high value, indicating that the year in which the crop is grown is the main determining factor for lavender production. The major difference between the years of

cultivation may reflect the maturation of the plants and the establishment of a deeper and more efficient root system.

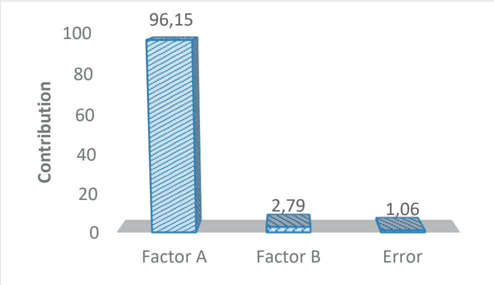

Figure 3. Contribution of factors A [crop year], B [crop locality] on the quantity of flowers/ha

Farmers need to pay close attention to crop management every year, adapting their strategies according to climatic conditions and the age of the crop. Anticipating significant growth in the years following planting is essential for crop planning.

The locality of cultivation contributes only 2.57% to the variation of flower production/ha. This is an insignificant contribution compared to the year of culture.

Minor differences between locations suggest that lavender is a resilient crop that adapts well to a variety of environmental conditions. The edaphic (soil type and structure) and microclimatic (temperature, local humidity) conditions in the studied locations seem to be similar enough not to significantly influence production. Uniform agricultural practices in the three locations (Deta, Vinga and Mailat) Are Another factor that has helped reduce variation between locations. This gives farmers flexibility in choosing the available land.

Experimental errors account for only 0.65% of the total variation. This extremely low value indicates a high degree of precision of the experiment and suggests that the results are reliable. The rigorous control of the experimental variables and the well-implemented data collection methodology helped to minimize errors. Homogeneity of samples and consistent application of experimental treatments reduced also uncontrolled variations.

Table 7 shows the amount of herb (total biomass) harvested per hectare by crop year. The data highlights a significant difference between production in 2023 and 2024.

Table 7. Amount of herb/ha by crop year

Year of Culture	Herb/ha (Kg)	%	Difference	Meaning
A1 - 2023	2682	100		
A2 - 2024	7922	295	5240	**
Dl 5% = 1672.11, Dl 1% = 3856.76, Dl01% = 5868.21				

The production of herb/ha in 2024 (7922 kg/ha) is 5240 kg/ha higher than in 2023 (2682 kg/ha). This represents an increase of 295%, almost triple compared to the first year. The difference of 5240 kg/ha exceeds the materiality limit at 1% (3856.76 kg/ha), but is below the limit of 0.1% (5868.21 kg/ha).

The statistical assurance of the difference between years as distinctly significant indicates that this difference is very statistically significant ($p < 0.01$). This means that there is less than a 1% chance that this difference is the result of random variation, confirming that the crop year has a decisive impact on herb production. Therefore, the crop year has a significant impact on the amount of herb/ha, the production in 2024 being almost triple compared to 2023. Distinctly significant difference, confirming that this result is not random. Plant maturation and favorable climatic conditions are the main likely causes of this growth.

Table 8 shows the amount of herb/ha (total harvested biomass) according to the locality of cultivation. The data compares production from three locations: Deta, Vinga and Mailat, assessing whether the differences are statistically significant.

Table 8. Amount of herb/ha depending on the cultivation location

Locality	Herb/ha (kg)	%	Difference	Significance
b1 - Deta	4684	100		
b2 - Vinga	5499	117	815	Ns
b3 – Mailat	5723	122	1039	Ns
Dl 5% = 2047.9, Dl 1% = 4723.55, Dl01% = 7208.12				

The Vinga experimental field (5499 kg/ha) has a production 815 kg/ha higher than Deta (4684 kg/ha), which represents an increase of 17%. In Mailat (5723 kg/ha) with a production of 1039 kg/ha higher than Deta, equivalent to an increase of 22%. The differences of 815 kg/ha

(Vinga vs. Deta) and 1039 kg/ha (Mailat vs. Deta) are below the materiality limit at 5% (2047.9 kg/ha). This means that there is a high probability that these variations are due to random factors or natural variations and not to actual differences in growing conditions. Although Vinga and Mailat had slightly higher yields than Deta, these differences are within the limits of natural variations and cannot be attributed to specific environmental or management factors.

The Duncan test to analyze the influence of crop locality (Factor B) on the amount of herb/ha (total biomass) demonstrates that all three locations (Deta, Vinga and Mailat) are classified in the same significance group "A". This indicates that the differences between locations are not statistically significant at a significance level of 5% (LD5%). And through this test it is demonstrated that the locality of cultivation does not significantly influence the production of herb/ha.

Although there are slight differences between Deta, Vinga, and Mailat, they are statistically insignificant, suggesting that lavender biomass production is relatively constant across different locations.

DUNCAN TEST - factor B, LD5% = 2048

```
Original dates
 Mean 1 = 4684.   A Mean 3 = 5723.   A
 Mean 2 = 5499.   A Mean 2 = 5499.   A
 Mean 3 = 5723.   A Mean 1 = 4684.   A
```

Mean 1 – b1 – Deta
Mean 2 – b2 – Vinga
Mean 3 – b3 – Mailat

Figure 4 shows the percentage contribution of the two main factors – Year of cultivation (Factor A) and Locality of cultivation (Factor B) – on the amount of herb/ha (total biomass of lavender). The contribution of experimental error is also included.

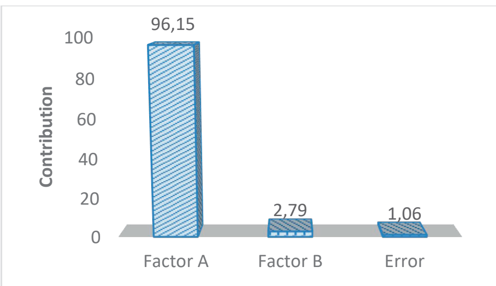

Figure 4. Contribution of factors A [crop year], B [crop locality] on the amount of herb/ha

The year of cultivation contributes 96.15% to the variation in the amount of herb/ha, which indicates an overwhelming impact of this factor on production.

The locality of cultivation contributes only 2.79% to the variation in the amount of herb/ha. This value is extremely low, suggesting minimal impact of location on production. The differences between locations are statistically insignificant, which indicates that lavender has a high ability to adapt to different soil conditions and microclimates.

Experimental errors account for only 1.06% of the total variation, indicating a high degree of accuracy and reliability of the data collected. The rigorous control of the experimental variables and the well-implemented methodology contributed to minimizing errors. Also, the homogeneity of the samples and the consistent application of experimental treatments reduced uncontrolled variations.

The relationship between the amount of flowers/individual and the amount of herba/individual (total biomass, including stems, leaves and flowers) for the lavender crop at Deta in 2023, is shown in Figure 5.

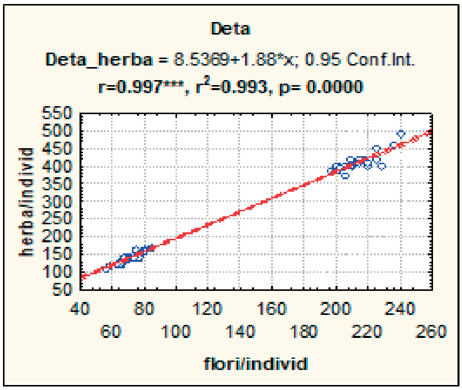

Figure 5. The correlation between flowers/individual and herb/individual at Deta

This relationship is expressed by a linear regression equation and is supported by a series of statistical parameters that indicate the strength and significance of the relationship. For each additional flower unit/individual (g), the amount of herba increases by an average of 1.88 units (g). The negative value of the intercept suggests that, theoretically, at 0 flowers/individual, the amount of herb would be negative. It does not have a direct biological interpretation and may indicate that the model is valid only in the analyzed range. The correlation coefficient (r=0.997) indicates a very strong positive correlation between the amount of flowers/individual and the amount of herb/individual. The r-value can range from -1 to +1. A coefficient close to +1 suggests a near-perfect linear relationship between the two variables. The coefficient of determination (r^2= 0.8798) means that 87.98% of the variation in the amount of herb/individual can be explained by the variation in the amount of flowers/individual. In other words, the regression model explains almost 88% of the total variation, which suggests an excellent fit of the data on the linear model. The p-value ($p < 0.0001$) indicates that the results are extremely statistically significant. This confirms that the observed relationship between the amount of flowers and the amount of herb is not due to chance. There is a very strong and positive linear relationship between the amount of flowers/individual and the amount of herb/individual at Deta in 2023. It can be concluded that for each additional flower, the amount of herba increases significantly, suggesting that the number of flowers is a good predictor of total biomass. The model explains almost 88% of the variation in herb production, which indicates a close relationship between the two variables and a very good fit of the model. The results are extremely statistically significant ($p = 0.0000$), which confirms the validity of the identified relationship. Practical implications of this determination - the optimization of harvesting is given by the fact that farmers can use the number (or quantity) of flowers/individual as a quick and efficient indicator for estimating the amount of herb before harvesting. This relationship can be used to select varieties or plants that produce more flowers, thus having a greater potential for herb production

The results of the correlation between flowers/individual and herb/individual in Vinga are shown in Figure 6.

The graph looks at the relationship between the amount of flowers/individual and the amount of herba/individual (total biomass, including stems, leaves, and flowers) for the lavender crop at Vinga. The relationship is expressed through a linear regression equation, accompanied by statistical indicators that highlight the strength and significance of this relationship. For each additional flower unit/individual (g), the amount of herba increases by an average of 1.9413 units (g), The negative intercept value suggests that theoretically, at 0 flowers/individual, the amount of herba would be negative, this is the theoretical amount of herba when the number of flowers is zero.

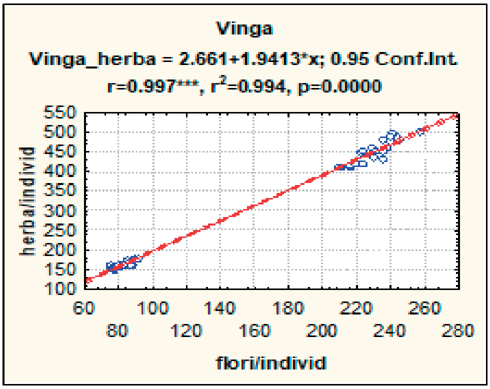

Figure 6. The correlation between flowers/individual and herb/individual in Vinga

The correlation coefficient (r= 0.997) indicates an almost perfect positive correlation between the amount of flowers/individual and the amount of herb/individual. The value is extremely close to 1, which suggests a direct and very close linear relationship between the two variables.

The coefficient of determination (r^2= 0.994) means that 99.4% of the variation in the amount of herb/individual is explained by the variation in the amount of flowers/individual. This represents a near-perfect fit of the data on the linear model, suggesting a highly accurate predictable relationship.

The p-value < 0.0001 indicates that the results are extremely statistically significant. This confirms that the relationship between the amount of flowers and the amount of herba is not the result of random variation. We notice that most of the points are very close to the regression line, suggesting minimal data dispersion and a highly accurate relationship. And for this location we can conclude that there is an extremely strong and positive linear relationship between the amount of flowers/ individual and the amount of herb/individual in Vinga. For each additional flower, the amount of herba increases significantly, suggesting that the number of flowers is an excellent predictor of total biomass. The model accounts for 99.4% of the variation in herb production, indicating near-perfect data matching and high predictability. The results are extremely statistically significant ($p = 0.0000$), confirming the validity of the relationship.

Analyzing the results with Deta (Figure 5), it is observed that in Vinga, the correlation is closer ($r = 0.997$) than in Deta ($r = 0.938$).

The coefficient of determination r^2 at Vinga (0.994) suggests a higher predictability of the relationship with Deta ($r^2=0.8798$). These differences could indicate more uniform growing conditions in Vinga or less genetic variation between individuals.

The results of the correlation between flowers/individual and herb/individual in Vinga, are presented in Figure 7.

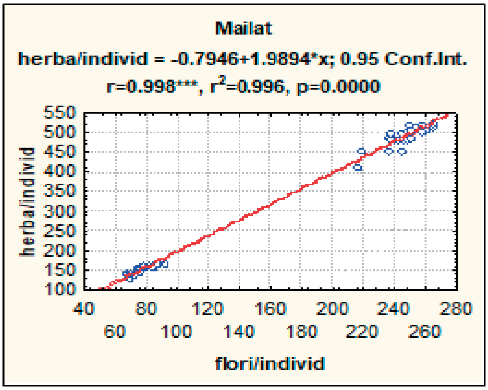

Figure 7. The correlation between flowers/individual and herb/individual in Vinga

The results of the regression equation show that for each additional flower unit/individual (g), the amount of herb increases by an average of 1.9894 units (g). The correlation coefficient (rr=0.998) indicates an almost perfect positive correlation between the number of flowers/individual and the amount of herb/individual. The value is extremely close to 1, which suggests a direct and very close linear relationship between the two variables. The coefficient of determination ($r^2=0.996$) shows that 99.6% of the variation in the amount of herb/individual is explained by the variation in the amount of flowers/individual. This represents a near-perfect fit of the data on the linear model, suggesting a highly accurate predictable relationship.

The p-value < 0.0001 indicates that the results are extremely statistically significant. This confirms that the relationship between the number of flowers and the amount of herba is not the result of random variation.

Most of the points are very close to the regression line, suggesting minimal data dispersion and a highly accurate relationship.

The results obtained in the Mailat field confirm that there is an extremely strong and positive linear relationship between the amount of flowers/individual and the amount of herb/individual at Mailat.

Comparing the results from Mailat with those from Deta and Vinga, we notice that:

The strength of the correlation at Mailat ($r = 0.998$) and Vinga ($r = 0.997$) shows an almost perfect correlation, while Deta ($r = 0.938$) has a strong but noticeably weaker correlation.

The explanatory power (r^2) in Mailat ($r^2 = 0.996$) explains 99.6% of the variation in herb production, followed by Vinga ($r^2 = 0.994$) and Deta ($r^2 = 0.8798$), and in Deta, there is a greater variability that is not explained by the number of flowers, possibly due to environmental factors or genetic variations.

Differences in the slope of the regression at Deta, the slope is slightly higher (2.0227), which indicates a greater increase in biomass per flower, but with a greater dispersion of data, and at Mailat and Vinga, the slope is similar (~1.94-1.99), suggesting a more constant efficiency in converting flowers into total biomass. The comparative results show that:

Mailat has the most accurate linear relationship, followed by Vinga. This indicates a high uniformity of plants and environmental conditions. Deta exhibits greater variability, which could be caused by genetic differences between plants or more variable environmental factors. In all locations, the amount of flowers/individual is an excellent predictor of the amount of herb/individual, but Mailat and Vinga provide more accurate estimates.

CONCLUSIONS

The results of the research clearly indicate that the year of cultivation is the main factor influencing flower production in lavender, highlighting the importance of plant adaptation and inter-annual climatic variations. The locality of cultivation does not have a significant impact, which suggests that lavender can be successfully grown in various locations, provided that the appropriate agrotechnical technologies are observed.

The results of the research demonstrate that the crop year also has a significant impact on the production of lavender herb, with a spectacular increase in biomass in the second year of cultivation. The locality of cultivation has a low, statistically insignificant influence, emphasizing the ability of lavender to adapt to a variety of edaphic and climatic conditions. The choice of the cultivation location can be based on logistical and economic considerations, without the risk of a negative impact on production.

The results of the regression function suggest that Mailat could be an optimal location for genetic selection programs due to the high uniformity of the relationship between flowers and herbs.

These conclusions are essential for the optimization of cultivation practices and for the choice of management strategies, emphasizing the need to monitor and adapt to annual climatic conditions in order to maximize production.

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PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY IN IRONWORT (*Sideritis syriaca* L.) FROM STRANDZHA MOUNTAIN

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Abstract

The current investigation aimed to evaluate total carotenoids, chlorophylls, phenols, flavonoids, as well as the individual phenolic compounds and antioxidant potential in ironwort (Sideritis syriaca L.) collected from Strandzha mountain in Bulgaria and Turkey. Five phenolic acids (chlorogenic, caffeic, p-coumaric, ferulic and sinapic acids) and ten flavonoids (lavandulifolioside, verbascoside, forsythoside A, isoscutellarein 7-O-allosyl(1→2)glucoside, apigenin 7-O-allosyl(1→2)glucoside, isoscutellarein 7-O-allosyl-(1→2)-[6''-O-acetyl]-glucoside, hypolaetin 7-O-allosyl-(1→2)-[6''-O-acetyl]-glucoside 3'-O-Methylhypolaetin 7-O-[6'''-O-acetyl]-allosyl(1→2) glucoside, 4'-O-methylhypolaetin 7-O-[6'''-O-acetyl]-allosyl(1→2)glucoside, apigenin 7-(6''-p-coumaroylglucoside) and apigenin 7-4''-p-coumaroylglucoside) were detected. The samples collected from the Bulgarian part of Strandzha mountain showed 1.5 to 2.5 times higher values for individual phenolic compounds and twice higher antioxidant activity and total carotenoids content in comparison to Turkish samples. Ferulic acid (8173 µg/g dw) and isoscutellarein 7-O-allosyl-(1→2)-[6''-O-acetyl]-glucoside (4452 µg/g dw) were detected in the highest content in Bulgarian ironwort.

***Key words**: Sideritis syriaca L., antioxidant activity, phenolic acids, flavonoids.*

INTRODUCTION

Sideritis syriaca L. (syn.: *Sideritis imbrex* auct. bulg., non Juz.; *Sideritis taurica* auct. bulg.; *Sideritis catillaris* Juz.; *S. taurica* Steph) known also as Strandzhanski chaj, Strandzhanski bilkov chay, Siriiski mirizliv buren, Crimean tea is a perennial herb, 10-50 cm tall that grows on dry calcareous, stony terrains, strongly eroded, in places with an outcropping of the bedrock from 0 to 400 m. It is more common on the karst rocky areas and is massively harvested (Stoyanov et al., 2022) https://www.tuns.eu/flora/; Prodanov, 2019). Strandzhanski bilkov chay (*S. syriaca*) is a protected species and is listed in the Red Book of Bulgaria, volume 1 - category "Endangered". It blooms in yellow during May-June, and its seeds ripen at the end of July-August. In Strandzha it is distributed to the south of Malko Tarnovo in the Turkish part of the mountain (Prodanov, 2019; Veli et al., 2023). Under the synonym *S. taurica* Stephan ex Willd, this species grows in Bulgaria, Turkey, Crimea and Asia (Fraga Citation, 2012; Prodanov, 2019). *Sideritis syriaca* (known also as Cretan mountain tea, Μαλοτήρας Τσαι του βουνού) grows also in Crete, Lebanon, Syria, Turkey (Aneva et al., 2019) and Italy (Menghini et al, 2005). Crimean tea is sold as dried flowers on the market in Kirklareli https://www.tuns.eu/flora/. Strandzha tea reduces the risk of a number of cancers, stroke and heart disease. It has a beneficial effect on high blood pressure, protects against atherosclerosis, strengthens the immune system, has anti-aging properties and has an invigorating effect. Strandja tea is endemic - a type that in Bulgaria is found only in Strandja. It is resistant to mountain climates and does not need care such as digging or spraying, besides it has a natural resistance to cold, frost and ice. (Prodanov, 2019; https://www.tuns.eu/flora/).

Sideritis syriaca has been used as an anti-inflammatory, antimicrobial (espcially significant activity against *S. aureus*, *E. coli, E. faecalis*), antioxidant and analgesic agent in folk medicine (Menghini et al, 2005, Kostadinova et al., 2008, Goulas et al. 2014, Aneva et al.,2019; Veli et al., 2023). It possesed also antiviral (against paramyxovirus) (Sattar et al., 1995) and anti- analgesic effects (Menghini et al., 2005). It is used as a tonic (Menghini et al., 2005), in Greece it is applied for the treatment of dyspepsia, in Turkey for the treatment of chesty cough and especially in western part of Anatolia as diuretics (Hanlidou et al., 2004; Çarıkçı et al., 2023).

Previous reports on *S. syriaca* were mainly focused on decoction, 95% ethanol, methanol, ethyl acetate, hexane, petroleum ether, butanol, diethyl ether and dichloromethane extract as well as its essential oil (Koleva et al., 2003; Armata et al, 2008; Goulas et al., 2014; Veli et al., 2023). In most of the papers DPPH was used as method to evaluate the antioxidant potential (Armata et al., 2008).

However, even the numerous studies about Balkan endemic *S. syriaca* there also missing gaps about its phytochemical compounds and antioxidant potential especially in plants growing wildly in the protected and unprotected area of Strandzhda mountains. The current research aimed to evaluate total carotenoids, chlorophylls, phenols, flavonoids, as well as the individual phenolic compounds and antioxidant potential in ironwort (*Sideritis syriaca* L.) collected from Strandzha mountain in Bulgaria and Turkey.

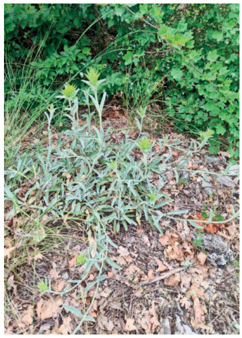
MATERIALS AND METHODS

Plant material

The samples of ironwort (*Sideritis syriaca* L.) were collected in summer 2023 in the flowering phase of the plants from natural habitats in the Strandzha mountains in Bulgaria (Strandzha Nature Park, Pic.1; Permit № 977/26.07.2023 from the Ministry of Environment and Water, Republic of Bulgaria) and Turkey (Kozulu, Pic. 2). The localites of the *Sideritis* population in Strandzha Nature Park are the same as reported by Aneva et al. (2012).

The fresh plant material was air-dried at 25°C and finely ground using laboratory homogernizer BN1200AL (Gorenje). The ground material was kept in tightly closed plastic containers for further analysis. The moisture of ironwort (%) was analyzed on moisture analyzers balance Kern DAB 100-3 (Germany).

Picture1. *Sideritis syriaca* L. (Bulgaria)



Picture 2. *Sideritis syriaca* L. (Turkey)

Photo credits: Pl. Zorovski

Ultrasound-assisted extraction

The samples were weighted in a centrifuge tube of 50 ml and extracted with 95% ethanol in solid to liquid ratio 1:20 (w/v) for dry samples. The extraction was performed in an ultrasonic bath (IsoLab 621.05.001, Germany), operating with ultrasonic frequency 40 kHz and ultrasonic power 60W for 20 mins, at 75°C. The obtained extracts were filtered, and the residues were extracted once again under the above mentioned conditions. The both extracts were combined and used for further analysis.

Total chlorophylls and carotenoids

Total chlorophylls and carotenoids were evaluated in 95% ethanol extracts *Sideritis syriaca* using spectrophotometer using the equations described by Lichtenthaler and Wellburn (1983). The concentrations of chlorophyll a (Chla), chlorophyll b (Chlb), total chlorophyll and total carotenoids were calculated and presented as μg/g (dry weight).

Total phenols and flavonoids

The total phenolic content in ironwort (*Sideritis syriaca* L.) extracts was evaluated using the Folin-Ciocalteu reagent (Stintzing et al., 2005), as the ironwort extract (0.2 mL) was mixed with 1 mL Folin-Ciocalteu reagent diluted 1:4 and then 0.8 mL 7.5% Na_2CO_3 was added then measured after 20 min at 765 nm against a blank sample. The results were expressed in mg equivalent of gallic acid (GAE) per g sample (Ivanov et al., 2014).

The total flavonoid content was determined by $Al(NO_3)_3$ reagent (Kivrak et al., 2009) with small modification (Ivanov et al., 2014). The results were reported as mg equivalents quercetin (QE)/g dry sample.

Antioxidant activity

The DPPH radical-scavenging ability, ABTS assay, ferric reducing antioxidant power (FRAP) assay and Cupric reducing antioxidant capacity (CUPRAC) assay of 95% ethanol extracts from *Sideritis syriaca* were performed as previously described (Ivanov et al., 2014). Antioxidant activity was expresse as mM Trolox equivaelt (TE)/g dry weight.

HPLC analysis of phenolic compounds

Individual phenolic acids and flavonoids were analyzed on a HPLC system equipped with Waters 1525 Binary Pump (Waters, Milford, MA, USA), Waters 2484 Dual Absorbance Detector (Waters, Milford, MA, USA), and a C18 column (Supelco Discovery HS, 5 µm, 25 cm × 4.6 mm), and Breeze 3.30 software. For flavonoids, separation gradient mode was used with a mobile phase composed of 2.0% (v/v) acetic acid (solvent A) and methanol (solvent B). The injected volume was 20 µL (Vrancheva et al., 2021). The results were calculated according to calibration curves.

Statistical analysis

Statistical analysis was performed using MS Excel 2010. The data were presented as mean values ± standard deviation (SD).

RESULTS AND DISCUSSIONS

Moisture content

The moisture content was 10.74±0.36% for Bulgarian representatives of *Sideritis syriaca collected* from Strandzha mountain. The Turkish polulaton showed slightly higher moisture content 11.18±0.12%. The results were needed for further presentation of the other results to dry weight basis.

Total chlorophylls and carotenoids

The results for presence of chlorophylls and carotenoids were summerizedin Table 1. This the first detailed study about photosynthetic pigments in *Sideritis syriaca* L. collected from Strandzha mountain Bulgaria. The results showed that total chloropylls (230.05±0.1 µg/g dry weight) and total carotenoids (54.96±0.52 µg/g dry weight) dominated in samples collected from Strandzha mountain Bulgaria. Their content was almost twice higher than in the Turkish samples. In all samples chl a dominated among chlorphyll b, as the ratio between Chla/Chlb is 1:7 or 1:8. Usually, Chla/Chlb varies in the range of 1.5-3.0, where lower values indicate adaptation to stress by activating Chlb, while higher values are maintained under normal conditions, as previously decribed for lavender samples growing in organic and conventional farming (Dobreva et al., 2024), Total carotenoids was almost four times lower than values of total chlorophylls. It is well known that foliar Chl content is a good indicator of variousbiotic and abiotic stresses (Li et al., 2017), that could explain the differences in chlorophyll content in both samples.

Phenolic compounds

The results for total phenolic content and total flavonoids were summarized in Table 2. It was obvious that samples collected from location Propada Bulgaria, showed more than 1.5 times higher values than the samples collected from Turkey. It was found that *Sideritis syriaca* from Strandzha mountain, Bulgaria contained total phenols 11.87±0.25mg GAE/g dw and total flavonoids 5.17±0.15 mg QE/g. The levels of total flavonoids were comparable with a previous report for cultivated *Sideritis scardica* from Bulgaria (Yanchev et al., 2022). However, in the current study the values of total phenols were twice lower in comparison with prevous data (Yanchev et al., 2022). Our data for total phenols in *Sideritis syriaca* from Strandzha mountain, Bulgaria were comparable with those reported by Goulas et al. (2014) values for a phenolic content in infusions prepared from Greek *Sideritis syriaca* (Epirus, Greece) equivalent to 1863 mg of GAE per 100 g of dry material. Prodanov (2019) reported for close to our results for cultivated *Syderitis syriaca* samples from Malko Tarnovo total flavonoids 5.73 and 5.65 mg/g, and 6.07 mg/g for cultivated samples on limestone, however the results for wild free growing samples from Golyam Valog and Dokuzak (2.79 and 3.35 mg

RE/g) were more than twice time lower than ours results for samples collected from Propada and comparable with samples from Turkish part of Strandzha. The results for total phenols from these places (Golyam Valog and Dokuzak) were comparable with our results (18.39 and 16.65 mg CAE/g).

Table 1. Natural pigments in the aerial parts of *Sideritis syriaca* L. collected from Bulgaria and Turkey

Sample	Chla, μg/g dry weight	Chlb, μg/g dry weight	Total chloropylls, μg/g dry weight	Total carotenoids, μg/g dry weight	Cha/Chlb
Sideritis syriaca from Strandzha mountains, Bulgaria	149.07±0.15	80.98±0.20	230.05±0.15	54.96±0.52	1.84
Sideritis syriaca from Turkey	92.28±0.14	50.02±0.25	145.29±0.20	27.16±0.26	1.74

Table 2. Total phenols, flavonoids, antioxidant potential of *Sideritis syriaca* L. collected from Bulgaria and Turkey

Sample	Total phenolic content GAE/g	Total flavnoids, mg QE/g	DPPH	ABTS	FRAP	CUPRAC
			mM TE/g dw			
Sideritis syriaca from Strandzha mountains, Bulgaria	11.87±0.25	5.17±0.15	163.09±2.11	226.18±2.62	45.27±0.21	224.55±2.20
Sideritis syriaca from Turkey	7.67±0.12	3.04±0.05	83.07±1.18	73.98±3.15	24.33±0.10	132.87±0.95

Table 3. Content of individual phenolic acids and flavonoids in the aerial parts of *Sideritis syriaca* L., μg/g dry weight

Compounds	*Sideritis syriaca* from Strandzha mountains, Bulgaria	*Sideritis syriaca* from Turkey
Phenolic acids		
Chlorogenic acid	509.8±1.2	201.6±0.9
Caffeic acid	61.1±0.5	29.0±1.1
p-Coumaric acid	447.4±1.6	222.4±0.6
Ferulic acid	8172.5±2.6	7634.7±2.1
Sinapic acid	379.9±0.5	257.9±0.8
Flavonoids		
Lavandulifolioside	281.2	114.6
Verbascoside	201.5	53.9
Forsythoside A	143.5	68.8
Isoscutellarein 7-O-allosyl(1→2)glucoside	2180.3	689.8
Apigenin 7-O-allosyl(1→2)glucoside	471.1	658.4
Isoscutellarein 7-O-allosyl-(1→2)-[6″-O-acetyl]-glucoside	4452.4	2450.7
Hypolaetin 7-O-allosyl-(1→2)-[6″-O-acetyl]-glucoside	2343.2	1300.6
3'-O-Methylhypolaetin 7-O-[6‴-O-acetyl]-allosyl(1→2)glucoside	3985.3	3749.5
4'-O-Methylhypolaetin 7-O-[6‴-O-acetyl]-allosyl(1→2)glucoside	246.2	219.5
Apigenin 7-(6"-p-coumaroylglucoside)	411.7	289.9
Apigenin 7-(4"-p-coumaroylglucoside)	485.1	343.2

The detailed content of individual phenolic acids and flavonoids were in Table 3. Five phenolic acids (chlorogenic, caffeic, p-coumaric, ferulic and sinapic acids) were detected in all *Sideritis syriaca* sampes. Ferulic acid was the dominating phenolic acid, found in the highest amount 8172.5 and 7634.7μg/g. Caffeic acid was detected in the lowest values. From flavonoids ten representives were detected, as follows: lavandulifolioside, verbascoside, forsythoside A, isoscutellarein 7-O-allosyl(1→2)glucoside, apigenin 7-O-allosyl(1→2)glucoside, isoscutellarein 7-O-allosyl-(1→2)-[6″-O-acetyl]-glucoside, hypolaetin 7-O-allosyl-(1→2)-[6′′-O-acetyl]-glucoside 3'-O-Methylhypolaetin 7-O-[6′′′-O-acetyl]-allosyl(1→2) glucoside, 4'-O-methylhypolaetin 7-O-[6′′′-O-acetyl]-allosyl(1→2)glucoside, apigenin 7-(6"-p-coumaroylglucoside) and apigenin 7-4"-p-coumaroylglucoside). Isoscutellarein 7-O-allosyl-(1→2)-[6′′-O-acetyl]-glucoside were dominating compounds in samples collected from Bulgarian part of Strandzha mountain 4452.4 µg/g, followed by 3'-O-Methylhypolaetin 7-O-[6′′′-O-acetyl]-allosyl(1→2) glucoside with content 3985.3 µg/g. 3'-O-Methylhypolaetin 7-O-[6′′′-O-acetyl]-allosyl(1→2)glucoside was the dominating compound in samples collected from Turkish part of Strandzha (3749.5 mg/g dw) and the second in the highest amount in samples collected from Bulgarian part of the mountain. Isoscutellarein 7-O-allosyl-(1→2)-[6′′-O-acetyl]-glucoside, hypolaetin 7-O-allosyl-(1→2)-[6′′-O-acetyl]-glucoside, 3'-O-methylhypolaetin 7-O-[6′′′-O-acetyl]-allosyl(1→2)glucoside, apigenin 7-(6"-p-coumaroylglucoside), forsythoside A and lavandulifolioside in *S. syriaca* collected from Bulgarian part of Strandzha mountain was with values almost twice time higher than their content in Turkish samples collected from this mountain. This is first detailed study about individual phenolic and flavonoids compounds in endemitic species samples collected from Strandzha mountains. A recent study reported that in the aerial parts of *Sideritis syriaca* ssp. *syriaca* endemic species in Crete were identified 1-rhamnosyl, 1-coumaroyl, dihydrocaffeoyl, protocatechuic tetraester of quinic acid, as well as chlorogenic acid, apigenin 7-O-glucoside, apigenin, 4′-O-methylisoscutellarein 7-O-[6′′′-O-acetyl-β-D-allopyranosyl-(1→2)-β-d-glucopyranoside], isoscutellarein 7-O-[6′′′-O-acetyl-β-D-allopyranosyl-(1→2)-β-d-glucopyranoside], 4′-O-methylisoscutellarein 7-O[β-d-allopyranosyl-(1→2)-β-d-glucopyranoside] and 4′-O-methylisoscutellarein 7-O-[β-d-allopyranosyl-(1→2)-6′′-O-acetyl-β-d-glucopyranoside] (Armata et al, 2008). Moreover, in extracts from Bulgarian representatives of *S. syriaca* and *S. montana* were identified the following compounds: verbascoside, hypolaetin-4 -methylether-7-O-[6-O-acetyl-β-Dallopyranosyl-(1 →2)-β-D-glucopyranoside] and hypolaetin-4 -methylether-7-O-[6-O-acetylβ-D-allopyranosyl-(1 →2)-β-D-6-O-acetyl-β-Dglucopyranoside] (Koleva et al., 2003). It was reported that hypoelatin and isoscutellarein diglucosides, chlorogenic acid, and three phenylpropanoids were the major constituents of the decoction of *S. syriaca* collected from Greece as isoscutellarein-7-O-[6’’0-O-acetyl-β-D-allopyranosyl-(1 → 2)-β-D-glucopyranoside was detected in the highest amount 184.2 ± 16.6 mg/100 g dw) (Goulas et al., 2014). The detected values or this compound were twice lower than found in our current research. Isoscutellarein-7-O-[6′′′-O-acetyl-β-D-allopyranosyl-(1→2)-β-D-glucopyranoside and 4′-Methylhypolaetin-7-O-[6′′′-O-acetyl- *β*-D-allopyranosyl-(1→2)-b-D-glucopyranoside were the dominating compounds that bring about almost 50% of antioxidant potential of decotion from Greek *S. syriaca* (Goulas et al., 2014). The main compounds were found in *S. perfoliata* were verbascoside, chlorogenic acid and apigenin 7-glucoside (Çarıkçı, et al.,2023). In our case 4'-O-Methylhypolaetin 7-O-[6′′′-O-acetyl]-allosyl(1→2)glucoside and isoscutellarein 7-O-allosyl-(1→2)-[6′′-O-acetyl]-glucoside were both dominating compounds in both *S. syriaca* samples collected from Bulgarian and Turkish part of Strandzha mountain.

Antioxidant potential

The antioxidant activity of *Sideritis syriaca* from Bulgarian samples was faintly studied. There are a research which states that the methanolic extracts of its aerial parts were equal antioxidants to rosmarinic acid, when antioxidant power was measured with the DPPH radical dot•test (Koleva et al., 2003). This authors state that the inhibitory effect on β-carotene bleaching of the polar extracts and rosmarinic acid was much lower than that of BHT. Goulas et al., (2014) used DPPH, FRAP and phosphmolybdenum assay to evaluate the antioxidant potential of decoction prepared with Greek *Sideritis syriaca.* Veli et al., 2023 used DPPH method to evaluate antioxidant potential of the extracts prepared with cultivated *Sideritis syriaca* from village Varovnik, Sredets Municipality, Bulgaria. All this showed the gap in analysis of antioxidant potential of wild growing plants from *Sideritis syriaca* from their natural habitat Strandzha mountain. In the current study the results for antioxidant potential of 95% extracts prepared with samples from Bulgarian and Turkish part of the mountain were listed in table 2. Four methods (DPPH, ABTS, FRAP and CUPRAC) based on different mechanism were used to evaluate the antioxidant potential of *Sideritis syriaca.* CUPRAC method that is based on single electron transfer demonstrated the highest andtioxiant potential from both samples (224.55±2.20 and 132.87±0.95 MTE/g dw). ABTS with a mixed mechanism showed the highest results for samples collected from Bulgaria. The lowest results were found for FRAP assay, that is based on single electron transfere and metal ferric reducing properties - 45.27 and 24.33 mMTE/g dw. In general, samples collected from Bulgarian part of Strandzha demonstrated almost twice higher antioxidant potential than Turkish samples (Table 2). This could be explained with the levels of total flavonoids and phenols in these samples.

Veli et al. (2023) reported that methanol and ethanol extracts from cultivated Bulgarian *Sideritis syriaca* demonstrated - 579.3 and 524.3 μM EVCAA/100 μg extract by DPPH method, while Goulas et al. (2014) reported FRAP antioxidant activity for *Sideritis syriaca* decoction (13.4 mmoL AsA/100 g DM). In our case 95% ethanol extracts especially form Propada Bulgaria gave the highest values 163.09±2.11 mMTE/g (DPPH assay) and 45.27±0.21 mM TE/g (FRAP assay). This study demonstrayed that *Sideritis syriaca* showed highest cupper reducing properties and good radical scavenging abitily, than ferric reducing capacity.

CONCLUSIONS

The current study contributes to the better understanding and utilization of the bioactive compounds found in *Sideritis syriaca* L. growing in Strandzha mountain on territry of Bulgaria and Turkey. To the best of our knowledge this is the first detailed study about natural pigments, phenolic acids profile and flavonoid content, antioxidant potential in representatives collected from this protected aria in Strandzha mountain (Bulgaria). Hypolaetin 7-O-allosyl-(1→2)-[6″-O-acetyl]-glucoside, O-methylhypolaetin 7-O-[6‴-O-acetyl]-allosyl(1→2)glucoside and isoscutellarein 7-O-allosyl-(1→2)-[6″-O-acetyl]-glucoside were there dominating compounds in both *S. syriaca* samples collected from Bulgarian and Turkish part of Strandzha mountain. The detected phenolic acids and flavonoids bring about and enlarge the knowledge about healthy properties of this plant and engourge their cultivation and further utilization in food and cosmetics, due to its antioxidant potential and rich flavonoids profile.

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STUDIES ON THE ADAPTABILITY OF SOME ROMANIAN VARIETIES OF AUTUMN WHEAT TO THE CURRENT CLIMATE CHANGES IN NORTHERN BĂRĂGAN

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Abstract

The paper presents the climate evolution in Northern Baragan Plain and the impact of current climate changes on the winter wheat crop, with the formulation of some recommendations for farmers in this agricultural area. Five Romanian varieties of wheat were studied, which are multiplied at the Agricultural Research and Development Station Braila, in the last five agricultural years, which were the most difficult because of climate changes, due to the pedological drought recorded in Northern Baragan Plain. Atmospheric heat negatively influences the physiology of agricultural plants, through deficiencies in root absorption and photosynthesis, increasing evapotranspiration and having the effect of drying leaves, deficient pollination of flowers and lack of fruiting, i.e. seed formation. The study carried out aims to support the revitalization of the Romanian seed market to ensure better access for farmers to the most efficient seed material in the zonal pedo-climatic conditions. Recommendations were formulated for choosing the best performing wheat varieties, in the pedo-climatic conditions of Northern Baragan.

Key words*: wheat, productivity, climate changes, pedological drought, wheat varieties.*

INTRODUCTION

Wheat (*Triticum aestivum* L.) is considered the most important agricultural species worldwide and in Europe, as it occupies the largest cultivated area and has the largest share in human nutrition (Shewry et al., 2013).

In the world, out of all 195 sovereign and independent states, wheat is cultivated in over 100, having an essential importance in the food industry by producing flour from caryopses, in the manufacture of cellulose by processing plant residues remaining after harvesting, as well as in animal feeding with bran remaining after the milling industry or as organic fertilizer by processing all residues remaining after the cultivation and processing of wheat (Shewry & Hey, 2015).

It is statistically proven that in the European Union wheat is the main cereal crop, obtaining more than half of the cereal production annually. The remaining 50% of the cereal production in the EU is represented by maize and barley (17% each) and the rest brings together cereals grown on smaller areas, such as rye, oats, triticale, and spelt (https://agriculture.ec.europa.eu/farming/crop-productions-and-plant-based-products/cereals_ro). Climate change manifested in the last 20 years has had a series of repercussions on wheat production, both at the global, European, and national levels, but especially in South-Eastern Romania, where soil aridification has become increasingly evident.

According to current statistics, in 2024, wheat was cultivated in Romania on an area of 2.19 million ha, and the average yield was 4.99 t/ha (Figure 1; https://ogor.ro/prognoza).

Under current climate change conditions, studies have been conducted on the physiology of wheat plants, leading to the conclusion that the growing season of wheat will gradually decrease, due to the increase in temperature and solar radiation rates in the current situation (Olesen et al., 2011; Valizadeh et al., 2014). In 2016, Bing et al. demonstrated that global wheat production could decline by 4.1-6.4% if global temperatures increased by 1°C. This would mean that of the more than 700 million tons of wheat produced annually worldwide and processed into various products for human and animal consumption, if there were a reduction of just 5% in production, the estimated loss would be 35 million tons each agricultural year.

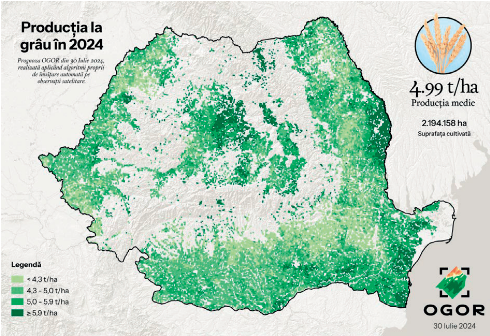

Figure 1. The map of wheat production in 2024 in different areas of Romania

From a temperature point of view, the physiological active zone for wheat is delimited by the temperature of 5ºC, and the requirement for the total sum of useful temperature degrees is 1800 - 2000 GDU (Drăguleasa et al., 2023). In terms of moisture, wheat crop has moderate and balanced requirements during the growing season, needing a minimum of 225 mm from sowing to maturity, but the average optimum being 600 mm. From a soil point of view, wheat is the cereal with the highest requirements, preferring a loamy or clay-loamy texture, with increased permeability, neutral pH, high fertility and a groundwater depth of over 90-100 cm, as it cannot tolerate soils where water stagnates, or sandy, too acidic or too alkaline soils. Planting density is another factor affecting crop yield (Zimeng et al., 2024). The paper presents the climatic evolution of the last five years and the status of wheat production for seeds, affected by these conditions, for the selection of the most performing genotypes for cultivation in this area.

MATERIALS AND METHODS

The research was carried out in the Chiscani Experimental Centre according to the satellite map in the Figure 2.

At the Chiscani Experimental Centre, climatic data are monitored with a meteorological station every agricultural year, and in 2023, a soil profile was carried out for a more detailed pedoclimatic characterization.

The diagnosis of the soils and their classification in the classification system was carried out in accordance with the Romanian Soil Taxonomy System (SRTS-2012), considering the morphological properties of the soils. For physical and chemical characterization of the soil, soil samples were collected for laboratory analysis, for samples in natural and modified settings.

Figure 2. The satellite map with the Experimental Centre Chiscani

The collection of samples from the pedological profile was carried out on genetic horizons and sub horizons, respectively 6 agrochemical soil samples, 12 soil samples in undisturbed structure.

RESULTS AND DISCUSSIONS

The soil profile characterizes the soil as CALCAREOUS CHERONZIOM ALUVIC (SRTS, 2012) located in Experimental Centre Chiscani from Braila Plain (Northen Bărăgan), at Latitude: N 45.20363, and Longitude: E 027.92138, with absolute altitude: 20 m, and the main relief form is plain, with horizontal or quasi-horizontal surface (with predominantly less than 2% slope) with unevenness.
Parental materials are loess and carbonate loess deposits, and the groundwater depth: > 5 m.
Overall natural drainage is good, humidity regime is ustic-seric, and temperature regime is mesic.
Soil landscape: Alluvial calcareous chernozems are associated with saline alluvial chernozems (Figure 3).

Figure 3. Images of the soil profile from CE Chiscani of the Agricultural Research and Development Station Braila, Romania

Morphological characteristics of soil (SRTS, 2012) are:
Apk 0-11 cm: clay, very dark brown (10YR 2/2) and very dark gray, brown (10YR 3/2) in dry state, structure modified by cultivation, reavon, weakly compact, weakly cohesive, weakly plastic, hard in dry state, thin roots frequent; coprolites frequent, moderate effervescence, gradual transition.
Aptk 11-32 cm: clay, very dark brown (10YR 2/2) and very dark gray, brown (10YR 3/2) in dry state, grain structure medium developed, reavon, moderately compact, weakly cohesive, weakly plastic, friable in wet state, hard in dry state, thin roots rare, coprolites frequent, strong effervescence, wavy transition.
Am/Ck 32-48 cm: dusty clay, very dark grey, brown (10YR 3/2) in wet state and dark grey, brown (10YR 4/2) in dry state, poorly developed grain structure, loose, poorly compact, friable in wet state, hard in dry state, weakly cohesive, weakly plastic, very strong effervescence, gradual wavy transition.
Ck 48-67 cm: dusty clay, dark grey, brown (10YR 4/2) in wet state and grey, brown (10YR 5/2) in dry state, massive, loose, hard, weakly cohesive, weakly plastic, frequent friable $CaCO_3$ concretions, violent effervescence, gradual straight transition.
Cca1 67-100 cm: dusty clay; yellowish brown (10YR 5/6) in the wet state and brownish yellow (10YR 6/6) in the dry state, massive, loose, weakly cohesive, weakly plastic, frequent friable $CaCO_3$ concretions, violent effervescence, gradual wavy transition.
Cca2 100-120 cm: dusty clay, pale yellow (10YR 7/4) in the wet state and yellow (10YR 7/6) in the dry state, massive, wet, weakly cohesive, weakly plastic, violent effervescence.
The physicochemical properties of the soil profile are highlighted in Tables 1 and 2.
Figure 4 shows the evolution of average annual temperatures, for each agricultural year, in Northern Baragan Plain, observing a significant increase compared to the multiannual average, in the last five agricultural years.

Table 1. Physical properties of the soil profile from the Chiscani Experimental Centre, Braila County

Horizon	UM	Apk	Aptk	Am/Ck	Ck	Cca1	Cca2
Deep	cm	0-11	11-32	32-48	48-67	67-100	100-120
wi	% g/g	15.1	15.9	16.7	11.7	10.4	9.7
Dawi	g/cm^3	1.18	1.38	1.18	1.17	1.31	1.28
RP	Kgf/cm^2	14	25	18	19	32	27
IC	-	0.0090	0.0044	0.0042	0.0064	0.0077	0.0078
ksat	mm/h	6.86	8.57	35.16	54.36	14.92	3.60
PTwi	% v/v	55.5	48.1	55.5	56.1	50.8	51.9

Table 2. Chemical properties of the soil profile from the Chiscani Experimental Centre, Braila County

Horizon Deep	UM cm	Apk 0-11	Aptk 11-32	Am/Ck 32-48	Ck 48-67	Cca1 67-100	Cca2 100-120
pH	pH unit	8.15	8.19	8.32	8.37	8.43	8.59
Humus (Cx1.72)	%	2.86	1.91	1.37	1.07	0.89	0.48
N total	%	0.156	0.136	0.103	0.091	0.064	0.044
P AL	mg/kg	115	74	36	25	21	15
P AL corected	mg/kg	50	31	13	8	6	15
Kmobile	mg/kg	174	257	160	255	201	169
Residue	mg/kg	49	51	44	41	43	40

Compared to the period 1945 – 2000, with average thermal values mostly below 11°C (average for 118 years), from 2000 to the present, thermal values have increased progressively, at a rate of 0.05°C/year.

Starting from this rate, a perspective of an increase in the average temperature by 0.4°C by 2025 (respectively reaching the average value of 12.1°C), and by 1.6°C in 2050 (respectively reaching the average value of 13.3°C) results (Patriche et al., 2024).

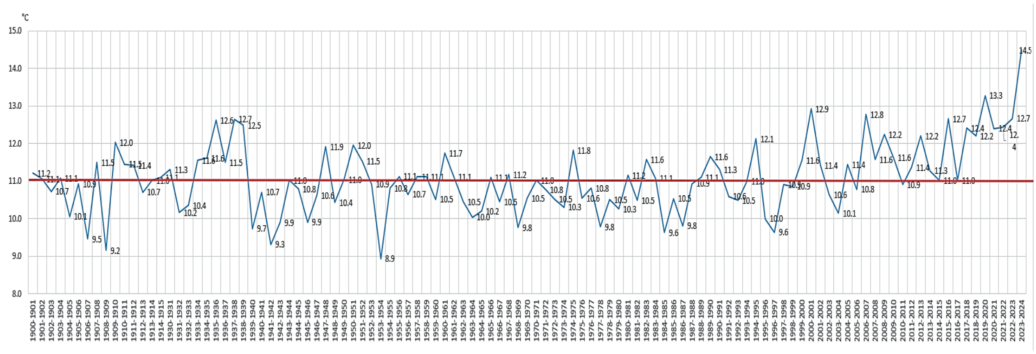

Figure 4. Dynamics of average annual temperatures in Northern Baragan Plain, from 1900 until 2024

Solar radiation has high values, of 125 kcal/cm^2/year, being linked to the duration of sunshine which in the areas of interest, namely in Chiscani area, records a number of 2200 h/year (only 75 days in a year without sun). During the year, the average monthly temperatures register a continuous increase from February to July, then a decrease from August to January, highlighting the thermal contrasts between winter and summer.

The Figure 5 presents the situation of annual cumulative precipitation over the last 20 years in Northern Baragan Plain, compared to potential evapotranspiration.

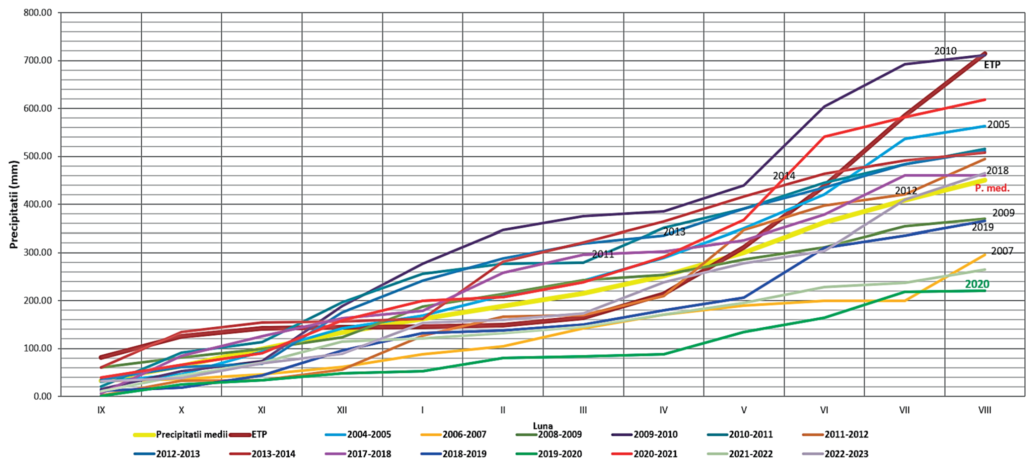

Figure 5 Dynamics of annual precipitation cumulated over the last 20 years in Northern Baragan Plain

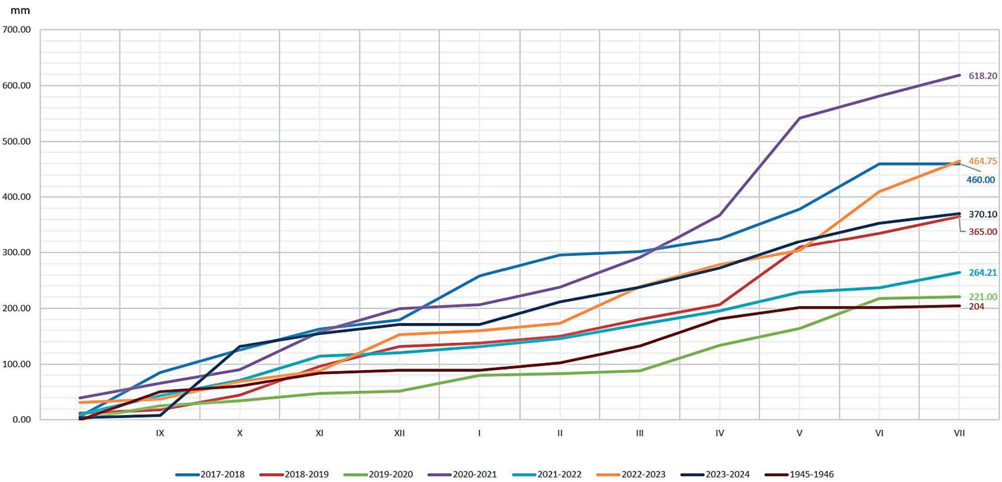

Figure 6. The situation of annual cumulative precipitation in the last 7 years, compared to the driest agricultural year 1945-1946

The driest agricultural year in historical data was the agricultural year 1945-1946, in which the cumulative precipitation was 204 mm, and Figure 6 presents the graph with the cumulative precipitation in the last 7 agricultural years, noting that the agricultural year 2019-2020 accumulated 221 mm, followed by the agricultural year 2021-2022 with 264.2 mm, 2018-2019, with 365 mm and 2023-2024, with 370.1 mm.

A wide range of adaptation options exists in most European regions to mitigate many of the negative impacts of climate change on crop production in Europe (Olesen et al., 2011).

Climatic conditions in the last 5 years have drastically affected wheat production due to soil drought, as well as biotic and abiotic stress. Thus, high atmospheric temperatures, lack of precipitation, as well as the need for irrigation in some years even from March, due to the water deficit in the soil, have affected the physiology of wheat plants and increased the attack of diseases and pests in some vegetation phenophases.

The average wheat yield obtained within the Agricultural Research and Development Station of Braila, in the last five years, ranged between the minimum value of 2166 kg/ha in the agricultural year 2019-2020 and the maximum value of 6174 kg/ha in the agricultural year 2020-2021 (Figure 7).

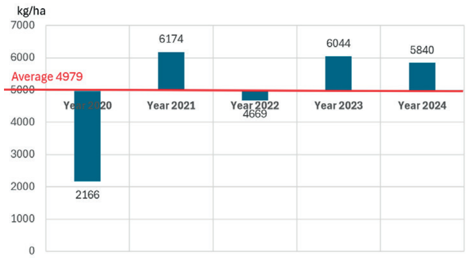

Figure 7. The wheat average yield in the last five year, at ARDS Braila

In 2020, the wheat yield was very low for all varieties tested, and compared to the average of experience, by 2166 kg/ha, the best results were obtained by the variety Ursita, followed by Miranda and Voinic, with insignificant differences, from 151 to 49 kg/ha, and Glosa obtained the lowest production compared to the average, with a difference of -271 kg/ha (Figure 8).

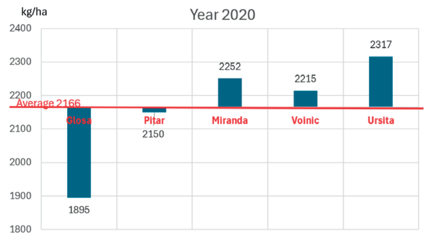

Figure 8. Wheat yield obtained by the five varieties tested in agricultural year 2019-2020

In 2021, compared to the experience average of 6174 kg/ha, the best production results were obtained by the Miranda variety, with a difference of +926 kg/ha and the Pitar variety, with a difference of +686 kg/ha, the other three varieties having below average production, the weakest variety being Glosa, followed by Voinic and Ursita (Figure 9).

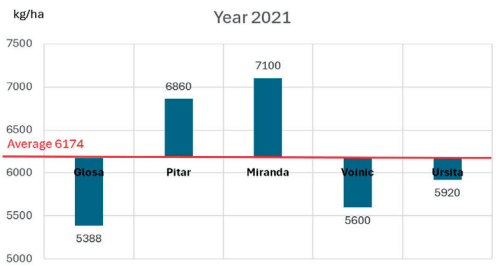

Figure 9. Wheat yield obtained by the five varieties tested in agricultural year 2020-2021

In 2022, the best production results compared to the average experience were obtained by the Miranda variety and the Pitar variety, with differences of +2294 kg/ha for Miranda and +1081 kg/ha for Pitar (Figure 10).

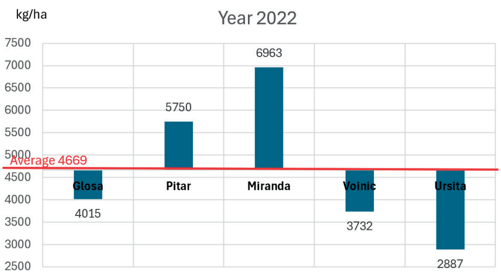

Figure 10. Wheat yield obtained by the five varieties tested in agricultural year 2021-2022

For 2023, the most productive wheat variety was Glosa, followed by Miranda and Voinic, with production differences compared to experience average of +826 kg/ha for Glosa, +556 kg/ha for Miranda, and +536 kg/ha for Voinic (Figure 11).

In 2024, the best production results for winter wheat were obtained by the Miranda variety, with a difference of +610 kg/ha, followed by the Glosa and Ursita varieties, with a difference of +260 kg/ha compared to the average of the experience. The lowest production compared to the average of the experience was obtained by the Pitar variety in 2024, with a difference of -1140 kg/ha (Figure 12).

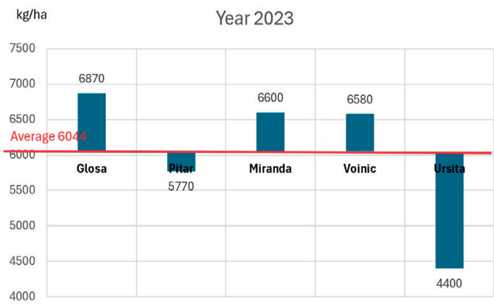

Figure 11. Wheat production obtained by the five varieties tested in agricultural year 2022-2023

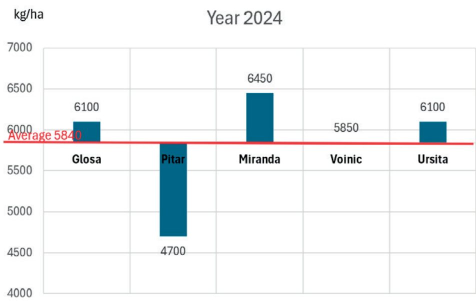

Figure 12. Wheat production obtained by the five varieties tested in agricultural year 2023-2024

The average wheat yield over the last 5 years was 4979 kg/ha, and the best production results were obtained by the Miranda variety, compared to the average over the last five years, with production values in the range of 7100 kg/ha in 2021, 6963 kg/ha in 2022, 6600 kg/ha in 2023, and 6450 kg/ha in 2024 (Figure 13).

Figure 13. The average productions of the five wheat varieties in the last five years at ARDS Brăila

The common winter wheat variety MIRANDA was obtained at INCDA Fundulea from the complex hybrid combination ERYT26221/ 96869G1-// GLOSA by individual selection following rapid homozygosity through the *Zea* system (https://www.incda-fundulea.ro/fise/miranda.pdf).

CONCLUSIONS

- Atmospheric heat negatively influences the physiology of wheat plants, through deficiencies in root absorption and photosynthesis, increasing evapotranspiration and resulting in leaf drying, poor pollination of flowers and lack of fruiting, i.e. seed formation.
- On the other hand, where intensive irrigation is used, disease and pest attacks frequently occur, weed invasion occurs and implicitly technology costs increase through the application of irrigation and phytosanitary treatments.
- Even if satisfactory production is maintained on irrigated lands, economic and operational challenges significantly limit the profitability of wheat cultivation for seed production.
- In the experience with five winter wheat varieties multiplied at ARDS Brăila in the last five agricultural years, the best performing variety proved to be Miranda, with better adaptation to biotic and abiotic stress conditions.

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REVIEW ON FERTILIZATION IN ORGANIC PRODUCTION OF THE SPECIES *Lavandula angustifolia* Mill.

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Abstract

Lavandula angustifolia Mill. (lavender), is an aromatic plant that is part of the Order Lamiales, Family Lamiaceae (Mint), Genus Lavandula L., having various uses among which we can mention: medical and therapeutic, cosmetic and pharmaceutical, dendrological and ornamental, food, industrial. The genus Lavandula includes approximately (47 species), and the most important in culture is Lavandula angustifolia Mill. This paper reviews the specialized literature and research conducted on the fertilization of the species Lavandula angustifolia Mill, under organic cultivation conditions.

Key words*: biological parameters, fertilization, Lavandula.*

INTRODUCTION

Medicinal and aromatic plants are widely studied worldwide (throughout the world), due to the advantage that the essential oils extracted from these plants represent a very important field in both the food industry and agriculture (Elguea-Culebras, 2022)

The genus *Lavandula* is the best known genus of the *Lamiaceae* family (includes/including 39 species) being (and) found in the Mediterranean Sea basin, in Southern Europe, North-East Africa, the Middle East, South-West Asia and South-East India, in the North Atlantic islands, the Arabian Peninsula (Benabdelkader et al., 2011; Camen, 2016, Giannoulis, 2020, Kıvrak, 2018; Tuttolomondo et al., 2015), but the most important species on which the (witch are also the subject of the) most research is done on the essential oil with aromatherapeutic and pharmaceutical values are: *Lavandula angustifolia* Mill., *Lavandula dentata* (lavender French), *Lavandula hybrida*, *Lavandula latifolia, Lavandula stoechas* (Castro-Vázquez et al., 2014)

Lavandula angustifolia Mill. (lavender), also known by the synonyms *Lavandula officinalis* L. Chaix et Vill., *Lavandula vera* DC (English lavender, true lavender), is part of the (belong to the) *Lamiaceae* family (formerly *Labiatae*) which includes over 236 genera (of which the genus *Lavandula* spp. is also part/including the genus *Lavandula* spp.), and over 7.000 species, where *Lavandula angustifolia* Mill. (lavender) known as a medicinal (aromatic) plant with an intense fragrance, is the most cultivated and traded (comercialized) species worldwide (Peçanha, 2021; Pirzad, 2018).

The *Lamiaceae* family is described in the specialized literature as the family of plants that are rich in essential oil (Giannoulis et al., 2020)

Lavandula angustifolia Mill., is a perennial, aromatic, Mediterranean shrub, known since ancient times for its multiple uses, especially in the field of alternative medicine or alternative medicine (*phytotherapy*) and aromatherapy (Mihalașcu, 2021), balms, creams, detergents, ointments, cosmetics, etc. (Chrysargyris et al., 2016), the food industry (special culinary properties) (Greff et al., 2023).

A rustic species (lavender), growing in areas where other plants cannot grow (thrive), while also having low nutritional requirements (Peçanha, 2021)

Medicinal and aromatic plant of particular importance for the pharmaceutical industry, the perfume industry, but also for landscaping (for land use), where the mineral content of the soil affects the yield and quality of medicinal plants (Chrysargyris et al., 2016).

Lavender (*Lavandula angustifolia* Mill.), an evergreen (woody) subshrub, with bush heights ranging from 20-60 cm, is a perennial species, with a typical productive lifespan of approximately 10 years (Giannoulis et al., 2020).

Aromatic plants from the *Lamiaceae* family represent a viable and safe ecological strategy for sustainable soil phytoremediation, due to their multiple benefits (ecological and socioeconomic) (Misha and Chandra, 2022)

Medicinal and aromatic plants are a rich source of nutrients and natural remedies, with some crops of these medicinal plants (*Lavandula angustifolia* Mill.) are becoming industrial crops worldwide due to their nutritional and medicinal effects (Bouzid et al., 2023).

***Fertilization of Lavandula angustifolia* Mill.**

Plants need nutrients to grow, and these (nutrients) are absorbed from the soil through the plant's root system (https://www.fertilizerseurope.com/fertilizers-in-europe/types-of-fertilizer/).

Fertilization is carried out both with (using both) chemical compounds (N, P, K) and with the application of biological compounds/ and biological compounds (compost, Cropmax, BlackJak).

Fertilization is applied radicularly (at the level of the root system - roots) and foliarly (at the level of the leaf system - leaves)/ Fertilizers are applied to the root system (roots) and foliage (leaves).

Advantages of using organic fertilizers

Organic fertilizers are currently widely used due to advantages such as: slow release, which means that fertilization is not carried out in excess and thus destroying plants (is not excessive and thus does not destroy the opplants); the risk of accumulation of toxins (toxin accumulation) in the soil is very low or even non-existent (when growing vegetables, fruits - food in general); they are renewable and biodegradable, sustainable and environmentally friendly; they improve the structure of the soil (soil structure) (where the organic fertilizer is applied) over time, together (along) with the microbial ecosystem (the one (found) in the soil); they include secondary nutrients and micronutrients that chemical (synthetic) fertilizers do not have. (https://mylittlegreengarden.com/organic-fertilizers/).

Ecological fertilization

Ecological fertilization integrates agricultural and environmental objectives and is adapted to current environmental conditions, based on the principle that it should only be applied to the soil only in the quantities needed (necessary) and at the optimal time of the crop, thus avoiding environmental damage. (https://link.springer.com/chapter/10.1007/978-94-007-0186-1_7)

Also, ecological soil fertilization (Organic soil fertilization) strictly (also) refers strictly to cultivating soil and plant health through natural processes and materials, thus avoiding synthetic inputs, while also representing a fundamental element of sustainable agriculture reflecting (that reflects) nature's methods of nutrient circulation (cycling) and soil enrichment. (https://energy.sustainability-directory.com/term/ecological-fertilization/)

At the same time, organic fertilization places great emphasis on practices that mimic natural nutrient cycles, and here we can mention (such as): the use of cover crops to fix nitrogen, the incorporation of organic matter into the soil (substrate) to improve its health.

The specialized literature shows us (The literature shows) that organic (ecological) fertilization integrates agricultural and environmental objectives and is adapted to current environmental conditions, based on the principle that it should be applied to the soil only in the quantities needed (necessary quantities) and at the optimal time of the crop, thus avoiding environmental damage. (https://link.springer.com/chapter/10.1007/978-94-007-0186-1_7)

Organic fertilizers

Organic fertilizers differ from mineral, organo-mineral and organic fertilizers.

Thus, organic (ecological) fertilizers can be classified into:

Organic fertilizers - include materials such as: compost, manure, sludge, and plant residues, which improve soil structure, water retention and nutrient availability.

Mineral fertilizers - can be used in an organic context only when they are derived from natural sources or formulated to release nutrients slowly, thus reducing the risk of nutrient runoff (leaching) and pollution (https://www.fertilizerseurope.com/fertilizers-in-europe/types-of-fertilizer/).

Organic fertilizers, organo-mineral fertilizers and mineral fertilizers, inhibitors, calcareous (limestone) materials, growing (growth) media and plant biostimulants can help farmers adapt their fertilization practices to environmental and crop conditions (https://www.fertilizerseurope.com/fertilizers-in-europe/types-of-fertilizer/).

Different crops and soil types may require specific (well-designed) fertilization strategies. For example, some soils may benefit from the addition of specific micronutrients, while others may require adjustments to the overall NPK ratio.

Thus, organic fertilization aims to reduce the use of synthetic fertilizers and pesticides, minimize greenhouse gas emissions and protect water quality.

Among the organic fertilizers we can list: compost, manure, vermicompost, blood meal, comfrey tea, fish emulsion, alfalfa meal, phosphate rock and azomite rock dust, liquid seaweed (https://mylittlegreengarden.com/organic-fertilizers/).

We will show in the table below (Table 1.) some examples of organic fertilization practices (lists several examples of ecological fertilization practices)

Table 1. Different organic fertilization practices

Name	Effect
No-till farming	Minimizing soil disturbance to improve soil structure and water retention
Biochar	Adding biochar (a coal-like substance produced from biomass) to the soil to improve fertility and water retention
Composting	Transforming (turning) organic waste into a nutrient-rich soil amendment
Cover crops	Planting crops to improve soil fertility and suppress weeds
Organo-mineral fertilizers	Combining organic and mineral fertilizers to provide (ensurre) a balanced supply of nutrients (nutrient supply)

Plant biostimulants

Plant biostimulants are any substance or microorganism applied to plants with the aim of improving (to improve) their nutritional efficiency, tolerance to abiotic stress and crop quality characteristics regardless of their nutrient content (Etesami et al., 2022)

The microflora found in the soil (naturally existing/occurring) attach to (attaches itself) the roots of plants, thus having a favorable effect on plant growth and productivity (Pole et al., 2022).

The use of organic fertilizers induces positive effects on soil organic carbon (Morugán-Coronado et al., 2020).

The incorporation of biostimulants into cropping systems is proving to be a very beneficial and ecological strategy at the same time for sustainable (and environmentally friendly strategy for both sustainable) agriculture and food security (food is no longer contaminated) (Etesami et al., 2023)

Compost

Composting is the controlled biological (aerobic - requires oxygen) decomposition of organic materials (plant waste/debris, grass, leaves, garden waste, food scraps) by microorganisms. (https://www.epa.gov/recycle/composting-home)

Vermicomposting (composting with worms) is a method of composting (is a composting method) that takes up little space, requires few materials, is cheap (inexpensive) and can be done very easily (both indoors and outdoors), improving the structure and health of the soil by adding organic matter, while also helping the soil to retain moisture and nutrients. (https://www.epa.gov/recycle/composting-home)

Research on compost fertilization (fertilization with compost) as organic fertilizers (Balkrishna et al., 2024) (results from wineries and distilleries (grape pomace/marc, citrus waste, manure), are beneficial for crops of species from the *Lamiaceae* family, adapting very well, strongly influencing the production of essential oil (essential oil productions), significantly increasing the concentration of volatile compounds as well as the total yield of volatile compounds.

Vermicompost can also be referred (defined) to as: it represents the operation (the process) by which waste from medicinal plants is transformed into vermicompost, which offers numerous benefits to agriculture at the present time (Balkrishna et al., 2024).

Vermicompost currently represents (is) a valuable source of fertilizers, significantly improving the physico-chemical (physical and chemical) composition of the soil (culture substrate/growing medium) in the case of agricultural work, due to the high level (content) of essential minerals, as well as those that lead to plant growth and development, while (it) also being able to mention the fact that it ensures good soil health, significantly increasing the yield of plant growth and development, both quantitatively and qualitatively (Balkrishna et al., 2024).

The effect of fertilization on antioxidant activity, essential oil production and plant height has not been widely (extensively) studied (Chrysargyris et al., 2016).

Lack of water in Lavandula angustifolia Mill. crops (Water shortage in lavandula angustifolia* Mill.*)

The effect of drought in agricultural crops on plant growth and development has been extensively studied, and previous studies show that irrigation has an effect on the morphological and physiological characteristics of plants, thus determining their yield (Pirzad and Mohammadzadeh, 2018).

Lavender (*Lavandula stoechas*) present in a research/wicch was part of the study (extensive green roofs) along with other Mediterranean plants, showed increased sensitivity to water availability, so the species was subjected to water stress (was stressed out by water), being the species that had a hard time adapted/adapting with difficulty to the total lack of water, which disadvantaged flowering/made it hardet to bloom (advancing the moment according to the specialized literature/ahead of the time specified in the literature) (Bellini A. et al., 2024).

In hydroponic cultures, high levels of P significantly affected plant growth, and if the level of N is low, it had a negative effect because it reduced the chlorophyll content in the plants (Chrysargyris et al., 2016).

It is known that plant fertigation, absorption and accumulation of minerals represent one of the most important factors in plant growth and development, this leads to very good quality and quality through high production of plants and vegetative mass plus inflorescences. It is well known that plant fertigation, mineral absorption, and accumulation are among the most important factors in plantt growt and development, leading to high quality and yield through high plant and vegetative mass production plus inflorescence (Chrysargyris et al., 2016).

Lavender plants subjected to water stress, showed reduced growth compared to those that did not suffer, but also a completely different chemical composition from plants that were not properly irrigated (Chrysargyris et al., 2016).

Chemical Fertilizers

The excessive use of chemical fertilizers in conventional agricultural crops (systems) has drastically reduced the efficiency of nutrient use, causing serious environmental problems by depleting soil minerals, acidifying (acidifying) the soil (through soil mineral depletion and soil acidification) (Ostadi et al., 2020).

Currently, new measures are being sought to replace the avoidance (use) of fertilizers and pesticides in agricultural work, offering an alternative through the use of beneficial microorganisms, thus avoiding soil pollution (Pole et al., 2022).

In the case of some plants belonging to the *Lamiaceae* family, phosphate-based fertilization (fertilizers) helps to increase the carbon fixation rates, water use efficiency, and improve the eco-physiological performance of plants (referring strictly to the genus *Lavandula* spp.), this being highlighted in the (as evidence by the) high productivity of lavender species (Crysargyris et al., 2019; Ruiz-Navaro, 2019).

Phosphorus fertilization was (has been) effective, resulting thus bringing the lavender crop a significantly higher production of plant mass production in lavender crops, as well as a much higher yield of extracted essential oil (Peçanha, 2021)

The excessive application (excessive application) of chemical fertilizers to crops (especially those with high levels of nitrogen

and phosphorus) can modify the plant structure of the plant (can alter the plants vegetative structure), thus reducing species diversity (Ostadi et al., 2020).
Nitrogen, phosphorus and potassium affect both growth and essential oil synthesis in medicinal plants, influencing the levels of enzymes that are very important in terpenoid biosynthesis (Chrysargyris et al., 2016).

Nutrient requirements for good soil fertility and beneficial plant growth and development

Plant growth requires a very good (excellent) compatibility between the plant, the atmosphere and the soil on which it is grown (cultivated), the latter providing the location, support, foundation, and nutrients necessary for growth and development. (https://forages.oregonstate.edu/nfgc/eo/onlineforagecurriculum/instructormaterials/availabletopics/fertilization/elements).
Table 2 shows the elements necessary for the good development of a plant (https://forages.oregonstate.edu/nfgc/eo/onlineforagecurriculum/instructormaterials/availabletopics/fertilization/elements).

Table 2. Chemical elements necessary for plant development and growth

Where are	Chemical Elements
Air	Carbon, Hydrogen, Oxygen
Soil	Boron, Calcium, Carbon, Cobalt, Chlorine, Copper, Iron, Phosphorus, Hydrogen, Magnesium, Manganese, Molybdenum, Nickel, Nitrogen,Oxygen, Potassium, Silicon, Sodium, Sulfur, Zinc

The present research was based entirely on the study (on studying) of the specialized literature on the fertilization of the *Lavandula angustifolia* Mill species.
Thus, the latest research was consulted, following the research (focussing on studies) carried (conducted) out by specialists in the field.
A study carried out in Greece shows that Lavender is sensitive to fungal infections with *Rhizoctonia* and *Fusarium*, which in most cases of infection with these diseases, the plants are completely destroyed (Giannoulis et al., 2020).
The waste generated (from plants of the *Lamiaceae* family) following the (after) harvesting, processing, or extraction (extracting) of the essential (volatile) oil can retain their nutritional value, and these can be transformed into compost and vermicompost with beneficial effects on crops.
Studies on medicinal (aromatic) plants show that they are very good candidates for the hyperaccumulation of heavy metals that they neutralize through chelation and soil phytoremediation (Mishra and Chandra, 2022)
Lavandula angustifolia Mill. is a species that presents increased interest in terms of the resulting plant mass, used in the production of essential oil and perfume, as well as the fact that it is useful in soil phytoremediation technologies (Lázaro et al., 2006)
The results obtained from the analysis of the specialist literature (te specialized literature) on the ecological fertilization of the *Lavandula angustifolia* Mill. species show that the species adapts very well to both types of fertilization, with excellent results in terms of production and quality.
Both plant development and essential oil production in medicinal plants can be affected both positively and negatively depending on the ratio and quantity of minerals applied to the crop.
Water deficit restricts both plant growth and development, with negative effects on production and especially of essential oils that are affected in terms of existing chemical compounds.
The use of biostimulants can increase both the production of inflorescences (spike/spices) and the production of essential oil, if the crop was (has been) infested with diseases and an organic (biological) culture (farming) was (has been) used.

CONCLUSIONS

The species *Lavandula angustifolia* Mill. it is very well suited to both organic and chemical fertilization, with very good results. Thus, production is higher in the case of fertilization where organic products are applied. The *Lavandula angustifolia* Mill. species adapts very well to both organic and chemical fertilization. Fertilization with organic products leads to very good productive results, in some cases even superior to conventional fertilization. The application of organic fertilizers contributes to obtaining high yields, highlighting their efficiency and the sustainability of organic practices in lavender cultivation. Fertilization contributes significantly to the growth and development of plants, and the type of fertilization chosen can directly influence their quality, development rate and productivity. Given the importance and multiple uses of the *Lavandula angustifolia* Mill. species, the increasingly widespread use of organic fertilization is recommended.

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VARIATION IN CBDA AND CBD CONTENT IN SOME INDUSTRIAL HEMP GENOTYPES

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Abstract

The content of CBDA and CBD was determined in three hemp genotypes, sown in two different seasons. Six experimental variants resulted, V1 (Silvana, S1), V2 (Silvana, S2), V3 (Loja, S1), V4 (Loja, S2), V5 (Finola, S1), and V6 (Finola, S2). The CBDA content varied between CBDA = 0.43±0.04% (V4) and CBDA = 0.70±0.04% (V5). The CBD content varied between CBD = 0.52±0.06% (V1) and CBD = 0.88±0.06% (V2). The CBDA/CBD ratio varied between CBDA/CBD = 0.5774±0.0688 (V2) and CBDA/CBD = 1.0577±0.0688 (V2). The CBD/CBDA ratio varied between CBD/CBDA = 0.0455±0.1115 (V1) and CBD/CBDA = 1.7320±0.1115 (V2). Compared to the mean value of the experiment, in the case of the CBDA, variant V5 presented positive differences, and variant V4 negative differences ($p<0.05$). In the case of the CBD, compared to the mean of the experiment, variant V1 presented negative differences ($p<0.05$). Based on PCA, PC1 has explained 64.27% of variance, and PC2 has explained 35.134% of variance. Cluster grouping and a ranking of the experimental variants were generated, in relation to the considered indices.

***Key words**: clustering, cannabidiol (CBD), cannabidiolic acid (CBDA), industrial hemp, principal component analysis (PCA), ranking.*

INTRODUCTION

Industrial hemp is a promising plant with high ecological and economic efficiency (Nath, 2022). Recent studies have highlighted the development potential of an industry based on industrial hemp, due to the diversity of resources provided by this plant (Burton et al., 2022). Industrial hemp has ecological importance (carbon sequestration), agronomic importance (crop structure, beneficial effects on the soil), and economic importance (fiber production, seed production), with beneficial effects for farmers and various economic sectors (Burton et al., 2022; Puiu et al., 2023; Visković et al., 2023; Popa et al., 2024).

Products and by-products derived from industrial hemp are used in the food industry, particularly for functional foods with health benefits (Rupasinghe et al., 2020).

Hemp belongs to the category of "gluten-free" plants, producing highly nutritious seeds (similar to soy) that are used in various functional foods. Moreover, it is a fully utilizable crop across multiple economic sectors (Yano & Fu, 2023).

From the perspective of climate change, which is increasingly affecting agricultural crops, industrial hemp is a plant of interest for fiber and food production due to its high drought resistance (Ahmed et al., 2022; Gill et al., 2023; Kaur & Kander, 2023).

Over the past decade, research on industrial hemp cultivation has focused on aspects such as yield in relation to climatic stress, seed quality (high-quality seeds), and optimal fertilizer doses (Dudziec et al., 2024). At the same time, studies have analyzed bioactive compounds regarding their selective activity or differences between male and female plants.

The active principles in hemp have been studied for their potential use in medicine and pharmaceuticals (Rupasinghe et al., 2020; Hossain & Chae, 2024; Simei et al., 2024).

The profile and content of active compounds (e.g., CBDA, CBD) in hemp plants are determined by genotype, as well as the interaction of genotype × environment ×

cultivation technology (De Meijer et al., 2003; Adesina et al., 2020).
In industrial hemp, phytocannabinoids (e.g., CBD, THC) are present in all genotypes but in varying amounts - with some genotypes exhibiting very low THC levels and others showing higher concentrations. Studies have reported that northern-region industrial hemp tends to have lower THC levels and higher CBD content (Leizer et al., 2000; Russo and Taming, 2011).
The common catalog includes many hemp genotypes, but recent studies indicate that the cannabinoid content is not yet well known for all varieties (Glivar et al., 2020). HPLC analysis has been used by researchers to investigate the profile and content of 13 cannabinoids in a collection of 15 hemp varieties. Under uniform cultivation conditions, the tested genotypes showed significant variations in cannabinoid content, according to the authors.
The CBD profile and essential oil content were also investigated in wild hemp genotypes (nine accessions) and 13 registered genotypes, breeding lines, and CBD-rich hemp strains (Zheljazkov et al., 2020). The authors reported variations in CBD profile and oil content depending on the genotypes studied.
Due to the growing importance and interest in CBD, studies have been conducted to identify high-CBD genotypes, as well as research on cultivation technologies that ensure high CBD yield (Chiluwal et al., 2023). Depending on the region and eco-climatic conditions, the sowing date has been evaluated for its influence on CBD production in different hemp varieties (Chiluwal et al., 2023).
For fiber hemp cultivation, high plant density is favorable for fiber production, whereas for seed and CBD production, a wider spacing between plants (between rows) is recommended, as it promotes branching and flower production (Adesina et al., 2020).
Regarding sowing date, a higher CBD content has been associated with earlier sowing (Chiluwal et al., 2023). The authors recorded CBD content variations between genotype groups, depending on their origin (Colorado, Kentucky).
Cannabidiol (CBD), derived from industrial hemp, is a promising bioactive compound for the food industry (functional foods) and the pharmaceutical and medicinal industry (pharmaceutical products and medications) (Deguchi et al., 2020; Charles et al., 2024).
This study evaluates the content of cannabidiolic acid (CBDA) and cannabidiol (CBD) in three industrial hemp genotypes, sown at two different sowing dates under uniform cultivation conditions, in the plains of Western Romania.

MATERIALS AND METHODS

The research was conducted at ARDS Lovrin during the 2023–2024 agricultural year. The field experiments were carried out on Plot 12, under chernozem soil slightly gleyed, medium clay loam with moderate fertility. The climatic conditions for the 2023-2024 agricultural year were recorded at the Meteo Station within ARDS Lovrin (Figure 1).

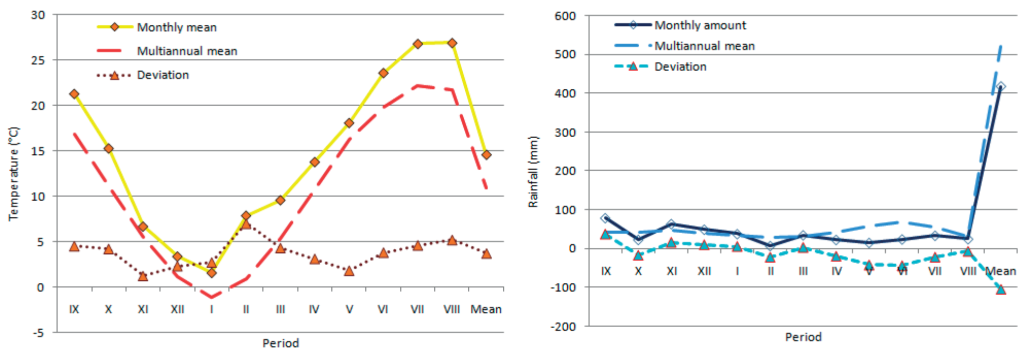

Figure 1. Climatic conditions, 2023-2024 agricultural year, ARDS Lovrin

Three genotypes were cultivated (Silvana, a variety developed at ARDS Lovrin, and Loja and Finola, foreign varieties) in two sowing periods (April 29, 2024, and May 15, 2024) and at four different row spacings (12.5 cm, 70 cm, 100 cm, and 150 cm).
Due to the lack of precipitation in the spring months, plant emergence was uneven. Emergence was recorded starting on May 17, 2024, for the first sowing period and on June 3, 2024, for the second sowing period.
The experimental variants analyzed for CBDA and CBD content were at 1-meter row spacing: conditions, characterized by medium fertility.
Three hemp genotypes were cultivated at two different sowing times (sowing time 1 and sowing time 2), with a row spacing of 1 meter: Silvana, sowing time 1 (V1), Silvana, sowing time 2 (V2), Loja, sowing time 1 (V3), Loja, sowing time 2 (V4), Finola, sowing time 1 (V5), Finola, sowing time 2 (V6).
To determine the CBDA and CBD content, plant samples were collected on July 19, 2024, from inflorescences. The harvested samples were placed in paper bags and sent for analysis. The plant samples were analyzed at Chromatec Plus Laboratory, a nationally certified SRAC and internationally certified IQNet facility. The CBDA and CBD content was determined using accredited laboratory methods, employing an HPLC system. Multiple replicates were prepared and analyzed for each sample, and the results were reported as mean values.
Based on the obtained CBDA and CBD indices, the CBDA/CBD and CBD/CBDA ratios were calculated.
The experimental results were analyzed to assess differences among experimental variants. Statistical analyses were performed using ANOVA, regression analysis, and multivariate analysis, conducted in Excel and PAST software (Hammer et al., 2001; Wolfram Alpha, 2020).

RESULTS AND DISCUSSIONS

The industrial hemp plant samples (inflorescences) were analyzed, and the CBDA and CBD content values were obtained (Table 1). Based on these values, the CBDA/CBD and CBD/CBDA ratios were calculated (Table 1). CBDA content ranged between 0.43±0.04% (V4) and 0.70±0.04% (V5), CBD content varied between 0.52±0.06% (V1) and 0.88±0.06% (V2), The CBDA/CBD ratio recorded values between 0.5774±0.0688 (V2) and 1.0577±0.0688 (V1), The CBD/CBDA ratio ranged between 0.9455±0.1115 (V1) and 1.7320±0.1115 (V2). The reliability of the experimental values was confirmed by the results of the ANOVA test (Table 2).

Table 1. CBD, CBDA values, and calculated ratios in industrial hemp

Experimental variant	CBDA	CBD	CBDA/CBD	CBD/CBDA
	(%)	(%)	Ratio	Ratio
V1	0.55	0.52	1.0577	0.9455
V2	0.51	0.88	0.5774	1.7320
V3	0.65	0.84	0.7698	1.2990
V4	0.43	0.59	0.7247	1.3798
V5	0.70	0.80	0.8792	1.1374
V6	0.52	0.77	0.6753	1.4808
SE	±0.04	±0.06	±0.0688	±0.1115

Table 2. ANOVA Test

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1.9906	3	0.6635	19.843	3.29E-06	3.098
Within Groups	0.6688	20	0.0334			
Total	2.6594	23				

For each parameter, the position of the variants was analyzed in comparison with the mean value.
In the case of CBDA, the results are presented in Table 3. Compared to the mean ($\overline{CBDA}$ = 0.56%), variant V4 presented negative differences, and variant V5 positive differences ($p<0.05$).

Table 3. Significance of differences in CBDA for industrial hemp variants

Variants	Given mean	Difference and Significance	95% conf. interval	t	p (same mean)
V1	0.55	-0.01^{ns}	(-0.093677 0.11368)	0.2479	0.814
V2	0.51	-0.05^{ns}	(-0.053677 0.15368)	1.2397	0.270
V3	0.65	0.09^{ns}	(-0.013677 0.19368)	-2.2315	0.076
V4	0.43	-0.13^{o}	(0.026323 0.23368)	3.2233	0.023
V5	0.70	0.14*	(0.036323 0.24368)	-3.4712	0.018
V6	0.52	-0.04^{ns}	(-0.063677 0.14368)	0.9918	0.367

The results for CBD content are shown in Table 4. The mean CBD value across all variants was $\overline{CBD}$ = 0.73%. V1 showed negative deviations compared to the mean (p<0.05).

Table 4. Significance of differences in CBDA for industrial hemp variants

Variant	Given mean	Difference and Significance	95% conf. interval	t	p (same mean)
V1	0.52	0.21°	(0.061449 0.36522)	3.6106	0.015
V2	0.88	0.15ns	(-0.0052178 0.29855)	-2.4823	0.056
V3	0.84	0.11 ns	(-0.045218 0.25855)	-1.8053	0.131
V4	0.59	0.14 ns	(-0.0085511 0.29522)	2.4259	0.060
V5	0.80	0.07 ns	(-0.085218 0.21855)	-1.1283	0.310
V6	0.77	0.04 ns	(-0.11522 0.18855)	-0.6206	0.562

In the case of the CBDA/CBD ratio, V1 showed a positive difference (0.2770), p = 0.0101, while V2 showed a negative difference (-0.2033), p = 0.0318 (Figure 2).

In the case of the CBD/CBDA ratio, V1 showed a negative difference (-0.3836), p = 0.0184, while V2 showed a positive difference (0.4029), p = 0.0153.

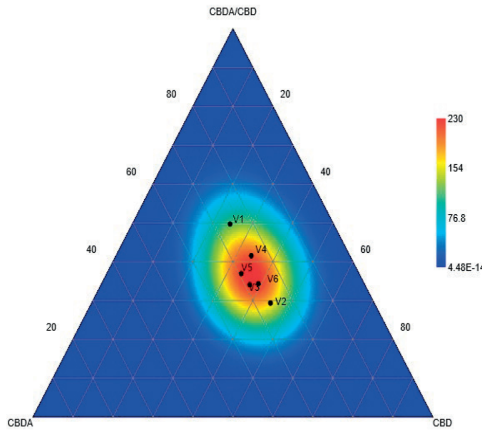

Figure 2. CBDA/CBD ratio in relation to primary parameters

The variation of the CBDA/CBD ratio, depending on the basic indices (CBDA, CBD), was described by the equation (1), R^2 = 0.998, p < 0.001, with the graphical distribution shown in Figure 3, and Figure 4. A divergent contribution of CBDA and CBD to the calculated ratio values was observed.

Based on the coefficients of equation (1) as well as the 3D distribution (Figure 3), it was found that the CBDA parameter had a higher amplitude in forming the ratio (CBDA/CBD) compared to CBD.

Additionally, a "scissor-like" interaction of the CBDA and CBD indices in forming the calculated ratio was observed.

$$CBDA/CBD = ax^2 + by^2 + cx + dy + exy + f \quad (1)$$

where: CBDA/CBD – the calculated ratio;
x – CBDA content; y – CBD content;
a, b, c, d, e, f – coefficients in equation (1);
a = -0.45245034; b = 1.49325136;
c = 3.41182257; d = -2.18829850;
e = -2.00205901; f = 0.62478504

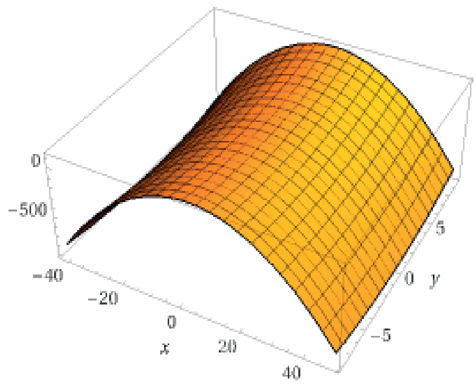

Figure 3. 3D Model for the distribution of the CBDA/CBD ratio, industrial hemp

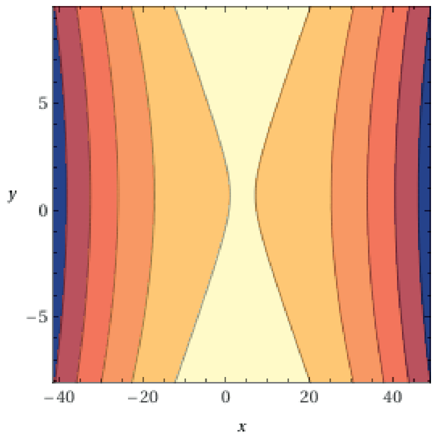

Figure 4. Isoquant model for the distribution of the CBDA/CBD ratio, industrial hemp

The multivariate analysis explained the PCA loadings, in relation to the determined parameters and principal components, as shown in Table 5.

The PCA diagram is presented in Figure 5. Variants V3 and V5 were positioned in association with CBDA and CBD, while variants V6 and V2 were positioned in association with the CBD/CBDA ratio. V1 and V4 displayed an independent position.

Table 5. PCA loadings based on indices for industrial hemp

	PC 1	PC 2	PC 3	PC 4
CBDA	-0.19722	0.79964	-0.22482	0.52072
CBD	0.44635	0.58765	0.30894	-0.59999
CBDA/CBD	-0.61747	0.06373	0.78398	0.00675
CBD/CBDA	0.61693	-0.10575	0.48928	0.60730

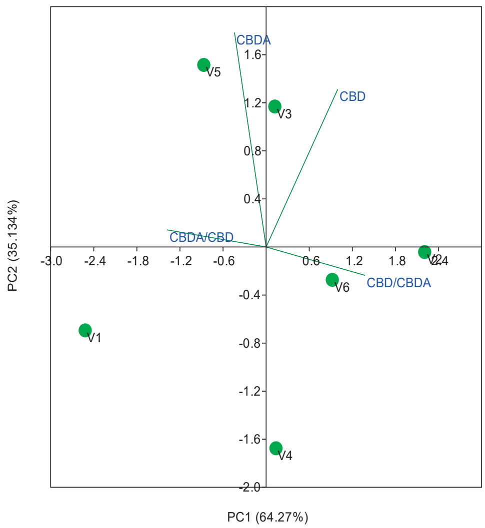

Figure 5. PCA diagram, industrial hemp parameters

The cluster analysis facilitated the grouping of variants based on similar values of the indices and calculated ratios (Coph. corr. = 0.723) (Figure 6).

Variant V1 was positioned independently. The other five variants were grouped into a subcluster, with different associations: V2 was positioned separately, while the other variants were grouped into two subclusters: (V3, V5) and (V4, V6).

The calculated similarity level is presented in Table 6.

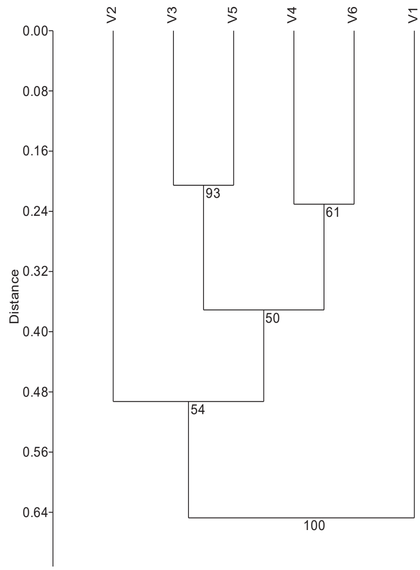

Figure 6. Cluster dendrogram of industrial hemp variants

Table 6. SDI values, industrial hemp variants

	V1	V2	V3	V4	V5	V6
V1		0.9902	0.5659	0.5646	0.4118	0.7044
V2	0.9902		0.4957	0.4861	0.6980	0.2914
V3	0.5659	0.4957		0.3456	0.2054	0.2526
V4	0.5646	0.4861	0.3456		0.4468	0.2305
V5	0.4118	0.6980	0.2054	0.4468		0.4391
V6	0.7044	0.2914	0.2526	0.2305	0.4391	

The influence of row spacing on CBDA and CBD content

The study considered a row spacing of 1 meter for the analyzed variants, but the specialized literature suggests that a greater row spacing can favor more intense branching and an increase in the number of inflorescences, which could explain the observed variations in CBDA and CBD content.

If we extrapolate this idea, the higher CBDA values in variant V5 (Finola, first sowing season) may be associated with a more vigorous plant structure, with more active inflorescences, allowing for a greater accumulation of cannabinoid acids.

This hypothesis could be confirmed by correlating plant biomass with CBDA/CBD content, an aspect that was not included in this analysis but is worth investigating.

Similarly, the higher CBD content in V2 (Silvana, second sowing season) suggests a possible effect of sowing time on CBD biosynthesis, which may be more efficient in the second season due to the influence of higher temperatures during the vegetation period.

The impact of climatic conditions on $CBDA \Rightarrow CBD$ conversion

Based on the presented data, we know that the plants were harvested on July 19, 2024, meaning they were exposed to high temperatures and potentially intense thermal stress during the final weeks of vegetation.

Variant V2 (Silvana, second sowing season), which had the highest CBD content, was sown later, meaning it had a shorter vegetation period but developed under higher temperature conditions.

Previous studies (Chiluwal et al., 2023) have shown that high temperatures can accelerate CBDA → CBD conversion by increasing the activity of enzymes responsible for this transformation.

This phenomenon could explain why the CBDA/CBD ratio is lower in variant V2 and why CBD/CBDA is higher. In other words, the sowing time influences not only cannabinoid production but also the balance between CBDA and CBD.

Variation among genotypes and possible correlations with their genetic origin

Looking at the raw data, we observe that Finola (V5 and V6) has the highest CBDA content, while Silvana (V1 and V2) shows the greatest variations in CBDA/CBD ratios. This could indicate that:

- Finola is more stable in CBDA accumulation, regardless of sowing time. This may be explained by its genetic origin, as Finola is known as a northern variety adapted to colder conditions, which favors the accumulation of cannabinoid acids.
- Silvana, a native Romanian variety, is more sensitive to climatic factors, making sowing time a critical factor in optimizing its CBD production.
- Loja (V3 and V4), despite having moderate values, seems to be the most affected by changes in sowing time, suggesting that this genotype may be less stable and more dependent on environmental factors.

These observations suggest that the analyzed genotypes responded differently to environmental factors, and future selection efforts could consider resistance to climate stress and the stability of cannabinoid biosynthesis.

Possible implications for optimizing CBD production

If the goal is to maximize CBD production, the results suggest that:

- Silvana should be sown later (second sowing season) to achieve a high CBD content, but it needs to be monitored to prevent losses due to the degradation of active compounds.
- Finola provides stable CBDA production, making it an ideal candidate for CBDA-based extracts, but it may require different processing methods to maximize CBD conversion.
- Loja exhibits high variability, suggesting that further studies are needed to determine its optimal cultivation conditions.

These findings provide new insights for optimizing CBD and CBDA production based on genotype selection, sowing time, and climatic conditions.

CONCLUSIONS

Industrial hemp genotypes sown at two different times and cultivated under uniform conditions generated variable content of bioactive compounds (CBDA and CBD)

Variant V5 (Finola, sowing time 1) ensured a higher CBDA content compared to the other variants, with statistical significance ($p < 0.05$) relative to the experiment's mean.

In the case of CBD, variant V2 (Silvana, sowing time 2) exhibited a higher content, but without statistical significance when compared to the experiment's mean.

For the CBDA/CBD ratio, compared to the experiment's mean, variant V1 showed a positive difference (0.2770; $p = 0.0101$), while variant V2 exhibited a negative difference (-0.2033; $p = 0.0318$).

In the case of the CBD/CBDA ratio, compared to the calculated mean, variant V1 showed a negative difference (-0.3836; $p = 0.0184$), while variant V2 showed a positive difference (0.4029; $p = 0.0153$).

A polynomial model ($R^2 = 0.998$, $p < 0.001$) and graphical models (3D, isoquants) described the variation of the CBDA/CBD ratio in relation to the basic indices (CBDA, CBD). A divergent contribution of both indices to the calculated ratio values was observed.

The multivariate analysis highlighted the association of the variants with the indices and the calculated ratios, as well as the grouping of the variants based on similarity.

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EXPLORING MORPHOLOGICAL VARIABILITY IN CHICKPEA CULTIVATION

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Abstract

This study aimed to evaluate the performance and morphological variability of ten chickpea (Cicer arietinum) lines, utilizing biological material from the Vegetable Research and Development Station Bacău. Data collection included key phenological and agronomic parameters: germination days and rate, days to first flowering, 50% and 100% flowering, and pod maturity stages (50% and 90%). Morphological traits assessed included flower colour, number of flowers per peduncle, plant growth habit, pigmentation, leaf type, leaflet count, and plant height. Additionally, yield-related traits such as pod insertion height, number of pods per plant, seeds per pod, total seeds per plant, seed mass per plant, seed shape, surface texture, and colour were evaluated. Results highlighted significant variability among the lines in flowering time and pod maturity, with L4 showing early, synchronized flowering and L6 exhibiting the longest flowering period. Pod maturity analysis showed that most lines reached 50% maturity between 115-119 days, indicating a consistent development rate. This comprehensive evaluation provides valuable insights into chickpea genetic diversity, supporting future breeding programs, with phenotyping playing a key role in understanding the genetic potential and adaptability of the accessions.

***Key words**: agronomic parameters, morphological traits, genetic diversity, breeding programs.*

INTRODUCTION

Chickpea (*Cicer arietinum*) is an economically valuable, diploid legume crop that is self-pollinating. With a genome size of approximately 740 Mbp, it is recognized for its rich nutritional composition and is widely grown for its substantial dietary benefits (Kumar et al., 2011).

Leguminous crops offer substantial nutritional benefits to humans, serving as rich sources of protein, omega-3 fatty acids, and essential macro- and micronutrients. Moreover, legumes contribute to agricultural sustainability by enhancing soil nitrogen levels. Globally, chickpeas rank as the second most important legume staple food crop, following dry beans. However, despite their notable advantages, legumes lag significantly behind cereals in genetic advancement. This disparity is primarily attributed to limited research efforts, constraints imposed by a narrow genetic base, and various biotic and abiotic challenges exacerbated by changing climatic conditions (Singh et al., 2022). Globally, two primary types of chickpea cultivars Kabuli and Desi are cultivated. Kabuli chickpeas are mainly grown in temperate regions, particularly in areas such as the Mediterranean, encompassing Western Asia, Northern Africa and Southern Europe. In contrast, Desi chickpeas are primarily cultivated in semi-arid tropical regions, including the Indian subcontinent and Ethiopia (Muehlbauer and Singh, 1987). Desi chickpeas are typically characterized by small, angular seeds with a rough, pink or purple flowers and dark seed coat, which result from the presence of anthocyanin pigments. In comparison, Kabuli chickpeas have larger, smooth-surfaced, beige seeds that are owl-shaped and produce white flowers due to the absence of anthocyanin pigments (Pundir et al., 1985). Additionally, Desi-type chickpeas tend to mature earlier and exhibit higher yields than Kabuli types. Achieving high yields in various agroecological regions requires rapid seedling establishment and uniform plant stands, both of which are influenced by seed vigour. Variations in seed vigour among legume crops

have been linked to differences in seed coat pigmentation (Dickson and Petzoldt, 1988; Kantar et al., 1996). Cultivated chickpeas, including Desi and Kabuli types, are derived from diverse genetic pools with distinct agronomic characteristics and are thought to trace their origins to the wild progenitor *Cicer reticulatum*. Recently, draft genome sequences for these varieties have been completed (Jain et al., 2013). A critical agronomic trait in chickpeas is their growth habit, which shows significant variation among Desi, Kabuli, and wild accessions (Upadhyaya et al., 2006). Typically, Desi and Kabuli chickpea cultivars display an erect to semi-erect growth pattern, while wild species accessions often exhibit a prostrate growth form (Wang and Li, 2008). The growth habit is a fundamental factor in determining plant architecture. It significantly influences key traits related to seed and pod yield and plays an essential role in the adaptation of chickpea plant types to diverse agroecological conditions (Benlloch et al., 2015; Upadhyaya et al., 2017). In chickpeas, the leaves, stems, and pods are covered with glandular trichomes that secrete acidic aqueous exudate, acting as a potential defence mechanism against *Helicoverpa armigera* infestation (Brar & Singh, 2017). Chickpeas exhibit superior performance in cooler climates due to their classification as C3 plants, which are generally better adapted to winter conditions (Pânzaru et al., 2022). Nevertheless, the harvest index (HI) for pulses, ranging between 15% and 20%, remains considerably lower than that of cereals, which typically fall within the 45-50% range a disparity that presents a significant challenge. This issue primarily arises from excessive vegetative growth, which may be mitigated through the early allocation of dry matter toward seed development (Saxena and Johansen, 1990). The determination of the significance and efficiency of yield components represents a primary objective in agricultural research. Additionally, the interplay between yield attributes and overall yield can vary across different trials, agronomic practices, and breeding initiatives. Identifying and optimizing the influential yield components, as well as understanding their interrelationships, can result in substantial yield improvements and enhanced outcomes (Kayan & Adak, 2012).
Research conducted by İslam and Begüm (1985), Khan et al. (1989), Gravaes & Helms (1992), and Toker & Cagirgan (2004) has demonstrated a positive association between grain yield and traits like plant height, branch number, and pod count per plant. Conversely, grain yield exhibits a significant negative correlation with traits such as days to maturity and flowering time (Ali & Ahsan, 2012; Mallu et al., 2015). Grain yield in chickpea is a quantitative trait influenced by a combination of genetic factors and environmental variations (Muehlbauer and Singh, 1987). Kayan and Adak (2012) further identified plant height, biological yield per plant, and the number of pods per plant as the most critical yield determinants in chickpea production.

Despite persistent efforts from national and international chickpea enhancement initiatives over the past several decades, notable improvements in chickpea yield and productivity have yet to be realized (Singh et al., 2022). In this context, the study aimed to evaluate the performance and morphological variability of ten chickpea lines. This information supports future breeding programs, highlighting the crucial role of phenotyping in understanding the genetic potential and adaptability of these accessions.

MATERIALS AND METHODS

The experiment was conducted at the Vegetable Research and Development Station in Bacău during the year 2024. The biological material consisted of ten chickpea lines (15 seeds per line) sourced from the same research station. The trial setup utilized seedlings cultivated in alveolar trays containing 70 alveoli. Sowing took place on April 11th, employing a textured substrate with moderate fertilization enriched with micronutrients. The resulting seedlings were subsequently transplanted on June 5, into pots with a diameter of 30 cm and a height of 22 cm. The plants were cultivated outdoors and protected with a shading net to regulate environmental conditions. The descriptors for developping evaluation sheets and characterizing the breeding material for the chickpea species were selected and standardized from the following sources: Descriptors for Chickpea (*Cicer arietinum* L.) (IBPGR/ICRISAT/ICARDA,

1993) and Biology of *Cicer arietinum* L. (Chickpea) (Office of the Gene Technology Regulator, 2019), in accordance with UPOV guidelines (UPOV, 2020). The descriptors used for analysing chickpea germplasm was assessed based on the following criteria:

Germination and Growth Stages

The germination and growth stages of chickpea were evaluated based on several key indicators. The germination onset was recorded as the number of days from sowing until seedling emergence. Flowering onset was determined by the number of days from sowing until the appearance of the first open flower. The 50% flowering stage was defined as the number of days from sowing until half of the plants exhibited flowers, while the 100% flowering stage was marked by the number of days required for all plants to produce flowers (IBPGR/ICRISAT/ICARDA, 1993).

Reproductive Development

The reproductive development of chickpea was assessed using several key parameters. Flower colour was recorded for newly opened flowers and categorized as dark pink, pink, blue, or white (Figure 1).

Figure 1. Flower color for chickpea: A - white; B - pink (original)

Pod appearance was determined by noting the number of days from sowing until the formation of the first pods. The 50% pod maturity stage was defined as the number of days from sowing until half of the pods reached maturity, while the 90% pod maturity stage indicated the time taken for 90% of the pods to mature. Additionally, the number of pods per plants was counted, and the number of pods per peduncle was recorded based on observations of the majority of peduncles.

Morphological Characteristics

The morphological characteristics of chickpea were evaluated based on several criteria. Anthocyanin pigmentation was observed at the base of the branches and categorized into three levels: no anthocyanin, moderate anthocyanin, and high anthocyanin (Figure 2).

Figure 2. Anthocyanin pigmentation for chickpea: A - No anthocyanin pigmentation; B - Anthocyanin pigmentation (original)

Pubescence intensity on the shoots was visually assessed and scored as low, moderate, or high. Growth habit was determined by measuring the angle of the main branches during the pod-filling stage, and classified into five categories: erect (0-15° from vertical), semi-erect (16-25°), semi-spreading (26-60°), spreading (61-80°), and prostrate (with branches spreading along the ground) (IBPGR/ICRISAT/ICARDA 1993).

Leaf and Plant Structure

The leaf and plant structure of chickpea was analyzed using several key descriptors. Leaf type was observed before maturity and classified into three categories: normal (pinnate), simple (with an undivided blade), and multipinnate (with multiple rachis divisions). The number of leaflets per leaf was determined by calculating the average from nine leaves, collected from three randomly selected plants at the pod-filling stage (IBPGR/ICRISAT/ICARDA 1993). Plant height was measured from the ground to the tip of the tallest stem when 50% of the pods had matured. Additionally, the first pod insertion height was recorded as the distance from the ground to the lowest pod, also measured at the 50% pod maturity stage

Pod and Seed Traits

Seed texture, assessed in mature seeds, is classified as smooth, rough, or tuberculate. Seed color: evaluated in mature seeds stored for no more than five months (Figure 3). Seed shape, observed in mature seeds and categorized as: angular (Desi type), pea-shaped, irregular (Kabuli type) (IBPGR/ICRISAT/ICARDA 1993, UPOV 2020).

Figure 3. Seed characteristics for chickpea (original)

Number of pods per plant: the average number of pods per plant.
Number of seeds per plant: the total number of seeds harvested from three representative plants.
Number of seeds per pod: the average number of seeds per pod.
Seed mass per plant (g): the total weight of all dried seeds.
The data were statistically analyzed in Excel, using means and standard deviations for result interpretation.

RESULTS AND DISCUSSIONS

The chickpea lines show considerable variation in germination percentage. Lines L1, L4, L5, L6, L9, and L10 have a high germination percentage, ranging from 80% to 100%. Lines L3 and L7 show lower germination percentages, with 46.7% and 26.7%, respectively. Line L8 has the lowest germination percentage, at 6.7%. Most lines started germination in a relatively short period, between 5 to 6 days. Line L8 had the longest time to first germination, 9 days, suggesting slower germination compared to the other lines. Lines L1, L4, L5, L6, L9, and L10 are the most efficient in terms of germination, with high germination percentages and relatively quick germination times (6 days or less). Lines L3, L7 and L8 require further attention, as they performed poorly in terms of germination. This may indicate they are more sensitive to environmental conditions or seed quality (Figure 4).

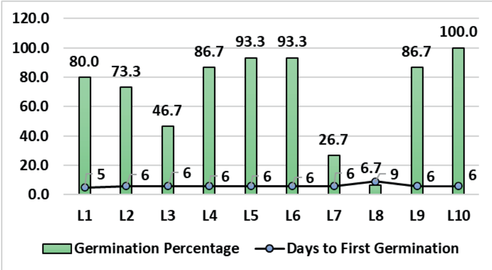

Figure 4. Comparison of Germination Days and Germination Rate in Chickpea Lines

The earliest flowering onset is observed in L4 (61 days after sowing), indicating a faster transition to reproductive phase (Figure 5). Most lines (L3, L5, L6, L7, L9) exhibit flowering onset around 71 days, suggesting a clustering of lines with a similar flowering pattern. Lines such as L1, L2, and L8 fall in the intermediate range between 66-68 days. L2, L4, and L8 exhibit the earliest completion of flowering (75 days), indicating a highly uniform flowering pattern across these lines.

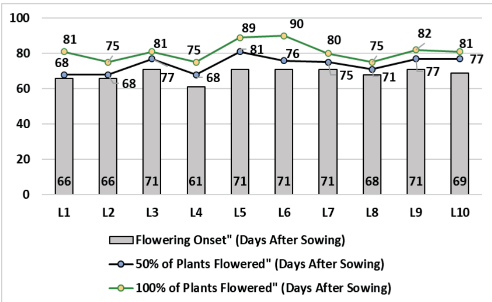

Figure 5. Chikpea flowering

L6 reaches 100% flowering at 90 days, demonstrating the highest variability among plants within this line, or a prolonged flowering duration. L4 shows early and synchronized flowering, making it suitable for environments requiring rapid maturation. L5 and L6 have the most extended flowering duration, indicating

possible variability within the population, which could affect consistency in yield.
Most lines (L2, L4, L6, L7, L9, L10) mature around 115-119 days, showing a moderate and consistent pod development rate across these genotypes. Lines like L5, L6, L9, and L10 demonstrate prolonged pod maturity (90% at 144 days), which might be advantageous for yield potential but requires a longer growing season (Figure 6).

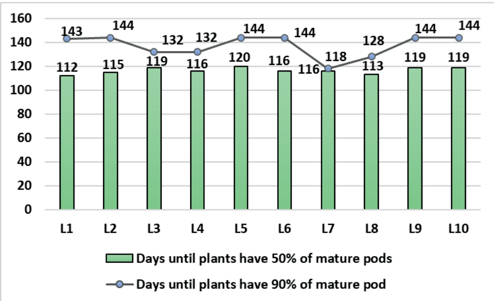

Figure 6. Pod Maturity Dynamics in Chickpea Lines

The variation in maturity duration among lines suggests genetic diversity, which can be utilized for developing varieties adapted to different climatic conditions or agricultural practices. This analysis highlights the importance of selecting chickpea lines based on their pod maturity profiles to optimize yield, resource use, and adaptability to specific environmental conditions (Figure 7).

Figure 7. Chickpea pods: A- green pods; B- mature pods (original)

All lines consistently produce one flower per peduncle. Lines with white flowers exhibit no anthocyanin pigmentation, while those with pink flowers show low anthocyanin levels (Table 1).

Table 1. Flower characteristics and pigmentation

Lines	Flower color	Number of flowers per peduncle	Plant: pigmentation
L1	white	1	No anthocyanin
L2	white	1	No anthocyanin
L3	white	1	No anthocyanin
L4	white	1	No anthocyanin
L5	pink	1	Low anthocyanin
L6	pink	1	Low anthocyanin
L7	white	1	No anthocyanin
L8	white	1	No anthocyanin
L9	pink	1	Low anthocyanin
L10	pink	1	Low anthocyanin

Most lines exhibit moderate pubescence, except for L8, which has low pubescence (Table 2).

Table 2. Morphological characteristics of chickpea lines

Lines	Plant pubescence	Plant: growth habi	Leaf: type
L1	Moderate pubescence	Semi- spreading	Normal (uni-imparipinnate)
L2	Moderate pubescence	Semi- spreading	Normal (uni-imparipinnate)
L3	Moderate pubescence	Semi- spreading	Normal (uni-imparipinnate)
L4	Moderate pubescence	Semi-spreading/Semi-erect	Normal (uni-imparipinnate)
L5	Moderate pubescence	Semi-spreading/Semi-erect	Normal (uni-imparipinnate)
L6	Moderate pubescence	Semi- spreading	Normal (uni-imparipinnate)
L7	Moderate pubescence	Semi- spreading	Normal (uni-imparipinnate)
L8	Low pubescence	Spreading/Semi-spreading	Normal (uni-imparipinnate)
L9	Moderate pubescence	Semi-spreading/Semi-erect	Normal (uni-imparipinnate)
L10	Moderate pubescence	Semi-spreading/Semi-erect	Normal (uni-imparipinnate)

The majority of lines exhibit a semi-spreading growth habit, with some lines (L4, L5, L9, L10) displaying semi-spreading to semi-erect tendencies. L8 stands out with a spreading to semi-spreading habit.
The consistent number of flowers per peduncle and leaf structure across all lines suggest stability in these traits, while variations in flower color, pigmentation, and growth habit highlight potential genetic diversity.

This variability, particularly in pubescence and growth habit, may influence adaptation to different environmental conditions and overall plant performance.
Plant height varies across lines, with L9 exhibiting the highest value (70.5 cm), while L7 show the lowest (41.5 cm) (Figure 8).

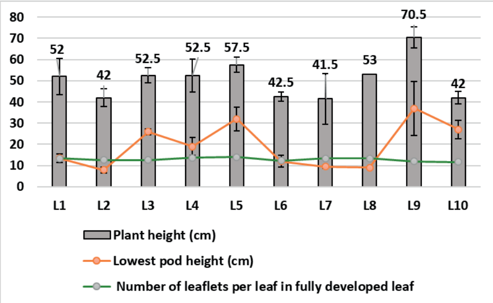

Figure 8. Comparative Analysis of Plant Height, Lowest Pod Height, and Leaflet Number in 10 Chickpea Lines

The number of leaflets per leaf remains relatively consistent across all lines, indicating stability in this trait. In contrast, lowest pod height fluctuates more significantly, suggesting potential differences in plant architecture or flowering pattern that may impact harvestability (Figure 9).

Figure 9. Chickpea lowest pod height (original)

L2 and L5 show the highest number of seeds per plant (94.7 and 95.0, respectively), while L9 has the lowest (39.0). The number of pods per plant varies across the lines, with L2 showing the highest value and L9 the lowest. The seed yield per plant remains relatively low, with L2 demonstrating the highest yield (27.5 g), suggesting potential correlations between pod number, seed number, and overall yield efficiency (Figure 10).

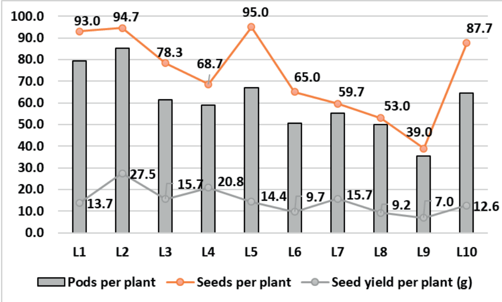

Figure 10. Pod Production, Seed Count, and Seed Yield per Plant Across 10 Chickpea Lines

The distinction between Kabuli and Desi types is evident in seed shape, surface texture, and color, with Kabuli lines characterized by smooth, beige seeds and Desi lines by tuberculate, darker-colored seeds (Table 3).

Table 3. Seeds characteristics of chickpea lines

Lines	Seed shape	Seed surface	Seed: colour	Seeds per pod
L1	Pea-shape - Irregular (Kabuli types)	Smooth	Beige	1/2
L2	Irregular (Kabuli types)	Tuberculate	Beige	1/2
L3	Pea-shape - Irregular (Kabuli types)	Smooth	Beige	1/2
L4	Pea-shape - Irregular (Kabuli types)	Smooth	Beige	1/2
L5	Angular (Desi types)	Tuberculate	Brown	1/2
L6	Angular (Desi types)	Tuberculate	Black	1/2
L7	Pea-shape - Irregular (Kabuli types)	Smooth	Beige	1/2
L8	Pea-shape - Irregular (Kabuli types)	Smooth	Beige	1/2
L9	Angular (Desi types)	Tuberculate	Brown	1/2
L10	Angular (Desi types)	Tuberculate	Black	1/2

This variability suggests different genetic backgrounds, with kabuli types typically preferred for culinary applications, while Desi types may offer greater resilience in diverse environments.
Despite differences in plant height, pod characteristics, and growth habits, the consistent number of flowers per peduncle and seeds per pod across all lines indicates genetic stability for these traits. However, variability in pod yield, plant architecture, and pubescence levels

suggests opportunities to select or breed for enhanced performance in specific environments or cultivation practices.

Desi lines (angular seeds, tuberculate surface, darker colours) and Kabuli lines (smooth, beige seeds) show distinct seed traits. However, seed yield per plant varies widely among the lines, with L2 having the highest yield and L9 the lowest. This indicates that seed surface and colour may not directly correlate with yield, highlighting the need to consider other agronomic factors when selecting high-yielding lines.

L5, L9, and L10, with their prolonged pod maturity (144 days) and angular "Desi" type seeds, could be suited for regions with extended growing seasons, contributing to enhanced yield stability under specific environmental conditions. Conversely, lines like L7 and L8, which mature faster, may be valuable for breeding early-maturing varieties to address water-limited or shorter-season environments.

CONCLUSIONS

Chickpea lines exhibit varied pod maturity, with 50% pod maturity ranging from 112 to 120 days and 90% pod maturity from 118 to 144 days, highlighting differences in growth cycle length.

Certain chickpea lines demonstrate valuable traits for future breeding and cultivation strategies. Lines such as L2 and L4 exhibit high seed yields combined with desirable plant architecture, making them potential candidates for developing high-yielding cultivars.

The highest seed yield per plant was recorded in L2, while the lowest yield was observed in L9, showing the influence of genetic traits and pod production.

The variability in seed shape, surface, and pigmentation across the lines indicates a broad genetic base that can be exploited for improving both yield and seed quality, aligning with market and environmental demands.

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THE IMPACT OF HERBICIDE TREATMENTS IN THE CONTROL OF WEED SPECIES PRESENT IN THE MAIZE CROP IN THE PEDOCLIMATIC CONDITIONS AT NARDI FUNDULEA

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Abstract

Maize (Zea mays) is the most valuable and important crop, the sown surfaces show a high degree of infestation with annual and perennial weeds. Weeds are usually characterized by rapid and abundant seed production, spread easily and grow quickly. They usually have very well developed root systems and are adapted to most pedoclimatic conditions. The researches were carried out in the experimental field from NARDI Fundulea, in the pedoclimatic conditions existing in the south of the country. The main objective of the work is to study the selectivity and effectiveness of herbicide treatments on the fight against annual and perennial weeds present in the maize crop. The use and application of herbicide treatments must be correlated with the degree of infestation, the spectrum and dominance of weed species and the pedoclimatic conditions in the research area.

Key words*: maize, weed, herbicide, efficacy, selectivity.*

INTRODUCTION

Maize (*Zea mays* L.) is the seed of a plant that is native to Central America but is widely cultivated around the world and has numerous health benefits.

Maize is the most important and valuable agricultural plant, occupying the third place in the world in area and the first in terms of production, being used in human nutrition, animal feed, raw material in industry and, more recently, for the production of fuels to replace gasoline and diesel.

In Romania, corn holds the most important place, having the largest contribution to the total grain grain production, the cultivated area represents approx. 49-52% of the area sown with cereals. Maize occupies the third place, in terms of importance, among the plants cultivated on the globe, and in Romania it is the most cultivated plant. The importance of corn cultivation is motivated by the following particularities:

- It has a high production capacity, about 50% higher than other cereals;
- It is a creeping plant, good forerunner for most crops;
- Supports monoculture for several years;
- Having a later sowing in the spring, allows a better stagger of agricultural works.

In Romania, the areas cultivated with corn show an extremely high degree of weeding, over 80%, with a varied range of annual and perennial monocotyledonous and dicotyledonous weeds, depending on the zonal pedoclimatic conditions. The most significant weed species are: mocotyledonates: *Setaria* sp., *Echinochloa crus-galli, Sorghum halepense (from seed and rhizomes), Digitaria sanguinalis, Elymus repens, Eriochloa villosa* and dicotyledonates: *Amaranthus retroflexus, Chenopodium album, Solanum nigrum, Xanthium strumarium, Polygonum* sp., *Sinapis arvensis, Raphanus raphanistrum, Stellaria media, Thlaspi arvensis, Hibiscus trionum, Datura stramonium, Abutilon theophrasti, Cirsium arvense, Convolvulus arvensis, Sonchus arvensis, Lepidium draba, Galinsoga parviflora, Capsella bursa - pastoris, Erigeron canadensis* (Popescu et al., 2009).

In the field of weed control in field crops, the main objective is to permanently eliminate weed competition below the damage threshold throughout the growing season, in order to reduce water and nutrient consumption by them, so that plants continue to grow. Culture to have a normal development, which will lead, in the end, to obtaining high yields, qualitative and at the level of the biological potential of the hybrids and cultivated varieties (Popescu A., 2007).

Weeds have the greatest negative impact, around 37%, compared to insects (18%), fungi and bacteria (16%) and viruses (2%) (Oerke, 2006). Weeds have a negative effect on the level of production and its quality, the presence of a large number of weeds makes harvesting difficult, they also increase drying costs, they can have toxic effects on people and animals, and they can also favor the transmission of diseases and pests to plants (Chirilă, 2001).

The number and spectrum of weeds depends on different factors such as soil type, crop rotation, tillage, crop density, fertilization level, etc. (Hanzlik and Gerowitt, 2011; Partal et al., 2023).

The magnitude of the loss is related to the composition of the weed flora, weed emergence timing in relationship to the crop, weed density, intensity, and crop development stage in relation to the period of competition (Singh et al., 2016). The competition with maize in the stage of 5 fully expanded leaves has the most negative interference to the crop, since it is in this phase that the components related to grain yield are established (Duarte et al., 2002).

Herbicides will remain in future agriculture an efficient tool for control of weeds as part of an integrated weed control. The application of herbicides requires only a quarter of the fuel used than one passage over the same surface with a row crop cultivator (Hanna, 2001, cited by Gianessi, L., 2013).

First, there is a growing concern at national and international level for the development of integrated management of weed control in agriculture. This involves taking a holistic approach to weed problems and using multiple and complementary methods to effectively control them. The main objective is to reduce the negative impact of weeds on agricultural production, while respecting the principles of sustainability and environmental protection. Regarding the existing state of research, significant progress has been made in identifying and developing effective methods of weed control.

The aim of the research was to identify technological solutions to control the weeds present in the maize crop by using the herbicide treatments, aiming to broaden the control spectrum, synergism, persistence and without negative impact on the environment. The main objective of this paper focused on the study of selectivity and effectiveness of the application of herbicide treatments in the control the weeds from the maize crop.

MATERIALS AND METHODS

The research was carried out in the period 2022-2023, at the National Institute for Agricultural Research and Development - Fundulea, being studied the application of the herbicide treatments at the maize crop. The research was carried out in the experimental field, the experiment being located on a soil of cambic chernozem type (3.2% organic matter, 37% clay, 6.5 pH), using the Felix maize hybrid created by the institute from Fundulea.

The Felix hybrid is a hybrid with high production potential, with good production stability in various climatic conditions, with a fast rate of water loss from the grains at harvest and a high starch content.

The organization of the experiment was done according to the method of randomized blocks, with a plot area of 25 m^2, in 3 replications and the amount of water used was 300 l/hectare.

In this experiment, we observed the degree of selectivity of maize plants and the degree of control of annual and perennial monocotyledonous and dicotyledonous weeds by applying herbicide treatments (table 1): Principal plus (50 g/kg dicamba + 92 g/kg nicosulfuron + 23 g/kg rimsulfuron) + Trend (adjuvant); Diniro (prosulfuron 40 g/kg + dicamba 400 g/kg + nicosulfuron 100 g/kg) + Trend (adjuvant); Radial 40 (40 g/l nicosulfuron) + Dicopur Top (344 g/l 2.4 D acid from DMA salt and 120 g/l dicamba); Radial 60 (60 g/l nicosulfuron) + Hudson (fluroxypyr 200 g/l).

The control of treatments with herbicides applied post-emergence (the growth and development stage of the corn being 4-6 leaves, BBCH 14-16) depends on the degree of weed infestation, the spectrum and dominance of the species present in the culture, the dose applied and last but not least the pedoclimatic conditions characteristic of each year from Fundulea.

Table 1. The herbicide treatments applied in the maize crop Experimental variants

No var.	Herbicides treatments	Active ingredient	Dose g,l /ha	Time of application
1	Untreated	-	-	
2	Principal plus + Trend (Adj.)	50 g/kg dicamba + 92 g/kg nicosulfuron + 23 g/kg rimsulfuron	440 g + 0.25 l	Postem BBCH 14-16 (maize 4-6 leaves)
3	Diniro + Trend (Adj.)	40 g/kg prosulfuron + 400 g/kg dicamba + 100 g/kg nicosulfuron	500 g + 0.25 l	
4	Radial 40 + Dicopur Top	40 g/l nicosulfuron + 334 g acid 2.4 D from salt of DMA + 120 g/l dicamba	1.0 l + 1.0 l	
5	Radial 60 + Hudson	60 g/l nicosulfuron + 200 gr/l fluroxypyr	0.7 l + 1.0 l	

The herbicide treatments were applied in the post-emergence (growth and development stage of maize cultivation: BBCH 14-16, 4-6 leaves) and weed development stage (monocotyledons: BBCH 11-14 and dicotyledons: BBCH 12-15). After the application of herbicide treatments, the observations of selectivity (%) for the maize plants were made at different intervals (7 -14- 28 days after the application of treatments) and the degree of control (%) of weeds at different intervals (14-28 days from the application of treatments).

The climatic conditions (Figure 1) recorded during the research period were extremely different, especially the amount of precipitation recorded. The average sum of precipitation (mm) for the 2022 year was 258.4 mm and for the 2023 year was 423.4 mm.

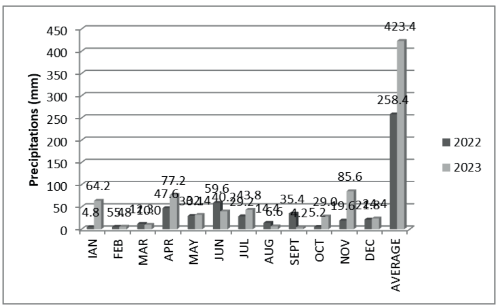

Figure 1. The climatic conditions (precipitations) of 2022-2023 years

The difference of precipitations (2022, 2023) was - 165.0 mm.

The average temperature (°C) in 2022 was 13.3°C and in the year 2023 was 14.3°C.

RESULTS AND DISCUSSIONS

In the maize experience carried out in the experimental field, the culture presented an average infestation degree – 90%, with mono-cotyledonous and dicotyledonos weeds, extremely diversified, depending on the predecessor plant, the local climatic conditions. The most representative (Figure 2) weed species were monocotyledons: *Setaria viridis, Echinochloa crus-galli, Sorghum halepense*, annual dicotyledonous: *Amaranthus retroflexus, Chenopodium album, Xanthium strumarium*, and perennial: *Cirsium arvense*.

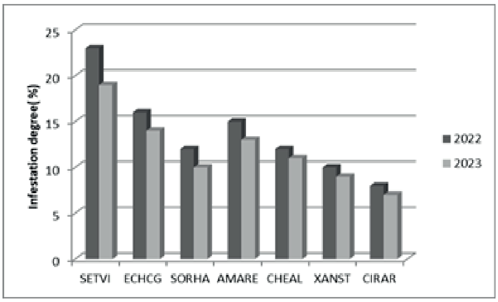

Figure 2. The infestation degree (%) with annual and perennial weeds from the untreated maize plots.

Monocotyledons: *Setaria viridis* is an annual grassy weed that forms sparse bushes. It prefers soils low in calcium, light, rich in nutrients, in warm regions. The flowering period is summer - early autumn.

Echinochloa crus-galli is a heat-loving annual grassy weed. This species is found on moist, humiferous soils, rich in nutrients, loamy -

sandy. The germination period is at the beginning of summer (it germinates in the heat). The flowering period is July - October.

Sorghum halepense weed that forms rhizomes and prefers warm areas. The stem is straight, glabrous, with slightly hairy nodes and up to 200 cm high. The germination period is spring and summer. The flowering period is summer - autumn.

Annual dicotyledonous

Amaranthus retroflexus is an annual seed-borne weed that is widespread, especially on permeable soils rich in humus and nutrients. Late germination period, typical summer germination. Flowering period June - September.

Chenopodium album - Annual weed, monoecious, propagated by seeds, with many forms. The root is pivoting and strong. It ispopularly called wild spinach. The young leaves (cotyledons) are covered with a whitish dust. It can be found on any type of soil, but it prefers loose, nitrogen-rich, humiferous, usually clayey and sandy soils. Germination time is late spring - autumn. Flowering period in full summer – autumn.

Xantium strumarium - Annual seminifera weed, present on heavy soils, rich in nutrients, in areas with hot summers. Germination time is spring. Flowering period late summer-autumn.

Perennial dicotyledonous

Cirsium arvense is a perennial weed with a deep taproot, vertical at first, then horizontally branched, nitrogen-loving. Germination time being spring, germination in the superficial soil layer. The flowering period is summer.

In the experimental field, all the selectivity observations made for the cultivated maize hybrid- Felix, not recorded phytotoxic phenomena (EWRS scale = 0).

In the maize crop, the herbicide treatments applied post-emergence (BBCH 14-16, maize 4-6 leaves) had a good control effect, highlighting their effectiveness through a single application. By applying the treatment with herbicides, good results were obtained regarding the effect of combating annual and perennial weeds, depending on: the climatic conditions, the degree of infestation, the spectrum and the dominance of the species present in this crop.

Figure 3 shows the average efficacy results (%) recorded after the postemergence application of the treatment with Principal plus (440 g/ha) + Trend (0.25 l/ha). This herbicide is absorbed through the leaves and is quickly systemically distributed in all the organs of the plant and is specially designed to combat monocotyledonous and dicotyledonous weeds in the maize crop.

In the 2 years of research, the results obtained show a good control effect (96- 97%) for annual monocotyledons: *Setaria viridis*, *Echinochloa crus-galli* and *Sorghum halepense*.

Following this treatment for the annual dicotyledonous species, a good degree of control was obtained, respectively *Amaranthus retroflexus, Chenopodium album* - 96% and *Xanthium strumarium* - 93%. The perennial dicot *Cirsium arvense* presented a good efficacy - 95%.

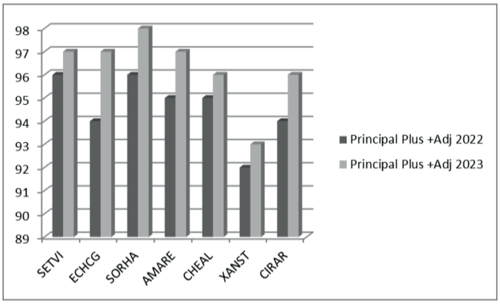

Figure 3. The efficacy (%) of the herbicides Principal plus (440 g/ha) + Trend (0.25 l/ha) for the weeds control from the maize crop

Figure 4 shows the average effectiveness results recorded after the post-emergence application of the treatment with Diniro (500 g/ha) + Trend (0.25 l/ha - adjuvant).

The activity of the product is based on two different modes of action: prosulfuron and nicosulfuron are sulphonylurea herbicides whose activity is based on blocking cell division in the growth tips, while dicamba is a growth regulator. Due to the application of this herbicide, a high degree of control was obtained for the following species: *Setaria viridis* - 96%, *Echinochloa crus-galli* - 95% and *Sorghum halepense* - 92%. Regarding the annual dicot species: *Amaranthus retroflexus* - 95%, *Xanthium strumarium* - 94% and *Chenopodium album* - 92%, the control was very good.

On the other hand, in the plots under this treatment the weed species *Cirsium arvense* (perennial dicotyledon), the average effectiveness was 94%.

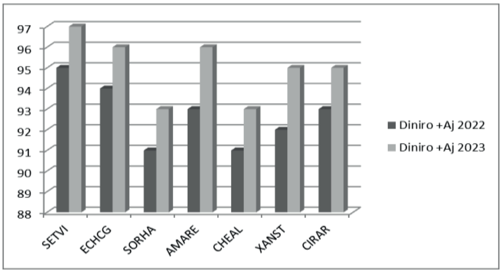

Figure 4. The efficacy (%) of the herbicides
Diniro (500 g/ha) + Trend (0.25 l/ha)
for the weeds control from the maize crop

The next variant treated was with the combination of Radial 40 (1.0 l/ha) and Dicopur top herbicides (1.0 l/ha). Radial 40 is applied only post-emergence and is quickly absorbed especially by the leaves, but also by the roots of the weeds. The weeds stop growing, then after they turn red, etiolate and necroses appear. Dicopur top is a combination of two active substances, it is a selective and systemic herbicide that acts on weeds through absorption, both at the level of the leaves and at the root level. Following the application of this treatment, a good control effect was recorded for the weed species *Setaria viridi - 92%*, *Echinochloa crus-galli - 90%* and the species *Sorghum halepense* it showed a good effect of 88%.

In the plots where this treatment was applied, a good control effect of was obtained for the annual dicotyledon - *Amaranthus retroflexus* 95%, *Chenopodium album* - 96%. For the other species *Xanthium strumarium* - annual dicotyledon 94% and *Cirsium arvense* - perennial dicotyledon, the control effect was 95% (Figure 5).

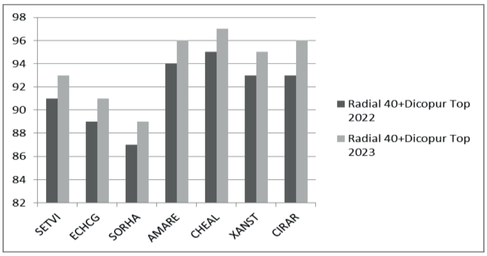

Figure 5. The efficacy (%) of the herbicides
Radial 40 (1.0 l/ha) + Dicopur Top (1.0 l/ha)
for the weeds control from the maize crop

Figure 6 shows the average efficacy results recorded after postemergence (BBCH 14-16, stage of growth and development of maize 4-6 leaves) applications of the herbicide combination Radial 60 (0.7 l/ha) + Hudson (1.0 l/ha). Hudson is absorbed in plants through the leaves and is quickly translocated throughout the plant. Sensitive weeds stop growing, discolor and die).

The results obtained show a superior control effect (96-98%) for the monocotyledonous species *Setaria viridis, Echinochloa crus-galli and Sorghum halepense*.

Following this treatment for the annual dicotyledonous species: *Amaranthus retroflexus* - 96% and *Chenopodium album* - 97%, a higher degree of control was obtained. For the other species *Xanthium strumarium* - annual dicotyledon, the effect was 92%.

This herbicide combination had a moderate efficacy of 69% on *Cirsium arvense*, this perennial dicotyledonous weed shows a certain degree of resistance to this herbicide combination.

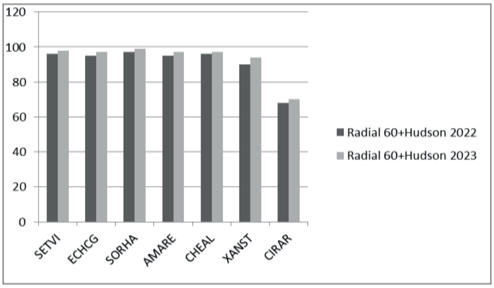

Figure 6. The efficacy (%) of the herbicides
Radial 60 (0.7 l/ha) + Hudson (1.0 l/ha)
for the weeds control from the maize crop

The chemical control of the weed species existing in the maize culture, on the type of cambic chernozem soil (3.2% organic mater, 37% clay, 6.5 pH) from Fundulea, represents an especially important and necessary technological measure.

In our country, special attention is paid to the control of annual and perennial monocotyledonous and dicotyledonous weed species by using and applying new herbicide treatments due to the degree of weeding, dominant and diversified spectrum of annual and perennial weeds present in the corn crop.

CONCLUSIONS

The maize crop showed a high degree of weeding and diversified with characteristic monocotyledonous weed species: *Setaria viridis, Echinochloa crus-galli, Sorghum halepense* and annual and perennial dicotyledons: *Amaranthus retroflexus, Chenopodium album, Xanthium strumarium,* and *Cirsium arvense.*

Treatments with post-emergence herbicides applied (BBCH 14-16, stage of growth and development of corn 4-6 leaves) did not register phytotoxic phenomena for the cultivated maize hybrid – Felix.

In the 2022-2023 research years, the use and application of new treatments with post-emergent applied herbicides (BBCH 14-16, stage of growth and development of corn 4-6 leaves) had a good control effect, highlighting their effectiveness through a single application.

The degree of control of herbicide treatments depends on the level of infestation, dominance, weed spectrum, applied dose and climatic conditions.

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COMPARATIVE STUDY OF THE INVASIVE ENTOMOFAUNA ASSOCIATED TO THE SPECIES *Phaseolus vulgaris* L. and *Glycine max* (L.) Merr. ON EXPERIMENTAL PLOTS IN THE "ALEXANDRU CIUBOTARU" NATIONAL BOTANICAL GARDEN (INSTITUTE), REPUBLIC OF MOLDOVA

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Abstract

Soybean and common bean are widely grown as food and fodder plants, but also have high estimated energy potential. They are among the most significant field crops, cultivated annually in the Republic of Moldova. These species are economically feasible, versatile crops, but their major disadvantage is their vulnerability to the impact of invasive entomofauna. The phytosanitary monitoring conducted on the experimental sectors of the Botanical Garden, in the collection of fodder, honey and energy plants, in comparison with the productive sectors in agro-industrial areas, resulted in the estimation of the invasive impact, in 2024, of the most harmful insect species for these crops. The phytosanitary monitoring revealed the presence of a significant complex of parasitic insects, represented by 12 species included in four orders (Coleoptera, Diptera, Hemiptera, Lepidoptera) and nine families. The most harmful and invasive pests for both researched plant species were: Acanthoscelides obtectus Say., *Agrotis spp., Sitonia liniatus L., Aphis fabae Scopoli, Myzus persicae Sulzer., Aphis glycines Matsumara; Vanessa cardui L., Delia platura Meigen. The obtained data are useful for adapting pest control measures to the integrated protection system.*

Key words: *common bean, experimental sectors, parasitic insects, phytosanitary monitoring, soybean.*

INTRODUCTION

The annual climatic instability in the Republic of Moldova and its interconnection with the structure and state of biodiversity in natural and anthropic ecosystems, also connected to global warming, results in the fact that distortions are reported in the quality and composition of flora and fauna, both useful and invasive (Busuioc, 2004; Starodub et al., 2013; Starodub, 2015). This system also includes complexes of parasitic insects, pathogens etc., some of which may be completely out of control, but with their evolution over time can become very dangerous for practically all field and horticultural crops (Ghizdavu et al., 1997; Lazări & Busuioc, 2002). In the Republic of Moldova, the common bean – *Phaseolus vulgaris* L. and soybean – *Glycine max* (L.) Merr. are cultivated annually, for the agri-food and zootechnical sectors, as raw material to produce canned and dried beans and pods, also useful for the chemical and pharmaceutical industries, as well as for the pedo-ecological significance of these plants. Plants of these species possess relative capacities of resistance and tolerance to environmental stressors, species of harmful organisms, which is why they are included in modern agricultural systems, with numerous advantages in optimal crop rotation structures and technologies of usage (Busuioc, 2004; Timuș, 2015; Țugulea & Derjanschi, 2015). The advantages noted are also connected to some disadvantages, which also require efforts to be overcome and, subsequently, to obtain high yields of beans and fodder. Thus, the control measures start with conducting annual and seasonal phyto-sanitary surveys, useful for further specific research on the parasitic impact of invasive organisms, including the analysis of the diversity of harmful insects affecting the crops.

The investigations we initiated are important to ascertain the dynamics of the phenological stages of common bean and soybean plants, the diversity, the parasitic impact of the most dangerous insect species, the local invasive potential, which could affect these local crops, traditionally cultivated both in industrial production associations and in the private sector. All these aspects and elements are necessary to provide the necessary cultivation practice in order to choose and apply the most appropriate control measures to all invasive insect associations in bean and soybean crops, as important links that are included in integrated protection management (Lazări & Busuioc, 2002; Tălmăciu & Tălmăciu, 2014).

The introductory data denote the research conducted within the "Alexandru Ciubotaru" National Botanical Garden (Institute) of Moldova State University (NBGI MSU), according to the institutional project and research subprogram (2024-2027). Investigations are conducted annually according to the multilaterally adopted research program, including research on complex biological phytosanitary control, where entomological surveys are also associated, being aimed at establishing insect complexes, especially harmful ones in experimental land plots planted with Fabaceae crops, including common bean and soybean. This program focuses on conducting research, which includes entomological surveys with a phytosanitary focus, on *Phaseolus vulgaris* L. and *Glycine max* (L.) Merr. The goal is to identify the most invasive insect species that pose a threat to the above-mentioned plants, under natural conditions, on experimental plots at NBGI MSU. The main objectives for achieving the proposed goal are: to establish the structure and diversity of invasive insect species affecting in common bean (*Phaseolus vulgaris* L.) and soybean (*Glycine max* (L.) Merr.) crops and to determine the values of major parasitic impact, through comparative analyses of the frequency and abundance indices of pests detected on the experimental plots, during various phenological stages of the plants.

MATERIALS AND METHODS

The research program referred to in the present article was conducted at the "Alexandru Ciubotaru" National Botanical Garden (Institute) of Moldova State University, Chișinău, Republic of Moldova, (NBGI MSU) during the growing season of 2024. The investigations included setting the experiments on the experimental plots of the "Plant Resources" Laboratory, which were planted with a wide range of species, varieties and hybrids of Fabaceae, meant to be cultivated for grains, pods and forage, on which, subsequently, complex study of morpho-taxonomic and bio-ecological characteristics was done. Activities such as demarcation of plots, sowing, soil maintenance and plant care woks were performed from mid-March until August, ending with harvesting and storage of the beans (Figures 1, 2).

A B

Figure 1. Experimental plots planted with Fabaceae species: a - soybean, b - common bean, the stage of 5-8 adult leaves (May, 2024, NBGI)

Figure 2. Common beans in the flowering stage (June, 2024, NBGI)

According to ecological characteristics, common bean and soybean seeds are incorporated into the soil later, as compared with other popular crops such as peas, because they need the soil temperature to be on average +7...+10°C, being unable to tolerate lower temperatures. The seeds were planted at a depth of 5-6 cm in the soil, the distance between rows being 70 cm. Further, the cultivation requirements and maintenance measures were observed. Once the plants germinated, visual phenological observations were made on the emerging organs, besides, periodic methodological maintenance works were performed on the entire experimental sector. Weed removal, soil loosening and irrigation, making periodic phytosanitary records and analyzing plant sensitivity to the impact of drought and heat, which are frequent phenomena in the period May – July, were key activities performed during the growing season of the studied plants. Phenological and complex phytosanitary records were initiated with the appearance of diseases and pests on all mounted Fabaceae forms, including common bean and soybean plants (Figures 3, 4).

A

B

Figure 3. Experimental plots with common bean and soybean (July, 2024, NBGI)

In the dynamics of phenological stages, periodic records were made to visualize the growth and development of plants according to the comparatively estimated potential, by species and plots, according to the guidelines of Beideman, 1974. Phytosanitary records for detecting the diversity of harmful organisms, in particular insect species, were made by taking samples, pictures, field notes, subsequently being analyzed in the laboratory, and determined taxonomically with the help of handbooks for entomological identification (Bei-Bienco, 1962; 1966; Plaviliscicov, 1997). The current methodological guidelines were also used to research and test new chemical and biological products with insecticidal and fungicidal action for the integrated protection management of crops in the Republic of Moldova, 2020-2022.

As part of the investigations into the extent of damage caused by harmful insects to the studied species, samples of affected organs and soil were collected for subsequent analysis under laboratory conditions. According to the results of entomological surveys, indices of the population *density* (D), *frequency* (F%) and *intensity* (I%) of diseases caused by harmful species were obtained and compared by species, repetitions, plots and phenological phases. To determine the degree of pest attack across different variants and repetitions, plant samples were collected and analyzed in the laboratory. Based on phytopathological research, the frequency of attack **(F %)** and the intensity of development **(I %)** were established. The frequency of attack **(F %)** represents the relative proportion of attacked plants or plant organs **(n)** compared to the total number of analyzed plants or organs **(N)** and is calculated using a standard formula. The intensity of attack **(I %)** represents the extent, as a percentage, to which a plant or organ is affected. The following formula is used to calculate the intensity of attack:

$$I\% = \frac{(n_1 . 1) + (n_2 . 2) + (n_3 . 3) + (n_4 .4)}{N \times 4},$$

where: n_1, n_2, n_3, n_4 – the number of plants or organs attacked at the respective grade; N – the total number of plants or organs examined; 4 – the maximum grade on the scale used (Îndrumări metodice pentru testarea produselor chimice şi biologice de protecţie a plantelor în Republica Moldova, 2012).

Scales with varying numbers of grades may be used to assess pest attack intensity. In our experiments, four-grade scale was used, corresponding to certain percentage ranges of the affected areas, as follows: 0 – no visible symptoms; 1 – up to 10% of the leaf blade affected; 2 – 10-25% of the leaf blade affected; 3 – 25-50% of the leaf blade affected; 4 – more than 50% of the leaf blade surface affected.

At the same time, the degree of attack was determined according to the estimation scale after the reaction of soybean and bean plants to the impact of parasitic insects (Perju, 1995; Starodub, 2015; Timuș, 2016).

A B

Figure 4. A - analysis of soybean roots for diseases; B - visualization of complex diseases in beans (July, 2024)

RESULTS AND DISCUSSIONS

The outbreaks of diseases caused by polyphagous parasitic insect associations specific to common bean and soybean plants growing under the environmental conditions of the Republic of Moldova are primarily caused by unstable environmental factors, creating difficulties in preventing their emergence and invasive impact (Lazăr & Busuioc, 2002; Timuș, 2015).

Not all species of harmful insects detected on these crops cause large economic losses, and in the case of common beans and soybeans grown for grains, the most important issue is the protection of the vegetative organs of the plant. This part of the plants is mostly responsible for the photosynthetic activity during the early stages of the growing season, and the damage caused by insects considerably reduces the productivity of this physiological process, also affecting significantly the subsequent formation of flowers, pods and grains. These associations of harmful insects targeting soybeans and common beans are scrutinized by researchers, and complex phytosanitary surveys are frequently conducted, focusing on entomological aspects to establish the level of resistance and tolerance to the impact of the most harmful insect species and other pests.

Both the analysis of published bibliographic sources and our own research findings indicate that, in the Republic of Moldova, the damage caused by some invasive insect species, along with some pathogen associations to the investigated crops, is of major agro-economic importance. The insects detected annually as potentially invasive pests, found in various phenological phases on common bean and soybean plants, are some common, polyphagous species, attacking plants since the germination stage: *Agriotes* spp. L., fam. Elateridae; bean weevil – *Acanthoscelides obtectus* Say, fam. Chrysomelidae; pea leaf weevil – *Sitona lineatus* L., fam. Curculionidae; turnip moth – *Agrotis segetum* Den. et Schiff, silver Y - *Autographa gamma* L., cotton bollworm – *Helicoverpa armigera* (Hubner), fam. Noctuidae; *Ophiomyia phaseoli* (Tryon) fam. Agromyzidae, silverleaf whitefly – *Bemisia tabaci* Genn. fam. Aleyrodidae, soybean aphid – *Aphis glycines* Matsumura, black bean aphid – *Aphis fabae* Scopoli fam. Aphididae.

The researched plants were monitored on the experimental sector of NBGI MSU, divided into plots, in several repetitions, randomly placed, on a natural background, with the presence of soil and terrestrial insect species, under equal environmental and time conditions. Table 1 includes the insect species that caused damage to the monitored plants, differentiated by the development stages of insects which caused damage, affected plant organs and indices of population density (D), intensity (I%) and frequency (F%) of attack. Some species were detected even during the earliest phenological stages of plants, as invasive polyphagous pests found in the soil, which have significantly affected the germinated seedlings, such as: *Agriotes* spp. L., *Acanthoscelides obtectus* Say, turnip moth - *Agrotis segetum* Den. et Schiff. As the plants matured, these pest associated with larvae of moths of the family Noctuidae (*Agrotis segetum* Den. et Schiff, *Autographa gamma* L., *Helicovepra armigera* Hubner), with an attach intensity of 15-30%, varying among the experimental plots. It is significant to note that during the research period, the following invasive species were reported in the monitored plots planted with common bean and soybean, such as; silverleaf whitefly – *Bemisia tabaci* Genn., soybean aphid – *Aphis glycines* Matsumura, black bean aphid – *Aphis fabae* Scopoli, greenfly – *Myzus persicae* Sulzer., painted lady – *Vanessa cardui* L., bean seed fly – *Delia platura* Meigen., which invasively attack the leaves, flowers, pods and grains during their formation. As shown in Table 1, diseases caused specifically by 12 species of harmful insects of four orders – Coleoptera, Diptera, Hemiptera, Lepidoptera, with polyphagous phytoparasitic specialization, specific to field crops as well as plants from the spontaneous flora were detected as a result of the entophytosanitary monitoring carried out during the growing season of 2024, on the experimental plots of NBGI MSU planted with soybean and common bean. Practically all plants analyzed are subject to pest invasions, which were highlighted during various stages of plant development, starting with plant germination, progressing to serious parasitic impact until the harvest of the grains, the damage expanding to various plant organs and tissues.

Thus, the earliest detected species are beetles of gen. *Agriotes*, bean weevil – *Acanthoscelides obtectus* Say, pea leaf weevil – *Sitona lineatus* L., followed by diverse caterpillars of the species *Agrotis segetum*, Den. et Schiff, *Autographa gamma* L., *Helicoverpa armigera* (Hubner) from fam. Noctuidae. The process of plant colonization, with the appearance of the generative organs, by various species of highly invasive aphids is of particular importance, since these pests seriously affect plants and are vectors of diseases, such as rot, affecting leaves and pods throughout the growing season, such as: black bean aphid - *Aphis fabae* Scopoli, greenfly – *Myzus persicae* Sulzer, soybean aphid – *Aphis glycines* Matsumura. Other significant pests causing severe damage to pods and grains are Pulse pod borer moth, *Etiella zinckenella* Triet., Painted lady – *Vanessa cardui* L., Bean seed fly – *Delia platura* Meigen., associated with species of thrips, bedbugs, wasps etc.

At the same time, during our research, some considerable populations of species of entomophagous insects that hunt on aphids that invade plants were also detected; the most frequent and effective were: *Trichogramma evanescens, Aphelinus flavipes*, associated with various species of ladybugs – *Coccinella* spp., which had an effective biological impact, especially on the regulation of aphid populations.

Table 1. Characteristics of phytosanitary indices of harmful insect species detected in common bean and soybean crops during the 2024 growing season, on experimental plots within the NBGI MSU

Species name, trophic specialization	Development stage of the insect	Affected plant organs	(D) Density / 100 g soil/m^2 / plant	(F) Frequency %	(I) Intensity %	Nr. of generations per year
Species of polyphagous insects found on common bean and soybean plants						
1. Species of beetles of gen. *Agriotes,* fam. Elateridae ord. Coleoptera	Larvae	They gnaw at the root collar, make cavities in the roots, stems	2-4 larvae/m^2	7-10	5-10	1 in 3-4 years
2. Species of moths of fam. Noctuidae, Ord. Lepidoptera: *Agrotes segetum*, Den. et Schiff, *Autographa gamma* L.	Larvae	Eat first the underground parts, then the aerial parts of the plants	2-3 larvae/m^2	10-15	7-12	1 -2 per year
3. Bean weevil - *Acanthoscelides obtectus* Say, fam. Chrysomelidae , ord. Coleoptera	Larvae, adults	'cut' the root collar, eat young plants	5-7 adults/m^2	12-17	10-15	1 per year
4. Pea leaf weevil - *Sitona lineatus* L. fam. Curculionidae, ord. Coleoptera	Larvae, adults	Eat young plants	5-10 adults/m^2	15-18	12-15	1 per year
5. Cotton bollworm - *Helicoverpa armigera* (Hubner); fam. Noctuidae, ord. Lepidoptera	Larvae	Eat intensively the whole plant	5-12 adults/m^2	15-30	17-25	7 per year
6. Whitefly, *Bemisia tabaci* Gen. ord. Hemiptera, fam. Aleyrodidae	Larvae, adults	Suck the sap from young soybean leaves	1-2 colonies / 10 plants	7-10	5-7	7-8 per year
7. Black bean aphid – *Aphis fabae* Scopoli., fam. Aphididae, ord. Hemiptera	Larvae, adults	Colonies located on the underside of the leaves, severely deform the leaf blade	2-3 colonies /10 plants	10-13	7-10	5 per year
8. Greenfly – *Myzus persicae* Sulzer., fam. Aphididae, ord. Hemiptera	Larvae, adults	Colonize the plant, young leaves	1-2 colonies / 10 plants	5-7	3-5	5-6 per year

Species name, trophic specialization	Development stage of the insect	Affected plant organs	(D) Density / 100 g soil/m^2 /plant	(F) Frequency %	(I) Intensity %	Nr. of generations per year
9. Soybean aphid – *Aphis glycines* Matsumara, fam. Aphididae, ord. Hemiptera	Larvae, adults	Colonize young soybean leaves	3-5 colonies / 10 plants	2-3	1-3	4-5 per year
10. Pulse pod borer moth, *Etiella zinckenella* Triet. – fam. Pyralidae, ord. Lepidoptera	Larvae, adults	Severely affect soybean pods	4-5 affected pods / plant	12-15	7-10	2 per year
11. Painted lady – *Vanessa cardui* L., fam. Nymphalidae, ord. Lepidoptera	Larvae	Consume the leaf blade of soybeans and beans	5-7 larvae in colonies / 10 plants	10-13	7-10	3 per year
12. Bean seed fly – *Delia platura* Meigen*., fam.* Anthomyiidae, ord. Diptera	Larvae	Severely affects bean grains	3-4 affected grains of 10 analyzed	5-10	3-7	3 per year

CONCLUSIONS

The results of the entomological and phytosanitary investigations on soybean and common bean crops, carried out on the experimental plots of NBGI MSU, in 2024, highlighted the range of invasive insect species that caused significant plant damage, resulting in the estimation of the invasive impact indices, the diversity and structure of polyphagous and specific insect communities, compared by crops, phonological stages and plots.

The phytosanitary monitoring revealed the presence of a significant complex of parasitic insects, represented by 12 species included in four orders (Coleoptera, Diptera, Hemiptera, Lepidoptera) and nine families. The most harmful and invasive pests for both researched plant species were: *Agrotis* spp., *Acanthoscelides obtectus* Say, *Sitona lineatus* L., *Aphis fabae* Scopoli, *Aphis glycines* Matsumara; *Myzus persicae* Sulzer., *Etiella zinckenella* Triet., *Vanessa cardui* L., *Delia platura* Meigen. The obtained data are useful for adapting pest control measures to the integrated protection system.

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SUSTAINABLE POTATO PEST AND DISEASE MANAGEMENT: GLOBAL INNOVATIVE PRACTICES WITH A FOCUS ON ROMANIA

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Abstract

Potato crops worldwide face significant challenges from pests and diseases that impact yield, quality, and economic value. This paper provides a comprehensive overview of current pest and disease pressures on potato production, focusing on both global and Romanian contexts. Key pests and pathogens, including the late blight, early blight, dry rot, potato virus Y or Colorado potato beetle are addressed, with discussions on their biology, epidemiology, symptomatology, and economic impacts. For each pest and disease, various control strategies are explored, emphasizing cultural practices as effective, low-cost preventive measures. Detailed tables summarize successful biological, chemical, and plant extract-based treatments. Additionally, the review highlights the importance of selecting resistant varieties and identifies proven chemical treatments for effective management. Through an integrated approach combining cultural, biological, and genetic methods, this paper underscores the critical role of sustainable practices and ongoing innovation in managing pests and diseases in potato crops.

Key words: *potato crop, pest, disease, IPM, Romania.*

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the world's most vital staple crops, playing a crucial role in global food security. It ranks worldwide as the sixth most significant food crop (0.9 billion tons – roots and tubers) after cereal crops, sugar crops, vegetables, oil crops and fruit (FAO, 2023). Potatoes are a primary source of carbohydrates, vitamins, and minerals, particularly vitamin C, potassium, and dietary fiber (Górska-Warsewicz et al., 2021). Due to its adaptability to diverse climates and soils (Chun-ling et al., 2015), the potato is cultivated in over 150 countries, making it a cornerstone of both subsistence farming and commercial agriculture, serving also as a vital alternative to major cereal crops in feeding the global population (Haas et al., 2009). Its versatility extends beyond direct consumption, contributing to various processed foods and industrial products, thereby supporting economies and livelihoods across the globe. The crop's importance is underscored by its ability to produce high yields in a relatively short growing season, making it a key player in addressing global challenges related to food availability and agricultural sustainability.

Potato crop production is constantly put under pressure by pests and diseases, which can cause annual losses of up to $6.7 billion due to late blight disease, caused by *Phytophthora infestans* (Haas et al., 2009). While pesticides can pose environmental risks, their careful and precise use, through targeted application, integrated pest management (IPM) strategies, and adherence to best practices, can significantly minimize these impacts. However, social pressure on pesticide use in agriculture has been growing due to increasing awareness of the environmental and health impacts associated with chemical pesticides. Concerns about soil degradation through affecting community of organisms (Tripathi et al., 2020), water contamination (Syafrudin et al., 2021) loss of biodiversity (Sánchez-Bayo & Wyckhuys, 2019), and the health risks to farmers (Islam et al., 2022) and consumers (Ssemugabo et al., 2023) have led to a demand for more sustainable farming practices. This societal push necessitates the adoption of new Integrated Pest Management (IPM) approaches, which emphasize reducing chemical use through a combination of biological controls, cultural practices, and technological innovations.

The area required for producing seed potatoes in Romania initially included 5 regions: Hărman and Râşnov in Braşov County, Ciuc and Lăzarea in Harghita County, Suceava in Suceava County, Târgu Secuiesc in Covasna County, and Neamţ in Neamţ County (Figure 1).

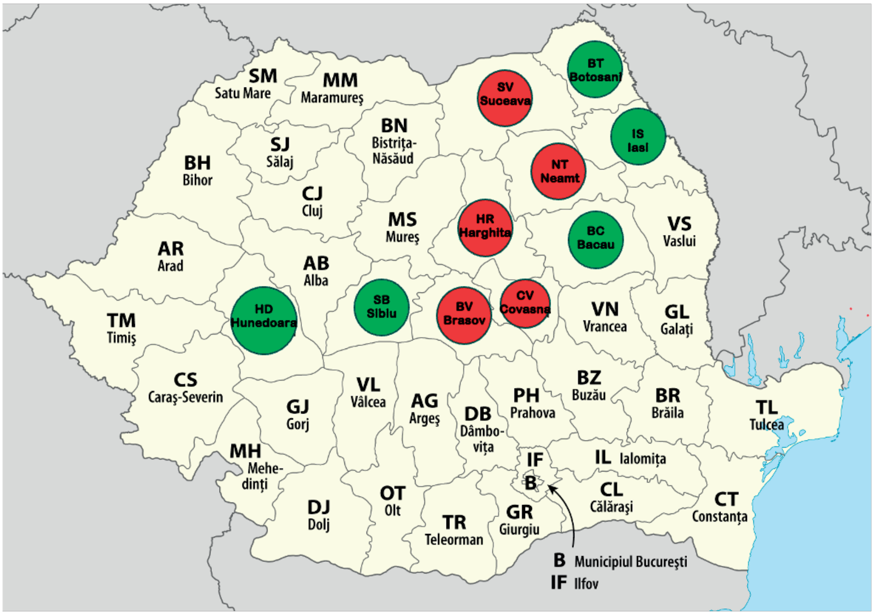

Figure 1. Most important regions in Romania for potato seed production

Subsequently, it expanded to include areas in Botoşani, Bacău, Iaşi, Sibiu, and Hunedoara counties, particularly in moist and cold regions. Yet, in the past two decades, Romania's potato production has seen a significant decline, with output dropping from 3.5 million tons in 2000 to just 1.34 million tons in 2022. This dramatic decrease in production is mirrored by a substantial reduction in the area harvested, which fell from 281,466 hectares to 80,770 hectares over the same period.

These statistics reveal a stark reality: potato farming in Romania is contracting both in terms of yield and cultivated area. Despite this downturn, potatoes continue to be a vital crop for Romania, ranking sixth among the country's top 10 commodities in 2022 (FAO, 2023). The correlation between the decreased production and the reduced harvested area suggests several underlying issues. It points to potential factors such as declining profitability, changes in agricultural policy, higher impact of pests and disease, variations of imports/exports or shifts in farmer preferences toward other crops. This context highlights the need for innovative approaches to revitalize potato farming in Romania.

The objective of this review paper is to explore advanced methods for managing pests and diseases in potato cultivation. The study aims to address the following areas: current pest and disease challenges in potato crops, fungicides, insecticides and biological control used, examples of best practices in integrated pest management potato production, emerging global and local challenges related to pests and diseases in potato crop, particularly in Romania.

CURRENT CHALLENGES AND CONTROL STRATEGIES OF PESTS AND DISEASES IN ROMANIA AND BEYOND

Potatoes are a staple crop globally, providing essential nutrition and economic value. However, their cultivation faces significant challenges from pests and diseases, which can severely impact yield and quality. The prevalence and impact of these issues vary across different regions, with specific pest and disease threats emerging in diverse climatic and geographic contexts. In potatoes, due to vegetative propagation, the quality of the planting material greatly determines the phytosanitary status of the crop, as well as the level and quality of the yield. This is because most diseases and pests are transmitted from one year to the next primarily through infected planting material. Potato crops face significant threats from various pathogens, attacking both in field and storage, including early blight (*Alternaria solani*) (Ivanović et al., 2022), dry rot (*Fusarium* spp.) (Prasetyo et al., 2024), late blight (*Phytophthora infestans*), (Guenthner et al., 2001) Potato Virus Y (PVY), and Potato Virus X (PVX) (Verchot, 2022), each of which can cause severe damage, reducing yields and quality.

In Romania, among the diseases that cause significant damage to potato crops are late blight (*Phytophthora infestans)* and early blight (*Alternaria solani)*, with the former being important in cooler, wetter areas and the latter in warmer, lowland regions. In spring, the incidence of tubers infected with various pathogens, particularly the most harmful ones like potato blight, dry rot, and wet rot, ranges from 3 to 20%, and occasionally even higher (Berindei, 2009). While general control strategies such as crop rotation, use of resistant varieties, and proper sanitation are essential in managing key potato diseases and pests, including early blight, late blight, dry rot, potato virus Y, and the Colorado potato beetle; targeted treatments play an equally significant role in mitigating their impact. These treatments include fungicides, biological control agents, botanical oils, and other specialized approaches. Each method has been thoroughly studied, showing varied levels of effectiveness depending on the active substances used and application conditions.

Controlling viral diseases is a crucial step in the multiplication process of planting material, potato growers only need to ensure they procure guaranteed, virus-free seed. A special attention should be paid to the significant importance of monitoring aphid populations in seed potato production to understand their structure, seasonal activity, and the risk of viral transmission.

Pests are a major threat to potato crops, with significant impacts on yield and quality. Among the most damaging is *Leptinotarsa decemlineata*, commonly known as the Colorado potato beetle. This pest is notorious for its ability to rapidly defoliate potato plants, leading to substantial reductions in crop yields. The beetle's larvae and adults feed aggressively on potato foliage, and their widespread resistance to many chemical insecticides further complicates control efforts. As a result, managing *L. decemlineata* has become a critical challenge in potato cultivation.

1. *Alternaria solani* – Early blight

Symptoms and Impact. Pathogenic species of the *Alternaria* fungus can lead to significant economic losses in crops (Berca & Cristea, 2015). On potato crop, early blight caused by the fungus *A. solani*, manifests as dark brown to black spots on the leaves, often surrounded by a yellow halo (Agrios, 2005; Dhaval et al., 2021). These lesions can expand, causing the leaves to wither and die prematurely, in some cases disease causing total defoliation (Zhao et al., 2023). This disease not only restricts the photosynthetic area of the plant (Horsfield et al., 2010), leading to smaller and fewer tubers but also makes the crop more susceptible to other stresses. The disease can harm both the foliage and tubers of potato plants, yield losses being recorded from 5% to 50% (Chaudhary et al., 2021), up to 40% in India (Suganthi et al., 2020), or high as 20-30% across U.S., due to severe epidemics (Shtienberg et al., 1990). Recent research highlights even more severe impact of *A. solani* on potato crops. One study (Meno et al., 2021) reveals that the Frisia and Daifla varieties, when subjected to early blight in control plots, exhibited substantial susceptibility, with nearly 75% of plants affected. Complementarily, another study reports a maximum Disease Severity Index

(DSI) of 24.95% recorded on the 13th day, which reflects the intensity of disease manifestation (Soni et al., 2023).

In all potato production areas, weather conditions favor the development of early blight disease. Epidemics can occur at any stage of potato growth but are more severe during the later stages of growth. In Romania the disease progresses rapidly when wet and dry weather alternate, a situation that is created in the southern part of the country under drought conditions through irrigation, national authorities warning that losses can reach from 20% up to 50% (MADR, 2018).

To develop effective strategies for controlling this disease, it is essential to first correctly diagnose and identify the pathogen affecting the potatoes (Borca & Carmen, 2013).

Pathogen Biology and Epidemiology. *Alternaria solani* is a fungal pathogen that thrives in warm, humid conditions (Kapsa, 2004; Batista et al., 2006). Sporulation occurs within a temperature range of 2-4°C to 28-30°C, with optimal conditions between 15 to 25°C (Saha & Das, 2013), more precisely at presented 20 °C (Chaudhary et al., 2021). This process requires a relative humidity (RH) above 90% or the presence of wet leaves. The germination of *A. solani* conidia is a critical factor in the onset and severity of early blight in potato crops. Research has demonstrated that *A. solani* conidia can germinate across a broad temperature range,. as low as 4°C to as high as 34°C, with initiation of germination occurring within 30 minutes under these favorable conditions, while optimum being recorded at 25°C (Thomidis et al., 2023). The fungus produces large-spores that are dispersed by wind and rain, infecting potato plants through natural openings or wounds, infection occurring usually after one week. Moreover, the pathogen can persist in the soil for prolonged periods, either as mycelia or as conidia on infected plant debris (Runno-Paurson et al., 2015).

Control Strategies. To effectively manage early blight caused by *A. solani*, it is crucial to understand the environmental conditions that favor disease development and the pathogen's life cycle. Insight into these factors can inform the development of comprehensive and sustainable approaches to reduce the impact of the disease on potato crops.

Across both Romania and worldwide, various cost-free and smart methods and practices have been developed to limit the occurrence and development of early blight. A sensitive point is the precision with which we analyze signs of primary infection. Initially, many of the lesions caused by early blight on potato leaves are less than 1 mm in diameter, making them difficult to observe with the naked eye. It is recommended to use a magnifying glass with appropriate magnification for monitoring. In the absence of warnings, when climatic conditions favor disease development, the potato crop should be inspected daily, given that the incubation period of the disease is 5-7 days.

Cultivation is appropriate in fields with good drainage and aeration, in which nutrients deficiencies are prevented. Application of chemical fertilizers must be carefully monitored, as high doses of phosphorus make plants more susceptible to this disease, while nitrogen increases resistance (MADR, 2018; Abuley et al., 2019). Implement a crop rotation plan, rotating to non-host crops for a minimum of three years, ideally three to four years, to reduce disease risk.

During cool, cloudy weather conditions it is advised to avoid irrigation.

During harvest, take care to avoid injury and skinning of the tubers. Post-harvest, ensure that all plant debris, remains of solanaceous crops, and weeds are thoroughly plowed under or collected.

Regarding varieties, there are no immune ones to early blight; however, early varieties are known to be more sensitive than mid-maturity or late-maturity varieties (Abuley et al., 2018). It is also recommended not to plant both early and late varieties in the same field (Wharton & Kirk, 2007).

The following table provides a detailed overview of these specific control strategies, summarizing key studies, their findings, and references (Table 1).

Table 1. Overview of control methods for *Alternaria solani*

Category/ Control method	Active substance	Results/Efficiency	References
Fungicides	combined use of difenoconazole, boscalid and pyraclostrobin	lower severity of the disease	Odilbekov et al., 2019
	azoxystrobin	did not improve disease control/ limited activity	Landschoot et al., 2017; Odilbekov et al., 2019
	dithiocarbamate mancozeb	the effectiveness of the dithiocarbamate mancozeb was high	Landschoot et al., 2017
	boscalid	completely inhibited disease development when applied one day before inoculation	Horsfield et al., 2010
Biological	*Trichoderma viride* (10^7 CFU/ml)	27.52% PDI (percent disease index)	Varma et al., 2008,
	Trichoderma spp.	"a significant reduction of early blight in the field was only observed in one out of four years of field trials"	Metz & Hausladen, 2022
	Bacillus amyloliquefaciens, B. cereus, Stenotrophomonas rhizophila	a) detached tomato leaflets – reduced lesion development by over 30% b) greenhouse - reduced early blight severity by over 50% c) field - slowed early blight disease progress and reduced disease severity	Riaz et al., 2024
	Bacillus velezensis SEB1	decrease of disease severity form 52.47 ± 3.8% to 9.59 ± 2.1%	Gorai et al., 2021
	Bacillus subtilis (alone or in combination with other treatments)	90% reduction in lesion size	Stridh et al., 2022
	Clonostachys chloroleuca, C. pseudochroleuca, C. rhizophaga	reduced disease severity from 88.7% to 92.9%	Da Silva et al., 2021
	Pseudomonas gladioli B25	controlled the early blight disease by 60.2%	Jagadeesh & Jagadeesh, 2009
Plant extracts	Clerodendron leaf extract	minimised PDI (23.22%)	Varma et al., 2008,
	Active extract of marine algae *Chaetomorpha antennina*	Affects *A. solani* spore germination (92.13%)	Chanthini et al., 2023
Oil extracts	Carnation, caraway, thyme oils	carnation oil had the strongest and most extensive inhibitory effect on fungal growth.	El-Mougy, 2009;
	Citronella essential oil	100% inhibition of *A. solani* mycelial growth at 2417 µL/L concentration	Hendges et al., 2020
Combined methods	Clerodendrum leaf extract + mancozeb	22.73% PDI	Varma et al., 2008,
	Bacillus subtilis (BS-01) + selective plant nutrients (NPK and Zn)	Relative fungal load reduced significantly by ~90%.	Awan et al., 2022

2. *Fusarium* spp. - Dry rot

Symptoms and Impact. Potato fusarium dry rot, caused by the fungus *Fusarium solani*, is one of the most damaging diseases affecting potatoes worldwide. Previously, 13 species of *Fusarium* were reported to cause dry rot (Cullen et al., 2005). This list has been updated to 17 species (Tiwari et al., 2020), indicating that these pathogens evolve and increasingly affect potato crops. This pathogen primarily targets the tubers, where the initial symptoms manifest as dark depressions, that internally continues with dark-brown necrotic areas (Wharton et al., 2007). Recent research (Gavrilova et al. 2024) reported that some *Fusarium* strains cause tissue necrosis ranging in size from 12.9 to 33.9 mm, confirming the aggressiveness of these new strains and their potential impact on potato crops.

Dry rot does not alter the shape of potato tubers in the initial weeks following infection, being visible only 3-4 weeks after (Hooker, 2001). Symptoms manifest later as slightly sunken areas. The affected tissues are distinctly separated from healthy ones by a layer of dark-coloured cells. As the disease progresses, the centre of the tubers decays, causing extensive rotting. Eventually, the affected areas dry out and harden, leading to significant loss of quality and marketability. Indirect symptoms have been reported, with primary and secondary roots also being affected (Tiwari et al., 2020). However, the most severe damage to potato crops occurs postharvest (Monjil et al., 2021). Since it was first recorded, dry rot has caused significant losses to potato crops, ranging from lower percentages observed in surveys across various districts of Bangladesh:

0.91% in Mymensingh, 0.96% in Dhaka, and 0.99% in Rajshahi (Masum et al., 2011), to broader estimates of 6.25% to 25%, with losses reaching up to 60% under certain conditions, particularly in injured tubers (Fan et al., 2021). Higher losses were recorded across the world, like 88% of total post-harvest potato losses were attributed to *F. sambucinum* and *F. solani* infections in China (Du et al., 2012), while another record indicated that *Fusarium species* infected 50% of tuber lots in Michigan storage units (Merlington et al., 2014).

Pathogen Biology and Epidemiology. Dry rot, mainly caused by *Fusarium* species, manifests as a dry, internal decay within potato tubers. This rot can range in colour or appearance at the surface or beneath the skin. Initial coluors of the rot can range from light brown (Xue et al., 2021) to dark black and typically begins at injury sites, such as bruises or cuts, which often occur during harvesting or transportation. These injuries serve as entry points for dormant spores on the tuber's surface. Once the pathogen penetrates the tuber, it often rots out the central portion, causing the tissue to shrink and collapse (Aydin et al., 2016). This leads to dark, sunken spots on the tuber's surface and internal cavities. Additionally, the rot may be accompanied by yellow, white, or pink mold (Vatankhah et al., 2019).

Regarding environmental conditions, even low temperatures of 3°C and 10°C ensure the survival of the fungus. Dry rot fungi thrive at temperatures of 20-25°C for mycelial growth, while growth slows down at 30°C and completely stops at 35°C or 40°C (Tiwari et al., 2020; Gavrilova et al., 2024). On the other side, some scientific results (Mejdoub-Trabelsi et al., 2012; Stefańczyk et al., 2016), showed that pathogenity is higher and varieties are highly susceptible to dry rot pathogen at 15-16°C than 20°C, for *Fusarium* strains tested, causing larger average lesions. While temperatures above 10°C in cold stores promote fungal growth, being reported to produce large tuber rots (Peters at al., 2008), the temperatures below 5°C reduce infection rates.

Control Strategies. Since symptoms of dry rot become visible only 3-4 weeks after infection, selecting high-quality tubers and planting resistant varieties are crucial steps in management. Using resistant varieties reduces the risk of infection, while high-quality, healthy tubers minimize initial disease presence, ensuring better storage outcomes and crop resilience. The reaction of different potato varieties to various *Fusarium* species reflects in differing degrees of susceptibility and resistance (Wastie et al., 1989). This variation is due to the genetic makeup of each potato variety, which influences its ability to fend off or succumb to specific *Fusarium* pathogens.

As *in vitro* tests have shown, some varieties possess inherent resistance traits, reducing the incidence and severity of dry rot, while others may be more vulnerable, leading to more significant disease impact and post-harvest losses. Moreover, no variety has been found to be fully resistant to dry rot. Among the highly resistant varieties, some have been reported: Atlantic, Barvin, Belmando, Bella Rossa, LaBelle (German selection), Glazurny, Cimmeria, Flooding, Tiras (Ukrainian selection), Russet Norkatah, Carrera, Marlene (Dutch selection), Snowden and Sorai (Belgian selection) (Bomok, 2019). Some tested cultivars exhibited partial tolerance to inoculation treatments; for instance, cultivars Spunta and Oceania demonstrated lesser susceptibility (Mejdoub-Trabelsi et al., 2012).

Similarly, no cultivars were entirely resistant to all *Fusarium* isolates, with only one cultivar, Broke®, displaying reduced susceptibility (Aydin & İnal, 2018).

Specific genotypes also demonstrated varying levels of resistance to different *Fusarium* species; for instance, Seuminar was highly susceptible to *F. sulphureum*, while Saturna, Desiree, and Ariana exhibited resistance. Conversely, Panda, Fregate, Folva, Ariana, and Saturna were resistant to *F. solani*, whereas Scort and Monalisa were more susceptible (Esfahani, 2005). Managing Fusarium diseases effectively requires a multi-faceted approach, incorporating fungicides, biological control agents, plant extracts, oil extracts, and combined methods (Table 2).

Table 2. Overview of control methods for *Fusarium* spp.

Category/ Control method	Active Substance	Results/Efficacy	References
Fungicides	Fludioxonil	effectively control tuber seed disease and sprout rot	Malyuga et al., 2022
	Azoxystrobin and fludioxonil	disease incidence decreased to 50%	Tiwari et al., 2020
	Metalaxyl hemexazol at 100 ppm	reduction of dry rot disease incidence by 82.5%	Awad et al., 2020
	Imazalil and thiabendazole (40 and 5 ppm respectively)	completely stopped the mycelia growth and reduced *F. solani* FPO-67 development by 68 and 71.69% respectively (tuber treatment).	Vatankhah et al., 2019
	Benomyl	suppressed growth of *F. sambucinum* and *F. solani* strains by an average of 76 ± 4%	Orina et al., 2024
	Azoxystrobin	least effective – 35 ± 5% averaged inhibition of *Fusarium* growth	Orina et al., 2024
	Carbendazim/ Benomyl/ Thiophanate methyl/ Triadimefon	carbendazim and benomyl significantly inhibited the fungal growth (86.72 and 87.03 respectively), even at its lower concentration (100 ppm), compared to thiophanate methyl and triadimefon (43.73% and 46.87%)	Sandipan et al., 2017
Biological	*Serratia grimesii* 4-9 and *S. plymuthica* 5–6	a) potato tuber slice assay - diameter of the infection sites was reduced 91 and 96%, compared to control b) potato tubers - suppressed development of *Fusarium* dry rot by 60 and 77%, respectively, at 15°C	Gould et al., 2008
	Burkholderia cepacian	*in vitro* antagonism test revealed inhibition zone expanded to 47.37 mm	Recep et al., 2009
	Pseudomonas fluorescens and *Enterobacter cloacae*	dry rot reduction in tubers averaged over the two years (35% and 26.5%, respectively)	Al-Mughrabi, 2010
	Clonostachys rosea IK726	45% reductions in the mean number of rot compared to the non-treated ones	Jima, 2013
Plant extracts	Garlic extract in 3%, 5% and 10% concentration	fungal growth inhibition	Awad et al., 2020
	Punica granatum L. (peels extract) 20 mg/ml	methanol extract exhibited 75.5% inhibition on *F. sambucinum* mycelial growth, and complete inhibition of spore germination	Elsherbiny et al., 2016
Oil extracts	*Beta vulgaris* essential oil (1000 µl)	inhibition rate of 29.1% on *F. sambucinum,* and 27.3% on *F. solani*	Zöngür, 2024

Most of the methods tested, both in vitro and in the field, begin with managing symptoms from the pre-plant phase and continue through to storage. This comprehensive approach is essential because *Fusarium* dry rot can infect potatoes at multiple stages of production and storage.

Managing *Fusarium* dry rot starts with the selection of high-quality, disease-free seed potatoes. Certified seed potatoes are less likely to harbour *Fusarium* pathogens. Certified seed potatoes must meet the stringent standards set by a certification agency. This entails that the seeds were produced, inspected, graded, and handled in compliance with the agency's regulations (Bohl at al., 1992).

European law mandates that all seed potatoes must be officially inspected and certified before they can be marketed. This ensures they meet the EU's quality and health standards. Member states (including Romania) are required to comply with the Plant Health Regulation Regulation (EU) 2016/2031 and Directive 2002/56/EC for seed potato marketing. Seed treatment with fungicides before planting can further reduce the

risk of initial infection. Good results have been reported from pre-treating tubers with various fungicides before planting. This practice not only reduced disease occurrence but also led to a noticeable improvement in yield and quality (Duellman & Olsen, 2019; Udalova et al., 2021). In addition to these measures, other strategies can also be effectively utilized. Use healthy seed and maintain strict hygiene when handling and planting tubers, including sterilizing tools and cleaning equipment. Employ proper storage and treatment practices, such as gradual temperature adjustment and timely planting, while ensuring long crop rotations and applying registered fungicides to minimize disease spread and soil contamination. Ensure tubers have a good skin set before harvest, and adjust harvester speed to minimize damage. Avoid wet conditions during harvest, keep tubers out of direct sunlight, and dry them quickly to aid in soil removal.

3. *Phytophthora infestans* - late blight

Symptoms and Impact. Since its devastating role in the Irish Potato Famine of the 1840s, late blight caused by *P. infestans* has remained one of the most economically damaging diseases affecting potatoes, causing annual losses of up to $6.7 billion worldwide. Once infected, entire foliage can collapse within a few days, and under favourable conditions, the disease can lead to complete crop loss if left unchecked, within 7 to 10 days (Yuen, 2021). Additionally, all asexual and sexual forms of the disease, such as mycelium, oospores (which can survive for up to four years), zoospores, and sporangia, can cause infection, making it even more dangerous to potato crops (Alrudainy & Mshari, 2022). The widespread distribution of the two mating types, A1 mating type before 1980, along with the emergence and rapid spread of the A2 mating type from Mexico to other regions, has enhanced the pathogen's capacity for sexual reproduction (Al Harethi et al., 2023). Late blight first symptoms usually begin 3 days after infection with small, brown lesions on the lower leaves and stems (Kool & Evenhuis, 2023) which quickly develop into brownish-green blotches or black lesions. Under high humidity conditions, these lesions can spread rapidly, with white mildew growth often appearing on the abaxial surface of the leaves (Al Harethi et al., 2023). The disease typically spreads across all parts of the plant. Infected tubers, due zoospores penetration through lenticels exhibit irregular, copper-dry brown or reddish lesions that can lead to secondary bacterial infections, leading mostly to a soft rot (Ristaino et al., 2018).

Pathogen Biology and Epidemiology. Late blight in potatoes, caused by the oomycete *Phytophthora infestans* (de Bary, 1876) is one of the most devastating diseases affecting this crop globally. The pathogen is highly adaptable, with a complex life cycle that includes both asexual and sexual reproduction. Mycelium, which is non-septate, can survive through winter, making it a primary cause of potato plant infections. Meanwhile, sporangia, typically found on the lower leaf surface, have the ability to germinate when water is present and temperatures range between 18-24°C (Ristaino et al., 2018). Alternatively, if temperatures drop below 16°C, sporangia can release zoospores (6-8), which are also capable of causing infection (Ristaino et al., 2018). The pathogen can sporulate on infected tubers in inadequately managed storage areas with excessive humidity. Moisture from condensation forms droplets on the tuber surfaces, prompting sporangia formation, which can then infect nearby tubers, potentially leading to the entire stock being devastated by soft rot bacteria.

Control Strategies. Effective management of late blight, a serious disease affecting potatoes, requires a multifaceted approach due to the pathogen's aggressive nature and ability to spread rapidly under favourable conditions. Key strategies include the use of resistant potato varieties, which can reduce the impact of the disease. So far, no immune potato cultivar has been reported, yet resistance to late blight has been observed in tetraploid breeding clones, cultivars, and wild diploid relatives of potatoes (Xue et al., 2021), or obtained by transferring resistance genes (*Rpi* genes) from wild potato relatives (Paluchowska et al., 2022). Research on potato cultivars' resistance to late blight has been conducted globally, typically highlighting cultivars that are highly susceptible to the disease, while susceptible cultivars got foliage distructed in less than 15 days. Runno-Paurson et al. (2019) tested twelve potato cultivars and found that only two of them, Anti and Toluca, exhibited resistance. In contrast, Xue et al. (2021) tested over 200 cultivars, both new and old, and found that 32 cultivars showed moderate resistance.

Given that the pathogen can survive for many years in the soil due to oospores, crop rotation becomes a crucial component of integrated management strategies. This includes avoiding other host crops, such as tomatoes or ornamental plants, and ensuring that potatoes return to the same plot only after a two- to three-year interval, two years being the minimum needed to delay disease onset (Abuley et al., 2019), while a three-year rotation can enhance the crop's resistance to pathogen attack (Peters et al., 2005).

Irrigation methods play a crucial role in limiting late blight in potatoes by influencing the moisture levels on plant surfaces and in the soil, which are key factors in the development and spread of the disease. Irrigation methods play a crucial role in limiting late blight in potatoes by influencing the moisture levels on plant surfaces and in the soil, which are key factors in the development and spread of the disease. While some studies (Olanya et al., 2007), showed no significant difference between different irrigation methods, other studies highlight importance of timing, frequency and duration in diseases emergence (Bohl et al., 2003), values up to 87.77% in disease incidence being observed with more frequent irrigations (Peerzada et al., 2013).

Planting potato tubers at the correct depth is vital in managing late blight, as it influences soil moisture and temperature, which are critical for both plant health and disease prevention. Proper depth helps maintain stable soil conditions around the tubers, promoting vigorous plant growth and reducing stress, which can make plants more resistant to late blight. Deeper planting, not more than 6 inches (Bohl et al., 2003), also minimizes the exposure of foliage to excessive moisture and pathogens that can accumulate at the soil surface, thereby lowering the risk of infection. Furthermore, it protects tubers from direct exposure to rain and irrigation, which can carry pathogen spores, and supports better water management by preventing waterlogging around the tubers. Overall, correct planting depth contributes to a more resilient plant and enhances the effectiveness of integrated disease management strategies.

Proper management of soil moisture through careful irrigation scheduling can also limit late blight. Avoiding excessive watering and maintaining consistent moisture levels without over-saturating the soil can reduce the likelihood of creating conditions favourable to the pathogen.

Fertilization significantly influences the emergence and severity of late blight in potatoes by affecting plant growth and susceptibility. Adequate fertilization promotes strong, healthy plants with robust foliage and root systems, which can better withstand disease pressure. However, excessive or imbalanced fertilization, particularly with high nitrogen levels, can lead to lush, dense foliage that creates a moist environment favourable for the pathogen *P. infestans* (Juárez et al., 2000). Properly balanced fertilization enhances plant resistance and reduces the likelihood of late blight outbreaks by avoiding conditions that support pathogen proliferation. Additionally, research has shown that potassium and calcium can play a significant role in disease management. Higher applications of phosphorus have been associated with smaller leaf lesions caused by late blight, while spraying calcium nutrients has been found to lower the incidence of late blight (Seifu, 2017).

Scouting is essential for managing late blight in potatoes as it facilitates early detection of disease symptoms, allowing for prompt intervention before the disease spreads extensively. Regular inspections help monitor disease progression, enabling timely adjustments to treatment strategies. Additionally, scouting provides critical information for targeted interventions, focusing resources on the most affected areas and reducing unnecessary treatments.

By maintaining consistent scouting practices, growers can effectively manage late blight, improving overall crop health and yield. The following table (Table 3) summarizes the most effective curative methods for managing dry rot.

Table 3. Overview of control methods for *Phytophthora infestans*

Category/ Control method	Active Substance	Results/Efficacy	Reference
Fungicides	Dimethomorph, fenamidone + mancozeb	area under disease progress curve (AUDPC) was consistently reduced by dimethomorph (90%) fenamidone + mancozeb (68%)	Khadka et al., 2020
	Mancozeb	lowest disease severity (38.50%) recorded on Belete variety	Teshome et al., 2022
	Mancozeb 75% WP (0.2%) + dimethomorph 50% WP (0.2%)	Applying mancozeb 75% WP (0.2%) before disease appearance, followed by two additional sprays with mancozeb 75% WP (0.2%) combined with dimethomorph 50% WP (0.2%) at 7-10 day intervals,	Lal et al., 2017

Category/ Control method	Active Substance	Results/Efficacy	Reference
		resulted in lower terminal disease severity (24.55%).	
	Mancozeb 640 g/kg + cymoxanil 80 g/kg	suppressed blight symptoms by 54%	Kilonzi et al., 2024
	Metalaxyl	failed to protect the potato crop from late blight in temperate highlands, resulting in 40–70% crop losses.	Lal et al., 2018
Biological	*Streptomyces* sp. FXP04	inhibition of colony of *P. infestans* was reduced by 32.4% and 58.2%	Fu et al., 2022
	Willaertia magna C2c Maky lysate	a) greenhouse - up to 80% disease reduction b) 77% protection in field in the case of low infestation (28%)	Troussieux et al., 2022
	Trichoderma spp.	in dual-culture assays – inhibited *P. infestans* growth with 53 to 95%	Alfiky et al., 2023
	Penicillium aurantiogriseum, P. viridicatum, Trichoderma viride and *Acremonium strictum*	all the four bio-agents significantly reduced *P. infestans* sporangial germination, *T. viride* and *P. viridicatum* being better than the others	Gupta et al., 2004
Plant extracts	*Syzygium cumini* leaves extract	very effective in controlling the late blight disease incidence and severity (up to 58 days after sowing (DAS) and increased the potato yield by 71.29%	Islam et al., 2021
	Garlic (*Allium sativum*), Neem (*Azadirachta indica*), Turmeric (*Curcuma longa*), Mint (*Mentha* sp.) at 5, 10, and 15% concentrations	a) *in vivo* test - *A. sativum* and *A. indica* at 15% concentration were found to be more effective in inhibiting *P. infestans* mycelial growth by 58.4% and 43.9%, respectively b) greenhouse trials – minimum potato late blight disease incidence (5.81%), due to *A. sativum extract*	Mehmood et al., 2022
Oil extracts	Juniper, tea tree, clove, thyme, cinnamon, turmeric, pepper and rosemary essential oils (EO), in 0.41, 0.83, 1.66, 3.33 and 6.66 µL/mL concentrations	rosemary, thyme EO and Timbor® (*Thymus vulgaris*) were the most effective compounds in reducing late blight with more than 80%	Najdabbasi et al., 2020; Gheorghe et al., 2022
	Cinnamaldehyde, carvacrol, and eugenol	Inhibited *P. infestans* by hindering its mycelial radial growth, zoospore release, and sporangiospores germination	Tian et al., 2024
Combined methods	Biochar + *Streptomyces* strains	decreased the disease index by 10 % to 26 %, in field trial, exhibiting better disease control than the use of either agent alone	Jin et al., 2023

4. Potato Viruses – Potato Virus Y

Plant viruses are widely recognized for their significant impact on crops worldwide, causing detrimental effects on major agricultural products and resulting in substantial losses, up to $60 billion (Sinha et al., 2024). Among the most dangerous viruses affecting potatoes, Potato virus Y (PVY) poses a widespread problem in potato-growing regions (Onditi et al., 2022). While its molecular structure is well studied, there is still limited understanding of the virus's evolutionary pathways (Gao et al., 2020).

Symptoms and Impact. PVY can significantly impact potato crops with a range of symptoms (depending also on the virus strain) that often start with the appearance of mosaic patterns on the leaves. These mosaic patterns typically present as alternating light and dark green areas, creating a mottled effect. As the infection progresses, the leaves may begin to curl either upwards or downwards, leading to noticeable deformities. This curling is often accompanied by chlorosis, where the leaves turn yellow and eventually become necrotic, which can result in premature leaf drop. Infected plants may also exhibit stunted growth, with a noticeable reduction in size compared to healthy plants. Several other more specific symptoms have been reported during virus-plant interactions, including significant metabolic changes such as alterations in fatty acids, amino acids, and energy pathways (Manasseh et al., 2023), lower concentrations of sugar metabolites (Kogovšek et al., 2016), a general reduction in photochemical efficiency (Stare et al., 2015) or decreased starch content and dry matter (Ospankulova et al., 2023). Tubers from affected plants can show various deformities, including necrotic ringspots (Chikh-Ali et al., 2020), irregular shapes or smaller sizes, and may have fewer eyes. Additionally, the overall yield of the potato crop can be markedly reduced, as the virus impacts the plant's ability to produce healthy tubers.

Due to the limited resistance in many potato cultivars, PVY has become one of the most economically significant viruses affecting potato crops. The widespread susceptibility of these cultivars allows PVY to easily infect and damage plants, leading to significant losses in yield and quality across Europe, with annual economic losses estimated at 187 million euros (Dupuis et al., 2024) or up to 80% of the entire yield (de Bokx & Huttinga, 1981).

Pathogen Biology and Epidemiology. PVY is a member of the *Potyvirus* genus in the *Potyviridae* family. Its virions are filamentous, measuring 730 nm in length, 11 nm in diameter, and contain a single-stranded RNA genome approximately 9.7 kb in length (Urcuqui-Inchima et al., 2001). PVY is a member of the *Potyvirus* genus, which is one of the largest groups of plant viruses (Wylie et al., 2020). It can infect a minimum of 495 species across 31 families (Abd El-Aziz, 2020). Three main strains have been initially reported for PVY: O (ordinary), N (necrotic), and C (common) (Wani et al., 2021; Samarskaya et al., 2023), but recombinant strains have been recently reported such as $PVY^{N:O}$, $PVY^{N\text{-}WI}$, and PVY^{NTN} (Karasev & Gray, 2013).

PVY is primarily transmitted mainly by mechanical actions (Fegla et al., 2001; Hamza et al., 2018), infected tubers (Rahman & Akanda, 2009), or aphid vectors (see Table 4), in a non-persistent manner, meaning the virus does not replicate within the aphid but can be transmitted rapidly during brief feeding periods.

Table 4. Reported aphid species transmitting Potato Virus Y (PVY)

PVY strains	Host range	Aphid species	References
PVA	Potato	*Aphis fabae, Metopolophium dirhodum, Sitobion avenae, Acyrthosiphon pisum* and *Cavariella aegopodii*	Fernández-Calvino et al., 2006; Fox et al., 2017
PVY	Potato, pepper	*C. aegopodii*	
PVY^{O}/ PVY^{N}/ PVY^{NTN}/ PVA	Potato	*M. persicae*	
PVY^{N-Wi}	*N. benthamiana*	*M. persicae*	Kamangar et al., 2019
PLRV and/or PVY.	Potato	*M. persicae*, *M. euphorbiae, A. gossypii*	Machado-Assefh et al., 2023
PVY^{O}/$PVY^{N\text{-}WI}$	Potato	*Myzus persicae*	Mello et al., 2011
PVY^{NTN}/PVY^{NW}	*Physalis floriana*	*Myzus persicae*	Kostiw & Trojanowska, 2011
PVY	Tobacco	*Brachycaudus helichrysi, Myzus persicae, Phorodon humuli*	Harrington et al., 1986

Control strategies. Effective control of PVY in potato crops requires a comprehensive, multi-faceted approach due to the virus's genetic diversity and the complex dynamics of its transmission.

A key component of this strategy is the use of diagnostic tools like ELISA (enzyme-linked immunosorbent assay), widely employed for detecting and differentiating PVY strains using commercially available polyclonal and monoclonal antibodies, although it has limitations. While serological assays can distinguish different serotypes of the virus, they are not capable of identifying recombinant isolates. This requires the integration of more advanced molecular techniques, such as RT-PCR, for precise detection (Baebler et al., 2020).

In addition to accurate diagnostics, breeding for PVY-resistant potato cultivars is a cornerstone of disease management. However, the constant evolution of the virus, particularly the emergence of recombinant strains that can overcome existing resistance, underscores the importance of ongoing research and development of new resistant varieties. Yet, some varieties showing moderate resistance (Villetta Rose, Eva, Rio Grande), have been reported (Schramm et al., 2011). In addition to genetic resistance achieved by transferring resistance genes like Ry_{chc} (Li et al., 2022), effective control of aphid vectors through IPM strategies is crucial. Although insecticides are commonly used, their efficacy is limited because they do not act quickly enough to prevent aphids from probing and transmitting the virus to healthy plants. Additionally, establishing thresholds for aphid populations is vital for the effective management of insecticide use (DiFonzo et al., 1996).

Therefore, integrating cultural practices such as crop rotation, the use of reflective mulches, and the regular removal of infected plants is essential for reducing aphid populations and minimizing virus spread. Dupuis et al. (2017), presents some promising new cultural methods of controlling or reducing spreading of PVY, using mulching, oil spraying and intercropping. Additionally, the use of certified virus-free seed potatoes plays a critical role in reducing the initial inoculum, further strengthening the overall control strategy. By combining these diverse approaches, farmers can more effectively manage PVY, mitigating its impact on potato yields and quality.

***5. Leptinoptarsa decemlineata* - Colorado potato beetle**

Symptoms and Impac. *Leptinotarsa decemlineata* L., or the Colorado potato beetle, is a highly destructive pest that significantly impacts potato crops. In Romania, the Colorado potato beetle was first reported in Săpânța, Maramureș, in the years 1952, 1953, and 1954, and in Uivar, Timiș, in 1955, in the form of isolated outbreaks that were eradicated. However, in 1956, it became permanently established, being discovered in numerous locations in the southwest of the country (the counties of Timiș, Arad, and Bihor), entering in large numbers by flight from Serbia and Hungary. Currently, the Colorado potato beetle is found throughout the entire country.

The symptoms of infestation begin with the presence of small, yellow eggs laid on the underside of leaves, which soon hatch into larvae that voraciously consume the foliage. As the larvae mature, they cause extensive leaf damage, up to 40 cm^2 of potato leaves during larval stage (Ferro et al., 1985), leading to defoliation (Figure 2), that severely impairs the plant's ability to photosynthesize. Even though the plant's photosynthetic rate is not affected, the attack on the plants stunts their growth and can result in significantly lower tuber yields (Hoback et al., 2015). In severe infestations, entire fields can be defoliated, with defoliation levels exceeding 75% often leading to total crop loss. The beetle's ability to develop resistance to multiple insecticides, up to 52 compounds so far (Alyokhin et al., 2008), further exacerbates its impact, making it one of the most challenging pests to control in potato farming.

Adults of the Colorado potato beetle are 10 mm long, 7 mm wide with an oval, convex body that is yellow-orange in colour (Alyokhin et al., 2022). The head is yellow-red with a triangular median spot and two black lateral-posterior spots. The pronotum has 11 black spots of varying sizes, with the central ones forming a "V" shape. The elytra are marked with 5 black stripes, bordered laterally by rows of dots. One female can lay from 500 eggs (Radcliffe & Lagnaoui, 2007), up to 800 during its lifetime (Sablon et al., 2012). The egg is 1.2-1.5 mm long, oval-shaped, and yellow-orange in colour. The larva in its final stage, is 8-10 mm long and orange, with black spots along the sides. The head is black, and the legs are dark brown.

Figure 2. Combined attack of adults and larvae of Colorado potato beetle on potato plants grown in Dolj County, Romania

Life cycle. The Colorado potato beetle overwinters as an adult in the soil (EFSA Panel on Plant Health, 2020) at depths ranging from 10 to 90 cm, depending on the soil type, and sometimes for as little as 30 days (Capinera, 2001). In the conditions of Romania, in alluvial soils, the overwintering depth of adults is between 10 and 50 cm, while in sandy soils, it ranges from 30 to 90 cm. In spring, after a period of 10-12 days with average daily temperatures above 10°C, usually in the second half of March, the emergence of adults begins, continuing until the end of May. The peak flight period typically occurs at the end of April or the beginning of May, when average temperatures range between 14-21°C. After an intense feeding period necessary for sexual maturation, mating and egg-laying occur. The eggs are laid in clusters of on the underside of potato leaves.

Control strategies. Colorado potato beetle populations can be effectively managed through a variety of cultural and physical control methods. Cultural practices such as crop rotation, manipulation of planting time, the use of mulches, and the incorporation of trap crops have proven successful in reducing beetle populations. Crop rotation, first recommended in 1872, remains a key strategy, as it not only reduces beetle populations but also helps manage potato pathogens and weeds. Research has shown that rotated fields (with distance rotations >400 m), can experience a significant decrease in beetle adult infestations in the spring (Sexson & Wyman, 2005). Additionally, when potatoes are planted following non-host crops like rye or wheat, early-season beetle densities can be reduced by nearly 96% (Wright, 1984).

Trap crops can attract beetles away from the main crop, effectively intercepting both over-wintered beetles in the spring and those moving from senescing crops later in the season (Hoy et al., 1996). Mulching, particularly with straw, has been shown to significantly reduce larval populations and delay their peak by one to two weeks, while also decreasing defoliation and increasing the beetles' predation risk (Stoner, 1993; Brust, 1994).

CONCLUSIONS

In conclusion, alternative methods for disease and pest control in potato cultivation are showing promising potential. Integrated approaches, including the use of low-input pesticides, biological control agents, and resistant crop varieties, offer effective solutions while minimizing environmental impact. Continued research and field trials are essential to optimize these strategies, ensuring sustainable, long-term protection against key pathogens and pests. Adopting these methods can contribute to healthier crop yields and promote resilience in agricultural systems.

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BIOMORPHOLOGICAL AND PHYTOCHEMICAL STUDY OF SOME PROMISING AROMATIC PLANT SPECIES FROM THE *Lamiaceae* FAMILY INTRODUCED IN THE REPUBLIC OF MOLDOVA

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Abstract

The article summarizes the results of the biomorphological and phytochemical study of some introduced species of aromatic plants from the Lamiaceae family under the pedoclimatic conditions of the Republic of Moldova: Elsholtzia stauntonii Benth., Koellia virginiana (L.) MacM., Agastache urticifolia (Benth.) Kuntze, Monarda fistulosa L., Perovskia atriplicifolia Benth. The biomorphological peculiarities of these species have been highlighted and the content and phytochemical composition of the volatile oil have been revealed. The species E. stauntonii accumulates 0.43% volatile oil and is characterized by a rich content in cinerone (50.8%) and rosefuran (20.6%). K. virginiana contains 1.00 -1.05% volatile oil, the main compounds being pulegone (84.6%) and menthol (2.5%). A. urticifolia plants contain 1.65-1.70% volatile oil with the basic compounds estragole (41.1%), pulegone (20.4%), limonene (15.3%). In the species M. fistulosa, the volatile oil content is noted at 0.75-0.80%, the basic components being carvacrol (54.83%), p-cymene (23.15%). P. atriplicifolia contains 0.54-0.65% volatile oil, rich in limonene (40.13%) and α-pinene (17.87%). The highlighted species can serve as sources of indigenous raw materials for the production and diversification of the range of natural cosmetic and pharmaceutical products.

Key words: *aromatic plants, biomorphology, cosmetics, essential oil, perfumery.*

INTRODUCTION

Aromatic plants of the Lamiaceae family contain different proportions of biologically active substances, especially essential oils, which accumulate in secretory tissues located either outside the vegetative organs or inside them. The pharmacological value of the plants derives from the chemical compounds and their ratio in the volatile oil. The antibacterial, expectorant, diuretic, anti-inflammatory, spasmolytic, choleretic, carminative and sedative actions are some of the most common effects of the frequently used volatile oils (Ștefănescu et al., 2018). They are the most promising sources of antimicrobial drugs with low toxicity (Marino et al., 2001). The demand for such preparations, as well as directly for natural essential oils is constantly growing. In this regard, expanding the base of raw material obtained from plants with a high and qualitative content of essential oil, studying the biochemical characteristics of new promising essential oil bearing plants, as well as researching the seasonal dynamics of the accumulation of biologically active substances depending on organ, phase and age, remains a current issue. The study of native aromatic and medicinal plants, as well as those introduced from the flora of other geographical regions into the pedoclimatic conditions of our country constitutes a research priority within the Botanical Garden. In the process of introducing new species, for a given cultivation area, the complexity of the interaction of different vegetation factors is taken into account, so as to ensure an optimal balance between pedoclimatic conditions and the biological requirements of the plants. This avoids situations in which natural conditions may cause an increase in plant biomass production to the detriment of the content of active principles. The exploitation of new plant species expands the range of volatile oils, opens up new ways of identifying effective measures to promote health and prevent diseases naturally (Gonceariuc, 2008).

The medicines derived from non-toxic plant matter have a healing effect and exert a complex action on the body, boosting the immune system. The cultivation of medicinal and aromatic plants is favored by the pedoclimatic conditions of our country. The variety of natural conditions gives rise to a diverse range of species, which represent a source of plant material for medicine, cosmetics and food.

The collection of aromatic plants of the "Alexandru Ciubotaru" National Botanical Garden (Institute) brings together over 160 native and non-native aromatic taxa, with strong fragrance, perfect taste and unique flavor. Among the study subjects, the following aromatic plant species with a high content of high-quality volatile oil, introduced in recent years, were selected, researched and acclimatized: *Elsholtzia stauntonii* Benth., *Koellia virginiana* (L.) MacM., *Agastache urticifolia* (Benth.) Kuntze, *Monarda fistulosa* L., *Perovskia atriplicifolia* Benth. (Colțun et al., 2022). The therapeutic action of these plants is based on the high content of volatile oils, which are pleasantly smelling, effective in the prevention or prophylaxis of infectious diseases caused by bacteria or parasites, and as a treatment for people allergic to antibiotics. Aromatherapy, over the years, has become a branch of phytotherapy, which explains and shows how to use volatile oils from aromatic plants to prevent and cure many diseases.

The phytochemical compounds and their ratio in the volatile oil of species of the genus *Elsholtzia* determine their pharmacological effect, which frequently includes antibacterial, expectorant, diuretic, anti-inflammatory, spasmolytic, carminative, choleretic and sedative action (Tucker et al., 1995). Due to its antimicrobial and antifungal properties, the volatile oil of *Koellia virginiana* (L.) MacM. has been appreciated in the pharmaceutical industry as a remedy with antimicrobial and antifungal action, necessary in the treatment of diseases caused by pathogenic bacteria (Prisacaru et al., 2010). According to the data from the specialized literature, the volatile oil of the species *Agastache urticifolia* (Benth.) Kuntze due to its compounds, exert antimicrobial, antidiabetic, anticancer, anti-inflammatory, antioxidant and immunomodulatory activities (Wilson et al., 2023).

Interesting studies have also been conducted on the volatile oil of *Monarda fistulosa* L. In several literature sources, it is recommended for skin rashes and dermatitis, insect bites and sunburns. This study reports on the chemistry and biological activity of the essential oil and hydroalcoholic extracts obtained from flowers and aerial parts of plants, which exhibited high levels of p-cymene, thymoquinone and decreased levels of thymol, γ-terpinene in senescent flowers. (Tiffany et al., 2013, Colțun et al., 2018, Berthalia et al., 2022).

Perovskia atriplicifolia Benth. has long been used in Pakistan as a traditional herbal medicine, against anti-inflammatory processes. The phytochemical compounds present in *Perovskia* volatile oil include rosmarinic acid and other hydroxycinnamic acids, monoterpenes, diterpenoids, mainly from the abietane class - carnosol, rosmanol, which give Russian sage volatile oil a number of therapeutic properties, namely: antibacterial, antiviral, anti-inflammatory and antitumor (Pourmortazavi et al., 2003, Ijaz et al., 2015). According to some studies, the main compounds of the essential oil from the flowering branches of *Elsholtzia stauntonii* Benth. are rosefuran (41.73%) and rosefuran epoxide (40.36%) (Tusker, 1995). Pharmacological investigations on extracts and pure compounds from *E. stauntonii* cover antiviral, antibacterial, antimicrobial, anti-inflammatory, antioxidant, as well as other protective activities (Zhiqin et al., 2012).

MATERIALS AND METHODS

The research carried out covers the period 2018-2024, the experiences being set on the territory of the Collection of Aromatic Plants of the NBGI. The species included in the research: *Elsholtzia stauntonii* Benth., *Koellia virginiana* (L.) MacM., *Agastache urticifolia* (Benth.) Kuntze, *Monarda fistulosa* L., *Perovskia atriplicifolia* Benth. were mobilized and preserved as promising species, introduced from other geographical areas under the local pedoclimatic conditions. The seed material was received through the International Seed Exchange program, traditionally conducted with various specialized botanical institutions. The second stage included the study of the bioecological and morphological peculiarities of the plants grown under the new conditions. The experiments were focused on testing different reproduction methods and identifying the peculiarities of the seasonal development and biological potential of plant growth. The phase, organ, age and seasonal dynamics of the accumulation of raw materials and volatile oil were established, and the best harvesting period was determined. Thus, aromatic plant species that possess a high potential for adaptability and accumulation of volatile oil were selected. The plants were grown in an open field, under ecologically balanced conditions, on a general agrotechnical background. Phenological observations and biomorphological studies were carried out every 3 days, throughout the entire growing season (Sparks, 2009). The volatile oil content of plants was determined by the method of steam distillation (Gosudarstvennaia, 1968). The chemical composition of the volatile oil was determined by gas chromatography-mass spectrometry (GC-MS) using the Agilent Technologies 6890N gas chromatograph coupled to the (MSD) 5975 inert XL Mass Selective Detector. The phytochemical research was conducted at the "Stejarul" Biological Research Center, Piatra Neamț, Romania.

RESULTS AND DISCUSSIONS

The species *Elsholtzia stauntonii* Benth. from the Lamiaceae family, introduced in the Botanical Garden in 2018, researched to date as an aromatic and medicinal plant, is of particularly high value for introducing and exploiting new aromatic species. In the wild, it occurs mostly in China and Pakistan. In the NBGI, the initial material was received by international seed exchange – Hortus Botanicus Latvia. Under the climatic conditions of the Republic of Moldova, it behaves as a perennial plant, which develops a typical shrub reaching a height of 60-90 cm, with a diameter of 60 cm, made up of shoots up to 65-70 cm long. The leaves are opposite, oblong-oval, reaching a length of 9-12 cm and a width of 4-5 cm, falling off at the end of the growing season, in November. The inflorescences are large spikes. The length of the central inflorescence is 4-17 cm. There are more flowers in the lower whorl and fewer in the upper one. The flowers reach a length of 6-9 mm and a diameter of 2.5-3.0 mm. The weight of 1000 seeds is 0.2 g (Figure 1).

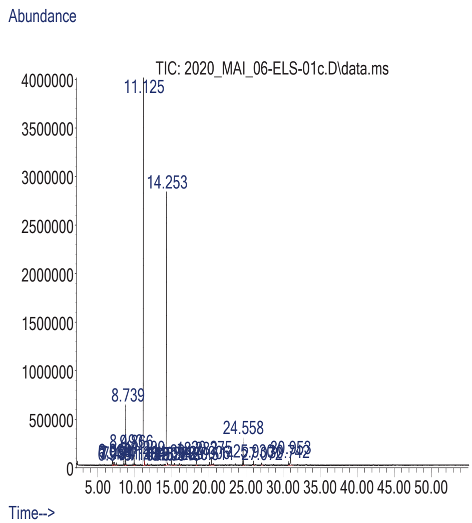
Figure 1. General appearance of the species *Elsholtzia stauntonii* Benth.

The plants are able to complete the full development cycle in the first year of life, however, the onset of phenological phases is delayed, so the fruits do not reach the full ripening phase. The plants grow the fastest in the second half of June. In late August - early September, inflorescences emerge at the top of the main and lateral buds. In the second and subsequent years, the growing season usually starts at the end of March. A plant develops up to 50 perennial 35-70 cm long shoots, the fastest growth occurs in May. The full flowering stage occurs at the end of July and lasts 31-42 days. From aesthetic point of view, the plants are the most attractive at the time of flowering, when they are completely covered with spike inflorescences of purple flowers, located at the ends of the branches. The fruits ripen at the end of September. The growing season lasts 125-145 days, depending on the weather conditions.

E. stauntonii prefers sunny areas with slightly fertile soils. Lands with groundwater located close to the surface and heavy clay soils are unsuitable for its cultivation. The bush is usually shaped by annual pruning, which plays a key role in increasing the productivity of the plant. The bush load should not exceed 35-40% of the shoots, with a nutrition area of 80 x 40 cm. Plants can be pruned annually in spring, starting from the second year after planting. Once every five years, the plant needs to be rejuvenated, by cutting it at a height of 15-20 cm above the soil surface. The biologically active substance contained by this plant is its volatile oil, the content of which increases before the start of flowering, reaching a maximum in this phase and then begins to decrease. A similar tendency was observed in previous years. The maximum content of volatile oil is found in the early flowering stage and constitutes 0.5-1.65%. The perfume note of the essential oil is 4.5 points and it is characterized by a rich content of cinerone (50.8%) and rosefuran (20.6%). Other important compounds, of the 25 identified, are eucalyptol (6.3%) and β-caryophyllene (6.2%) (Figure 2). The volatile oil is a thin, orange-colored fluid that harmonizes with all plant fragrances. Literature data and investigations on the chemical compounds of the volatile oil have confirmed a pronounced antibacterial activity against pathogenic bacteria. The dried raw material contains a number of vital micro- and macronutrients: iron, manganese, molybdenum, which makes it possible to use it in the treatment of anemia, as a diuretic and stimulant for digestion, as well as in the treatment of respiratory diseases. In conclusion, the allochthonous species *Elsholtzia stauntonii*, introduced and researched in the NBGI, can be recommended for cultivation, as an aromatic and medicinal species with aromatic and therapeutic potential. This plant has not yet revealed all its secrets. It is still very promising for study – there are great prospects for researchers and lovers of new organic herbal products.

RT (min)		Kovats index	Compounds	Area %
7.03		978	1-Octen-3-ol	0.3
7.19		985	3-Octanone	0.3
7.47		996	3-Octanol	0.2
8.49		1025	*p*-Cymene	1.4
8.74		1032	Eucalyptol	6.2
9.71		1060	γ-Terpinene	0.2
9.86		1064	Acetophenone	1.6
11.12		1099	(Z)-Cinerone	43.3
14.25		1178	Rosefuran epoxide	33.2
14.35		1180	4-Terpineol	0.2
14.89		1194	α-Terpineol	0.6
18.28		1272	Geranial	1.0
24.56		1421	β-Caryophyllene	3.9
25.93		1456	α-Humulene	0.6
27.07		1484	α-Curcumene	0.4
30.95		1583	Spathulenol	1.5
			Other compounds	*5.1*

1



2

Figure 2. The chemical composition of the *Elsholtzia stauntonii* Benth. volatile oil by GC-MS
1- identified chemical compounds; 2- chromatogram

Koellia virginiana (L.) MacM. native to North America, is another promising species. It has been researched in the Botanical Garden as an aromatic and medicinal plant (Figure 3).

In the first year of vegetation, under the conditions of our country, the plants develop a single stem and do not reach the germination phase. After the first autumn frosts, the aerial organs die. The perennial plants start the growing season at the beginning of March. From the rhizomes, 30-60 annual shoots appear on the soil surface. The plants bloom between July 20 and August 30.

The seeds ripen by the end of September. *K. virginiana* plants synthesize volatile oil in all organs: the maximum is produced in inflorescences 2.00-2.10% of the absolute dry matter. The content of volatile oil varies depending on the plant development stage, the maximum is noted at the end of the flowering stage: 1.41-1.52% (Figure 4).

Figure 3. General appearance of the species *Koellia. virginiana* (L.) MacM.

25 chemical compounds were identified in the volatile oil, the main ones being pulegone (84.6%), menthol (2.5%) and limonene (8.4%). The volatile oil, having a strong and pleasant fragrance, was appreciated by the specialists of the "Viorica-Cosmetic" company with a perfumery rating of 4.6 points out of 5. The menthol aroma is due to the presence of menthol and limonene among its compounds, therefore, and is of interest in the manufacture of fragrances for cleaning and laundry products. The research conducted in collaboration with the "Nicolae Testemițanu" State University of Medicine and Pharmacy, within the Epidemiology Laboratory, demonstrated that the volatile oil obtained from *K. virginiana* plants in the full flowering phase exhibits antimicrobial properties due to the high content of its main component – pulegone (84.6%) and can be used as an antimicrobial and antifungal substance in the production of preparations for the treatment of mycoses and other diseases caused by some gram-positive and gram-negative bacteria.

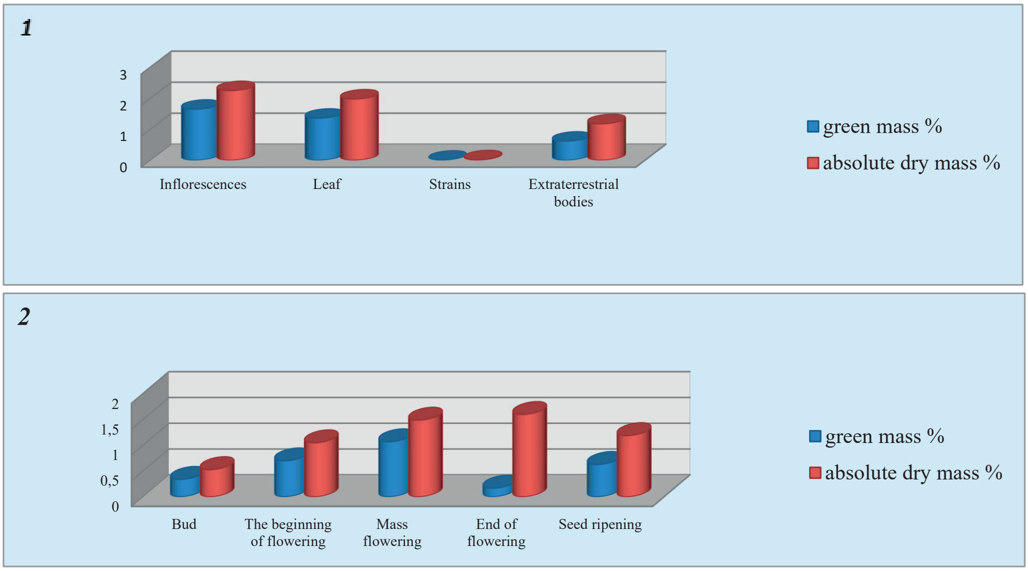


Figure 4. Dynamics of volatile oil accumulation in the species *Koelllia virginiana* (L.) MacM. :1- volatile content depending on the aboveground organs; 2- volatile oil content depending on the phenological phase.

Another promising aromatic and spice species is *Agastache urticifolia* (Benth.) Kuntze native to western North America, from California to Colorado, where it occurs in the wild flora. It is cultivated in Japan, China and some European countries. Under the conditions of the Republic of Moldova it behaves as a perennial plant, growing about 1.1-1.3 m in height (Figure 5). It may not tolerate temperatures below -18º C for a long time, prefers southern exposure, warm, sunny places with well-drained soils. In recent years, the species has tolerated well the weather conditions, completing its entire development cycle and forming viable seeds.

Figure 5. General appearance of the plant *Agastache urticifolia* (Benth.) Kuntze

The species was propagated by seeds sown directly into the soil in early spring and by seedlings stored initially indoors and then transplanted in the field, by division conducted in spring and, less often, by young rooted basal cuttings. The plants complete a full development cycle in the first year of vegetation. They start active growth at the end of April. During the summer, the plants develop slowly, forming 1-2 stems bearing 4-6 pairs of first-order branches, 4-5 pairs of leaves with a blade length of 4.5-6.5 cm and width of 3-4 cm. The plants reach a height of 50-55 cm at the end of August. In September, the first buds appear, some reaching the beginning of flowering. Under the local pedoclimatic conditions, *A. urticifolia* develops a bush composed of 4-6 erect, branched stems. Each branch ends with a compact spike inflorescence. It blooms from July to the end of summer, the flowers are small, the corolla ranging from lilac to blue-violet. It accumulates volatile oil throughout the entire growing season, which lasts 200-220 days. The maximum content is found in the full flowering phase, in 2-year-old plants, in inflorescences: 1.65-1.70% of the absolute dry matter. In the volatile oil of *A. urticifolia*, 17 chemical compounds were identified, the main ones being estragole (41.1%), pulegone (20.4%), limonene (15.3%), iso-menthone (12.0%), methyl eugenol (5.1%), menthone (1.7%) (Figure 6). Estragole determines the strong antibacterial and antifungal effect of the oil. Its presence brings the anise-like smell. Pulegone gives the volatile oil the insecticidal property, so that it can be recommended for the biological protection of plants.

RT(min)	Kovats index	Compounds	Area%
6.91	973	Sabinene	0.1
6.97	976	3-Cyclohepten-1-one	0.7
7.19	985	3-Octanone	0.1
7.39	993	β-Myrcene	0.5
8.67	1030	Limonene	15.3
11.21	1101	Linalool	0.2
11.68	1113	1-Octenyl acetate	0.3
12.02	1122	1,3,8-ρ-Menthatriene	0.2
12.59	1136	*cis*-ρ-Mentha-2,8-dien-1-ol	0.2
13.37	1155	Menthone	1.7
13.80	1166	*iso*-Menthone	12.0
14.26	1178	*cis*-Linalool oxide (furanoid)	0.4
15.24	1202	Estragole	41.1
16.93	1241	Pulegone	20.4
23.85	1404	Methyl eugenol	5.1
24.56	1422	β-Caryophyllene	0.7
		Other compounds	*1.0*

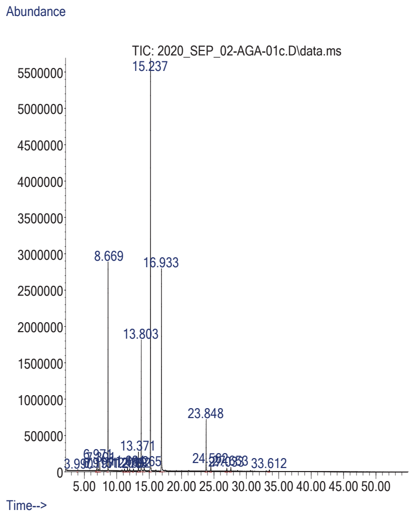


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Figure 6. The chemical composition of the essential oil of *Agastache urticifolia* (Benth.) Kuntze by GC-MS22
1 – chemical compounds; 2 – chromatogram

Monarda fistulosa L. (wild bergamot, bee balm) is a perennial herbaceous species, cultivated as an aromatic, medicinal and spice plant (Figure 7). It occurs in the spontaneous flora of North America and Canada.

Figure 7. General appearance of the species *Monarda fistulosa* L.

In our country, wild bergamot is cultivated on small areas, in gardens of aromatic herbs and in flower beds. In the Botanical Garden, it has been researched as an aromatic and medicinal plant, with a rich content of biologically active substances, especially volatile oil. Under the local conditions, the plant develops a bush composed of 16-20 stems with a height of 65-120 cm, which lignify towards the end of the growing season. The leaves are cordate-lanceolate, toothed, with fine hairs. The flowering period is quite long, lasting from mid-summer to October. The flowers are small, connected to the axillary false whorls, located at the main ends of the lateral buds. On a stem there are 5-9 inflorescences with a diameter of 6-9 cm, in each inflorescence – 230 -292 purple flowers. All parts of the plant contain volatile oil, which gives it a lemony scent and a refined taste.

The fruit is a nutlet. Wild bergamot starts the growing season early, in the second half of February - early March. It blooms from the second year, in June - July. The full flowering stage usually occurs in the first half of July. Heavy, marshy and acidic soils are unsuitable for wild bergamot. It prefers sunny, open places, possibly with a slight shade. Monarda is a promising aromatic plant, which is recommended to be grown outside the crop rotation system with annual plants. The species can be planted again on the same area at least after 5-6 years. Soil preparation involves plowing to a depth of 22-25 cm. The seedbed is prepared 4-5 days before sowing or planting, by leveling and smoothing the soil as much as possible and then by compacting it. Wild bergamot is propagated by seeds sown directly in the field or by seedlings obtained in greenhouses, as well as by division. Sowing right before winter begins is more advantageous, considering that this work is carried out in a period less busy with other agricultural works. Wild bergamot seeds must have a physical purity of 95% and a germination of 70%. They are sown directly into the field right before the beginning of winter, late November - early December, when the average daily temperature drops below -5°C and there is no longer a risk that the seeds will germinate until spring. During winter, temperature, humidity and other factors have a positive impact on seeds, stimulating and maintaining the complex processes preceding germination in spring. To create 1 ha of wild bergamot plantation, 1.5-2.0 kg of seeds is needed. The optimum soil temperature for seed germination in spring is +13.-+15 °C. Using the moisture accumulated in the soil during the winter, they sprout in the first days of May. In some years, with mild winters, this sowing time is not favorable because of the high temperatures, which favor early seed germination, and the possible return of low temperatures, which may destroy the young plants. Seed germination, regardless of the time of sowing in the field, is 55-75%, under laboratory conditions 70-80%. Wild bergamot plants can also be propagated by seedlings, being grown in a substrate of chernozem and sand (1:1). Sowing is carried out at the end of February or the beginning of March. In order to obtain the required amount of seedlings for 1 ha, 500-700 g of seeds and a greenhouse area of 100 m^2 are needed. This species is responsive to organic and mineral fertilizers. One of the simplest and most effective methods of plant propagation is division. The perennial plants can be divided both in autumn and spring. The plant portions planted in autumn start vegetating in spring, 10-15 days earlier than those divided and planted in March. Wild bergamot, during the growing season, may be affected by rust (*Puccinia menthae* Pers.), which appears as rust spots, especially on the leaves, and consequently causes damage by reducing the quality of the leaves and by premature defoliation of the stems. Rust attacks on plants can be avoided by strictly following crop rotation, planting seedlings free from other plant debris and using healthy propagation material. Wild bergamot plants accumulate volatile oil throughout the entire growing season in all aboveground organs. Wild bergamot plantations intended for obtaining volatile oil are harvested starting from the second year of vegetation, for 5-6 years. The maximum volatile oil content is found in the full flowering stage, mainly in leaves and inflorescences, up to 0.75-0.80% of the absolute dry matter. In the volatile oil of *Monarda fistulosa* L., 15 chemical compounds were identified, the main ones being carvacrol (54.83%), p-cymene (23.15%), carvacrol methyl ether (5.90%) etc. (Figure 8). Due to the compounds present in the volatile oil, the species possesses antifungal properties and it can be recommended to be included in phytotherapeutic remedies with antifungal action. The oil is in demand in the food industry, as it is used to flavor wines, soft drinks and confectionery products. The optimal harvest time is during the full flowering stage of the crop. If the area to be harvested is large, it is recommended that harvesting begins when 50-60% of the plants have bloomed, so that the entire area can be harvested during the optimal period. To obtain seeds, harvesting is carried out at the stage of full maturation – at the end of October. On average, 2-3 kg of seeds is obtained from 1 ha. After 5-6 years of exploitation of a wild bergamot plantation, the herb yield decreases, thus, the volatile oil content decreases too. New plantations need to be established on a different plot.

To sum up, it is necessary to mention that perennial *Monarda fistulosa* plants go through an entire vegetation cycle, which lasts 185-190 days, accumulate a large amount of high-quality volatile oil and also are of high aesthetic value, being promising in landscaping as an ornamental species. It can be successfully cultivated on industrial areas. Literature analysis and the investigations on the content and quality of the volatile oil demonstrate the need for further research on *Monarda* species, in order to establish their therapeutic effects. The research will allow for the diversification of effective and harmless antimicrobial preparations available in medical practice.

TR(min)	Compounds	Area %
5.59	α-Thujene	2.30
5.79	α-Pinene	0.59
6.92	3-Octenol	2.44
7.33	β-Myrcene	0.35
8.19	α-Terpinene	2.54
8.45	p-Cymene	23.15
8.61	Limonene	0.87
9.65	γ-Terpinene	1.42
14.29	4-Terpineol	0.66
17.09	Carvacrol methyl ether	5.90
17.28	Thymoquinone	1.50
19.60	Carvacrol	54.83
24.48	β-Caryophyllene	0.30
26.96	Germacrene D	0.47
29.80	Thymohydroquinone	1.33
	Other compounds	*1.36*

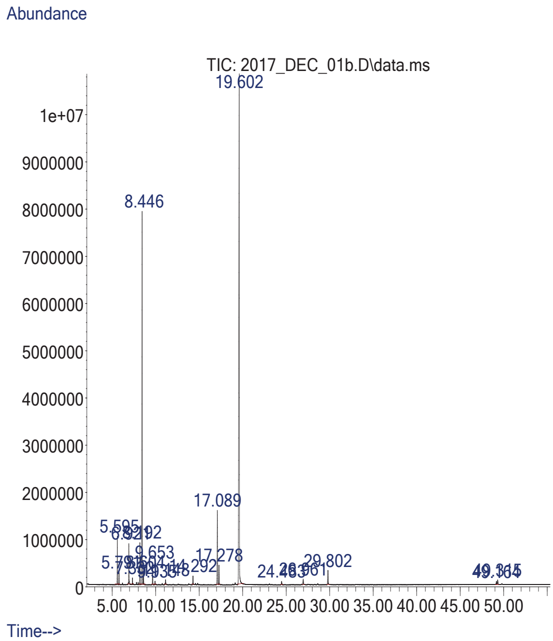

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Figure 8. Chemical composition of the volatile oil of *Monarda fistulosa* L. by GC-MS
1- chemical compounds; 2 - chromatogram

Figure 9: General appearance of the species *Perovskia atriplicifolia* Benth.

A complex study was also conducted on the species *Perovskia atriplicifolia* Benth., obtained from seeds received from Russia in 2018. Research shows that, under the climatic conditions of the Republic of Moldova, it behaves as a perennial plant, developing a bush of 17-18 whitish stems and lobed, deeply toothed, silver-gray leaves (Figure 9).

Mature stems are woody at the base and young stems are herbaceous. The plant exudes a specific sage scent when crushed. It is a quite undemanding plant in terms of growing conditions. It thrives on poor soils and, once established, it is able to tolerate rather long periods of drought. It prefers alkaline soils, not the acidic or swampy ones. It withstands low temperatures down to -15 °C, as well as high ones. It reacts favorably to large amounts of precipitation. It is a light-loving species. In early spring, last year's branches are cut at a height of 5-10 cm above the ground. Perennial plants start growing in early April. The flowering stage is long-lasting, sometimes until early October, which offers the possibility of including the species in the landscaping of green spaces of a curative-prophylactic and decorative type, as well as being used as a honey plant. *P. atriplicifolia* plants accumulate volatile oil throughout the growing season, in all aboveground organs. The maximum content is noted in the mass flowering phase 0.54-0.65%. Investigations on the chemical composition of the volatile oil of *P. atriplicifolia* resulted in the identification of 28 compounds that have a concentration above 0.5%. The compounds identified with the highest values are: d-limonene – 21.47%, eucalyptol – 16.19 %, α-pinene – 8.17%, caryophyllenes (α and β) - 11.91% (Figure 10). The above suggests us that the species *P. atriplicifolia* can be successfully cultivated under the local conditions, thus increasing the assortment of aromatic plants with a potential therapeutic effect in inflammatory diseases.

Nr. crt.	Kovats Index	Retention Time	Compound	Area %
1	914	5.16	α-Thujene	0.20
2	923	5.29	α-Pinene	8.17
3	939	5.51	Camphene	3.87
4	969	5.94	β-Pinene	3.93
5	980	6.09	β-Myrcene	0.98
6	1011	6.53	Δ-3-carene	0.23
7	1020	6.66	*p*-Cymene	0.89
8	1028	6.76	D-Limonene	21.47
9	1031	6.80	Eucalyptol	16.19
10	1043	6.98	*cis*-β-Ocimene	0.23
11	1057	7.17	γ-Terpinene	0.55
12	1099	7.76	Linalool	0.53
13	1146	8.41	Sabinol	0.56
14	1176	8.84	Borneol	4.34
15	1187	8.99	4-Terpineol	0.51
16	1200	9.18	alfa-Terpineol	0.54
17	1299	10.56	Bornyl acetate	6.06
18	1360	11.41	α-Terpinyl acetate	3.08
19	1434	12.46	β-Caryophyllene	6.20
20	1466	12.91	α-Caryophyllene	5.71
21	1490	13.24	Germacrene D	0.36
22	1519	13.65	τ-Cadinene	0.91
23	1527	13.76	Calamenene / Cadina-1,3,5-triene	0.22
24	1586	14.59	Caryophyllene oxide	3.43
25	1599	14.77	α-Bisabolene epoxide	0.20
26	1612	14.95	Cubenol	0.64
27	1637	15.30	τ-Cadinol	3.77
28	1648	15.45	α-Eudesmol	1.24
			Other compounds	*4.99*

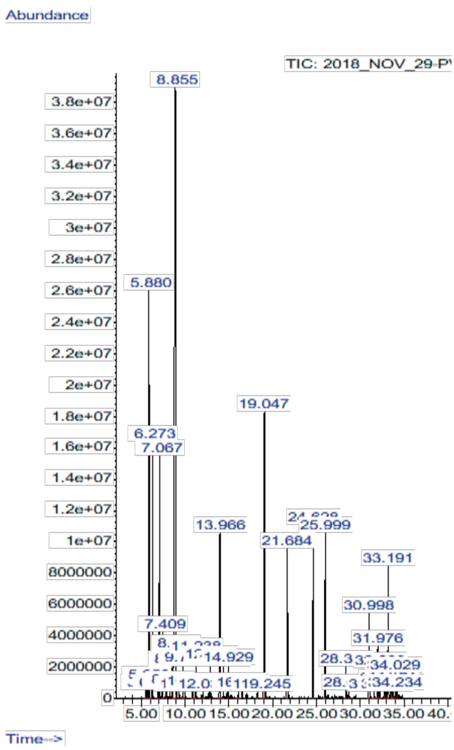

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Figure 10. Chemical composition of the volatile oil of *Perovskia atripicifolia* Benth. by GC-MS
1- chemical compounds; 2 - chromatogram

CONCLUSIONS

The pedoclimatic conditions of the Republic of Moldova are favorable for the growth and development of aromatic, medicinal and spice plants. All the highlighted species fully complete the ontogenetic cycle, are characterized by resistance to drought and frosts from -15°C of - 25°C.

The study of the peculiarities of volatile oil accumulation in plants shows that they depend on the age, phenological stage and plant organ. The maximum content was found in the species *Koellia virginiana* in the late flowering stage (1.41-1.52% of the absolute dry matter), followed by *Agastache urticifolia* (0.80-0.85%) in the full flowering stage. The researched species contain volatile oil and can serve as sources of local raw materials for the production and diversification of the range of plant-derived natural cosmetic and pharmaceutical products.

The laboratory research on the volatile oil obtained from *K. virginiana* and *Agastache urticifolia* plants confirms its antimicrobial properties are due to the high content of the main component – pulegone, which can be used as an antimicrobial and antifungal substance in the production of preparations for the treatment of mycoses and other diseases caused by some gram-positive and gram-negative bacteria.

The species *Elsholtzia stauntonii* Benth. *Koellia virginiana* (L.) MacM., *Agastache urticifolia* (Benth.) Kuntze, *Monarda fistulosa* L., *Perovskia atriplicifolia* Benth, which belong to the family *Lamiaceae,* are plants introduced and researched in the "Alexandru Ciubotaru" National Botanical Garden (Institute), with aromatic, medicinal and melliferous potential.

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BREAKING CYTOPLASMIC MALE STERILITY IN INBRED CORN LINES (*Zea mays* L.): A REVIEW

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Abstract

Cytoplasmic male sterility (CMS) in corn is an important aspect of hybrid seed production, enabling efficient crossbreeding by eliminating the need for mechanical and manual detasseling. However, breaking sterility is a phenomenon that appears often. Research highlights that the breaking of sterility is governed by genetic factors as restorers of fertility (Rf genes), interactions between nuclear and mitochondrial genomes, environmental factors such as temperature and light condition, physiological and biochemical factors, or anthropogenic interventions. While these mechanisms have advanced hybrid crop production, they can also introduce challenges such as reduced genetic adaptability and heightened susceptibility to environmental stressors. A detailed understanding of the factors implications in breaking sterility is vital to optimizing corn production systems while mitigating potential risks. This study consolidates information from the literature on studies of the factors that lead to the sterility breakage of CMS lines in corn.

Key words: *corn, sterility breakage, seed production, cytoplasmic male sterility.*

INTRODUCTION

Corn is considered a multifunctional agricultural crop around the world. Seed production is a very important activity for agricultural production as the quality of the cultivated biological material largely depends on the level of production achieved. Hybrid corn seed is produced in hybridization fields using two important methods. One method involves the use of normal inbred lines, while the other takes advantage of cytoplasmic male sterility.

In corn there are two general types of male sterility: genic and cytoplasmic.

Genic male sterility occurs when lesions in nuclear-encoded genes disrupt normal male gametogenesis. Genic male sterile mutants can be either dominant or recessive and typically exhibit Mendelian inheritance. (Skibbea & Schnablea, 2005). Cytological examination of a subset of these male sterile mutants showed that the lesions in the nuclear genes affect nearly all stages of another development, ranging from pre-meiosis to fully engorged pollen (Albertsen, 1997; Chaubal et al., 2003).

Cytoplasmic Male Sterility (CMS) in corn is a genetic characteristic that stops plants from producing fertile pollen. It is a consequence of changes in mitochondrial DNA and is a trait that is transmitted maternally (Williams, 1995). So male sterility is characterized by non-functional pollen grains, while female gametes function normally (Sunidhi & Versha, 2018).

CMS is reported to over 150 plant species existing as a spontaneous mechanism (Hanson and Bentolila, 2004) or can be created by experimental means such as induced mutations, hybridization, protoplasmic fusion, broad/inter-specific, or genetic engineering (Singh et al., 2015; Wang et al., 2013).

Thoroughly understanding the nucleocytoplasmic interactions between genes linked to fertility restauration and cytoplasmic male sterility offers valuable insights for agriculture and developing corn hybrids.

In corn hybrids, fertility restoration is crucial for the effective production of hybrid seeds, which are essential for achieving higher yields and improving crop performance, however, the same does not apply to the use of sterile lines in seed production.

There are situations in which the process of sterility breakage occurs in the case of certain CMS lines. This process of breaking sterility is very dangerous and can contaminate the entire field if it is not noticed in time. It is very important to understand this process of breaking sterility and the factors that influence it, in order to avoid the contamination of hybrid production fields. Additionally, it is crucial to implement measures that prevent this phenomenon from occurring.

THE ADVANTAGES OF USING CMS LINES IN HYBRID SEED PRODUCTION

The production of corn hybrid seed requires a directional cross to be performed between two inbred lines, where pollen from one inbred line (the male parent or pollen donor) is used to fecundate the silk from the second inbred line (the female parent or pollen receiver). Since corn is a monoecious plant is needed to perform the removal of female plant tassel manually or mechanized and in most cases performing both operations.
The use of CMS forms in corn seed production eliminates the need for castration operations. In the case of fertile maternal forms, this process is delicate, involves a special machine for detasseling, as well as manual labor for verification, making it very important from an economic perspective meaning that this phenomenon of male sterility is of great significance to produce cost effective hybrid seeds (Sunidhi &Versha, 2018).
Another major advantage of using CMS lines in production is that the plants are not stressed by the castration operations, resulting in significantly higher yields. For a fertile female form, by castration, losing part of the plant, automatically will decrease the production potential (Figure 1).

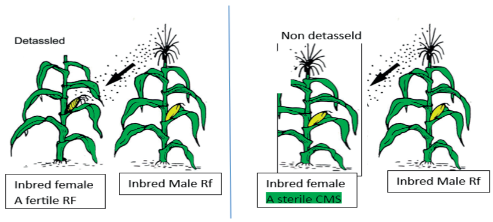

Figure 1. Influence of detasseling process on plant vs non-detasseling (original)

The quality of fecundation on CMS forms is much better, resulting in a higher percentage of large seeds, with a high TKW, and a very good germination and at the same time with a reduction in the percentage of waste (broken or small round seeds) in the seed lots after the final processing.

CMS LINES

The detailed study of CMS inbred lines before their introduction into the production process, as well as the identification of factors that lead to sterility breakage is an important objective for the research departments within the companies producing hybrid corn seed.
Utilization of male sterility involves following steps:
- Identification and obtaining the CMS form.
- Integration of the male sterility trait into desired variety through recurrent backcrossing, transforming it into female parent for hybridization.
- Multiplication of male sterile lines by developing maintainer line.
- Hybrid seeds production in open pollination under controlled conditions or via manual cross-pollination techniques. (Sunidhi&Versha, 2018).

Obtaining a cytoplasmic male sterility line (CMS) involves several steps that combine plant breeding techniques with knowledge of genetics.
Developing cytoplasmic male sterility (CMS) lines in corn involves a focus on two critical phases: discovery and isolation of CMS donors, which are typically identified within cultivated corn or related wild species.
The plants selected as sources of cytoplasmic male sterility exhibit an inability to produce viable pollen due to genetic alterations in the cytoplasmic (mitochondrial) genome. CMS genes are chimeric in nature and originate from recombination events that occur between mitochondrial genes and the flanking sequences (He et al., 2020). While these recombination events contribute toward complexity of the genomic structures, they also help maintain genomic stability along with increasing genetic variation (Tuteja et al., 2013).
A second step consists of the initial crossing between a fertile female elite line (also called the "A" line) with a plant that carries the sterile cytoplasm (the "S" line), to transfer the sterile cytoplasm to the A line.

The third stage is that of recurrent crossing (backcross), so the F1 line is repeatedly crossed with the original A line for several generations. This process aims to maintain the nuclear genetic background of the A line, but with sterile cytoplasm. CMS can be transferred easily to a given strain by using that strain as a pollinator (recurrent parent) in the successive generations of backcross programme (Sunidhi & Versha, 2018). After 6-7 backcrosses, the nuclear genotype of the male sterile line would be almost identical to the recurrent pollinator strain (A line) but contains sterile cytoplasm (Figure 2). "A sterile" line is the male-sterile female line with "S" cytoplasm and recessive fertility nuclear alleles (frfr).

The 4th stage continues with the confirmation of male sterility. Line A is tested to ensure that it does not produce viable pollen. This may involve morphological examination of the anthers and pollen testing for viability.

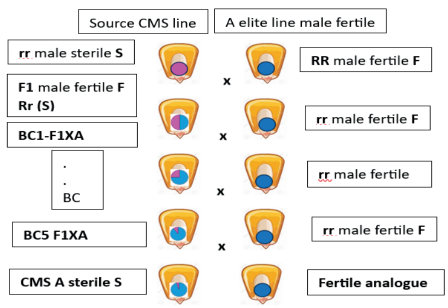

Figure 2. Obtaining a CMS line after crossing a CMS source with an Elite line (original)

Once obtained the CMS line, to complete hybrid system, involves another 2 distinct genotypes:

- "B" line is a maintainer of the female line, and it has fertile "F" cytoplasm and recessive nuclear alleles (frfr). When this line is crossed with "A" line, the entire progeny is male sterile.
- Third parent is designated as "R" line, and it contains dominant fertility-restoring gene (FrFr). This line can restore the male fertility of the hybrid plants produced by crossing with "A" line. (Saxena & Anupama, 2015).

Cytoplasmic Male Sterility (CMS) corn lines are of three main types, depending on differences in mitochondrial DNA:

All these three types of CMS are classified based on specific nuclear restorer genes (Rf), which can neutralize the CMS trait and restore fertility in the first generation of plants (F1). (Christophe Weider et al., 2009).

1.Type T (Texas cytoplasm, CMS-T) was widely used in the 1970s, but its use declined due to its susceptibility to *Helminthosporium maydis*, race T, which causes corn leaf blight disease (Levings, 1993). The T-cytoplasm was removed from breeding germplasm after a widespread and serious epidemic of Southern Corn Blight, which drastically reduced maize yields in 1969 and 1970 (WISE et al., 1999)

2. Type C (Charrua cytoplasm, CMS-C) is the most used source of sterility in corn seed production. Is less susceptible to disease compared to type T and is used as an alternative to avoid problems related to disease susceptibility. It has been discovered in maize lines in South America, and this form is caused by specific changes in the mitochondrial genome that affect pollen fertility (Laughnan, 1983).

3. Type S (USDA cytoplasm CMS-S) is used for its stability and resistance to diseases, being another option in maize breeding programs. However, plants with CMS-S show a moderate resistance to pathogens and have varied performances depending on environmental conditions and agronomic practices (Beckett, 1973).

These types are chosen according to the specific needs of the hybridization programs and the local growing conditions.

CMS lines are very important in hybrid corn production also because they increase productivity, ensure a controlled and more uniform pollination, which contributes to obtaining high-quality hybrids with desired genetic characteristics and minimizes the risk of unwanted or accidental pollination, ensuring the genetic purity of the hybrids and increasing the success rate of production (Saxena et al., 2013). The use of CMS lines has revolutionized the seed industry and significantly advanced modern agriculture, providing farmers with access to superior quality seeds that enhance production and contribute to global food security. However, the use of these lines is not without its challenges.

Problems reported by the production teams are sometimes related to the height of the plants, which is sometimes significant compared to the male form, and then the pollination of the central rows of mother is poor, the pollen not being able to pass between the rows of mother especially for sowing ratio (6Ax2Rf rows).

Another problem that may arise is related to the restoration of fertility in F1 for hybrids obtained with CMS lines due to the recessiveness of sterility genes respectively mitochondrial genes mutations that disrupt normal pollen development (Schnable &Wise, 1998), with the risk that a significant percentage of plants will exhibit androsterility and not produce pollen. In this case, the hybrids obtained with these female CMS forms are checked and tested very well before moving on to their commercial production.

The most important problem is the sterility breakage of CMS lines. If this breakage is not noticed in time, the seed production field is totally compromised because biological contamination with pollen from the female line form takes place.

If this phenomenon is observed in time, the field can be saved by urgently performing castration. In this case, the farmer must intervene quickly with the castration operation that requires the available machine, manual labour to pass and remove any tassels from the smaller plants that have not been removed by the castration machine and thus the costs increase significantly and unforeseen.

Breaking sterility factors

Breaking male sterility refers to the phenomenon where plants that are expected to remain male-sterile begin producing functional pollen.

This is a unique male sterility system where the expression of male sterility and fertility of the plants is controlled by environmental factors. Under this system the male sterility, gene expresses only under specific environment such as low or high temperature, short or long photoperiod, variable light intensity, different soilborne stresses, or their specific combinations (Kaul, 1988). This situation can arise both in genetic as well as cytoplasmic nuclear male sterility systems. The reversion of sterility is influenced by cytoplasmic rather than nuclear genetic factors, and loss of such factors is correlated with reversion of male sterility to male fertility (Levings et al., 1980). The conversion of male sterility to fertility and its reversal is a complex genetic phenomenon, and more research is required at genomics and physiological levels to understand it better.

Fertility restoration occurs through mechanisms that suppress the activity of CMS-related genes or mitigate their harmful effects. Like CMS itself, fertility restoration is a highly intricate process, taking place at multiple molecular levels.

Research indicates that this phenomenon is influenced by both genetic and environmental factors as is mentioned above.

Genetically, fertility restoration can happen at various stages, including genomic, post-transcriptional, translational, post-translational, or metabolic levels. Interestingly, all identified restorer genes are located within the nuclear genome. In corn, restorer genes such as Rf2 have been successfully cloned (Cui et al., 1996).

The restorer allele at Rf2 encodes a functional mitochondrial aldehyde dehydrogenase, suggesting that the restoration of fertility can occur through metabolic compensation. This compensation offsets the effects caused by the mitochondrial CMS-determining gene (Chase, 2007). From our perspective, this mechanism highlights the adaptability of plant genetic systems, as they develop complex strategies to ensure reproductive success even when challenged by cytoplasmic sterility.

In corn, the three major CMS types - T-CMS, S-CMS, and C-CMS - are defined by their unique restorer genes. For T-CMS, fertility restoration depends on the combined action of two restorer genes, Rf1 and Rf2, which must function together to fully restore the plant's ability to produce viable pollen (Duvick, 1965). However, particularly fascinating is that these genes are ineffective in restoring fertility in other CMS systems, such as S-CMS and C-CMS. This specificity underscores the nuanced relationship between nuclear restorer genes and mitochondrial CMS systems.

For S-CMS, fertility restoration is managed by a single major gene, Rf3, which is critical for enabling pollen fertility (Duvick, 1965). According to our understanding, the simplicity of the S-CMS system compared to T-CMS reflects the diversity of evolutionary adaptations

in corn, each tailored to meet specific environmental or genetic challenges. This insight not only emphasizes the intricacies of nucleocytoplasmic interactions but also provides a foundation for targeted breeding strategies aimed at optimizing hybrid seed production.
Compared to the restoration mechanisms of T-CMS and S-CMS, the process of restoring fertility in C-CMS is far more complex and remains largely unexplored. Evidence suggests the involvement of at least three nuclear restorer genes, known as Rf4, Rf5, and Rf6, which are believed to interact in a complementary manner. Interestingly, this trigenic system may also be duplicated elsewhere in the genome, adding another layer of complexity to the restoration process (Vidakovic, 1988; Vidakovic et al., 1997). In our opinion, this intricate interplay between multiple genes reflects the incredible sophistication of plant genetic systems and highlights the challenges researchers face in unravelling these mechanisms.
Complementary interactions between paternal and maternal factors appear to play a significant role in the fertility restoration of C-CMS. This conclusion was drawn from experiments involving crosses between C-CMS lines and maintainer lines with normal cytoplasm, where the progeny exhibited partial or even full restoration of fertility (Vidakovic, 1988; Sotchenko et al., 2007). This finding underscores the importance of studying the dynamic interactions between nuclear and cytoplasmic components, as they seem to be pivotal in determining the stability of CMS systems. A unique feature of C-CMS is its tendency toward "partial restoration", which often includes a phenomenon called the "late-break of sterility". In such cases, pollen shedding can still occur several weeks after the silks have emerged (Tracy et al., 1991; Kheyr-Pour et al., 1981; Vidakovic, 1988). This delayed response is fascinating, as it may point to underlying regulatory mechanisms that are not yet fully understood. Exploring these mechanisms could provide valuable insights for improving hybrid seed production and mitigating risks associated with unintended fertility restoration.
The restoration of CMS sometimes occurs spontaneously. This was found for T- and S- (Smith & Chowdhury, 1989).

In T-CMS, the CMS-inducing gene, urf13, can be lost through recombination, resulting in restored fertility (Fauron et al., 1990). In S-CMS, spontaneous reversion to fertility arises either through reorganization within the mitochondrial DNA (Small et al., 1988) or through mutations of the nuclear DNA resulting in new restorer genes (Gabay-Laughnan et al., 2004). Apart from the restorer genes, which fully restore the fertility, some genes lead to a partial restoration of fertility (Duvick, 1965). Partially restored plants bear fewer anthers than male-fertile plants, and the emergence of the anthers is usually delayed.

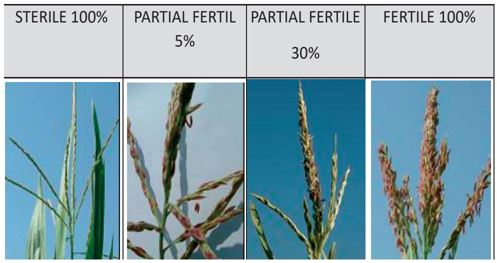

Figure 3. Partial restoration of fertility for tassel comparing with complete sterile and complete fertile

The anthers may be misshapen and have less pollen than a fully restored type (Tracy et al., 1991). The genetic basis of partial restoration is still unclear. It may be governed by multiple genes, which may have an effect in the absence of fully restoring genes (Tracy et al., 1991). Furthermore, partial restoration might result from incomplete homozygosity if the inbred line has been converted into CMS by backcrossing rather than maternal induction of haploidy (Gontarovskii, 1974).
Regarding the environmental factors, several scientific studies have been done to investigate the influence of environmental factors on the stability of male sterility in corn.
Wind, air temperature, solar radiation, and water evaporation appeared to influence, positively or negatively, the expression of male sterility 2 to 3 weeks before anthesis (Sarvella, 1966; Marshall et al., 1974). In another research 22 CMS variants of corn hybrids has been examined across 17 different environments in countries as France, Bulgaria and Switzerland. The results revealed significant variability in the stability of male sterility across the three CMS

types (T, S, and C) (Weider et al., 2009). Among these, T-cytoplasm hybrids show the best stability, while S-cytoplasm hybrids were more susceptible to partial fertility restoration. The study highlighted that the environmental factors, particularly temperature, evapotranspiration, and water vapor levels during the critical 10-day period before and during anthesis, were closely linked to partial reversion to male fertility (Weider et al., 2009). These findings emphasize the interaction between genetic factors and environmental conditions in determining CMS stability.

Another study examines how deficiencies in the Dicer-like 5 (DCL5 protein) gene render corn plants sensitive to temperature changes during meiosis. This genetic deficiency leads to stop tapetal development and subsequent male sterility under specific temperature conditions. (Teng et al., 2020).

Although these studies did not specifically address the impact of sowing density or water availability, the results strongly suggest that environmental conditions are a critical component in maintaining stable male sterility. In our opinion, this raises an important point: factors such as water stress and improper sowing densities, while not explicitly studied, likely play a role in influencing the expression of Cytoplasmic Male Sterility (CMS). Such insights are invaluable for breeding programs, as they highlight the need to consider a wide range of environmental parameters when developing and testing CMS lines for hybrid seed production.

CONCLUSIONS

Cytoplasmic male sterility (CMS) is an inherited trait passed through the maternal line, characterized by the plant's inability to generate functional pollen.

CMS lines are valuable instruments for hybrid seeds production used to facilitate the production of hybrid seeds and to minimize costs as detasseling operation is not needed.

Using CMS line in hybrids production increase not only yield but also the quality of hybrids seeds.

CMS breakage is a very dangerous phenomenon translated into the restoration of fertility and therefore the emission of viable pollen.

Male sterility breakage in corn can be associated with various factors, leading to unintended fertility and affecting hybrid seed production.

The most important factors studied linked to sterility breakage are genetic factors and environmental factors.

Genetic factors that lead to sterility breakage are Restauration of Fertility (Rf) genes, Mitochondrial Gene Mutation and Interaction between nuclear and cytoplasmic genes.

The environmental factors that influence breakage sterility are air temperature, evapotranspiration but the drought or inadequate sowing density are not excluded either. Understanding these factors is crucial for maintaining stable male sterility in corn breeding programs, ensuring the production of hybrid seeds without unintended fertility restoration.

The study of male sterility and factors that leads to breakage of sterility holds great promise for agriculture, biotechnology, as well as basic science.

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BEHAVIOR OF AN ASSORTMENT OF SPRING OAT VARIETIES DEPENDING ON THE LEVEL OF MINERAL FERTILIZATION AND SOWING

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Abstract

The study followed the behavior of an assortment consisting of eleven varieties of spring oats, depending on the level of mineral fertilization, on the level of production, crude protein content and fats. Mineral fertilization with nitrogen had five graduations: N0, N30, N60, N90 and N120, applied on a constant background of P60K60. The sowing density also had three graduations: 350 seeds/m^2, 450 seeds/m^2 and 550 seeds/m^2. The lowest production of 2961 kg/ha was recorded for the Lovrin 1 variety on the N0P60K60 agrofund and the density of 350 seeds/m^2, and the highest of 4879 kg/ha for the Gentiana variety, on the N120P60K60 agrofund and 550 seeds/m^2. The values of the protein content depending on the level of mineral fertilization ranged between 13.4 on the N0P60K60 agrofund and 15.05% on the N120P60K60 agrofund. As for the fat content, the highest value of 54.2% was also recorded on the agrofund fertilized with N120P60K60 and the Muresana variety.

Key words: *chernozem, fat content, nitrogen, protein content.*

INTRODUCTION

Oats have been cultivated for thousands of years due to their adaptability to various climates, nutritional benefits and versatile uses (Steward & McDougal, 2014; Kolmanic et al., 2022). As the global demand for oats is increasing, the optimization of fertilization technology along with the biological material becomes essential to increase productivity and ensure sustainable agricultural practices (Kiviharju et al., 1998; Hilli&Kapoor, 2023; Ruja et al., 2024). The climate changes of recent years, manifested by the lack of humidity and very high air temperatures, require the adaptation of cultivation technology by choosing resistant varieties, appropriate density and level of fertilization with nitrogen, phosphorus and potassium (Dvoracek et al., 2003; Midha et al., 2015; Fu et al., 2023). Mineral fertilization plays an essential role in optimizing oat production by ensuring adequate nutrient availability for optimal plant growth and development. Scientific studies highlight that nitrogen, phosphorus and potassium are the primary macronutrients required to improve oat yield and grain quality. Nitrogen is particularly important because it promotes vegetative growth, increases plant height, and improves the protein content of grains. However, excessive nitrogen can lead to deposition, reducing both yield and quality (Gordara et al., 2016; Bljahhina et al., 2023). Phosphorus supports root development, energy transfer and early plant vitality. Potassium contributes to water regulation, enzyme activation and disease resistance, while improving drought tolerance and grain filling (Ahmad & Zaffar, 2014; Singh et al., 2023). Precision in timing and application methods - such as split applications of nitrogen and phosphorus bands- improves nutrient use efficiency and minimizes environmental risks such as leaching and greenhouse gas emissions. In addition, balanced mineral fertilization improves the harvest index, straw yield and multi-element composition of oat grains (Murariu & Plăcintă, 2017; Ma et al., 2017; Warchol et al., 2023). Due to climate change, choosing the right oat variety is crucial to ensuring crop resilience and productivity (Gangoadhyay et al., 2015). Different oat varieties offer different levels of drought, heat and disease tolerance, allowing farmers to adapt to changing weather patterns. Climate-resistant oat varieties improve yield stability and grain quality under extreme conditions, increasing at the same time the nutrients use efficiency (Decker et al., 2014; Leszczynska et al., 2023). In addition, various genetic traits can help mitigate the risks associated with pests and soil degradation. Thus, choosing the right oat varieties plays an essential role in supporting agricultural productivity and environmental health in the current climate context (Sheoran et al., 2017; Smuleac et al., 2020; Quintarelli et al., 2022; Chițu et al., 2024).

MATERIALS AND METHODS

The research was conducted in a three-factorial experiment, where Factor A - the cultivated variety, with 11 graduations: a1 - Lovrin, a2 - Jeremy, a3 - Ovidiu, a4 - Muresana, a5 - GK Pillango, a6 - Prokop, a7- Effectiv, a8 - Overdrive, a9 - Venafor, a10 - Earl, a11 - Genziana; Factor B - sowing density with 3 graduations: b1 - 350 seeds/m^2, b2 - 450 seeds/m^2, b3 - 550 seeds/m^2; Factor C - nitrogen fertilization level, on a constant background of P60K60, with 5 graduations: N_0, N_{30}, N_{60}, N_{90} and N_{120}.

RESULTS AND DISCUSSIONS

Table 1 presents the production results for the 11 oat varieties tested. The Genziana variety achieved the highest production (3999 kg/ha), respectively an increase of 297 kg/ha compared to Lovrin 1 (the control sample), a difference statistically ensured as highly significant.

Table 1. Oat production according to cultivated variety

Variety	Yield kg/ha	%	Difference kg/ha	Significance
Lovrin 1	3702	100	-	-
Jeremy	3865	104	163	***
Ovidiu	3837	104	135	***
Muresana	3937	106	235	***
GK Pillango	3661	99	-41	00
Prokop	3593	97	-109	000
Effectiv	3839	104	137	***
Overdrive	3881	105	179	***
Venafor	3828	103	126	***
Earl	3948	107	246	***
Genziana	3999	108	297	***
DL 5% = 31.45 kg/ha, DL1%=41.46 kg/ha, DL 0.1%=53.53 kg/ha				

The Prokop variety had the lowest yield (3593 kg/ha), underperforming the control value (Lovrin 1) by 109 kg/ha, a difference statistically ensured as very significant in a negative sense. It should be noted that the other tested varieties Jeremy, Ovidiu, Muresana, Efectiv, Overdrive, Venafor and Earl also showed strong yield performances with high statistical significance over the Lovrin 1 control sample.

The results of oat production according to density are presented in Table 2. The density of 550 seeds/m² led to the highest yield (3980 kg/ha), significantly exceeding both lower densities, the difference of 306 kg/ha more than the lowest density, showing a highly significant relevance. To the density of 450 seeds/m² the difference of (151 kg/ha) compared to the density of 350 seeds/m², is also very significant.

Table 2. Oat production according to sowing density

Density	Yield kg/ha	%	Difference kg/ha	Significance
350 seeds/m^2	3674	100	-	-
450 seeds/m^2	3825	104	151	***
550 seeds/m^2	3980	108	306	***
DL 5% = 16.43 kg/ha, DL1%=21.73 kg/ha, DL 0.1%=28.12 kg/ha				

Oat production results depending on nitrogen fertilization level on a constant P60K60 background, prove the beneficial effect of nitrogen fertilization, on all 4 fertilization levels the production exceeding the N0 with differences statistically assured as highly significant. The largest difference in yield compared to the N0 control was recorded on the agrofund fertilized with N120, respectively an increase of 1199 kg/ha.

Table 3. Oat production as a function of nitrogen fertilization level

Nitrogen level	Yield kg/ha	%	Difference kg/ha	Significance
N0	3182	100	-	-
N30	3637	114	455	***
N60	3844	121	662	***
N90	4087	128	905	***
N120	4381	138	1199	***
DL 5% = 21.20 kg/ha, DL1%=27.95 kg/ha, DL 0.1%=36.09 kg/ha				

The results of Duncan's multiple range tests (Figure 1), used to compare the means in different groups for three factors: the cultivated variety (factor A), the sowing density (factor B) and the level of nitrogen fertilization (factor C), show us that regarding the varieties: The

Genziana variety (3999.9 kg/ha) has the highest average and is marked with A, indicating that it is statistically superior. The Lovrin (3702.5 kg/ha) and Venafor (3828.2 kg/ha) varieties are marked with F and E, respectively, showing lower yields and significant differences from Genziana. Varieties such as Muresana and GK Pillango fall into groups DE and CD, suggesting intermediate performances. Letter clusters (A, B, C) denote groups that are not significantly different from each other. Regarding the three seeding densities, it is observed that increasing the seeding density from b1 (350 seeds/m^2) to b3 (550 seeds/m^2) has a result in significantly higher yields. Density B3 (550 kg/m²) significantly outperforms the others, suggesting that a higher density increases yield. Fertilization levels c4 (N90) and c5 (N120) are statistically the highest yielding, both marked A. The lowest yield is from c1 (N0), showing that no fertilizer significantly reduces yield.

factor A[variety]

Variety	Yield kg/ha	
11. Genziana	3999.	A
10. Earl	3948.	B
4. Muresana	3937.	B
8. Overdrive	3881.	C
2. Jeremy	3865.	CD
7. Effectiv	3839.	DE
3. Ovidiu	3837.	DE
9. Venafor	3828.	E
1. Lovrin	3702.	F
5. GK Pillango	3661.	G
6. Prokop	3593.	H

DL 5% = 31.4 kg/ha

factor B[density]

Density	Yield kg/ha	
b3 – 550 seed/m^2	3980.	A
b2 – 450 seed/m^2	3825.	B
b1 – 350 seed/m^2	3674.	C

DL 5% = 16.4 kg/ha

factor C[density]

Nitrogen level	Yield kg/ha	
c5 – N120	4381.	A
c4 – N90	4087.	B
c3 - N60	3844.	C
c2 – N30	3637.	D
c1 – N0	3182.	E

DL 5% = 21.20 kg/ha

Figure 1. The results of Duncan's multiple tests regarding the influence of nitrogen fertilization

Figure 2 highlights the percentage contribution of three important factors on the achievement of oat production. Each factor is measured according to its influence on the total production, and experimental error is included to assess the accuracy of the results.

Nitrogen fertilization has the greatest impact on the oat production, contributing 83.84% to the variation in yield, and is the essential factor for achieving high yields. Nitrogen doses such as N90 and N120 are probably the most efficient in maximizing production. This indicates that, under the experimental conditions, nitrogen nutrition plays a critical role in stimulating plant growth and grain development.

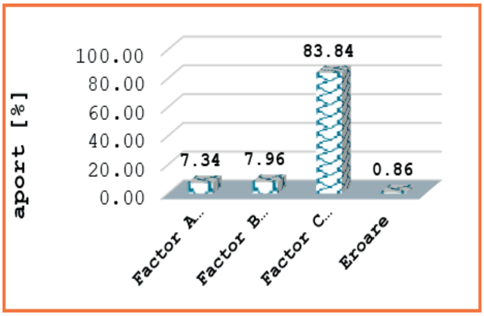

Figure 2. The contribution of factors A [cultivated variety], B [sowing density], C [nitrogen fertilization] to achieve production

Sowing density influences production in proportion to 7.96%. Adjusting seeding density can improve production, but the impact is much smaller compared to fertilization. It is important to choose an optimal density to avoid excessive competition between plants and to maximize the use of soil resources. Although the contribution is relatively small, a correctly chosen density can complement the effects of fertilization. The cultivated variety has a 7.34% influence on the production. While selecting the proper variety is important for certain quality characteristics (such as hectolitre mass - HLM), the impact on total production is less than that of fertilization. However, choosing a suitable variety can bring additional benefits in terms of disease resistance or adaptation to local climatic conditions.

The error value is 0.86%, indicating high precision of the experiment and precision of the results. Unexplained variability is minimal, suggesting that most of the variations in the data are due to the factors analyzed and not to external or random factors.

Table 4 presents the results regarding the hectolitre mass of the 11 oat varieties tested. The Jeremy variety has the highest hectolitre mass (48.05 kg/hL), with a significant difference of 0.52 kg/hL compared to Lovrin 1 (**).

Table 4. Hectoliter mass according to cultivated variety

Variety	Hectolitre mass kg/hL	%	Difference kg/hL	Significance
Lovrin 1	47.53	100	-	-
Jeremy	48.05	101	0.52	**
Ovidiu	45.94	97	-1.59	000
Muresana	46.51	98	-1.02	000
GK Pillango	47.1	99	-0.43	0
Prokop	47.6	100	0.07	ns
Effectiv	47.66	100	0.13	ns
Overdrive	45.54	96	-1.99	000
Venafor	47.49	100	-0.04	ns
Earl	45.61	96	-1.92	000
Genziana	47.41	100	-0.12	ns
DL 5% = 0.4 kg, DL1%=0.5 kg, DL 0.1%=0.7 kg				

The varieties with low hectolitric mass were Overdrive (45.54 kg/hL), Earl (45.61 kg/hL) and Ovidiu (45.94 kg/hL) have the lowest HLM values and are significantly weaker (000) compared to Lovrin 1. The varieties without significant differences: Prokop, Effectiv, Venafor and Genziana do not show significant differences compared to Lovrin 1 (ns), indicating a stability of the hectolitre mass between these varieties. Table 5 presents the results of hectolitre mass depending on the applied nitrogen dose. The results show that hectolitre mass increases as the nitrogen fertilization level increases. Without fertilization (N0), the hectolitre mass is 46.18 kg/hL. With maximum fertilization (N120), the hectolitre mass reaches 47.52 kg/hL, an increase of 1.34 kg/hL. All fertilization levels show significant differences (***), even at a low nitrogen level (N30), suggesting that fertilization has a significant impact on the quality of production.

Table 5. Hectoliter mass as a function of nitrogen fertilization level

Nitrogen level	HLM kg/ha	%	Difference kg/ha	Significance
N0	46.18	100	-	-
N30	46.68	101	0.50	***
N60	47.06	102	0.88	***
N90	47.31	102	1.13	***
N120	47.52	103	1.34	***
DL 5% = 0.3 kg/hL, DL1%=0.4 kg/hL, DL 0.1%=0.5 kg/hL				

The results of the Duncan test (Figure 3), prove that the best variety in terms of hectolitre mass (HLM) is Jeremy, it has the highest HLM (48.05 kg/hL) and is in group A, which indicates that it differs significantly from the other varieties. The Effectiv and Prokop varieties have high hectolitric mass, but are in different groups (B and C), indicating a significant difference from Jeremy. Varieties with low hectolitre mass are Overdrive (45.54 kg/hL) and Earl (45.61 kg/hL) are in groups J and I, having the lowest HLM values and being significantly inferior to the varieties in the higher groups. The Lovrin 1 and Venafor varieties are in the same group (D), indicating that there are no significant differences between them. Regarding the impact of nitrogen fertilization on hectolitre mass, the N120 fertilization level has the highest hectolitre mass (47.52 kg/hL) and is in group A, which indicates that it is significantly superior to the other fertilization levels. As the nitrogen level decreases, the hectolitre mass decreases progressively, with each fertilization level forming a distinct group (B, C, D, E). There are significant differences between all fertilization levels. Even a small increase from N0 to N30 brings a significant difference in hectolitre mass. The increase from N90 and N120 are close in value, but are in different groups, which shows a significant difference between them.

factor A[variety]

Variety	HLM	
2. Jeremy	48.05	A
7. Effectiv	47.66	B
6. Prokop	47.60	C
1. Lovrin	47.53	D
9. Venafor	47.49	D
11.Genziana	47.41	E
5.GK Pillango	47.10	F
4. Muresana	46.51	G
3. Ovidiu	45.94	H
10.Earl	45.61	I
8.Overdrive	45.54	J

DL 5% = 0.04 kg/hL

factor B[fertilization]

Nitrogen level	HLM	
c5 – N120	47.52	A
c4 – N90	47.31	B
c3 - N60	47.06	C
c2 – N30	46.68	D
c1 – N0	46.18	E

DL 5% = 0.03 kg/hL

Figure 3. The results of Duncan's multiple tests regarding the influence of hectolitre mass

Figure 4 shows the relative contribution of three experimental factors on hectolitre mass.

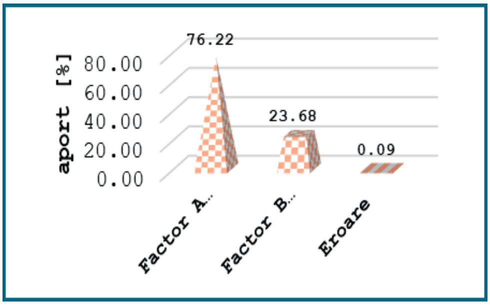

Figure 4. Contribution of factors A [cultivated variety], B [sowing density], C [nitrogen fertilization] to achieve the hectolitre mass

The cultivated variety has the greatest influence on the analyzed variable, with a contribution of 76.22%. Choosing the right variety is decisive for maximizing production or improving the quality of oats. Varieties such as Jeremy or Effectiv (according to previous analyses) would have the greatest positive impact on hectolitre mass or yield. Nitrogen fertilization contributes with 23.68% to the variation of the results. Even if fertilization has a significant impact, it is less influential than the variety.
However, applying optimal doses of nitrogen (for example, N90 or N120) can significantly improve the production and quality of oats. It is important to adjust fertilization according to the variety used and local conditions. The error value is almost insignificant (0.09%), indicating that the experiment was well controlled and the results are reliable. The unexplained variability is minimal, suggesting that most of the variation in the data is due to the factors tested and not random factors. Table 6 presents the fat content (%) in oats for different varieties, comparing the percentage values and the significant differences between them. The statistical significance limits for assessing the differences are also included.

Table 6. Fat content according to cultivated variety

Variety	Fat content %	%	Difference %	Significance
Lovrin 1	48.8	100	-	-
Jeremy	48.49	99	-0.31	00
Ovidiu	50.13	103	1.33	***
Muresana	52.08	107	3.28	***
GK Pillango	51.99	107	3.19	***
Prokop	48.24	99	-0.56	000
Effectiv	48.33	99	-0.47	000
Overdrive	50.91	104	2.11	***
Venafor	49.58	102	0.78	***
Earl	48.92	100	0.12	ns
Genziana	47.29	97	-1.51	000
DL 5% = 0.18 %, DL1%=0.24 %, DL 0.1%=0.32 %				

The varieties with the highest fat content were Mureșana (52.08%) and GK Pillango (51.99%) have the highest fat content, exceeding the control Lovrin 1 by 3.28% and 3.19%, respectively. The differences are significant at a high level (***). The varieties Overdrive (50.91%) and Ovidiu (50.13%) also have a significantly higher fat content than the control, with differences of 2.11% and 1.33%. Low-fat varieties: Genziana (47,29%) has the lowest fat content, with a significant negative difference of -1.51% compared to Lovrin 1 (000). The varieties Prokop (48.24%) and Effectiv (48.33%) also have a lower fat content than the control, with significant negative differences. Earl variety (48.92%) shows a very small difference from the control (0.12%) and is not significantly different (ns), and Jeremy (48.49%) has a slight decrease compared to the control, but the difference is significant at a less strict level (00). The fat content according to the level of nitrogen fertilization is presented in Table 7.

Table 7. Fat content as a function of nitrogen fertilization level

Nitrogen level	Fat content %	%	Difference %	Significance
N0	47.39	100	-	-
N30	49.00	103	1.61	***
N60	49.58	105	2.19	***
N90	49.97	105	2.58	***
N120	51,68	109	4.29	***
DL 5% = 0.12 %, DL1%=0.16 %, DL 0.1%=0.21 %				

As the nitrogen fertilization level increases, the fat content also increases significantly. From N0 (47.39%) to N120 (51.68%), the fat content increases by 4.29%, a significant difference (***). There is a direct correlation between the level of nitrogen applied and the fat content of oats. High nitrogen levels, especially N120, determine a maximum fat content (51.68%). Fertilization not only improves production (according to previous analyses), but also has a positive impact on the nutritional content, increasing the percentage of fat in oats. Depending on the purpose of production (human consumption, feed, industrial processing), fertilization levels can be adjusted to optimize fat content. Results of Duncan's Test (Figure 5), for Factors A (Varieties) and B (Nitrogen Fertilization) to compare the means of fat content (%) according to the cultivated variety (Factor A) and the level of nitrogen fertilization (Factor B).

The varieties with the highest fat content are Mureșana (52.10%) and GK Pillango (52.00%) are in group A, which means that they are statistically the best varieties in terms of fat content. The differences from the other varieties are significant. Varieties with average fat content are Overdrive (50.90%) and Ovidiu (50.13%) are in separate groups (B and C), indicating significant differences between them and from the varieties in group A, and the variety Venafor (49.58%) is in group D, close to the average. Varieties with low fat content: Genziana (47.29%) has the lowest fat content and is part of group H, significantly lower than all other varieties. Varieties without significant differences: Lovrin (48.80%) and Earl (48.92%) are in the same group (E), which means that there is no significant difference between them.

factor A[variety]

Variety	FAT	
4. Muresana	52.10	A
5.GK Pillango	52.00	A
8.Overdrive	50.90	B
3. Ovidiu	50.10	C
9. Venafor	49.60	D
10.Earl	48.90	E
1.Lovrin	48.80	E
2.Jeremy	48.50	F
7.Effectiv	48.30	G
6.Prokop	48.20	G
11.Genziana	47.30	H

DL 5% = 0.18 %

factor B[fertilization]

Nitrogen level	FAT	
c5 – N120	51.68	A
c4 – N90	49.97	B
c3 - N60	49.58	C
c2 – N30	49.00	D
c1 – N0	47.39	E

DL 5% = 0.12 %

Figure 5. The results of Duncan's multiple tests regarding the influence of fat content

The contribution of factors A (cultivated variety) and B (nitrogen fertilization) to achieving fat content is shown in Figure 6.
Cultivated variety accounts for 53.77% of the variation in fat content which suggests that the variety choosing has the greatest impact on the amount of fat in oats. Varieties such as Mureșana and GK Pillango, which were identified in the previous analysis as having high fat content, contribute significantly to the increase in this parameter.
Choosing an appropriate variety is essential for achieving optimal fat content, whether the goal is to increase or reduce it.

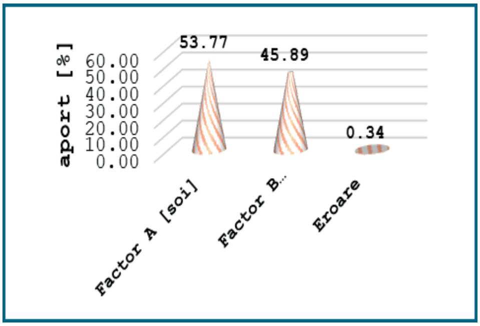

Figure 6. The contribution of factors A [cultivated variety], B [nitrogen fertilization] to achieve the fat content

Nitrogen fertilization contributes 45.89% to the variation in fat content, having an impact almost as important as the cultivated variety. High nitrogen levels (such as N120) have previously been shown to significantly increase fat content. Fertilization not only influences total production, but also plays a crucial role in modifying the nutritional composition, especially in increasing fat. The error value is 0.34%, indicating high measurement accuracy and negligible variability due to uncontrolled factors. The accuracy of the data is high, and most of the variations in fat content can be attributed to the analysed factors (variety and fertilization). The protein content according to the cultivated variety is presented in Table 8.

Table 8. Protein content according to cultivated variety

Variety	Protein content %	%	Difference %	Significance
Lovrin 1	13.97	100	-	-
Jeremy	14.26	102	0.29	***
Ovidiu	14.11	101	0.14	***
Muresana	13.91	100	-0.06	000
GK Pillango	13.93	100	-0.04	0
Prokop	14.17	101	0.20	***
Effectiv	13.96	100	-0.01	ns
Overdrive	14.03	100	0.06	***
Venafor	13.91	100	-0.06	000
Earl	14.39	103	0.42	***
Genziana	14.47	104	0.50	***
DL 5% = 0.03 %, DL1%=0.05 %, DL 0.1%=0.06 %				

The varieties with the highest protein content are Genziana (14.47%), followed by Earl (14.39%) and Jeremy (14.26%). The differences compared to the control Lovrin 1 (13.97%) are positive, but are not marked as statistically significant, which suggests that the variations are small and may not be relevant from a practical point of view. Varieties with low protein content are Mureșana (13.91%), Venafor (13.91%) and GK Pillango (13.93%). They have a slightly lower protein content than the control, but the differences are insignificant.

All varieties show very small variations in protein content. The differences are not marked as statistically significant, which indicates that the cultivated variety has a low impact on protein content. The protein content depending on the nitrogen fertilization level is presented in Table 9.

Table 9. Protein content as a function of nitrogen fertilization level

Nitrogen level	Protein content %	%	Difference %	Significance
N0	13.5	100	-	-
N30	13.95	103	0.45	***
N60	14.12	105	0.62	***
N90	14.24	105	0.74	***
N120	14.70	109	1.20	***
DL 5% = 0.02 %, DL1%=0.03 %, DL 0.1%=0.04 %				

The protein content increases progressively as the nitrogen fertilization level increases. The N120 dose (14.70%) has the highest protein content, with an increase of 1.20% compared to the unfertilized control (N0). The increases from N30 to N90 are gradual and consistent, suggesting a cumulative effect of nitrogen on protein content. However, none of these differences are marked as statistically significant, suggesting that the impact of fertilization on protein content is limited. Analyzing the data according to Duncan's Test (Figure 7), the varieties with the highest protein content are Genziana (14.50%) which has the highest protein content and is in group A, being significantly superior to all other varieties. Earl (14.40%) follows closely and is in group B, differing significantly from Genziana, but having a high content compared to other varieties. Varieties with medium protein content are Jeremy (14.30%) and Prokop (14.17%) in groups C and D, which indicates significant differences from the varieties in the higher groups, and with low protein content Venafor (13.91%), Mureșana (13.90%), GK Pillango (13.93%) and Effectiv (13.96%) are part of group G, having the lowest protein content, being significantly inferior to the varieties in the higher groups. Varieties Lovrin (14.00%) and Overdrive (14.03%) are in the same group (F), which means that there are no significant differences between them. Maximum fertilization significantly increases. N120 (14.70%) has the highest protein content is significantly superior to all other fertilization levels (Group A), followed by N90 (14.24%) and N60 (14.12%) levels found in different groups (B and C), indicating significant differences between them, but also compared to maximum fertilization. Fertilization with N30 (13.95%) has a moderate impact on protein content and is in group D. Fertilization level N0 (13.50%) has the lowest protein content and is significantly lower than all other fertilization levels (Group E).

The cultivated variety contributes 18.73% to the variation in protein content. Even if variety has a smaller influence than fertilization, choosing the right variety can add additional value in optimizing protein content. Varieties such as Genziana and Earl, previously identified as having higher protein content, contribute significantly to this variation.

factor A[variety]

Variety	Protein	
11.Genziana	14.50	A
10.Earl	14.40	B
2.Jeremy	14.30	C
6.Prokop	14.20	D
3. Ovidiu	14.10	E
8.Overdrive	14.00	F
7.Effectiv	14.00	F
1.Lovrin	14.00	F
4. Muresana	13.90	G
5.GK Pillango	13.90	G
9.Venafor	13.90	G

DL 5% = 0.03 %

factor B[fertilization]

Nitrogen level	Protein	
c5 – N120	14.70	A
c4 – N90	14.24	B
c3 - N60	14.12	C
c2 – N30	13.95	D
c1 – N0	13.50	E

DL 5% = 0.02 %

Figure 7. The results of Duncan's multiple tests regarding the influence of protein content

However, the contribution of variety is smaller compared to fertilization, which means that in the absence of adequate fertilization, differences between varieties are less relevant. Principal component analysis (PCA) simplifies the visualization of relationships between variables and observations. In figure 8, the projection of the variables in the plane of the factorial axes of the two main components [Factor 1/CP1 and Factor 2/CP2] is presented. This biplot illustrates the relationships between variables (Hectolitre mass, Protein, Fat) based on the first two principal components (Factor 1 [CP1] and Factor 2 [CP2]). Factor 1 explains 52.40% of the total variance, while factor 2 explains 32.93%, cumulatively representing 85.33% of the variability of the data set. Protein and fat are closely aligned, indicating a positive correlation between these two variables. Hectolitre mass (possibly related to mass or weight) is positioned in a different quadrant, suggesting a weaker or negative correlation with protein and fat. The length of the vectors indicates the strength of the contribution of each variable to the cultivated variety contributes 18.73% to the variation in protein content. Even if variety has a smaller influence than fertilization, choosing the right variety can add additional value in optimizing protein content. the principal components. Protein and fat have longer vectors, meaning they contribute significantly to the variation in the data set. The angles between the vectors show correlation: smaller angles indicate stronger positive relationships, while larger or perpendicular angles suggest a weak or no correlation.

The results of the principal component analysis (PCA) show that the protein and fat content of oats are closely related, while hectolitre mass behaves differently, probably influenced by other factors.

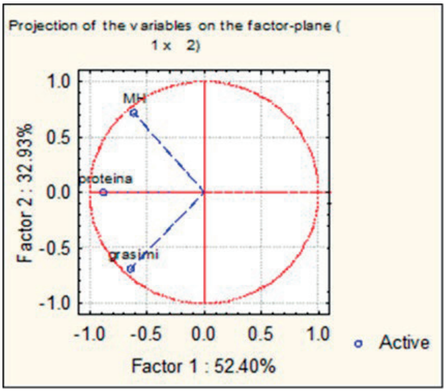

Figure 8. Projection of the variables in the plane of the factorial axes of the two principal components [Factor 1/CP1 and Factor 2/CP2]

Figure 9 shows the projection of the cases [variety x fertilization levels combinations] in the factorial axes plane of the two main components [Factor 1/CP1 and Factor 2/CP2]. This graph shows how individual cases (soil combinations, fertilization, and other factors) are distributed based on the first two principal components. Each point represents a specific combination, appropriately labelled (for example, s1N1, s4N3, etc.), where s represents the soil type and N denotes the fertilization level. The spread of points indicates the diversity of responses to different variety and fertilization treatments. Cases clustered closely together have similar characteristics, while those more distant are more distinct in their responses.

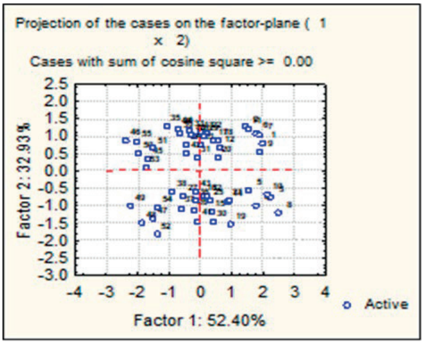

Figure 9. The projection of cases [variety combinations x fertilization levels] in the plane of the factorial axes of the two main components [Factor 1/CP1 and Factor 2/CP2]

PCA helps identify combinations of variety and fertilization that lead to similar results in terms of yield, protein, fat or hectoliter mass. This analysis helps identify the optimal soil and fertilization combinations that produce the desired results (for example, high protein content or improved yield).

The correlation between protein and hectoliter mass (Figure 10) and the correlation between protein and fat content (Figure 11), show that there is a moderate but statistically significant positive correlation between protein and fat content in oats.

While higher protein levels tend to coincide with increased fat levels, the relationship is not strong enough to be the only predictor, indicating that other variables probably play a role in the variability of fat content.

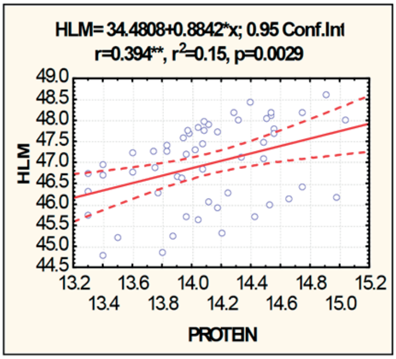

Figure 10. Correlation between protein and hectolitre mass

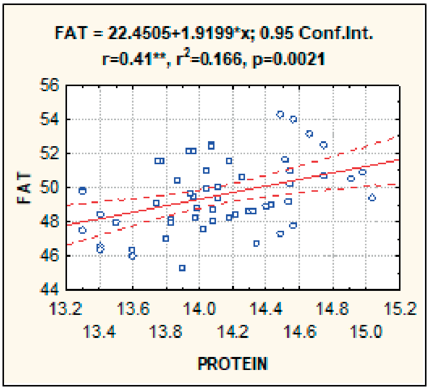

Figure 11. The correlation between proteins and fats

The correlation between nitrogen fertilization level and hectolitres mass is presented in Figure 12 and shows that nitrogen has a moderate effect on hectolitre mass, implying that other factors could play a more significant role in determining hectolitre mass, especially moisture.

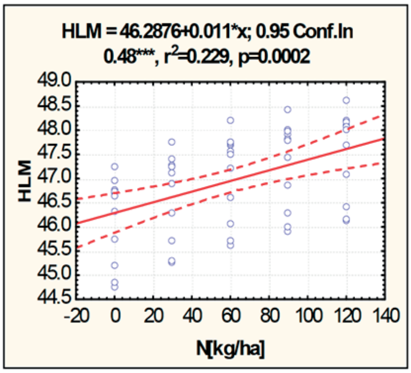

Figure 12. Correlation between nitrogen fertilization level and hectolitre mass

Nitrogen also has a strong positive effect on fat content, although not as pronounced as in the case of protein (Figure 13).

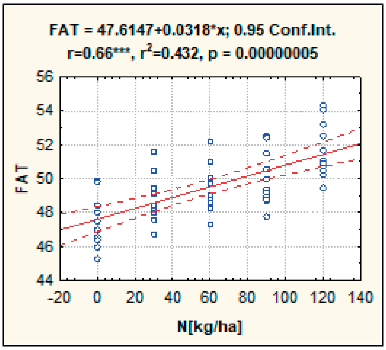

Figure 13. Correlation between the level of nitrogen fertilization and fat content

The strongest correlation is between nitrogen and protein content, suggesting that nitrogen fertilization is essential for maximizing protein levels in oats (Figure 14).

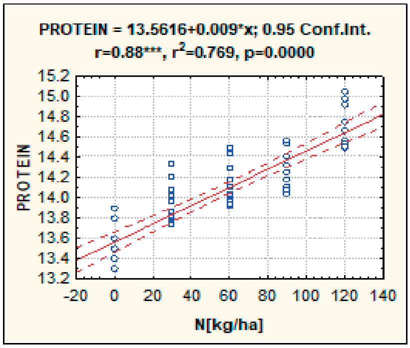

Figure 14. Correlation between nitrogen fertilization level and protein content

CONCLUSIONS

Fertilization plays a critical role in oat production, influencing yield, grain quality and environmental sustainability. By adopting best practices, valorisation the technological advances and integrating sustainable approaches, farmers can optimize oat production while minimizing environmental impact.
Continued research and innovation in the creation of valuable biological material and fertilization strategies will be essential to meet the growing global demand for oats and ensure the resilience of agricultural systems.

Protein and fat content are closely related, principal components analysis (PCA) confirms a strong positive correlation between protein and fat levels in oats, suggesting that fertilization strategies targeting protein may also influence fat content.

The behaviour of hectolitres mass in PCA indicates that it is influenced by different factors compared to protein and fat, possibly related to environmental or genetic variables.

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TECHNOLOGIES FOR GROWING MAIZE IN REPEATED AND CONTINUOUS CROPS UNDER IRRIGATION

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Abstract

The use of chemical ameliorant allows maintaining agrophysical soil parameters in dark chestnut medium loam soil with constant corn cultivation under drip irrigation at the initial baseline level. Without the use of ameliorant, the stocking density increased by 1.5...4.8% compared to the base year. The soil was most compacted in the 10-20 cm horizon. The use of ameliorant allowed to maintain the initial soil density during the constant cultivation of corn for 5 years 2020…2024. Soil porosity decreased by 1.5...1.8%, and in relative values by 2.5...3.3% under constant corn crops without the use of chemical ameliorants. The use of a soil improver allowed to slow down the decrease in porosity by almost an order of magnitude – to 0.2…0.3 relative percent. The humus content when using chemical ameliorant was half as low compared to the options without ameliorant. Fluctuations in humus content and a possible downward trend were at a low level - the percentage of fluctuation of the indicator to the base year 2020 was 0.7-1.4% without the use of ameliorant and 0.3% with the use of ameliorant.

Key words: *maize, humus content, soil bulk density, soil porosity, chemical ameliorant.*

INTRODUCTION

In the southern regions of Ukraine, maize is predominantly cultivated under irrigation, ensuring high profitability. A critical issue for researchers studying the cultivation of agricultural crops under irrigation in the Southern Steppe is the trend toward the degradation of the soil's agrophysical and physico-chemical properties due to prolonged irrigation with highly mineralized water (classified as Class II water quality) and long-term continuous maize cropping. Intensive cultivation technologies for profitable grain crops (maize, soybeans) require increased irrigation rates (up to 6,000–8,000 m³/ha) and impose greater technical pressure on the soil, leading to secondary salinization and the destruction of the agrophysical structure of the arable layer. The issue of soil fertility preservation and regeneration has become a matter of national significance. Regenerative agriculture should primarily be based on the use of chemical and phytomeliorants, as well as monitoring the agrophysical indicators of soil fertility.

Analysis of Recent Research and Publications. Even though some positive examples of crop tolerance to continuous cropping exist, they do not eliminate certain problems associated with this cultivation system. Maize serves as a prime example. Extensive practical experience with high crop concentration and continuous cropping of maize demonstrates that its cultivation should not exceed a period of 4-6 years. After this period, it becomes necessary to introduce a break of at least two years and cultivate crops from different botanical families that do not share common pests and pathogens (Bocsa et al., 2000).

Continuous cropping of any crop, including maize, requires specific environmental conditions for successful growth and development, particularly in terms of soil fertility. These conditions are also necessary for maize cultivation within classical crop rotation systems (Klymchuk, 2005; Cherney & Small, 2016). At first glance, there appears to be no significant difference. However, detailed analysis reveals otherwise. Specifically, in areas where continuous maize cropping occurs, there is an increasing presence of crop-specific insect pests and disease pathogens. In the early stages of concentration, these changes may be insignificant, but as the duration of continuous cropping increases, the trend toward the accumulation of harmful organisms intensifies (Fedorenko et al., 2016).

A significant challenge in continuous cropping systems is the activity of plant root systems, particularly the biochemical nature of their root exudates - colloquially referred to as allelochemicals - which contribute to soil fatigue. The allelopathic activity of rhizosphere soil and extracts from aerial plant organs can act as strong inhibitors of monoculture growth (Jalgaonwala & Mahajan, 2014).

The accumulation of such biologically active substances in the arable layer over the years can induce unfavorable changes in plant growing conditions. Numerous examples of soil fatigue under long-term monoculture cultivation have been documented, including in fruit orchards, flax, sugar beet, and sunflower (Skrypchenko et al., 2020; Grodzinsky, 1991).

At the same time, it is well known that maize, winter rye, potatoes, and certain other crops can withstand continuous cropping for decades without significant yield reduction (Fedotov & Shoba, 2019).

One of the key factors in mitigating the negative effects of continuous cropping is maintaining high nutrient availability. However, the rational application of organic and mineral fertilizers in production conditions can only be assessed by considering numerous factors that influence their efficiency. The dependence of crop yield on increased application rates of specific types of mineral fertilizers and their combinations can only be determined experimentally through vegetation and field trials (Werf et al., 1996).

Specialization in agriculture has been a key factor in increasing farmers' profitability. Production systems have become increasingly simplified as farmers cultivate only a small number of crops that command favorable market prices. However, monoculture systems require increased agrochemical inputs, leading to unsustainable environmental costs (Arrobas et al., 2015).

Corn is one of the agricultural crops increasingly grown in no-till systems. This approach enhances the efficient use of agricultural land, which, in addition to enabling high yields, improves overall farm management and economic efficiency. In Poland, studies have shown that the lowest average corn yield was recorded in monoculture under direct seeding. The yield obtained in monoculture was 17-27% lower than in a crop rotation system. The humus content in soil under monoculture corn grown with direct seeding or conventional tillage remained unchanged, whereas it increased in soil where corn was grown in rotation with other cereals (Księżak et al., 2018).

The reduction in yield under continuous maize cultivation can range from 0% to 30%, but typically falls between 5% and 15%. This decline is associated with nitrogen (N) immobilization, increased disease risk, and allelopathy. The elevated volume of corn residues exacerbates these negative effects. Continuous maize requires more nitrogen than maize grown after soybeans. Hybrid selection plays a crucial role in adapting to continuous maize cultivation (Licht, 2019).

A study on the impact of long-term monoculture and crop rotation on soil biological activity revealed a statistically significant increase in soil enzymatic activity and the total number of bacteria and actinomycetes in monoculture (Gałązka et al., 2017).

Intensive agroecosystem management may lead to soil degradation, negatively affecting the relationship between agricultural production and climate change. To increase soil organic carbon (SOC) and total nitrogen (STN) reserves, conservation tillage (i.e., zero and minimal tillage) is recommended. This practice positively impacts food security, biodiversity, water quality, and the environment. An eight-year comparison of tillage systems in irrigated monoculture maize showed that minimum tillage (MT) is a valuable alternative for increasing maize yield and biomass return compared to conventional tillage (CT). However, this does not apply to no-till (NT), which resulted in lower maize yields and biomass return during the initial five-year transition period. Implementing conservation tillage in intensive maize monoculture systems should be recommended to support maize yields (Fiorini et al., 2020).

In Ukraine's southern regions, maize is primarily grown under irrigation, ensuring high profitability. A key issue for researchers studying irrigated agriculture in the Southern Steppe is the degradation trend in agro-physical and physico-chemical soil properties due to prolonged irrigation with highly mineralized water (classified as Class II water quality) (Vozhehova et al., 2014).

Intensive technologies for growing high-profit grain crops (such as maize and soybeans) require increased irrigation rates (up to 6,000-8,000 m³/ha) and higher mechanical loads on the soil, leading to secondary salinization and the destruction of the agro-physical structure of the plow layer. Preserving and regenerating soil fertility has become a matter of national importance. Regenerative agriculture should be based primarily on the use of chemical and phytomeliorants (Vozhehova et al., 2020).

The economics of crop production in Ukraine dictate a specific structure of sown areas. High-profit crops dominate crop rotations, sometimes disrupting traditional (archaic) scientifically based crop rotation systems. The share of crops such as maize, soybeans, and sunflower in crop rotations has significantly increased. Scientists recommend and actively promote tripartite crop rotation with two maize fields, two-field rotation (maize-soybean), and four-field rotation with three maize fields (Zubets, 2010).

Research on continuous maize cultivation for grain on typical chernozem in the Left-Bank Forest-Steppe of Ukraine has shown varying impacts of anthropogenic and natural factors on yield levels and soil fertility. Maize yield depends more on weather conditions than on the duration of cultivation in the same location. A mathematical analysis of maize yield data and its correlation with fertilization systems, temperature, and water regimes demonstrated a broad spectrum of correlations, ranging from direct to inverse relationships. Weed surveys indicated that over four years, the average weed density in continuous maize fields was 84.9 plants/m², whereas in crop rotation systems, it was 59.1 plants/m² - a 30% reduction (Kohan et al., 2019).

The **objective** of this research is to determine the impact of soil ameliorants on the agro-physical properties of the arable layer of dark chestnut soil under drip irrigation in continuous maize fields in the Southern Steppe of Ukraine.

MATERIALS AND METHODS

The research was conducted using methodological approaches employed in international practice and compliant with the state standards of Ukraine.

The response of corn hybrids to different cultivation conditions was studied at the Institute of Climate-Smart Agriculture, National Academy of Agrarian Sciences of Ukraine, located in Kherson, Ukraine (46°44'33" N; 32°42'28" E; 50 m above sea level) (location A) during the period of 2020-2024. Agricultural cultivation techniques and research methods were consistent with generally accepted practices for irrigation conditions, in addition to the factors under investigation. Surface drip irrigation was applied, maintaining the pre-irrigation soil moisture level at 80% of the lowest moisture capacity in the 0-50 cm soil layer (Vozhehova et al., 2014). The mathematical processing of research results was performed using the dispersion analysis method with the Agrostat computer software package (Ushkarenko et al., 2013).

The cultivation practices were standard for irrigated conditions and aligned with the requirements of grain corn production technologies (Vozhehova et al., 2023). Plowing was carried out after the harvest of the preceding crop (grain corn) to a depth of 26-28 cm. Spring soil tillage included early spring harrowing at the stage of soil physical maturity. Pre-sowing cultivation was conducted to a depth of 6-8 cm, corresponding to the seeding depth. Gypsum was applied as a chemical ameliorant at a rate of 5 t/ha. Agronomic indicators of the arable soil layer were assessed after the corn harvest.

RESULTS AND DISCUSSIONS

The analysis of humus content dynamics in different soil layers over a five-year rotation revealed that the highest losses occurred in the 0-10 cm soil layer when no chemical ameliorant (gypsum at a rate of 5 t/ha) was applied (Table 1). In the 10-20 cm and 20-30 cm layers, the negative indicators were half as significant. The loss of humus was reduced by half when a chemical ameliorant was applied compared to the non-ameliorant variants. However, fluctuations in humus content and a potential decreasing trend were minimal - ranging from 0.7-1.4% without the ameliorant and 0.3% with the ameliorant, relative to the baseline year of 2020. Thus, the application of the chemical ameliorant reduced humus loss in the 0-30 cm soil layer by 0.01%. The greatest humus loss was observed in the 0-10 cm layer (up to 0.04%), likely due to increased moisture availability in this layer under drip irrigation and enhanced root system activity. Over the five-year period, humus content fluctuations were minor in the non-ameliorant variant (0.7-1.4%) and even smaller with the ameliorant (0.3%), indicating the feasibility of continuous corn cultivation under irrigation without significant humus loss in the arable soil layer.

Table 1. Dynamics of Humus Content in Dark Chestnut Medium Loamy Soil on Carbonate Loess Under Continuous Corn Cultivation with Drip Irrigation, %

Experiment Variant	Soil Layer, cm	Study Years					Changes in 2024 vs. 2020	
		2020	2021	2022	2023	2024	Absolute Values	%
Without Ameliorant	0–10	2.75	2.75	2.74	2.74	2.71	–0.04	1.4
	10–20	2.70	2.70	2.69	2.69	2.68	–0.02	0.7
	20–30	2.37	2.36	2.35	2.35	2.35	–0.02	0.7
	0–30	2.61	2.60	2.59	2.59	2.58	–0.03	1.1
With Ameliorant	0–10	2.75	2.75	2.73	2.74	2.74	–0.01	0.3
	10–20	2.70	2.70	2.71	2.70	2.71	+0.01	0.3
	20–30	2.37	2.36	2.36	2.36	2.36	–0.01	0.3
	0–30	2.61	2.60	2.60	2.60	2.60	–0.01	0.3
CV_{05}		0.11	0.12	0.09	0.10	0.09		

The dynamics of humus reserves followed a similar pattern. Without the ameliorant, humus reserves in the 0-30 cm soil layer were 24.4 t/ha in 2024, compared to 24.7 t/ha in 2020 (Table 2).

Table 2. Dynamics of Humus Reserves in Dark Chestnut Medium Loamy Soil Under Continuous Corn Cultivation with Drip Irrigation, t/ha

Experiment Variant	Soil Layer, cm	Study Years					Changes in 2024 vs. 2020	
		2020	2021	2022	2023	2024	Absolute Values	%
Without Ameliorant	0–10	26.1	26.1	25.9	25.9	25.7	–0.4	1.5
	10–20	25.6	25.6	25.5	25.5	25.4	–0.2	0.7
	20–30	22.4	22.2	22.2	22.2	22.2	–0.2	0.7
	0–30	24.7	24.6	24.5	24.5	24.4	–0.3	1.2
With Ameliorant	0–10	26.1	26.1	25.9	25.9	26.0	–0.1	0.3
	10–20	25.6	25.6	25.7	25.6	25.7	+0.1	0.3
	20–30	22.4	22.4	22.3	22.3	22.3	–0.1	0.3
	0–30	24.7	24.7	24.7	24.6	24.6	–0.1	0.3
CV_{05}		1.22	1.13	1.15	1.06	1.14		

Changes in humus reserves from 2020 to 2024 ranged from –0.2 to –0.4 t/ha in absolute terms, or 0.7-1.5%. The application of the ameliorant reduced humus reserve losses in the 0-30 cm layer. Humus reserves decreased from the 0-10 cm to the 20-30 cm layer, likely due to increased organic stem residues after grain harvest in the upper layers. This trend of decreasing humus reserves in lower layers was observed in all study years, both with and without the ameliorant.

The bulk density of dark chestnut medium loamy soil under continuous corn cultivation with drip irrigation was more pronounced for analysis (Table 3). Without the ameliorant, bulk density increased by 1.5-4.8% compared to the baseline year of 2020. The greatest compaction occurred in the 10-20 cm layer. The application of the ameliorant mitigated soil compaction during continuous corn cultivation. Bulk density increased from 1.340 to 1.405 g/cm³ over the five-year period without the ameliorant, while with the ameliorant, it increased from 1.270 to 1.281 g/cm³, six times less than the non-ameliorant variant. Soil bulk density was higher in the lower layers (10-20 cm and 20-30 cm) across all study years, regardless of ameliorant use. The ameliorant reduced soil bulk density in all layers by 1.1-4.0%, with the most significant effect observed in the 10-20 cm layer.

Table 3. Dynamics of Bulk Density in Dark Chestnut Medium Loamy Soil Under Continuous Corn Cultivation with Drip Irrigation, g/cm³

Experiment Variant	Soil Layer, cm	Study Years					Changes in 2024 vs. 2020	
		2020	2021	2022	2023	2024	Absolute Values	%
Without Ameliorant	0-10	1.298	1.303	1.301	1.342	1.350	+0.052	4.0
	10-20	1.340	1.351	1.374	1.386	1.405	+0.065	4.8
	20-30	1.371	1.376	1.380	1.391	1.392	+0.021	1.5
	0-30	1.336	1.343	1.351	1.373	1.382	+0.046	3.34
With Ameliorant	0-10	1.199	1.201	1.203	1.198	1.205	+0.006	0.5
	10-20	1.270	1.273	1.274	1.279	1.281	+0.011	0.8
	20-30	1.307	1.315	1.310	1.304	1.302	–0.005	0.4
	0-30	1.259	1.262	1.262	1.260	1.262	+0.003	0.2
CV_{05}		0.045	0.039	0.042	0.053	0.048		

The porosity of dark chestnut medium loamy soil under continuous corn cultivation tended to decrease without the ameliorant (Table 4). In absolute terms, porosity decreased by 1.5-1.8%, and in relative terms, by 2.5-3.3%. The application of the ameliorant slowed the reduction in porosity by nearly an order of magnitude to 0.2-0.3%. Soil compaction increased with depth, reaching 20-30 cm. Porosity in all soil layers was significantly higher with the ameliorant, indicating improved soil aeration under drip irrigation.

Table 4. Dynamics of Porosity in Dark Chestnut Medium Loamy Soil Under Continuous Corn Cultivation with Drip Irrigation, %

Experiment Variant	Soil Layer, cm	Study Years					Changes in 2024 vs. 2020	
		2020	2021	2022	2023	2024	Absolute Values	%
Without Ameliorant	0–10	58.3	58.0	57.4	56.9	56.8	–1.5	2.5
	10–20	54.5	54.2	53.2	52.9	52.7	–1.8	3.3
	20–30	50.6	50.3	49.7	49.5	48.8	–1.8	3.3
	0–30	54.4	54.2	53.4	52.8	52.7	–1.7	3.1
With Ameliorant	0–10	61.2	61.0	60.9	60.9	61.0	–0.2	0.3
	10–20	55.4	55.3	55.0	55.2	55.2	–0.2	0.3
	20–30	51.1	51.2	51.3	51.2	51.0	–0.1	0.2
	0–30	55.9	55.8	55.7	55.7	55.7	–0.1	0.2
CV_{05}		2.31	1.87	2.14	2.40	2.15		

Nitrate content in dark chestnut medium loamy soil under continuous corn cultivation was highly variable across study years (Table 5). Without the ameliorant, nitrate content decreased from 2020 to 2024 by 0.4-2.2 mg/kg soil, or 2.6-13.3%. However, in 2022, nitrate levels exceeded those of the previous year. Similar fluctuations were observed with the ameliorant. In 2024, nitrate content increased by 5.5-17.2% compared to 2020, indicating significant dependence on preceding weather conditions.

Table 5. Dynamics of Nitrate Content in Dark Chestnut Medium Loamy Soil Under Continuous Corn Cultivation with Drip Irrigation, mg/kg

Experiment Variant	Soil Layer, cm	Study Years					Changes in 2024 vs. 2020	
		2020	2021	2022	2023	2024	Absolute Values	%
Without Ameliorant	0-10	16.5	16.7	17.6	16.6	14.3	–2.2	13.3
	10-20	18.9	18.5	19.2	18.9	17.8	–1.1	5.8
	20-30	15.4	15.0	13.8	14.8	15.0	–0.4	2.6
	0-30	16.9	16.7	16.9	16.8	15.7	–1.2	6.1
With Ameliorant	0-10	16.2	16.0	18.4	17.2	15.3	–0.9	5.5
	10-20	18.1	18.7	19.5	19.3	20.0	+1.9	10.4
	20-30	15.7	16.2	17.8	18.1	18.4	+2.7	17.2
	0-30	16.6	17.0	18.5	18.2	17.9	+1.3	7.8
CV_{05}		0.66	0.58	0.71	0.69	0.57		

CONCLUSIONS

The application of a chemical ameliorant helps maintain the agro-physical properties of dark chestnut medium loamy soil under continuous corn cultivation with drip irrigation at a sufficiently high fertility level.

Variability in humus content was halved with the ameliorant compared to non-ameliorant variants. Fluctuations in humus content and a potential decreasing trend were minimal - 0.7-1.4% without the ameliorant and 0.3% with the ameliorant, relative to the 2020 baseline.

Without the ameliorant, soil bulk density increased by 1.5-4.8% compared to 2020, with the greatest compaction in the 10-20 cm layer. The ameliorant preserved initial bulk density levels during continuous corn cultivation.

Porosity decreased by 1.5-1.8% in absolute terms and 2.5-3.3% in relative terms under continuous corn cultivation. The ameliorant slowed porosity reduction to 0.2-0.3%.

Nitrate content in dark chestnut medium loamy soil under continuous corn cultivation was highly variable, depending on preceding weather conditions and years of continuous cropping.

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GRAPHICAL DIALLEL ANALYSIS FOR QUANTITATIVE TRAITS IN DURUM WHEAT

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Abstract

The aim of the study is to determine the genetic system controlling traits related to productivity in durum wheat and to identify the parents and their correct usage for achieving optimal results. A diallel cross without reciprocals was conducted with five modern durum wheat varieties at the Field Crops Institute - Chirpan. The inheritance of traits involves incomplete, complete and overdominance. Productivity tillering, spikelets number per spike and grain weight per spike are controlled by a simply additive-dominant genetic system with epistasis from complementary type. For the traits: plant height, spike length, and thousand kernel weight, the inheritance control is accomplished by a simply additive-dominant genetic system. All traits increased by accumulation of dominant genes, except productivity tillering. Complexly breeding valuable genotype with more dominant gene simultaneously several traits are the variety Progress. It can be successfully used to improve the traits related to productivity in durum wheat breeding programs.

Key words: *additive-dominance model, epistasis, traits rise, durum wheat.*

INTRODUCTION

Durum wheat (*Triticum durum* Desf.) is an important agricultural crop, widely cultivated for pasta production and other high-protein, gluten-rich food products. It is a raw material mainly used for human consumption. Over the past decades, the increasing global population has necessitated a significant rise in crop yields. Breeding programs for durum wheat predominantly rely on hybridization to generate genetic diversity, which is crucial for effective breeding and the creation of new genotypes. The selection of parents used for crossing is a fundamental step in achieving high breeding efficiency. The acquisition and assessment of these genotypes assist in creating new ones adapted to changing climatic conditions. Establishing the genetic control systems governing inheritance is fundamental for achieving optimal breeding outcomes. In the context of durum wheat, graphical diallel analysis is an underutilized approach that provides valuable insights. This method allows breeders to efficiently utilize parental lines as donors and optimize their breeding potential.

The genetic system controlling productivity-related traits exhibits various inheritance patterns. In most cases, an additive-dominant genetic system governs these traits, although some are also influenced by non-allelic interactions (epistasis). One of the key advantages of graphical diallel analysis is its ability to account for epistatic gene interactions. Epistasis can either enhance or reduce the degree of dominance (Hayman, 1957). In quantitative genetics, two primary types of epistasis are recognized: complementary (recessive gene) and duplicate (dominant gene) (Mather, 1967). Both types of epistasis play significant roles in the inheritance of quantitative traits in durum wheat. Hayman (1954a), Jinks (1955; 1956), and Whitehouse et al. (1958) demonstrated that the simplest way to extract information from diallel crosses is through the graphical representation of the covariance (Wr) of each row against its variance (Vr). Graphical diallel analysis provides valuable information regarding the genetic nature of the studied quantitative traits, as well as the nature of recessive and dominant genes in the parents.

Furthermore, it offers insight into which types of genes contribute to increased or decreased trait values.

Several researchers have conducted diallel analyses of productivity-related traits. However, studies focusing on durum wheat remain relatively scarce, and different genetic systems governing the traits have been observed depending on the parental lines used. Kashif et al. (2003) reported the predominant role of complete dominance in the inheritance of spike length and epistasis, which complicates selection efficiency. For thousand kernel weight, they reported incomplete dominance and a lack of epistasis. Ivanovska et al. (2003) investigated plant height in F_1 and F_2 generations of a seven-parent durum wheat diallel cross. They found that overdominance played a general role in inheritance in F_1, while incomplete dominance was reported in F_2. Ismail et al. (2003) reported incomplete dominance in F_1 and F_2, with dominant genes predominant in the parents for plant height and spike length, while overdominance was observed for thousand kernel weight. Aydogan & Yagdi (2004) concluded that overdominance was the principal inheritance pattern for all examined traits in their study. Inamullah et al. (2006) suggested that the accumulation of recessive genes was responsible for achieving higher results for productivity tillering and spike length, while dominant genes led to increased thousand kernel weight. They reported that incomplete dominance was the primary inheritance pattern for thousand kernel weight, while overdominance was predominant for the other traits. Gami et al. (2010) proposed that plant height and spike length were enhanced by the accumulation of recessive genes. Nazeer et al. (2011) observed overdominance for spike length and grain weight per spike, whereas incomplete dominance played a primary role in the inheritance of all other traits. Zaazaa et al. (2012) and Ijaz & Kashif (2013) conducted diallel analyses on grain weight per spike and highlighted its direct correlation with yield levels. They reported that this trait was controlled by an additive-dominant genetic system with epistatic interactions, emphasizing the importance of epistasis in trait expression. Adel & Ali (2013) performed graphical diallel analysis and concluded that incomplete dominance played a crucial role in the inheritance of productivity tillering, whereas overdominance was observed for plant height. Fellahi et al. (2015) investigated productivity tillering and found that it was controlled by a simple additive-dominant genetic system. Kultu & Olgun (2015) confirmed that spike length and grain weight per spike were controlled by an additive-dominant genetic system with non-allelic interactions. Sadeghzadeh-Ahari et al. (2015) noted that the examined traits increased as a result of the accumulation of either dominant or recessive genes. Shehzad et al. (2015) reported that most of the studied traits exhibited overdominance in their inheritance. All these studies identified genotypes carrying dominant or recessive genes that are suitable for improving specific traits. Some of these genotypes are globally recognized varieties. These varieties can be effectively utilized to enhance individual traits in breeding programs, ultimately improving durum wheat productivity. This approach accelerates the breeding process and facilitates the achievement of desirable outcomes for plant breeders.

The aim of this study is to determine the genetic system controlling productivity-related traits in durum wheat. Specifically, it seeks to identify the types of genes responsible for enhancing yield-related traits and, most importantly, to determine which parental lines carry these genes.

MATERIALS AND METHODS

Five modern durum wheat varieties were included as parents in a diallel cross scheme. The Victoria variety was developed at the Field Crops Institute (FCI) Chirpan and was certified by the Patent Office in 2008. It is a medium-stemmed variety with good lodging resistance and high tillering capacity. Its vegetative period is similar to that of the Predel variety - it heads simultaneously and matures 1-2 days later. It has a more erect flag leaf. The spike is medium in length, dense, white, and pointed in profile, belonging to var. *Valenciae*. Awns are distributed along the entire spike length and are longer relative to the spike. The variety is characterized by yellow, semi-elongated grains, with a thousand kernel weight ranging from 44 to 46 g and vitreousness of 85-95%. The test weight is 79-80 kg/hl. It exhibits good rust resistance and moderate resistance to powdery mildew. The variety has a high yield potential exceeding 8500 kg/ha, good winter hardiness, and phenotypic stability across different years. The Deni variety was developed at Field Crops Institute - Chirpan using the pedigree selection method and was certified by the Patent Office in 2012. It has a high yield potential and belongs to the group of medium-early varieties. The stem reaches a height of 90-95 cm. It has a white, pubescent spike (var. *Valenciae*) with long, white, uniformly pointed awns. The grains are medium-sized, with a thousand kernel weight of 48-50 g. The test weight is high, ranging from 80 to 82 kg/hl. Based on biochemical and technological parameters, the variety is considered a high-quality accession. It has a high vitreousness, reaching up to 87% in certain years. It possesses good resistance to powdery mildew and moderate resistance to rusts. The variety exhibits high tillering capacity and good winter hardiness. It is suitable for producing high-quality pasta products and pearled durum wheat. The Superdur variety was developed in Austria by the breeding company *ProbsdorferSaatzucht* and was listed in the European variety catalog in 2000. It is a winter-type variety with high cold tolerance. It belongs to *var. Leucomelan (ALEF) KOERN.* According to the varietal description, the spike is white with dark awns, elongated, dense, and flat. The variety is medium-stemmed with good lodging resistance. It is characterized by amber-yellow grains with very high virtuousness, exceeding 86%. The grains are large, with a thousand kernel weight of 48–54 g, and a test weight of 78–80 kg/hl. The variety demonstrates high resistance to rusts and moderate resistance to powdery mildew. Its technological properties are superior, with grain protein content reaching up to 15% and wet gluten content exceeding 32%. The Progress variety was recognized as an original variety in 1990. It belongs to var. *Leucurum Al.*, characterized by white to pale-yellow, non-pubescent spikes and awns. The spike is cylindrical, slightly tapered at the top, and approximately 8.5 cm in length. The grains are very large, with a thousand kernel weight of 55-60 g and a test weight of 80-82 kg/hl. The variety exhibits good drought and winter hardiness. It shows moderate resistance to brown and black rust as well as powdery mildew. It has favorable values for protein content and wet gluten in the grain. It is classified as a medium-early variety in terms of the vegetative period. The productivity potential exceeds 7500 kg/ha. Due to its large and well-nourished grains, it is highly suitable for producing pearled durum wheat and other pasta products. The Predel variety was recognized in 2010 and is currently the national yield standard in Bulgaria. It is characterized by high and stable yields across different years, with a productivity potential exceeding 8500 kg/ha. The variety is medium-stemmed (85-95 cm) with very good lodging resistance. It belongs to the medium-early group. The spike is white, slightly pubescent, with white awns (var. *Valenciae*). It is of medium length and has a moderate spikelets density. The variety demonstrates good resistance to rusts and powdery mildew and exhibits enhanced drought tolerance. The grains are yellow, medium-sized (thousand kernel weight 44-47 g), and slightly elongated. The test weight reaches up to 82.4 kg/hl. Vitreousness varies across years, consistently exceeding 85%. Predel has a high carotenoid content, which ensures a stable color in pasta products. It carries a genetic marker for high gluten quality – gamma gliadin 45 allowing for high and stable pasta quality over the years. In terms of technological and biochemical properties, it matches or surpasses the standard variety in quality. The combination of high yield and excellent pasta-making qualities makes it one of the most valuable durum wheat varieties in Bulgaria.

The diallel crosses among the five varieties were performed without reciprocal crosses. Hand-pollination was conducted at the beginning of heading under field conditions, using 30 spikes per cross. A total of ten crosses were performed. The genotypes were arranged in a randomized block design with three replications in the breeding garden of Field Crops Institute - Chirpan. The parents and F_1 hybrids were sown in two rows, while F_2 hybrids were sown in five rows handmade in beds. The bed length was two meters, with an inter-row spacing of twenty cm and an intra-row spacing of five cm. A total of 20 plants were selected per parent, F_1 crosses and F_2 crosses at full maturity. The experiment was conducted in the breeding trial field of Field Crops Institute - Chirpan over three consecutive years (2014-2016). The recommended technology for durum wheat cultivation was applied. The preceding crop was field pea harvested for green fodder. Nitrogen fertilization was applied once in spring as a top-dressing at a rate of 100 kg active nitrogen substance per hectare. Weed control was performed with a single treatment using a combination of two herbicides. No fungicides were applied for disease and pest control. The three study years were characterized by higher temperatures compared to the long-term period. The first and second years had normal precipitation during the vegetative period, whereas the third year had 20% less rainfall than the long-term average. Six quantitative traits were analyzed: plant height, productivity tillering, spikelets number per spike, spike length, grains weight per spike and thousand kernel weight.

The graphical diallel analysis was conducted following the methods of Hayman (1954a; 1954b; 1957; 1958; 1960), Jinks (1954), Mather (1967), and Mather & Jinks (1971). The diallel analysis program by Aksel& Johnson (1962) was used. The diallel graphs were presented without eliminating parents that cause non-allelic interactions to obtain adequate genetic information. The genetic inheritance systems of quantitative traits were determined using a graphical regression analysis following Hayman (1954a) and Jinks (1954). The interpretation was based on the following assumptions: homozygous parents, diploid segregation, no difference in reciprocal crosses, independent action of non-allelic genes and independent gene distribution among parents. Homozygosis of the parents was ensured as durum wheat is a self-pollinating crop. Diploid segregation was assumed based on typical disomic inheritance in durum wheat. According to Hayman (1954), measuring the average degree of dominance on the graph confirms the absence of multiple allelism and the independent gene distribution among parents. The graphical representation and interpretation were performed using the Vr/Wr coordinate system and the regression line Wr = a ± bVr. In the absence of inter-locus interactions, variety points on the diagram should fall within a limiting parabola calculated as $Wr^2 = Vr.Vp$ and be distributed along the regression line (b = 1). If the regression coefficient (b) significantly differs from unity, the presence of non-allelic interactions (epistasis) is assumed. A coefficient significantly lower than unity indicates complementary epistasis by recessive gene, while a coefficient greater than unity indicates duplicate epistasis by dominant gene. The degree of dominance is certin by the crossing of regression line with the Wr ordinate. When cross above the origin it indicates incomplete dominance. When cross around the origin it indicates complete dominance. If regression line cross below the origin it indicates overdominance. The location of genotypes along the regression line indicates which genes, recessive or dominant genes are more in parents of diallel cross. Points located close to the origin indicate parents with more dominant genes, while points located closer to the intersection of the regression line with the limiting parabola indicate parents with more recessive genes. The correlation coefficient $r_{Yr(Wr+Vr)}$ shows whether dominant or recessive factors control trait expression. A positive r indicates that higher trait values are associated with recessive genes, while a negative value suggests that dominant genes contribute to higher trait values. Variation in quantitative traits contains variation due to genetic causes, which is inherited, and variation due to environmental conditions, which is not inherited.

RESULTS AND DISCUSSIONS

A major contribution to the development of a successful breeding program for durum wheat is the proper utilization of genetic diversity. Identifying high-potential parents and crossing them is essential for the recombination of genes in newly created genotypes. The evaluation of the breeding value of parents as starting material for improving specific traits particularly productivity is crucial for achieving the ultimate goal. Graphical analysis provides a comprehensive explanation of the behavior of dominant and recessive genes in the parents and indicates the contribution of individual parents to inheritance. This helps in selecting parents as donors for improving newly developed genotypes. This study found significant differences between genotypes (analysis of variance not shown), and based on this, the following results have been presented:

Plant height

Figure 1 shows the graphical analysis for two generations: F_1 and F_2. The regression line for both generations crosses above zero, indicating that inheritance is governed by incomplete dominance. Incomplete dominance is thus the underlying genetic basis of this trait. In both graphs (Figure 1), the regression line shows a decline that significantly differs from zero but not significantly from unity.

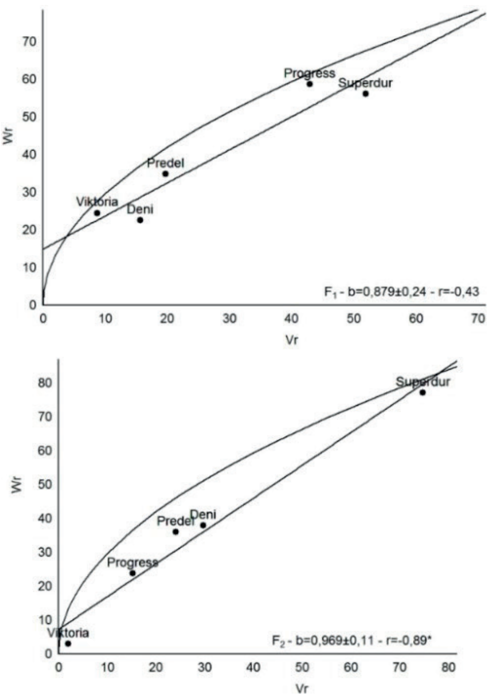

Figure 1. Graphical diallel analys for Plant height in F_1 and F_2; b – regression coefficient; $r_{Yr(Wr+Vr)}$- correlation coefficient; * - $p \leq 0.05$

Therefore, we can infer that plant height is controlled by a simple additive-dominant genetic system. Similar results have been reported by other researchers (Gami et al., 2010; Yao et al., 2011; Kutlu & Olgun, 2015; Shehzad et al., 2015). It is interesting to examine the relationship between the trait values in parents and their sum. The correlation coefficients (r) are negative in both generations, and in F_2, the correlation is statistically significant. This suggests that dominant genes are associated with increased plant height in durum wheat. In Figure 1, the configuration of the graphs in both generations shows that the Victoria variety is located near to the origin of the coordinate system. This indicates that Victoria likely contains more dominant genes. Conversely, the Superdur variety appears to possess the highest number of recessive genes, as shown consistently in both generations. Thus, we can assume that its short-stemmed nature is determined by the action of recessive genetic factors. It is worth highlighting the high breeding value of the Superdur variety. When used as a parent, dwarf plants appear as early as the F_2 generation and are likely to remain stable in subsequent segregating generations.

In the two generations, the other three varieties occupy an intermediate position and exhibit greater variation in the coordinate system across generations. This suggests that they contain an equal proportion of dominant and recessive genes.

Productivity tillering

The graphs for this trait are presented in Figure 2. In the figure, the regression line crosses below the starting point in F_1, indicating overdominance. In the F_2 generation, the regression line crosses the ordinate near the starting point, suggesting complete dominance. Overdominance in the expression of this trait was also reported by Shehzad et al. (2015). It is evident that the trait in durum wheat is determined by a genetic system involving complementary epistasis in both generations. This is demonstrated by the regression coefficient "b", which is significantly less than one, causing the regression line to bend downward to a circumstantial angle.

The correlation coefficient (r) is positive but not statistically significant. Therefore, the accumulation of recessive genes increases the values of the trait. This is consistent with the findings of Inamullah et al. (2006).

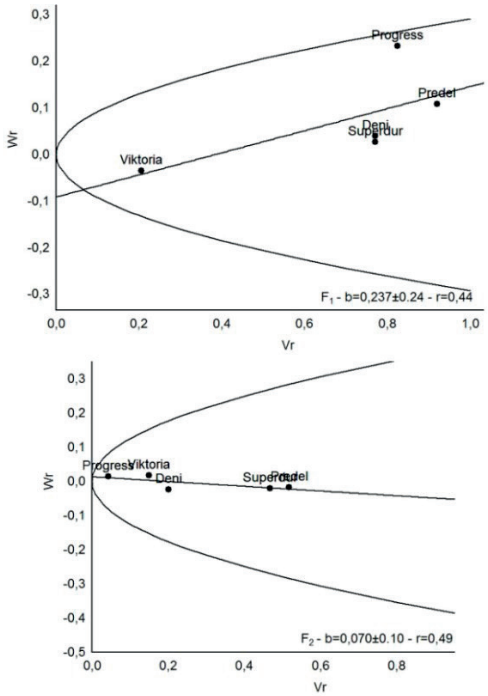

Figure 2. Graphical diallel analys for productivity tillering in F_1 and F_2; b – regression coefficient; $r_{Yr(Wr+Vr)}$- correlation coefficient

The parental points are significantly spaced apart and lie between the regression line and the parabola. This is attributed to the genotype-environment interaction in the expression of the trait. The positions of the parent points change across generations, which is explained by genotype - environment interaction. Based on the location of the parental points at the beginning and end of the crossing between the regression line and the parabola, it can be concluded which varieties have more dominant genes and which have more recessive genes. It is noteworthy that in both generations, the varieties are located near the origin of the coordinate system, indicating they have more dominant genes.

Spike length

The graphs for this trait are presented in Figure 3. In both generations (F_1 and F_2), the regression line intersects the origin of the coordinate system (Vr/Wr) above zero, indicating that incomplete dominance controls the inheritance of the trait. The regression lines in both generations suggest that inheritance is governed by a simple additive-dominant genetic system. This is consistent with the results obtained by other authors (Nazeer et al., 2011; Shehzad et al., 2015). The correlation coefficients (r) obtained for the F_1 generation (Figure 3) indicate that the increase in trait values is due to the accumulation of dominant genes. This correlation is significant in the F_1 generation and should be taken into consideration. However, in the second generation, the correlation coefficient is not significant and is negative. Therefore, the values of the trait increase with the accumulation of dominant genes. In the first generation, the Victoria variety contains more recessive genes, while Progress and Superdur varieties are located near the origin, indicating they have more dominant genes.

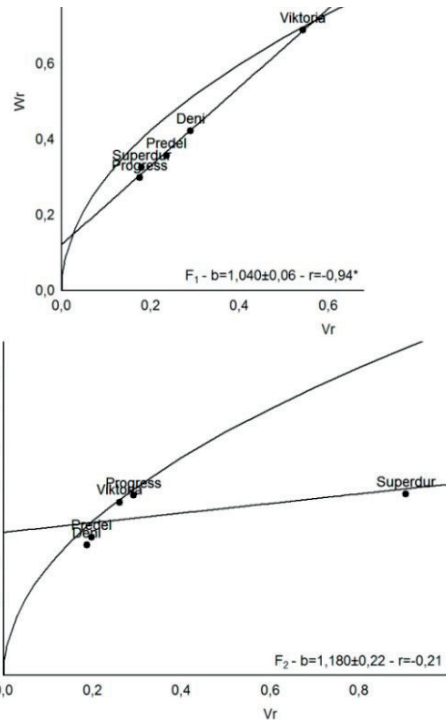

Figure 3. Graphical diallel analys for Spike length in F_1 and F_2; b – regression coefficient; $r_{Yr(Wr+Vr)}$- correlation coefficient; * - $p \leq 0.05$

The rest of the varieties are located in the middle, which indicates they are with equal dominant and recessive genes.

In the second generation, all varieties are located near to the beginning and have low values of (Wr + Vr), since the parabola remains open on the chart at the end. Therefore they have more dominant genes.

Spikelets number per spike

Graphical diallel analysis is presented in Figure 4. In generation F_1, the regression line intersects the coordinate system below the starting point, which means that overdominance is of essential importance. In the second generation, complete dominance have major role. The results are also in sync with other authors (Nazeer et al., 2011; Fellahi et al., 2015; Shehzad et al., 2015). The slope of the regression line indicates that in inheritance of this trait presence complementary epistasis.

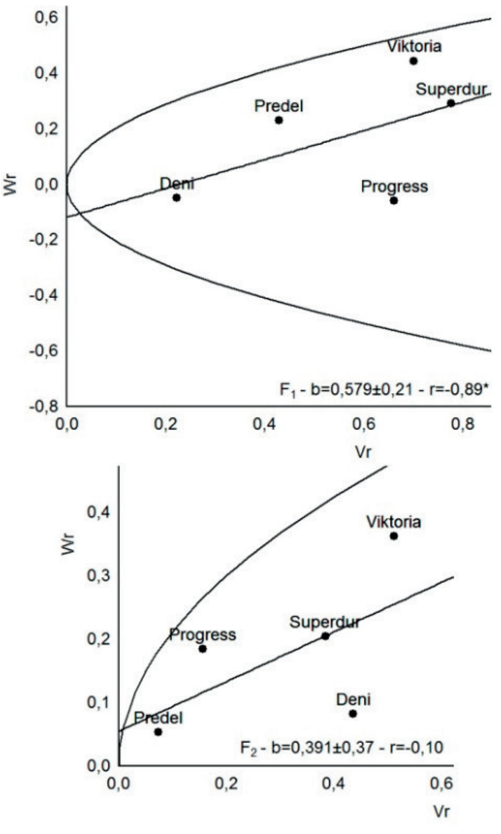

Figure 4. Graphical diallel analys for Spikelets number per spike in F_1 and F_2; b – regression coefficient; $r_{Yr(Wr+Vr)}$- correlation coefficient; * - $p \leq 0.05$

The correlation coefficient r shows dominant genes increasing values of the trait (Figure 4). In the first generation correlation coefficient is significant. Gami et al. (2010) reported the same statement for this trait and cunclude dominance genes are important for this trait. In second generation correlation coefficient is again negative and unproven. From the graphs for the F_1 generation, Deny variety have more dominant genes. In second generation with the most dominant genes is Predel variety. The dispersion (distance) of the points from the regression line is also impressive, which shows the great influence of the environment on the expression of the trait.

Grains weight per Spike

The graphs of graphical analysis of both generations is presented in Figure 5. The regression line intersects Wr below and show overdominance. Other authors also report that overdominance is involved in the inheritance of the trait (Vanda & Houshmand, 2011; Nazeer et al., 2011; Shehzad et al., 2015). In Figure 5, the regression coefficients b in both generations does not differ from zero and differs from unity indicates the participation epistasis of complementary type. Epistasis for this trait was reported before from other authors (Zaazaa et al., 2012; Ijaz & Kashif, 2013; Kutlu & Olgun, 2015). Dominant genes are responsible for increasing the trait. Correlation coefficients are significant and this statement remains valid. In the first generation, all varieties are near to the Wr and they have more dominant genes. The most dominant genes have varieties Victoria and Progress. In second generation in Predel and Progress dominant genes prevails when in Superdur prevails recessive genes. The Victoria and Deni varieties are with equal dominant and recessive genes. Progress variety have more dominant genes and that makes it extremely important.

Thousand kernel weight

The regression line intersects the ordinate of the coordinate system above the starting point in both generations, indicating that incomplete dominance plays a important role in the inheritance of the trait (Figure 6). Incomplete dominance in the inheritance of the trait have also been reported by other authors (Inamullah et al., 2006; Fellahi et al., 2015).

The values of the regression coefficients in both generations differed significantly from zero and did not differ significantly from unity (Figure 6).

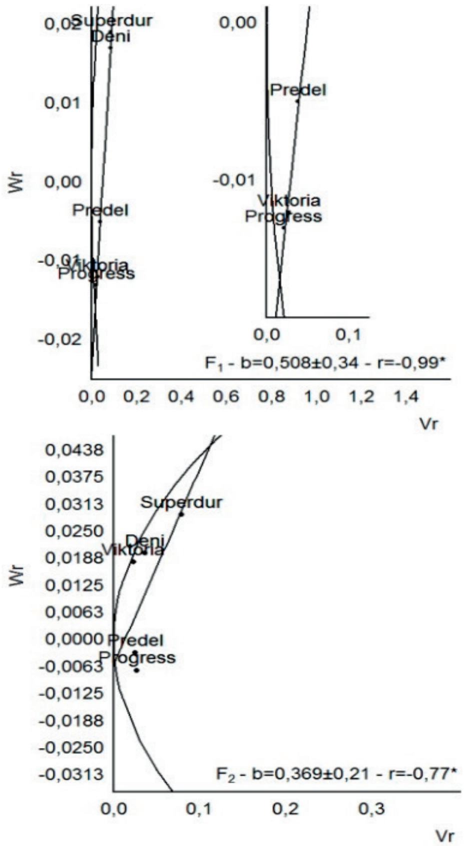

Figure 5. Graphical diallel analys for grains weight per spike in F_1 and F_2; b – regression coefficient; $r_{Yr(Wr+Vr)}$ - correlation coefficient; * - $p \leq 0.05$

Therefore, in both generations F_1 and F_2 in our set of parental varieties, control in inheritance of the trait has a simple additive-dominant genetic system. Shehzad et al. (2015) reported the same statement like in our case. The correlation coefficient r in the both generation is negative and the trait are increase with accumulation of dominant genes. In the case of our study, the correlation coefficients are significant and it can be relied on that this trait is increased by the dominant genes. In generation F_1, the Victoria and Progess varieties has greatest number of dominant genes. Other varieties are with equal dominant and recessive genes. In the F_2 generation with the most dominant genes is the Progress variety.

In generations F_1 and $F_2$the traits plant height, spike length and thousand kernel weight are under control of additive-dominant genetic system.

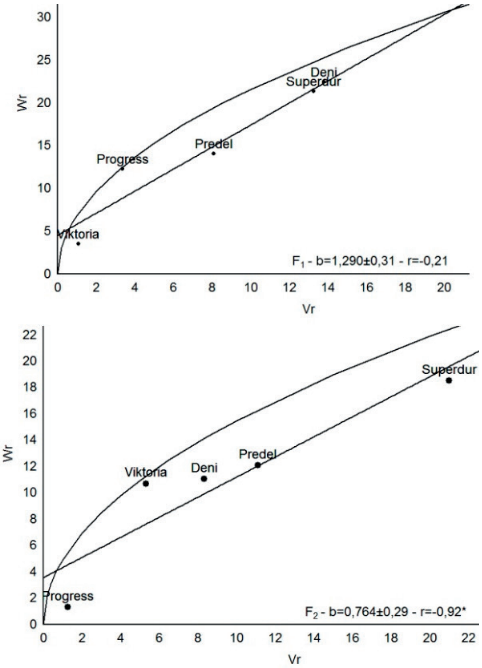

Figure 6. Graphical diallel analys for thousand kernel weight in F_1 and F_2; b – regression coefficient; $r_{Yr(Wr+Vr)}$ - correlation coefficient; * - $p \leq 0.05$

When this genetic system participates in inheritance, individual selection is very effective and improvement of traits can be expected. For other traits in both generations is observed epistatic interactions of genes from complementary type. Manifestations of complementary epistasis affect disintegration, and selection can continue into later segregating generations. It is possible to select different valuable forms in different years in the same cross. The results obtained indicate that the most suitable for individual selection using classical methods are the traits like plant height, spike length and thousand kernel weight. It should be noted that the main structural element of the yield - thousand kernel weight - has no manifestations of epistasis. For a significant increase of the traits, it is of great importance which genes accumulate. The correlation coefficients in some cases are significant and this information is essential. Despite the unproven values for correlation coefficient the traits related to the productivityin most cases are increase with dominant genes. In most cases these are the varieties Progress and Victoria. The Progress variety is extremely valuable in terms of breeding, as there are more dominant genes for the traits like grains weight per spike and thousand kernel weight. For productivity tillering which recessive genes are important for increasing the values of the trait, may have better results in the later segregating generations, which allows for a longer selection.

CONCLUSIONS

The trait plant height in durum wheat is controlled by a simple additive-dominant genetic system. Dwarf plants can be obtained with accumulation of recessive genes. Incomplete dominance plays an important role in the expression of this trait. The trait productivity tillering is controlled by complementary epistasis and recessive genes increase the values of this trait. Both complete dominance and overdominance are involved in its expression. The trait spike length is controlled by a simple additive-dominant genetic system. Dominant genes are responsible for its increase, and incomplete dominance plays a major role in its expression. The trait spikelets number per spike is controlled by complementary epistasis. The accumulation of dominant genes increases this trait. Both incomplete dominance and overdominance are essential for its expression. The inheritance of the trait grain weight per spike is governed by complementary epistasis. Trait values increase with the accumulation of dominant genes, and overdominance plays a major role. The trait thousand kernel weight in durum wheat is controlled by a simple additive-dominant genetic system. Dominant genes are associated with increased trait values, and incomplete dominance plays an essential role in its inheritance. The Progress variety is extremely valuable in breeding due to its higher proportion of dominant genes for both grain weight per spike and thousand kernel weight. Since dominant genes are responsible for increasing two of the main elements of productivity, this variety is of significant importance.

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PECULIARITIES OF GROWTH AND DEVELOPMENT OF LEGUMINOUS FODDER GRASSES IN THE SOUTH OF UKRAINE

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Abstract

In recent decades, the intensity of chemical use in agriculture has increased significantly, which threatens food security, contributes to the accumulation of toxic chemicals in the environment, leading to the destruction of beneficial organisms (entomophages) and soil microflora, and thus disrupting the balance of the ecosystem. Agroecology and organic farming have become priority topics integrated into the scientific and technological approach to sustainable agriculture. The inclusion of legumes in crop rotation can contribute to the restoration of soil fertility and phyto-improvement of saline soils in the southern region of Ukraine, desalination being possible due to natural drainage and carbonic acid root secretion. In addition, the root system of these plant species enriches the soil with nitrogen and improves its structure. This article presents the results obtained in a field experiment with 3 species of perennial and biennial forage legumes, under different technological conditions with the aim of: i) evaluating the phenological evolution and biological characteristics of the growth and development of the studied plants; ii) investigating the impact of their cultivation on the ecological state of the soil and iii) determining the optimal parameters regarding the production obtained under different cultivation conditions.

***Key words**: fodder leguminous grasses, soil fertility restoration, agroecology, crop rotation.*

INTRODUCTION

Scientists have found that the increased use of arable land over the last century has led to a total decrease in soil fertility on the planet. (Nyfeler, 2009; Clayton et al., 2004; Meyer, 1984; Armin, 2011; Parrish, 2005; Özköse & 2016). This problem is extremely relevant for Ukraine. Since plants and microorganisms are the mandatory and most active participants in soil formation processes, the issue of soil fertility formation should quite rightly be considered as largely biological. However, the biological state of many soils in the country today should be recognized as degraded. Dehumification processes in the soils are activated due to the absence of organic matter and unbalanced use of mineral fertilizers, ignoring crop rotation, minimizing the areas under legumes crops, and burning straw (Antonova et al., 2019; Gibson et al., 2006).

The composition of soil biocenoses is significantly impoverished as there is a reduction to a minimum and even a loss of certain species, some of them being beneficial organisms. Besides, many agrocenoses have turned into reservoirs of pathogens (Vozhehova et al., 2020; Vlashchuk et al., 2015). The magnitude of such phenomena causes serious concerns and there is an urgent need for improvement measures, adopted at the state level, in order to optimize the state of agrocenoses in general and soil-forming processes in particular (Rajput et al., 2008; Tristram, 2013).

Among the biological measures for the conservation and increase of soil fertility, the cultivation of plant species (e.g. fodder legumes) that are capable of having beneficial effects on the soil at the physical, chemical and biological levels is one of the most effective measures from an agro-ecological and economic point of view (for example, it is 5-10 times cheaper than chemical improvement). In addition, in today's conditions, it is also possible to solve the problem of vegetal protein deficiency by increasing the sown area, expanding the range and increasing the yield of perennial and annual fodder legumes. This also contributes to the natural restoration of soil fertility due to the plants' nitrogen-fixing ability, which is especially relevant today because of the increase in the mineral and organic fertilizers cost (Vozhehova et al., 2020; Rigal M. et al., 2016; Demydas' G. I. et al., 2017; Harker et al., 2012; Babich et al., 2000).

In general, legumes are some of the best preceding plants in crop rotation and also, they are effective soil improvers. They enrich the soil with nitrogen and improve its structure, which contributes to the growth of agricultural crops. Legume fodder grasses act also as improvers for saline soils that is the case of the southern region of Ukraine not only due to drainage, but also due to the root release of carbonic acid, which triggers the chemical process of desalination. So, these crops are able to provide soil reclamation (Wolf, 2004; Vozhehova et al., 2020; Zinchenko et al., 2003).

The South of Ukraine belongs to the risky agriculture zone, therefore, the cultivation of drought-resistant agricultural crops, capable of forming stable yields of high-quality seeds in extreme conditions, is of exceptional importance in the region (Bezugliy et al., 2012; Tsandur, 2006; Zinchenko et al., 2003; Denisow B., 2016).

Therefore, our project envisages conducting research to establish the characteristics of growth processes and productivity of perennial forage legumes, as well as studying their impact on the ecological state of the soil in the southern steppe zone of Ukraine. The research is relevant because it addresses the issues of improving agrocenoses in organic farming rotations through the use of environmentally safe perennial forage legumes, which will contribute to improving and restoring the ecological state of the soil and increasing the yield of agricultural crops within the rotation.

The aim of the research was to determine the optimal parameters that would ensure the best phenological indicators of plants and maximum productivity of perennial and biennial forage legume seeds under irrigated and non-irrigated conditions, as well as to investigate the impact of cultivating these species on the ecological status and soil restoration.

MATERIALS AND METHODS

The influence of the main tillage methods and irrigation on the peculiarities of growth processes and the formation of fodder legumes grasses seed productivity and the influence of their cultivation on the content of exchangeable cations in the soil were studied. The experimental part was carried out in the conditions of the experimental field of the Institute of Climate Smart Agriculture of the National Academy of Agrarian Sciences (NAAS), located in the south of Ukraine during 2022-2024.

The climate of the research area is continental with insufficient precipitation and its extremely uneven distribution throughout the year, low relative humidity, warm autumn and winter, and a long frost-free period. The region is also characterized by the greatest aridity and the greatest thermal resources (Figure 1).

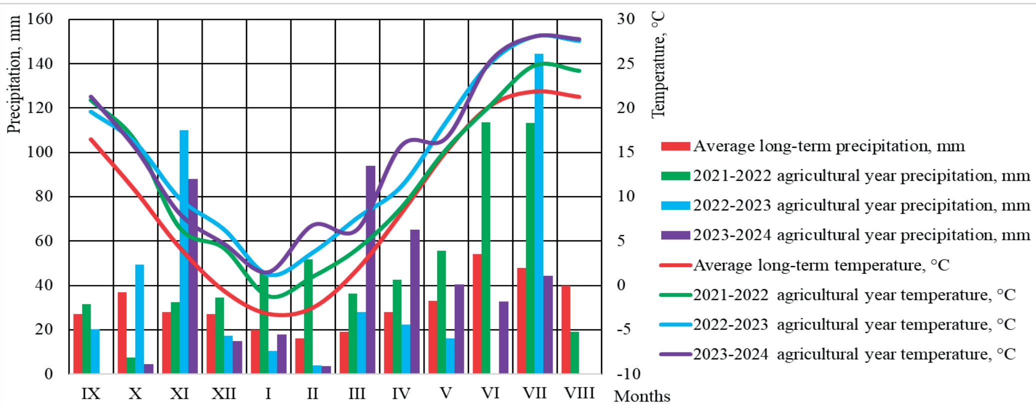

Figure. 1. Monitoring of weather conditions over the research years

Almost every year there are periods with strong winds, dust storms, and dry winds, which cause great damage for agriculture (Babich, A.O. et al., 2000; Tsandur, 2006). Thus, the soil and climatic conditions of southern of Ukraine are favorable for the formation of a high and stable harvest of fodder leguminous grasses seeds, but due to insufficient rainfall and to high temperatures, the potential of crops is not always able to be realized. In this regard, it is necessary to constantly improve the elements of cultivation technology.

The experiment was set up in randomized plots with using the split-plot method, with four replications. The experiment followed the single logical difference principle and the range of factors' gradations, which allows determining the optimal parameters of the action of each factor. The research was carried out sequentially, and the obtained data were systematized. The experiment was a three-factor experiment: Factor A was the presence of irrigation (without irrigation; irrigation), Factor B was the method of main tillage (plowing to a depth of 27-30 cm; chiseling to a depth of 22-44 cm; disking to a depth of 15-17 cm), Factor C was the fodder leguminous grasses (*Medicago sativa* L.; *Onobrychis viciifolia* Scop.; *Melilotus albus* Medik.). The generally accepted field experiment methods and methodological recommendations were used (Vozhehova et al., 2014; Ushkarenko et al., 2014).

The results of the harvest accounting were processed by methods of variance, correlation and regression analyses using the software and information complex "AGROSTAT" (Software Product Developer Institute of climate smart agriculture of the National Academy of Agrarian Sciences of Ukraine).

RESULTS AND DISCUSSIONS

Plant height

The influence of the studied factors on the dynamics of the height of leguminous forage crops was established. The results of observations indicate that the growth rates of plants in height are directly dependent on the growing conditions (Table 1).

The growth and development of plants is influenced by a significant number of factors, the main of which in the arid conditions of the Southern Steppe of Ukraine are the availability of irrigation and the main tillage method.

Table 1. Dynamics of plant height of fodder leguminous grasses depending on the studied factors, cm (average for 2022-2024)

Factor A, irrigation	Factor B, main tillage method	Factor C, fodder leguminous grasses	Phenological phases			Average by factor B		
			branching	budding	flowering	branching	budding	flowering
Without irrigation	plowing (27-30 cm)	*Medicago sativa* L.	18,1	45,6	58,7	17,9	55,2	81,7
		Onobrychis viciifolia Scop.	18,0	41,3	56,4			
		Melilotus albus Medik.	17,2	69,2	110,5			
	chiseling (22-24 cm)	*Medicago sativa* L.	17,9	48,5	56,6	17,8	54,8	79,1
		Onobrychis viciifolia Scop.	17,7	40,1	55,8			
		Melilotus albus Medik.	17,0	68,4	105,8			
	disking (15-17 cm)	*Medicago sativa* L.	17,6	40,0	54,7	17,5	51,7	75,9
		Onobrychis viciifolia Scop.	17,4	39,8	53,9			
		Melilotus albus Medik.	16,8	64,3	98,5			
Average by factor A			17,5	50,8	72,3	Average by factor C		
Irrigation	plowing (27-30 cm)	*Medicago sativa* L.	18,7	50,7	69,4	18,1	47,4	61,9
		Onobrychis viciifolia Scop.	18,4	49,5	66,8	18,0	44,5	60,5
		Melilotus albus Medik.	17,3	74,8	128,2	17,1	69,8	114,2
	chiseling (22-24 cm)	*Medicago sativa* L.	18,5	50,1	67,3			
		Onobrychis viciifolia Scop.	18,7	49,0	65,9			
		Melilotus albus Medik.	17,2	72,6	123,1			
	disking (15-17 cm)	*Medicago sativa* L.	18,0	49,2	64,9			
		Onobrychis viciifolia Scop.	17,9	47,4	64,0			
		Melilotus albus Medik.	17,0	69,5	119,2			
Average by factor A			17,9	56,9	85,4			
Assessment of the materiality of partial differences		LSD_{05}, cm	A=0,16 B=0,10 C=3,26	A=0,11 B=0,14 C=3,26	A=0,20 B=0,13 C=3,26			
Assessment of the significance of average (main) effects		LSD_{05}, cm	A=0,05 B=0,04 C=0,05	A=0,04 B=0,06 C=0,05	A=0,07 B=0,05 C=0,04			

Over the years of research, extreme weather conditions with rather high values of average daily temperatures and lack of precipitation did not allow fodder leguminous grasses plants to fully realize their biological potential, which usually affected the height of the plants.

According to Factor B (the main tillage method), in non-irrigated conditions, the best results were shown by plowing to a depth of 27-30 cm. In this variant, plants of *Medicago sativa* L. in the flowering phase had a height of 58.7 cm, which was per 3.65% higher than by chiseling to a depth of 22-44 cm and was per 6.81% higher than by disking to a depth of 15-17 cm. The plants of *Onobrychis viciifolia* Scop. in similar conditions had a height of 56.4 cm, which was per 1.07% higher than by chiseling to a depth of 22-44 cm and 4.46% higher than by disking to a depth of 15-17 cm. Plants of *Melilotus albus* Medik. in such conditions reached a height of 110.5 cm, which was per 4.25% higher than by chiseling to a depth of 22-44 cm and was per 10.86% higher than by disking to a depth of 15-17 cm.

Irrigation (Factor A) had a positive effect on the growth and development of fodder leguminous grasses' plants and contributed to an increase in the height of these plants' species by an average of 16.58-18.5%, depending on the crop and the main tillage method. At the same time, the trend in the response of plants to the influence of the main tillage system was maintained. Thus, the best results in irrigated conditions were shown by the use of plowing to a depth of 27-30 cm. In these conditions, the plants of *Medicago sativa* L. in the flowering phase had a height of 69.4 cm, which was per 3.02% higher than by chiseling to a depth of 22-44 cm and was per 6.48% higher than by disking to a depth of 15-17 cm. The plants of *Onobrychis viciifolia* Scop. in the flowering phase iv similar conditions reached a height of 66.8 cm, which was per 1.35% higher than by chiseling to a depth of 22-44 cm and was per 4.19% higher than by disking to a depth of 15-17 cm. The height of *Melilotus albus* Medik. plants in these conditions reached of 128.2 cm, which exceeded per 3.98% the variant with chiseling to a depth of 22-44 cm and exceeded per 8.97% the variant with disking to a depth of 15-17 cm. On average, the use of irrigation allowed to increase the height of plants of *Medicago sativa* L. per 18.23-18.91%, the height of plants of *Onobrychis viciifolia* Scop. per 18.10-18.74% and the height of *Melilotus albus* Medik. plants per 16.02-17.37%, depending on the main tillage method.

Seed productivity

Depending on the main tillage method in irrigated and non-irrigated conditions, fodder leguminous grasses' plants formed different seed productivity (Table 2).

During the research period, the maximum seed productivity (0.89 $t \cdot ha^{-1}$) was obtained by sowing of *Onobrychis viciifolia* Scop. and using plowing to a depth of 27-30 cm in irrigation conditions.

When combining these elements of agricultural technology (plowing and irrigation), *Medicago sativa* L. and *Melilotus albus* Medik. also formed the highest seed productivity (0.46 and 0.74 $t \cdot ha^{-1}$, respectively).

In general, irrigation (Factor A) contributed to an increase in the average seed productivity of fodder leguminous grasses to 0.63 $t \cdot ha^{-1}$. The values of the same indicator in the variants without irrigation were lower by 0.15 $t \cdot ha^{-1}$, or per 23.78% (LSD_{05} A - 0.014 $t \cdot ha^{-1}$, B - 0.017 $t \cdot ha^{-1}$, C - 0.033 $t \cdot ha^{-1}$).

The method of main tillage (Factor B) also affected the formation of the average seed productivity index of fodder leguminous grasses. For plowing to a depth of 27-30 cm it was obtained the highest average seed productivity (0.61 $t \cdot ha^{-1}$). With other methods of main tillage (chiseling to a depth of 22-44 cm and disking to a depth of 15-17 cm), the values of this indicator were lower.

The seed productivity of various fodder leguminous grasses depends more on their biological potential. According to the Factor C (fodder leguminous grasses), the average maximum seed productivity (0.74 $t \cdot ha^{-1}$) was obtained by sowing *Onobrychis viciifolia* Scop.

As a result of variance analysis, the share of influence of factors on the growth, development and formation of seed productivity of fodder leguminous grasses was determined (Figure 2).

All factors and their interaction were significant in this experiment. It was established that the studied factors to the greatest extent (72.4%) depended on the biological characteristics of the fodder leguminous grass (Factor C).

Table 2. Seed productivity of fodder leguminous grasses depending on the studied factors, t·ha^{-1} (average for 2022-2024)

Factor A, irrigation	Factor B, main tillage method	Factor C, fodder leguminous grasses	Average seed productivity	Average by factor		
				A	B	C
Without irrigation	plowing (27-30 cm)	*Medicago sativa* L.	0,46	0,48	0,61	0,34
		Onobrychis viciifolia Scop.	0,89			0,74
		Melilotus albus Medik.	0,74			0,59
	chiseling (22-24 cm)	*Medicago sativa* L.	0,39		0,55	
		Onobrychis viciifolia Scop.	0,82			
		Melilotus albus Medik.	0,67			
	disking (15-17 cm)	*Medicago sativa* L.	0,34		0,51	
		Onobrychis viciifolia Scop.	0,78			
		Melilotus albus Medik.	0,59			
Irrigation	plowing (27-30 cm)	*Medicago sativa* L.	0,31	0,63		
		Onobrychis viciifolia Scop.	0,69			
		Melilotus albus Medik.	0,58			
	chiseling (22-24 cm)	*Medicago sativa* L.	0,27			
		Onobrychis viciifolia Scop.	0,64			
		Melilotus albus Medik.	0,51			
	disking (15-17 cm)	*Medicago sativa* L.	0,25			
		Onobrychis viciifolia Scop.	0,61			
		Melilotus albus Medik.	0,47			
Assessment of the materiality of partial differences						
LSD_{05}, t·ha^{-1}		A =	0,014			
		B =	0,017			
		C =	0,033			
Assessment of the significance of average (main) effects						
LSD_{05}, t·ha^{-1}		A =	0,050			
		B =	0,060			
		C =	0,050			

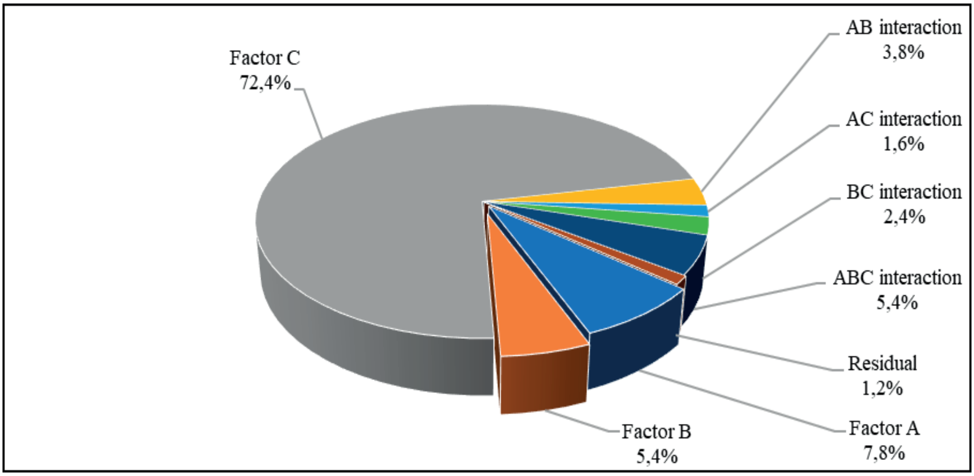

Figure 2. The share of the influence of factors and their interaction on the formation of the height of fodder leguminous grasses' plants and their seed productivity (average for 2022-2024); Factor A - irrigation; Factor B - main tillage method; Factor C - fodder leguminous grass

The presence of irrigation (Factor A) provided a share of influence of 7.8%. The method of main tillage (Factor B) provided a share of influence on the studied plant indicators of 5.4%.

Correlation between the height and seed productivity

It has been established that there is a certain correlation between the height of fodder leguminous grass plants and seed productivity (Figure 3).

For all studied fodder leguminous grasses, the correlation coefficients (r) between seed productivity and plant height in all variants of the experiment have a positive value and are within 0.907-0.993.

This indicates a strong positive correlation between these indicators.

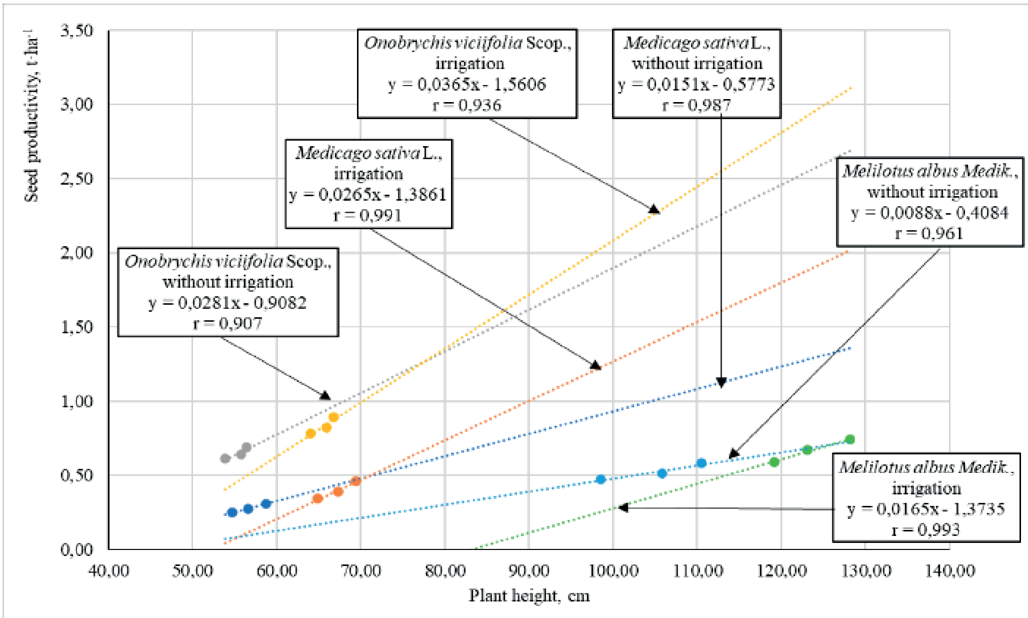

Figure 3. Correlation-regression models of the dependence of fodder leguminous grasses' seed productivity on plant height (average for 2022-2024)

At the same time, higher values of correlation coefficients (r) for the studied crops were established in variants where irrigation was used. The highest value of the correlation coefficient (r=0.993) was established for *Melilotus albus* Medik., during the cultivation of which irrigation was used. The lowest value of correlation coefficient (r=0.906) was established for *Onobrychis viciifolia* Scop. in non-irrigated conditions, which indicates the possibility of increasing the seed productivity of the studied crops by implementing optimal agrotechnical solutions that will contribute to increasing the height of these plants. The obtained regression equations have a linear relationship, which indicates that with an increase in the height of the plants, their seed productivity will increase within the biological potential of the crops. This confirms the possibility of increasing the seed productivity of these crops by choosing an appropriate main tillage system and introducing irrigation.

A feature of fodder leguminous grasses is that root secretions in a certain way affect the content of exchangeable cations in the soil. In the study, the dynamics of the content of exchangeable cations in the soil were studied during the growing of *Medicago sativa* L., *Onobrychis viciifolia* Scop., *Melilotus albus* Medik.

Changes in the content of exchangeable cations in the soil

Table 3 shows the content of exchangeable cations in the soil layer of 0.40 cm before sowing fodder leguminous grasses.

According to the experimental variants before sowing, no significant fluctuations in the content of exchangeable cations in the soil were found, therefore the average values of the indicators are given.

At the end of the growing season of crops, the content of exchangeable cations underwent changes in all experimental variants depending on the studied factors (Table 4).

It was found that in irrigation conditions, the total content of exchangeable cations was slightly higher (17.95-18.23 meq·100 g^{-1}) than without irrigation (17.86-18.08 meq·100 g^{-1}).

Table 3. Content of exchangeable cations in the soil before sowing of fodder leguminous grasses, meq·100 g^{-1} (average for 2022-2024)

Content of exchangeable cations			TCEC*)	Percentage of the cations sum, %		
Na^+	Mg^{+2}	Ca^{+2}	19,1	Na^+	Mg^{+2}	Ca^{+2}
0,56	5,6	12,7		3,9	30,2	65,9

*) TCEC: Total content of exchangeable cations

This trend indicates a slight increase in the content of exchangeable cations during irrigation due to irrigation water. But this increase is insignificant, within 0.25 meq·100 g^{-1}, which gives grounds to conclude that the use of fodder leguminous grasses helps to control the number of exchangeable cations that cause increased soil salinization.

Table 4. Content of exchangeable cation in soil before harvesting of fodder leguminous grasses, meq·100 g^{-1} (average for 2022-2024)

Factor A, irrigation	Factor B, main tillage method	Factor C, fodder leguminous grasses	Content of exchangeable cations			TCEC
			Na^+	Mg^{+2}	Ca^{+2}	
Without irrigation	plowing (27-30 cm)	*Medicago sativa* L.	0,56	5,4	11,6	18,04
		Onobrychis viciifolia Scop.	0,58	5,5	11,7	18,08
		Melilotus albus Medik.	0,55	5,3	11,5	18,01
	chiseling (22-24 cm)	*Medicago sativa* L.	0,55	5,2	11,7	17,94
		Onobrychis viciifolia Scop.	0,57	5,4	11,8	18,00
		Melilotus albus Medik.	0,53	5,2	11,7	17,93
	disking (15-17 cm)	*Medicago sativa* L.	0,53	5,2	11,9	17,87
		Onobrychis viciifolia Scop.	0,55	5,3	11,9	17,91
		Melilotus albus Medik.	0,52	5,1	11,8	17,86
Irrigation	plowing (27-30 cm)	*Medicago sativa* L.	0,60	5,7	12,0	18,14
		Onobrychis viciifolia Scop.	0,63	5,8	12,1	18,23
		Melilotus albus Medik.	0,59	5,6	12,0	18,09
	chiseling (22-24 cm)	*Medicago sativa* L.	0,57	5,6	12,0	18,10
		Onobrychis viciifolia Scop.	0,60	5,7	12,0	18,19
		Melilotus albus Medik.	0,57	5,6	12,0	18,04
	disking (15-17 cm)	*Medicago sativa* L.	0,56	5,4	12,0	18,03
		Onobrychis viciifolia Scop.	0,58	5,5	12,0	18,09
		Melilotus albus Medik.	0,53	5,3	12,0	17,95

CONCLUSIONS

Fodder leguminous grasses, such as *Medicago sativa* L., *Onobrychis viciifolia* Scop., *Melilotus albus* Medik., due to their biological characteristics can be successfully introduced into crop rotations in arid conditions of Southern Step of Ukraine. The influence of the main tillage methods on the growth and development and formation of seed productivity of these crops in irrigated and non-irrigated conditions, and influence of fodder leguminous grasses on the change in the ecological and reclamation state of chestnut soils, namely content of exchangeable cations, has been established.

It was found that according to Factor B (main tillage method), plants grew and developed best by plowing to a depth of 27-30 cm. On average, this main tillage method provided an increase in the height of plants in the experiment per 1.07-10.86%. At the same time, this main tillage method contributed most to the growth of *Melilotus albus* Medik. plants. The best response to irrigation (Factor A) was shown by *Medicago sativa* L., increasing the height of plants an average per 18.5%. *Onobrychis viciifolia* Scop. increased the height of plants an average per 18.43% and *Melilotus albus* Medik. increased the height of plants an average per 16.58%.

Studies have shown that the fodder leguminous grasses' seed productivity depended on the use of irrigation, main tillage method, and plant species. It was found that the use of optimal values of all studied factors contributed to obtaining maximum seed productivity of fodder leguminous grasses.

By Factor A (irrigation), the highest average seed productivity of fodder leguminous grasses (0.63 t·ha^{-1}) was obtained by using irrigation. By Factor B (main tillage method), the highest average seed productivity (0.61 t·ha^{-1}) was obtained by plowing (factor B). By Factor C (fodder leguminous grasses), the maximum seed productivity (0.74 t·ha^{-1}) was obtained by sowing *Onobrychis viciifolia* Scop.

The use of irrigation contributed to an increase in the fodder leguminous grasses' seed productivity by an average per 23.78%. The most sensitive to irrigation was *Medicago sativa* L., which showed the greatest increase in seed productivity per 32.61%. The least sensitive to irrigation was *Melilotus albus* Medik. with a maximum seed productivity increase per 21.62%. *Onobrychis viciifolia* Scop. showed intermediate results with a maximum seed productivity increase per 22.47%.

Observations of the dynamics of exchangeable cations in the soil indicate that growing of fodder leguminous grasses helps to control the number of cations that cause increased soil salinization.

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STUDY REGARDING THE MAIZE PRODUCTIVITY IN RESPONSE TO SHORT-TERM APPLICATION OF ORGANIC AND MINERAL FERTILIZERS IN A SOUTHEASTERN AREA OF ROMANIA

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Abstract

Maize (Zea mays L.) is the most widely produced food crop globally, providing essential nutrition for both humans and livestock, while also being an important raw material for industrial processes. However, factors such as climatic conditions and fertilization practices significantly affect maize yields. To address these challenges, this study explores the impact of short-term application of organic and mineral fertilizers on maize grain yield and its components. The field trial was conducted at the Moara Domnească Research and Development Station for Agronomy (RDSA) belonging to USAMV Bucharest, Romania, on a preluvosoil. Manure compost was applied in three doses (15 t/ha, 30 t/ha, and 60 t/ha), either alone or in combination with NPK complex fertilizers (20:20:0). Yield components and total production were evaluated prior to harvest, with results showing significant differences between treatments compared to the control (soil).

Key words*: maize yield, yield components, mineral fertilization, organic fertilization, sustainable production.*

INTRODUCTION

Maize (*Zea mays* L.) is essential for improving global food security and serves as a fundamental component of agricultural production in Romania, particularly in the southeastern regions, where climatic and soil conditions present both opportunities and challenges for crop growth (Prăvălie et al., 2020). Furthermore, maize is a significant source of macro-, micro- and phytonutrients including carbohydrates, fibres, protein, vitamin, minerals, phenolic acids and others, which makes it a suitable staple food (Gogoi et al., 2023).

Maize productivity can be influenced by the environmental conditions, as well as the technological factors. The water supply is an ecological factor that can influence the morphological, physiological, and biochemical processes, ultimately impacting the growth, development, and productivity of maize plants (Bășa et al., 2016). Despite the cultivation of high-performance hybrids, drought conditions during the maize growth stages that require maximum water input significantly impact yield components (Dumbravă et al., 2017).

Another key factor for crop growth is improving technologies to increase the production potential of maize hybrids. Thus, fertilization practices play a vital role in soil-based agricultural systems by improving soil fertility and quality, ultimately leading to increased crop yields (Kim et al., 2022). For instance, nitrogen is a decisive nutrient in agricultural ecosystems and an essential element for plant growth, playing a key role in various physiological and metabolic processes within plants and being the primary yield-limiting factor in crops (Lin et al., 2022; Ramesh Naik et al., 2022). Inefficient nitrogen utilization in maize cultivation is largely attributed to the excessive application of nitrogenous fertilizers by farmers, often without accounting for the nitrogen requirements of crops and growth stages. This mismanagement can result in nitrogen loss from the soil-plant system and low nitrogen use efficiency (Ramesh Naik et al., 2022). Inadequate fertilization practices have also been associated with nitrate leaching into aquatic ecosystems, causing considerable ecological impacts downstream of their source, increased production costs, and a decline in grain quality (Fang et al., 2013; Mahmood et al., 2017; Kong et al., 2022).

Applying organic amendments, such as cattle manure, is a viable alternative to the adverse impacts of inorganic fertilizers due to its widespread availability, its added benefits for soil carbon sequestration, and its ability to store and release nutrients over an extended period (Jjagwe et al., 2020). However, the slow-release rate of nutrients can pose problems in terms of meeting the timely nutritional requirements of plants (Zhai et al., 2023). In this context, the combined application of organic and mineral fertilizers offers a sustainable approach to enhancing nutrient utilization by improving the efficiency of chemical fertilizer, reducing nutrient losses and optimizing crop yields and quality (Schoebitz and Vidal, 2016; Chang et al., 2024), but their effects on maize productivity, especially in the short run, remain a subject of ongoing research.

This study aims at investigating the impact of short-term applications of organic and mineral fertilizers on maize productivity in a southeastern area of Romania, a region characterized by specific climatic and soil conditions. Understanding the interaction between these fertilizers and maize growth is crucial for developing sustainable agricultural practices that maximize yield while minimizing environmental impact.

This research findings aim to provide valuable insights into the efficiency of various fertilization strategies, contributing to the improvement of maize production in this region and potentially in similar agro-ecological areas.

MATERIALS AND METHODS

Study area and climatic conditions. The field experiment was carried out at Moara Domnească Research and Development Station for Agronomy (RDSA) during the 2022 growing season (Figure 1).

Figure 1. Maize experimental field, 2022 growing season, RDSA Moara Domnească

RDSA Moara Domnească is part of the University of Agronomic Sciences and Veterinary Medicine of Bucharest (USAMVB), and it is located in the Romanian Plain, in the southeastern part of Romania.

The climatic conditions in Moara Domnească area influenced the crop growth mainly due to the significant variations. In 2022, the minimum temperatures ranged from -7.9°C (in January and March) to 15.3°C (in August). April 2022, corresponding to the maize sowing period, was characterized by minimum temperatures of 0.1°C and maximum temperatures of 25.9°C.

The precipitation regime in April was very favourable, with a total monthly rainfall of 71.5 mm, which supported soil moisture levels necessary for proper seedling establishment and early growth of maize. This rainfall, along with generally mild temperatures in the spring, created optimal conditions for the initial stages of maize growth.

The summer months were characterized by high temperatures, with maximum values reaching 36.2°C in August. This hot period provided a favourable environment for maize growth, as crops thrive in warm temperatures. However, the relatively high maximum temperatures also posed a potential stress factor, particularly during the grain filling phase of maize.

The hot and dry conditions in late summer presented challenges for the maize crop, especially because irrigation was not available to supplement the water shortage. The precipitation regime and monthly temperatures can be seen in Figure 2.

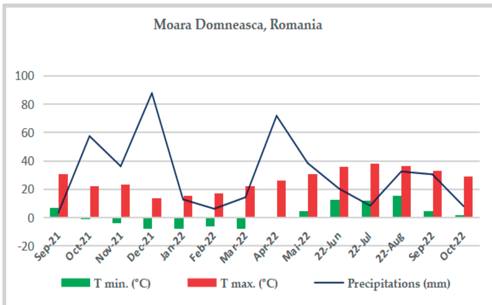


Figure 2. Precipitations (mm) and monthly temperatures (ºC) in the Moara Domnească area between September 2021 and October 2022

Soil conditions. The soil at Moara Domnească is classified as red preluvosoil. To evaluate its chemical properties prior to land preparation and maize sowing, soil samples were collected from six locations at a depth of 0-20 cm. The samples were air-dried, sifted, and subsequently analyzed to determine the agro-chemical characteristics (Table 1).

Table 1. Agrochemical characteristics of red preluvosoil from Moara Domnească, before maize sowing

Soil characteristics	Mean values ± SD*
pH	5.90 ± 0.244
C org. (%)	2.11 ± 0.178
N_{total} (%)	0.17 ± 0.007
N-NO_3 (mg/kg d.m.)	49.0 ± 8.041
N-NH_4 (mg/kg d.m.)	5.33 ± 4.027
P_{AL} (mg/kg d.m.)	113.6 ± 5.230
K_{AL} (mg/kg d.m.)	373.3 ± 8.556

*SD – standard deviation

Due to autumn drought conditions that hindered ploughing, the soil was scarified twice to a depth of 40 cm. To prepare the seedbed, two passes with a cultivator were carried out, and the preceding crop was alfalfa.

The compost utilized in the experiment was obtained from cattle manure and straw collected at the RDSA Moara Domnească farm. Maize hybrid, SY Carioca, was sown on April 15, 2022, at a seeding rate of 15 kg/ha and a row spacing of 70 cm.

Experimental design. The experiment was conducted using a randomized complete block design, comprising 8 treatments and 4 replications. Each plot measured 15 m² (5 m long and 3 m wide).

The fertilization treatment involved three different doses of manure compost: 15 t/ha, 30 t/ha, and 60 t/ha (Figure 3 and Table 2).

Figure 3. Different doses of manure compost applied on the experimental plots, Moara Domnească, autumn of 2021

These doses were applied at the end of September 2021, either individually or in combination with NPK complex fertilizers (20:20:0).

The NPK fertilizer was applied in multiple fractions, based on the nutrient requirements of the maize crop.

Table 2. Treatment variants and doses of mineral fertilizers applied for maize during the 2022 vegetation period

Treatment	Maize		
	Doses of mineral fertilizers		
	Fraction (kg/ha)		
	1	2	3
	15.04.2022	15.05.2022	15.06.2022
V1 - soil (Control)	-	-	-
V2 - NPK	28 N; 28 P_2O_5 0 K_2O	46 N; 46 P_2O_5; 0 K_2O	29 N; 29 P_2O_5; 0 K_2O
V3 - 15 t/ha MC*	-	-	-
V4 - 15 t/ha MC + NPK	29 N; 29 P_2O_5 0 K_2O	34 N; 34 P_2O_5; 0 K_2O	21 N; 21 P_2O_5; 0 K_2O
V5 - 30 t/ha MC	-	-	-
V6 - 30 t/ha MC + NPK	19 N; 19P_2O_5; 0 K_2O	22 N; 22 P_2O_5; 0 K_2O	14 N;14 P_2O_5; 0 K_2O
V7 - 60 t/ha MC	-	-	-
V8 - 60 t/ha MC + NPK	According to the dose calculation, in V_8, the quantity of MC should have covered the nutrient requirements (NPK) and it was decided not to supplement it with chemical fertilizers		

*MC - manure compost

Yield components and grain yield determination methods. At harvest time, 5 cobs from each treatment and replication were manually harvested at their physiological maturity to determine the yield components, i.e. cob length (cm), number of grain rows/cobs, number of grains/cobs, grain weight/cob (g), and thousand grain weight (TGW) (g).

The grain weight/cob was measured using an electronic weighing balance. Thousand grain weight was determined by weighing 1,000 grains. Grain yield was registered from each experimental plots. The data was adjusted at 14% moisture level and reported as grain yield kg ha^{-1}.

Statistical analysis. A statistical analysis was conducted using *Analyse-it software* for Microsoft Excel. A one-way analysis of variance (ANOVA) was applied to evaluate all parameters and determine statistically significant differences between treatments (p<0.05). Furthermore, the least significant differences (LSD) were calculated using Microsoft Excel.

RESULTS AND DISCUSSIONS

Maize yield components. The data analysis revealed variations in cob length across the different variants. The control variant (V1) had the shortest cob length (13.4 cm), while the longest was observed in V6, measuring 16.5 cm. This was followed by V7 (16.3 cm) and V5 (16.1 cm) (Table 3 and Figure 4). Mohsin et al. (2012) and Bhatt et al. (2020) also observed that the application of chemical fertilizers in combination with organic fertilizers led to an increase in cob length, compared to the use of chemical fertilizers alone.

Table 3. Maize yield components during the 2022 growing season, RDSA Moara Domnească experimental field

Yield components/ Vt	Cob length (cm)	No. grain rows/ cob	No. grains/ cob	Grain weight/ cob (g)	TGW (g)
V1	13.4^{c}	13.5^{c}	256.0^{d}	44.2^{d}	172.9^{d}
V2	14.7^{bc}	14.0^{b}	298.0^{cd}	55.3^{cd}	185.7^{cd}
V3	15.5^{b}	14.1^{b}	315.6^{c}	59.6^{c}	188.9^{c}
V4	15.7^{b}	14.3^{ab}	358.3^{bc}	76.7^{bc}	214.1^{a}
V5	16.1^{ab}	14.0^{b}	376.2^{b}	74.5^{b}	198.0^{bc}
V6	16.5^{a}	14.5^{a}	405.2^{a}	83.3^{a}	205.5^{b}
V7	16.3^{a}	14.5^{a}	393.6^{ab}	83.2^{a}	211.3^{ab}
V8	16.0^{ab}	14.3^{ab}	388.5^{ab}	80.6^{ab}	207.5^{b}
LSD 0.5	*0.29*	*0.19*	*20.53*	*3.02*	*4.62*
Similar letters show that there is no significant difference according to Duncan's test at the 5% probability level					

The number of rows per cob varied from 13.5 rows in the Control variant (V1), to 14.5 rows in V6 and V7. The differences between treated variants were statistically insignificant.

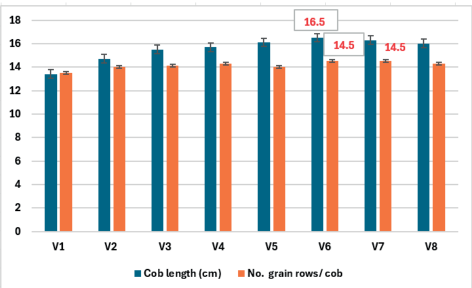


Figure 4. The cob length and number of grain rows/cob at maize under various doses of manure compost and chemical fertilizers, 2022 growing season. The bars stand for the standard errors

The number of grains per cob was influenced by the size of the grains, and it varied across treatments. During the 2022 cropping season, the results showed a decrease in the number of grains per cob when manure compost was applied alone. However, a significantly higher number of grains was noted when a combination of 30 t/ha of manure compost and NPK fertilizers was used, i.e. 405.2 grains, followed by 60 t/ha manure compost and NPK (V7), respectively 393.6 grains (Table 3 and Figure 5). The increased number of grains per cob might be attributed to the availability of more nitrogen and other nutrients from manure compost and chemical fertilizers to the maize plants through their life cycle. This finding is consistent with Bhatt et al. (2020), who suggested that the timely availability of nitrogen, along with improvements in moisture retention and soil structure through the application of organic manures, can enhance the number of grains per cob. The control treatment gave a statistically lower number of grains per cob (256), which can be attributed to the insufficient availability of nutrients in the soil.

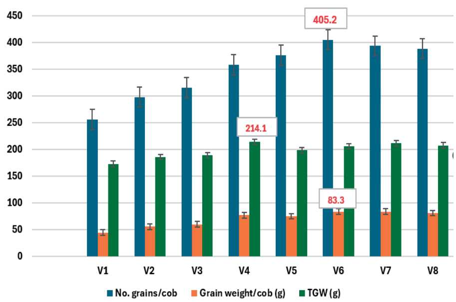
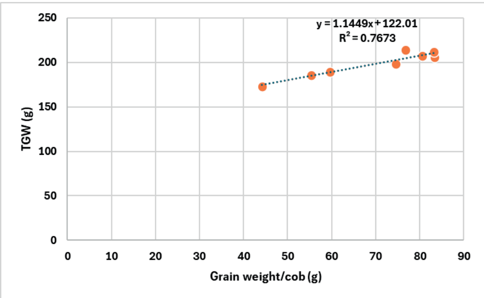


Figure 5. Number of grains/cob, grains weight/cob and TGW at maize under various doses of manure compost and chemical fertilizers, 2022 growing season. The bars stand for the standard errors

The grain weight per cob is an important factor in determining the overall productivity of maize crop. The highest value was recorded in the variant where 30 t/ha of manure compost and NPK fertilizer were applied (V6), i.e. 83.3 g, which is 47% higher than the control (only soil). This result can reflect the efficient utilization of nutrients by the plants and their subsequent translocation to the generative structures.
Thousand grain weight (TGW) is an important agronomic feature used to assess the size and weight of maize grains and is a significant

indicator of grain quality. TGW can vary depending on various factors such as variety, growing conditions, management practices, or climate.
The data showed that TGW were not significantly affected by different levels of organic and inorganic fertilizers. However, a higher TGW, i.e. 214.1 g, was found in V4 (15 t/ha manure compost and NPK), followed by V7 (211.3 g) and V8 (207.5 g) (Table 3 and Figure 5).
The correlation between grain weight per cob and thousand grain weight is positive (R^2 = 0.76) and can be attributed to the fact that as thousand grain weight (TGW) increases, resulting in larger individual grains, the total grain weight per cob also tends to rise. Additionally, there is a strong correlation between the number of grains per cob and the total grain weight (R^2 = 0.99), indicating that an increase in the number of grains per cob leads to a corresponding increase in the total grain weight. Specifically, the grain weight per cob is directly influenced by both the number of grains per cob and the average weight of each grain (Figure 6a and 6b).

a.



b.

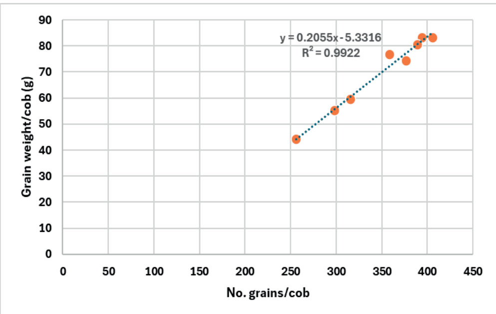


Figure 6. Correlation between the grain weight/cob and TGW (a) and the number of grains/cob and grains weight/cob (b)

Maize grain yield at 14% moisture. The drought conditions in 2022 resulted in a noticeable decrease in maize yield, and this effect was observed across the variants evaluated in our study.The data presented in Table 4 clearly showed that the highest grain yield of 3,264 kg/ha was registered in the variant where 30 t/ha of manure compost and NPK (V6) were applied, followed by V7 (3,186 kg/ha), V8 (3,160 kg/ha) and V4 (3,008 kg/ha). The values obtained for these treatments were statistically confirmed as distinct significant positive, suggesting that they are providing the optimal conditions for maximizing maize yields (Table 4).

Table 4. Maize yield under different doses of manure compost and chemical fertilizers, Moara Domnească, 2022 growing season

Variant	Yield (kg/ha) at 14% moisture	Differences from the Control		Significance
		kg/ha	%	
V1	1,735	-	100	-
V2	2,169	434	125	ns
V3	2,337	602	135	*
V4	3,008	1,273	173	***
V5	2,920	1,185	168	**
V6	3,264	1,529	188	***
V7	3,186	1,451	184	***
V8	3,160	1,425	182	***

DL5% - 504 kg/ha *ns-insignificant*
DL 1% -763 kg/ha
DL0.1% - 1,225 kg/ha

The lowest grain yield was obtained in the variant where there was no use of organic and chemical fertilizers (V1-1,735 kg/ha) (Table 4). Similar results were reported by Rajesh et al. (2014) and Mhoro et al. (2025).
In the context of global warming, where crop plants are heavily affected, maize being among the most vulnerable, it is becoming essential to find technical solutions to adapt to the effects of these climate changes. Irrigation and fertilizer management strategies that concurrently optimize yield, water use efficiency, and fertilizer efficiency are essential for ensuring sustainable agricultural production (Chen et al., 2024).

CONCLUSIONS

The combined application of organic and mineral fertilizers can offer a sustainable approach to enhancing nutrient utilization, improving the efficiency of chemical fertilizers and optimizing crop yields.

The yield components were influenced by the different fertilizer treatments as follows: the application of manure compost in combination with NPK fertilizers resulted in longer cob lengths compared to the control treatment (only soil).

As far as the number of rows per cob is concerned, the differences between treatments were statistically insignificant, indicating that the number of rows might be less responsive to the treatments compared to other factors like cob length or grain number/cob.

The increase in the number of grain/cobs can be attributed to the enhanced availability of nutrients (especially nitrogen) from the manure compost and NPK fertilizers and the variation between treatments is likely due to the varying levels of nutrient availability, soil organic matter, and the interaction between slow-release nutrients from compost and immediate nutrients from chemical fertilizers.

While the TGW did not show significant statistical differences across treatments, the trend suggests that a combination of organic and inorganic fertilizers may slightly enhance the size and weight of individual grains.

A strong positive correlation was observed between the grain weight per cob and the TGW, indicating that as TGW increases, the individual grains grow larger, leading to a higher total grain weight per cob. There was also a very strong correlation between the number of grains per cob and the total grain weight, which suggests that an increase in the number of grains per cob directly contributes to higher grain weight, further emphasizing the importance of both grain count and individual grain size in determining overall productivity.

The combination of manure compost and NPK fertilizers, particularly at the 30 t/ha manure compost and NPK rate (V6), had a significant positive impact on maize productivity, improving cob length, the number of grains per cob, grain weight per cob, and overall grain yield.

The 2022 climatic conditions at Moara Domnească had a mixed effect on maize productivity, with favourable temperatures and precipitation early in the season, followed by heat stress in the summer. This prolonged drought and climate variability diminished the crop yield.

Such climatic conditions emphasize the importance of water management strategies, as moisture stress during the later stages of maize development can reduce yield potential. Also, these factors underline the complex relationship between climatic conditions and maize productivity, highlighting the need for adaptive farming practices to mitigate adverse weather conditions.

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WATER SORPTION ISOTHERMS OF MINTS

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Abstract

The mints are aromatic plants with utilisation in human been food as aromatic plant as well as medicinal plant. The objective of that study was to provide fundamental data on experimental measurements of water sorption isotherms of different species of mints at various temperatures, using standardized static method. The obtained data of sorption characteristic of mints were fitted by mathematical models. The experiments results were also used in modeling the storage behavior and quality attributes of the dried mints. *Seven varieties of Mint have been analysed in this experiment: M. spicata L., M. aquatica L., M. arvensis L., M. longifolia L., M suaveolens Ehrh., M. pulegium L., and M. pipperita L. The isotherms experiments have been conducted using standard gravimetric method recommended by Spiess and Wolf (1983). Eight salts are selected to give different relative humidity's in the range of 0.11-0.84. The experiments have been conducted at the 20^{0}C, 30^{0}C, 40^{0}C, 50^{0}C and 60^{0}C constant temperatures. Have been describe the isotherms equation for adsorbtion and desorbtion for all mints varieties.*

Key words: *mints, isotherm of sorption, hysteresis, water activity, BET equation.*

INTRODUCTION

Water plays a very important and unique role in agricultural products. Being present in the highest concentration, it influences a wide range of physical, chemical and biological phenomena, which occur during processing storage. Most importantly, the concentration of water affects practically all-deteriorative processes that are microbiological in nature and enzymatic or non-enzymatic in origin. The rate of the various deteriorative processes depends mainly on water concentration. The potential of water to take part in the deteriorative processes can be characterized by the water activity (a_w) which is defined, according to the generalized Raoult's law, as the ratio between the water vapour pressure of the product at a given temperature and the saturation pressure of pure water at the same temperature (Wolf et al., 1985). At equilibrium, the water activity is related to the relative humidity of the surrounding atmosphere (Iglesias and Chirife, 1982). The importance of water sorption equations has been stressed by many researchers including the nutrient retention during dehydration and shelf life of product in a packaging material (Labuza, 1968). They are also needed for evaluating the thermodynamic functions of the water sorbed in foods (Iglesias and Chirife, 1976), prediction of drying time (Henderson and Perry, 1976) and simulation of drying systems (Park et al., 2002). Such drying simulations could be used to predict drying time, determine the effect of change in certain parameters on the drying efficiency, or minimize operating costs. It also constitutes an essential part of the drying theory (King, 1968), and in the measurement of the latent heat of vaporization (Murata, 1988; Tagawa et al., 1993). Currently, sorption isotherms are gaining in importance as the number of recommendations, official regulations and product specifications which use water activity as an evaluation criterion is growing continuously (Wolf et al., 1985). The objective of drying mints is to extend the shelf life and conserving the fresh characteristics. This is achieved by reducing the water activity (a_w) of the product to a value which will inhibit the growth and development of pathogenic and spoilage microorganisms, significantly reducing enzyme activity and the rate at which undesirable chemical reactions occur. The removal of most of the water from the product reduces the weight to be carried per unit product value. This can lead to substantial savings in the cost of handling and transporting the dried product as compared with the fresh material. A reduction in volume of the dried material, as compared with the fresh, can lead to savings in the cost of storage and transport.

The sorption curves express the hygroscopic equilibrium states of a given product (Kane et al., 2008). Their determination constitutes an indispensable stage for better understanding the problems of modelling the drying processes (Akawasi, 1997; Vaios et al., 1999). Using an experimental approach, these equilibrium curves are determined by the saturated salt solutions method. The experimental sorption curves are described by (GAB), modified Halsey and Peleg equations. The GAB model is recognised as the most widely utilised and versatile (Timmerman et al., 2001), and was recommended by European COST 90 Project (Wolf et al., 1985)

Data of water desorption isotherms are very important in that aspect to properly select the final moisture content which the product is safe for storage and to determine the optimum storage conditions (Aguerte et al., 1989). Due to the complex food composition, theoretical prediction of sorption isotherms is not possible and experimental measurements are necessary (Epure et al., 2005; Gal 1985). The objective of that study was to provide fundamental data on experimental measurements of water sorption isotherms of onion at various temperatures, using standardized static method (Epure et al., 2005).

The depression of water activity in foods is due to a combination of factors each of that may be predominant in a given range of the water activity (Karel, 1973); the sorption properties may change as a consequence of physical and chemical interaction induced by heating or pre-treatments (Iglesias and Chirife, 1976); changes of water sorbtion it usually undergoes changes of constitution, dimensions and other properties of the product (McLaren and Rowen, 1952). Water sorption leads to phase transformations of the sugar contained in the food (Karel, 1973; Iglesias et al., 1975). Due to that, it is no possible of having a unique mathematical model, either theoretical or empirical for describing accurately the sorption isotherms in the whole range of water activity and different type of foods. The GAB equation was applied by Labuzza et al. (1985) to model moisture sorption isotherm in the range of 0,1 to 0,9 water activity at various temperatures who reported an excellent fit to data. Spiess and Wolf (1987) used the GAB model to adsorbtion isotherms at 25°C. Van den Berg and Bruin (1981) have compiled and discussed some of empirical isotherm equations that have been reported in the literature for fitting water sorption isotherms of food. Chirife and Iglesias (1978) made a research of the most of the isotherm equations for fitting moisture sorptions isotherms of foods (Epure et al., 2005).

MATERIALS AND METHODS

For this research 7 species from genus Mentha have been used: *Mentha piperita* L. (Peppermint), *Mentha spicata* L. (Spearmint), *Mentha aquatica* L. (Water mint), *Mentha arvensis* L. (Wild mint), *Mentha longifolia* L./ Huds (Horse mint), and *Mentha suaveolens* Ehrh (Apple mint), and *Mentha pulegium* L. (Pennyroyal mint) and the material (herba) have been harvested just before flowering from Botanical Garden fields. Representative samples were taken randomly and cut manually into small pieces. The samples of 5 grams were taken randomly and placed in sorbostats. A small quantity of thymol was placed in each hygrostat in order to prevent fungal activity, Wolf et al. (1985). The sorption isotherms of mints were determined using standard gravimetric method recommended by Spiess and Wolf (1983) and AOAC (1995). Eight salts are selected to give different relative humidity's in the range of 0.11-0.84. All the salt solutions used in the experiment were prepared with the reagent grade salts and distilled water, accordingly with Spiess and Wolf method (1983). Saturated salts solution have the advantage of maintaining a constant relative humidity of the air as long as the salt present is above saturation level (Karel, 1975). The effect of pressure on adsorption isotherm is negligible at reasonable levels (Okos et al., 1992). The salts solution used are presented in Table 1. The sorption containers were placed in a temperature-controlled cabinet (HERAEUS type B 5090E Germany) (Figure 1) at 20, 30, 40, 50, and 60^{0}C. Each sample was replicated two times. First weighing was done one week from the start of the experiment using an analytical balance (SARTORIUS type BP

221S of the SARTORIUS AG Göttingen Germany) with 0,1 mg accuracy. Successive weightings were done after every three days. The equilibrium was reached when the sample weight difference between two successive measurements was less than the balance accuracy of 0.1 mg. (Saravacos et al., 1986). The time required for the mints to reach equilibrium moisture content varied with the relative humidity and the temperature. The moisture content of the equilibrated samples was determined by drying at 105^{0}C using hot air oven until the moisture content became constant.

Table 1. Equilibrium of relative humidity of the saturated salts solutions at six temperatures used in the experiments (Wolf and Spiess, 1983)

Salt Solutions	Temperature of saturation salts, ^{0}C				
	20	30	40	50	60
LiCl	0.1240	0.1128	0.1121	0.1110	0.1095
CH_3COOK	0.2330	0.2161	0.2040	0.1920	0.1800
$MgCl_2$	0.3360	0.3244	0.3160	0.3054	0.2926
K_2CO_3	0.4400	0.4317	0.4299	0.4265	0.4211
$Mg(NO_3)_2$	0.5490	0.5140	0.4842	0.4544	0.4727
$NaNO_3$	0.6530	0.7314	0.7100	0.6904	0.6735
NaCl	0.7547	0.7509	0.7468	0.7443	0.7450
KCl	0.8511	0.8362	0.8332	0.8120	0.8025

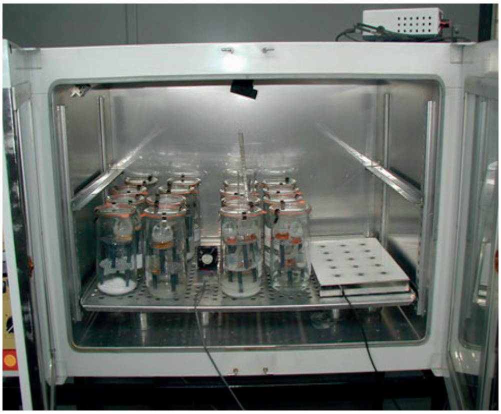

Figure 1. Temperature controlled cabinet HERAEUS type B 5090E with sorbostats

The equilibrium moisture content (U_e) of the tomato, expressed in wet basis, was calculated using Eq. (1).

$$U_e = \frac{m_e - m_D}{m_e}100 \quad (1)$$

For the analysis and presentation of sorption data, the moisture content obtained were converted to dry basis expressed in kg water / kg solid (Iglesia and Chirife ,1982), using Eq. (2).

$$X_e = \frac{U_e}{100 - U_e} \quad (2)$$

The data were graphically presented by plotting X_e versus a_w, computed using Eq. (3).

$$a_w = \frac{P_f}{P_0} = \frac{H_e}{100} \quad (3)$$

The data were further analyzed by fitting them to two-parameters isotherm equations (Table 1). Using regression analysis, each equation was linearized to solve for the constant and the degree of the linear relationship between X_e and a_w was determined for each temperature by solving the coefficient of correlation R using Eq. (4).

$$R = \frac{n\sum X_e a_w - (\sum X_e)(\sum a_w)}{\sqrt{\left[n\sum (X_e)^2 - (\sum X_e)^2\right]\left[n\sum (a_w)^2 - \left(\sum a_w^2\right)\right]}} \quad (4)$$

To determine the percent of variation of the X_e , the coefficient of determination (R^2) was computed. The accuracy of the fit was determined by calculating the root mean square error (RMS) cited by Gal (1981), Greenspan (1977), and Tagawa et al. (1983), using Eq. (5).

$$RMS = \sqrt{\left(\frac{\sum_{i=1}^{n}(X_{e_i} - X_{c_i})}{n-1}\right)} \quad (5)$$

The smaller the value of RMS, the better is the fitting of the equation to the experimental sorption data. The parameters of the BET equation were determined by plotting aw / X (1-aw) against aw. From the slope and intercept of the line, the constant Xm and C were determined (Aquerte et al., 1989; Timmerman et al., 2001; van den Berg, 1985).

RESULTS AND DISCUSSIONS

The results of the experimental measurements of water desorption and adsorption isotherms of mint for all ranges of temperatures analysed are presented in Figures 1 for desorption and Figure 2 for adsorption. The experimental points are based on a mean value of two replications. The obtained sorption isotherms of mint showed the typical sigmoid shape of type II, according to the BET classification (Brunauer et al., 1938) for all 7 species of genus *Mentha* (Peppermint, Spearmint, Water mint, Wild mint, Horse mint, Apple mint and Pennyroyal mint). Higher equilibrium moisture contents were found at the lower temperature for the same relative humidity for all mint species analysed.

The desorption isotherm is higher than the adsorption isotherm at the same temperature and relative humidity for all mint species analysed. The adsorption and desorption isotherms exhibited hysteresis for the entire range of relative humidity and for all species of mint analysed. That is in concordance to the theory of physical sorption (Iglesias et al., 1975), and have been reported also by Kane et al. (2008), Dalgic et al. (2011).

Hysteresis are found to be higher at lower temperatures than higher temperatures. It is also observed from the hysteresis curves that the hysteresis loop decreased with decreasing of relative humidity and have been reported also by Park et al (2002).

The experimental results of determination of isotherm of desorption for Peppermint are presented in Figure 2.

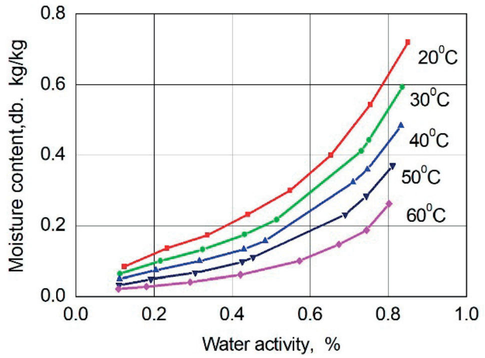

Figure 2. Water desorption isotherms of *Mentha pipperita* at different temperatures

After experiments have been conducted, using plotting equation the parameter X_m and C of BET equation have been calculated and also the coefficient of determination R^2, for isotherms of desorption of peppermint (Table 2) and for isotherms of adsorption (Table 3)

Table 2. BET equation parameters and goodness of fit of desorption of Peppermint at different temperatures

Temperature (°C)	X_m	C	R^2
20	0.1193	39.8203	0.9496
30	0.1053	13.6487	0.9846
40	0.0878	9.6443	0.9837
50	0.0752	4.5277	0.9963
60	0.0556	2.7119	0.9979

The experimental results of determination of isotherm of adsorption for Peppermint are presented in Figure 3. That correspond with data presented by Dolgic et al. (2012). Different values of coefficients of BET equation could occur due to different chemical composition of fresh and dried mints used in experiments due to different crop and harvest conditions accordingly with Iglesias and Chirife (1976), Wolf et al. (1985), Timmerman et al. (2001).

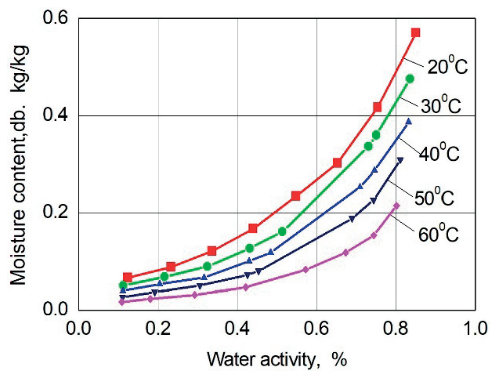

Figure 3. Water adsorption isotherms of *Mentha pipperita* at different temperatures

The values of coefficient of determination R^2, for isotherms of desorption of Peppermint (Table 2) and for isotherms of adsorption (Table 3) indicate that the BET equation could be use with a good estimation of water activity for Peppermint for all ranges of isotherms between 20 and 60 °C.

Table 3. BET equation parameters and goodness of fit of absorption of Peppermint at different temperatures

Temperature (°C)	X_m	*C*	R^2
20	0.0932	21.1306	0.9636
30	0.0856	8.0228	0.9815
40	0.0706	6.7580	0.9848
50	0.0632	3.2062	0.9970
60	0.0485	1.9913	0.9951

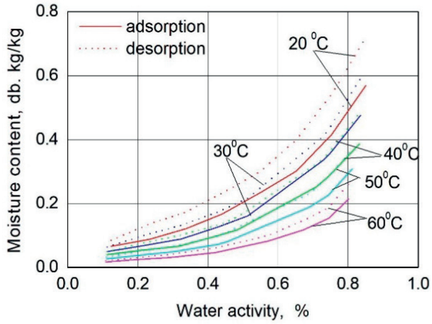

Figure 4. Water sorption isotherms of *Mentha pipperita* at different temperatures

The hysteresis loop decreased with decreasing of moisture content of Peppermint and with increasing of temperatures (Figure 4).

The experimental results of determination of isotherm of sorption for Peppermint are presented in Figure 5.

The values of coefficient of determination R^2, for isotherms of desorption of Spearmint (Table 4) and for isotherms of adsorption of Spearmint (Table 5) indicate that the BET equation could be use with a good estimation of water activity for Spearmint for all ranges of isotherms between 20 and 60^{O}C.

The isotherms present hysteresis for all range of isotherms and all values of water activity for Spearmint (Figure 5).

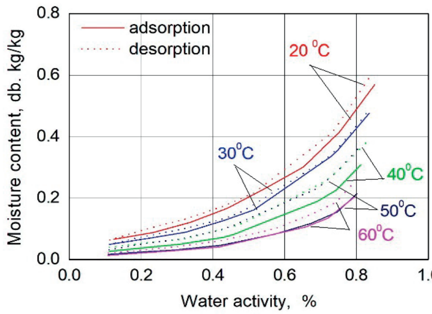

Figure 5. Water sorption isotherms of *Mentha spicata* at different temperatures

Table 4. BET equation parameters and goodness of fit of desorption of Spearrmint at different temperatures

Temperature (^{0}C)	X_m	*C*	R^2
20	0.0857	8.0225	0.9813
30	0.0875	9.6447	0.9834
40	0.0756	4.5278	0.9969
50	0.0481	1.9912	0.9953
60	0.0474	1.6955	0.9973

Table 5. BET equation parameters and goodness of fit of absorption of Spearrmint at different temperatures

Temperature (^{0}C)	X_m	*C*	R^2
20	0.0931	21.1309	0.9632
30	0.0704	6.7586	0.9845
40	0.0879	9.6444	0.9834
50	0.0750	4.5279	0.9961
60	0.0481	1.9919	0.9959

The experimental results of determination of isotherm of sorption for Water mint are presented in Figure 6.

The values of coefficient of determination R^2, for isotherms of desorption of Water mint (Table 6) and for isotherms of adsorption of Water mint (Table 7) indicate that the BET equation could be use with a good estimation of water activity for Water mint for all ranges of isotherms between 20 and 60^{O}C.

The isotherms present hysteresis for all range of moisture content and all values of water activity for Water mint (Figure 6).

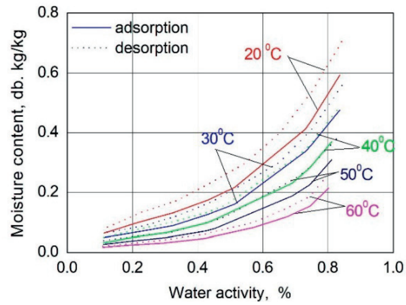

Figure 6. Water sorption isotherms of *Mentha aquatica* at different temperatures

The results for Wild mints are presented in Figure 7. The isotherms present hysteresis for all range of water activity. The calculated values of BET coefficients and coefficient of determination R^2, for isotherms of desorption of Wild mint are presented in Table 8, and for isotherms of adsorption in Table 9.

Table 6. BET equation parameters and goodness of fit of desorption of Water mint at different temperatures

Temperature (^{0}C)	X_m	C	R^2
20	0.1190	39.8200	0.9591
30	0.0933	21.1305	0.9635
40	0.0871	9.6451	0.9823
50	0.0707	6.7584	0.9854
60	0.0555	2.7118	0.9978

Table 7. BET equation parameters and goodness of fit of absorption of Water mint at different temperatures

Temperature (^{0}C)	X_m	C	R^2
20	0.1053	13.6487	0.9846
30	0.0856	8.0228	0.9815
40	0.0752	4.5277	0.9963
50	0.0632	3.2062	0.9970
60	0.0483	1.9916	0.9953

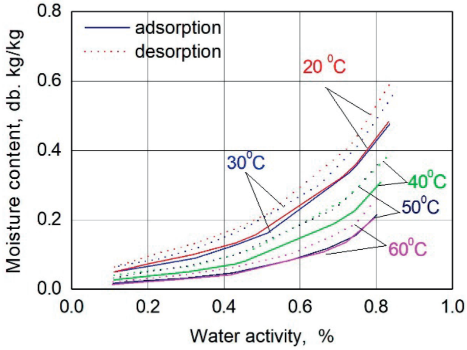

Figure 7. Water sorption isotherms of *Mentha arvensis* at different temperatures

Table 8. BET equation parameters and goodness of fit of desorption of Wild mint at different temperatures

Temperature (^{0}C)	X_m	C	R^2
20	0.1293	39.8401	0.9292
30	0.0956	9.0228	0.9515
40	0.0848	8.6441	0.9431
50	0.0751	5.5277	0.9583
60	0.0556	3.7119	0.9779

Table 9. BET equation parameters and goodness of fit of absorption of Wild mint at different temperatures

Temperature (^{0}C)	X_m	C	R^2
20	0.1083	13.6281	0.9641
30	0.0906	8.7580	0.9648
40	0.0753	4.5255	0.9921
50	0.0585	3.9913	0.9961
60	0.0499	1.9954	0.9777

The results for Horse mints are presented in Figure 8. The isotherms present hysteresis for all range of water activity. The calculated values of BET coefficients and coefficient of determination R^2, for isotherms of desorption of Horse mint are presented in Table 10, and for isotherms of adsorption in Table 11. The obtained values indicate that BET equation is suitable for describe isotherms of sorption for Horse mint.

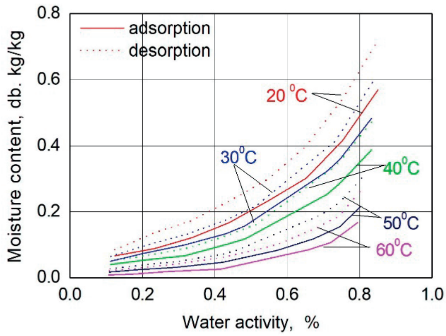

Figure 8. Water sorption isotherms of *Mentha longifolia* at different temperatures

Table 10. BET equation parameters and goodness of fit of desorption of Horse mint at different temperatures

Temperature (^{0}C)	X_m	C	R^2
20	0.1194	38.8203	0.9576
30	0.1053	14.6487	0.9911
40	0.0885	9.6459	0.9872
50	0.0588	2.7119	0.9132
60	0.0477	0.9942	0.9747

Table 11. BET equation parameters and goodness of fit of absorption of Horse mint at different temperatures

Temperature (^{0}C)	X_m	C	R^2
20	0.0939	20.1306	0.9456
30	0.0856	8.9228	0.9815
40	0.0716	6.7577	0.9748
50	0.0621	4.2062	0.9843
60	0.0488	2.1913	0.9641

The results for Apple mints are presented in Figure 9. The isotherms present hysteresis for all range of water activity for all analysed temperatures. The calculated values of BET coefficients and coefficient of determination R^2, for isotherms of desorption of Apple mint are presented in Table 12, and for isotherms of adsorption in Table 13. The obtained values indicate that BET equation is suitable for describe isotherms of sorption for Apple mint.

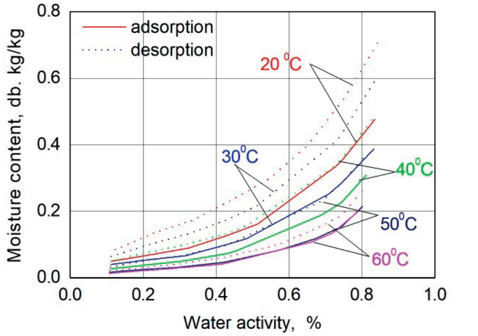

Figure 9. Water sorption isotherms of *Mentha suaveolens* at different temperatures

Table 12. BET equation parameters and goodness of fit of desorption of Apple mint at different temperatures

Temperature (°C)	X_m	*C*	R^2
20	0.1183	38.7721	0.9191
30	0.1086	13.9487	0.9346
40	0.0996	9.5243	0.9337
50	0.0756	4.5587	0.9873
60	0.0597	2.6134	0.9567

Table 13. BET equation parameters and goodness of fit of absorption of Apple mint at different temperatures

Temperature (°C)	X_m	*C*	R^2
20	0.0911	8.0328	0.9445
30	0.0736	6.8151	0.9847
40	0.0649	3.7162	0.9650
50	0.0584	1.9913	0.9431
60	0.0444	1.8313	0.9851

The results for Pennyroyal mints are presented in Figure 10. The isotherms present hysteresis for all range of water activity for all analysed temperatures. The obtained isotherms are similar with isotherms obtained by Kane et al., (2008). The calculated values of BET coefficients and coefficient of determination R^2, for isotherms of desorption of Pepper mint are presented in Table 14, and for isotherms of adsorption in Table 15.

The obtained values indicate that BET equation is suitable for describe isotherms of sorption for Pennyroyal mint.

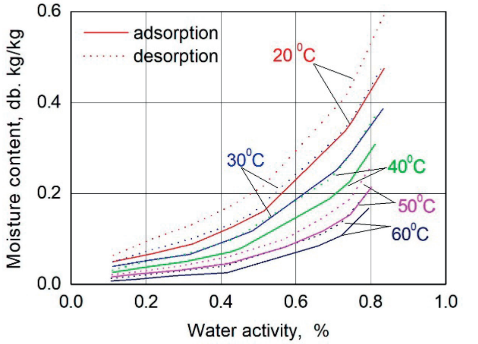

Figure 10. Water sorption isotherms of *Mentha pulegium* at different temperatures

Table 14. BET equation parameters and goodness of fit of desorption of Pennyroyal mint at different temperatures

Temperature (°C)	X_m	*C*	R^2
20	0.1054	13.6487	0.9847
30	0.0878	9.6442	0.9838
40	0.0754	4.5277	0.9963
50	0.0553	2.7119	0.9976
60	0.0473	1.6954	0.9977

Table 15. BET equation parameters and goodness of fit of absorption of Pennyroyal mint at different temperatures

Temperature (°C)	X_m	*C*	R^2
20	0.0858	8.0227	0.9816
30	0.0706	6.7580	0.9847
40	0.0633	3.2062	0.9971
50	0.0484	1.9912	0.9951
60	0.0438	0.9042	0.9974

The gap of hysteresis obtained in experiments differ from a mint species to another, but hysteresis are presented to all isotherms (adsorptions and desorptions) for all species of mint analysed.

CONCLUSIONS

The water sorption isotherms of mints were determined using the gravimetric method based on the recommendation of the European Cooperative Project COST 90. Results of the experimental measurement of sorption isotherms (adsorption and desorption), at temperature range between 20 and 60^0C, showed a typical sigmoid shape of type II according to the BET classification to all mint species analyzed. The temperature had the expected effect predicted by theory of physical adsorption, the quantity of sorped water at a given water activity increased as the temperature decreased. The quantity of sorpet water differ to one mint species to another. The amount of sorped water depends on the equilibrium temperature and mint species, maybe due to a different chemical composition. The increase of the temperature has as result the increase of the water activity for the same moisture content to all mints varieties. Hysteresis was found for all 7 mint species analysed: *Mentha piperita* L. (Peppermint), *Mentha spicata* L. (Spearmint), *Mentha aquatica* L. (Water mint), *Mentha arvensis* L. (Wild mint), *Mentha longifolia* L./ Huds (Horse mint), and *Mentha suaveolens* Ehrh (Apple mint), and *Mentha pulegium* L. (Pennyroyal mint); for the entire range of relative humidity, for both adsorption and desorption isotherms. Hysteresis loops decreased with an increase of temperature, but differ to one mint species to another. These indicate the irreversibility of sorption process and chemical and microbiological deterioration during adsorption or desorption of mints.

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INDIVIDUAL AND COMBINED EFFECTS OF FOLIAR IRON, ZINC AND BORON APPLICATIONS ON YIELD AND MINERAL NUTRITION OF SUGAR BEET

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Abstract

Foliar application is a succesful way for a plant microelement nutriton under calcareous conditions. Study was aimed to evaluate the effects of foliar micronutrient applications on yield, mineral nutrition and some quality parameters of sugar beet. For this, solutions containing 500, 500 and 250 ppm of Fe, Zn and B and their combinations (Fe+Zn, Zn+B, Fe+B, Fe+Zn+B) were foliar applied. Compared to control, Fe+B combinations increased the root and root+leaf yield by 54% and 68%. The highest leaf Fe was obtained with Fe application, and the highest Zn and B were obtained from the Zn+B. The highest root Fe, Zn and B were obtained from Fe+Zn, Zn and B applications, respectively. Positive effects of the applications on other nutrients were determined. The highest polar sugar and total soluble solids were obtained from the Fe+B and the highest reducing sugar was recorded from Zn and B applications. Zn, B, and all combinations containing B significantly increased the α-amino N content. As conlusion, foliar application of Fe, Zn and B positively affected sugar beet nutrition and thus increased yield and quality.

Key words: *foliar ferilization, micronutrients, sugar beet, yield and quality.*

INTRODUCTION

Sugar beet (*Beta vulgaris* L.) is a biennial herb in the Chenopodiaceae family (Song et al., 2019), and is the world's second-largest sugar crop and beet has also become a popular energy crop in recent years (Muir & Anderson, 2022). Approximately 180 million tons of sugar are consumed worldwide annually, and approximately 25% of this sugar is produced by processing sugar beets (Keller et al., 2021). More recently, sugar beet has gained prominence as a crucial resource for ethanol production as a biofuel (Rinaldi & Vonella, 2006). World sugar beet production was nearly 252.9 million tons and the most important producers of sugar beet were Russia (33.9 million tons), the United States of America (USA) (30.5 million tons), Germany (28.6 million tons), France (26.2 million tons), and Turkey (23 million tons) in 2020 (FAO, 2022). Over 30% of world soils suffer from the deficiency of one or more micronutrients, a trend which is deteriorating (Sillanpaa, 1982), with the passage of time. There are numerous reports about the role of micronutrients in enzymatic responses, plant metabolism and assimilation of carbon, nitrogen and different compounds, sugar translocation, cellular division, water regulation, conductivity, and consequently, higher photosynthetic capacity and productivity of the plants (Shiemshi, 2007). Plants require some nutrients for optimum growth, among which trace elements like boron (B), iron (Fe) and zinc (Zn) are important nutrient elements for the growth and develoment of sugar beet and their deficiencies in soil can affect the performance of macronutrients (Xue et al., 2014).

Zinc is essential element for ideal crop production and satisfying yield performances due to its involvement in many biochemical processes in plant metabolism. It take place or regulates many enzimatic reactions. Zinc is required for maintaining phytohormones, vitamins, and amino acids level in the plant tissues and chlorophyll biosynthesis (Marschner, 2011). By promoting the root and shoot growth of plant it aguements the uptake of nutrients through the roots (Leite et al., 2020). These modifications stimulate plant enzymes and hormones, suppress diseases, heat stress, and frost damage by promoting antioxidants activity (Seydabadi & Armin, 2014). Due to most enzymes that play key roles in carbohydrates metabolism are activated by Zinc, the most required elements in the carbohydrates metabolism is Zn. The activity of these enzymes decreases in Zn deficient conditions. In different studies, foliar application of Zn alone or together with B and Mo increased the photosynthetic pigments contents, yield and some quality parameters such as sucrose percentage, purity percentage, sugar yield of sugar beet (Zewail et al., 2020). Iron (Fe), has metabolic importance in plants due to it takes parts in many physiological roles. Because of it plays critical role in metabolic processes such as DNA synthesis, respiration, and photosynthesis Fe is an essential micronutrient for almost all living organisms. Further, many metabolic pathways are activated by iron, and it is a prosthetic group constituent of many enzymes (Rout & Sahoo, 2015). Iron is a component of the active groups of various enzymes. Its one of the best known function is to be a part of prostatic groups of hemin enzymes. Although Fe is not included in the structure of the chlorophyll molecule, it plays many roles in the synthesis of chlorophyll and the process of photosynthesis. It plays a role as an electron carrier in energy metabolism, especially in relation to oxidation and the respiratory chain (Marschner, 2011). Of all crops, sugar beet has one of the largest requirements of B. So, B is the most important trace element required by sugar beet, because when it is not supplied in sufficient quantities, root yield and quality are seriously reduced (Draycott & Christenson, 2003). It has significant role in plant cell wall formation and cell division (Miah et al., 2020). It accelerates the translocation of sugars to the storage and growing parts (Allen et al., 2007; Ewais et al., 2020; Kandil et al., 2020). As their importance on plants is briefly stated above, for a high quality and high yield, sugar beet must receive sufficient amounts of these nutrients. Roots are the main way for plants to uptake nutrients. However, they can also take in various nutrients through their leaves. Micro element availability is associated with soil types and many soil physicochemical properties. Although foliar fertilization is seen as an additional support application to root nutrition, it is an extremely indispensable fertilization method under some conditions. It is known that various fertilizers, especially micro element fertilizers, are successfully applied via leaves when the soil has unfavourable conditions. In soils with high pH and excessive calcareous soils, which generally limit the availability of micro elements from the soil, foliar application gives quite successful results. Again, with foliar fertilization, the negative interaction of micro elements with other elements found in excess in the soil is also avoided (Fahad et al., 2014; Lucena, 2000).

In this study it was aimed to investigate individual and combined effects of foliar Fe, Zn and B applications on mineral nutrition, yield and some quality parameters of sugar beet grown on a calcareous soil.

MATERIALS AND METHODS

Field experiment was conducted during the 2023 growing seasons under field conditions in Burdur, Turkiye. The soil of the experimental area has an clayey-loam texture having slightly alkaline pH and high $CaCO_3$ content. The experimental soil is sufficient in terms of macro nutrients and cooper. On the other hand, iron content is medium, zinc and boron contents are around the deficient levels. Some properties of the experimental soil were given in Table 2. Soil texture, $CaCO_3$ and organic matter were measured with methods of Bouyoucos (1951), Allison & Moodie (1965) and Walkley & Black (1934) methods, respectively. The pH and EC values of the soil were determined using pH-EC meter. Available P in the soil was determined spectirofotometricaly (Olsen, 1954). Exchangeable K, Ca, Mg (Jackson 1962), and DPTA-extractable microelements (Lindsay and Norvell, 1978) were determined using inductively coupled plasma (ICP). Soil B concentrations were measured using ICP after the hot extraction of soil in 0.01 M $CaCl_2$ (Kacar, 2009; Erdal et al., 2016).

As plant material, Cesira which is videlly used variety for sugar beet cultivation in the region was used. $FeSO_4.7H_2O$, $ZnSO_4.7H_2O$ and H_3BO_3 were used as Fe, Zn and B sources. The treatments and application dasages are given in Table 2. Weather conditions during the experiment are summarized in Figure 1.

Table 1. Some properties of experimental soil

Properties		Evaluation	References
Texture		Clayey-loamy	
pH (1/2.5)	7.9	Silightly alkaline	(Richards, 1954)
EC (1/2.5, ds/m)	0.3	No saline	
Organic matter (%)	2.4	Modarate	
$CaCO_3$ (%)	16	High	
P (ppm)	25	Sufficient	(FAO, 1990)
K (ppm)	600	Sufficient	
Ca (ppm)	7000	Sufficient	
Mg (ppm)	280	Sufficient	
Fe (ppm)	3.5	Modarate	(Lindsay & Norwell, 1969)
Zn (ppm)	0.6	Deficient	
Cu (ppm)	2.5	Sufficient	
B (ppm)	0.4	Deficient	(Keren & Bingham, 1985)

Table 2. Treatments and application dosages

Treatments	Application dosages
Control	Water spraying
Fe	500 ppm
Zn	500 ppm
B	250 ppm
Fe+Zn	500 ppm Fe + 500 ppm Zn
Zn+B	500 ppm Zn + 250 ppm B
Fe+B	500 ppm Fe + 250 ppm B
Fe+Zn+B	500 ppm Fe + 500 ppm Zn + 250 ppm B

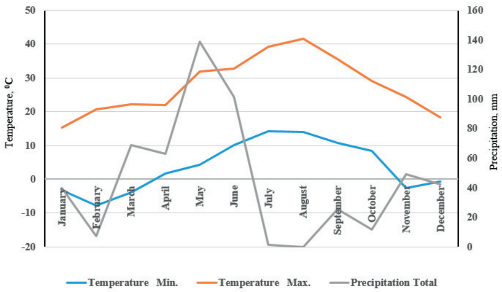


Figure 1. Temperatures and precipitations recorded during growing season

As basal fertilisation 6 kg N/da (Urea), 6 kg P/da (Tripl super phosphate) and 4.5 kg K/da (Potassium sulphate) were applied to the soil and mixed before sawing. Sawing was carried out with a five line mibzer arranged 45 x 15 cm spacings.

Before foliar fertilization, the experimental area was divided into 2 m long plots with 5 rows. Experiment was arranged with 3 replications, and the subjects were randomly distributed to the plots according to the randomized plots experiment design. Foliar applications of micro nutrients were performed 2 times with 15 days intervalls after the plants had 4-5 foliages. Water was sprayed to the control groups. In order to determine the effects of applications on the nutritional status of the plants, leaf samples were collected 3 weeks after following the second application. For leaf analysis, samples were washed with top and pure water then dried at 65±5°C and were grounded. Afterwards, samples were wet digested with microwave oven and filled up to 50 ml with pure water. Total nitrogen was analyzed according to Kjeldahl method. Phosphorus concentrations of samples were determined with a spectrophotometer (Shimadzu UV-1208) at 430 nm according to the vanadomolybdo phosphoric acid method. Potassium, Ca, Mg, Fe, Cu, Zn, and Mn concentrations were determined using atomic absorption spectrophotometer. Boron concentration of the leaf was measured using the same filtrate with ICP (Mills & Jones, 1996). To determine root weight and some quality characteristics, 10 plants from each plots were pulled out then brought up the laborotary immididatelly. In laboratory, roots were washed with water and weighted. After leves were separated from the root, some quality parameters were performed. Root weights were detemined by taking the avarages of ten roots.

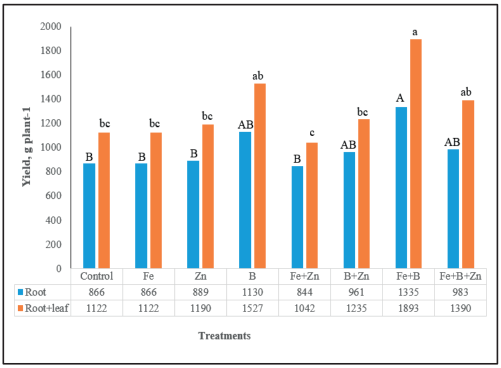
Total soluble solids (TSS) of beet juice were determined by using digital refractometer. The concentrations of sucrose and α -amino nitrogen were determined from beet brei. Sucrose percentage (%) was determined polarimetrically on lead acetate extract of fresh macerated roots by using automatic polarimeter (ATAGO AP 300, Japan), and α-amino nitrogen content was determined spectrophotometrically according to bluenumber method (ICUMSA, 2007).

Statistical analysis was performed using the MSTAT program for a one-way analysis of variance to determine significant differences at the 0.01% level. A Tukey test was conducted to identify significant differences among the treatments.

RESULTS AND DISCUSSIONS

Effect of treatments on yield of sugar beet

The yield of sugar beet has been affected by foliar applications of different micronutrients. As given in Figure 2, root and root+leaf weights were varied between 844 and 1335 g and 1042 and 1893 g per plant, respectively. While the most effective treatment was the combined application of Fe and B (Fe+B); control, Fe, Zn and Fe+Zn treatments were found to be the most ineffective treatments. The same treatments showed similar effect on the Root+leaf weights and Fe+B treatment was found the most effective. According to these results, it was observed that there was a difference of approximately 1.5 times for root and 1.8 times for root+leaf between the highest and lowest yield values. Results of both yield values showed that B and B combined other micro element combinations showed superior effects comparing to other treatments which B not included.



Fe: 500 ppm, Zn: 500 ppm, B:250 ppm, *means sharing the same latter are not significantly different (P>0.05)

Figure 2. Effect of foliar micro element treatments on the yield of sugar beet

Effect of treatments on mineral nutrition of sugar beet

Differences among the treatments on micro-element concentrations of leaf and root were found to be significant (Table 3). The lowest Fe, Mn, Zn, Cu and B concentrations in leaf were 60, 40, 31, 10.7 and 175 ppm, respectivelly. On the other hand the highest walues were 85 ppm for Fe, 53 ppm for Mn, 54 ppm for Zn, 16.3 ppm for Cu and 329 ppm for B.

Table 3. Effect of foliar micronutrient applications on microelement concentrations of sugar beet (mg kg^{-1})

Treatments	Fe	Mn	Zn	Cu	B
			Leaf		
Control	60 B	40 B	31 C	10.7 C	175 E
Fe	85 A	46 AB	33 C	16.3 A	202 DE
Zn	69 ABC	50 A	50 AB	13.7 B	183 E
B	72 AB	52 A	31 C	16.0 A	245 CD
Fe+Zn	77 AB	48 AB	43 B	16.3 A	266 BC
Zn+B	66 B	53 A	54 A	16.3 A	329 A
Fe+B	69 ABC	45 AB	31 C	12.7 BC	294 AB
Fe+Zn+B	61 B	46 AB	38 BC	11.3 C	313 A
			Root		
Control	313 AB*	12.5 A	41 B	24.5 B	14.7 B
Fe	313 AB	12.5 A	41 B	24.5 B	19.1 A
Zn	162 C	9.5 B	52 A	32.5 A	14.3 B
B	162 C	9.5 B	38 BC	26.5 B	19.8 A
Fe+Zn	335 A	9.5 B	51 A	35.0 A	14.5 B
Zn+B	132 C	12.5 A	37 BC	31.5 A	14.0 B
Fe+B	212 BC	7.0 C	36 C	21.0 B	16.5 AB
Fe+Zn+B	216 ABC	8.5 BC	47 AB	30.5 A	15.1 B

Fe: 500 ppm, Zn: 500 ppm, B:250 ppm, *means sharing the same latter are not significantly different (P>0.05)

When the results of sugar beet leaf micro elements were examined, it was revealed that the applications of Fe, Zn and B elements increased the amounts in the leaf. While the highest Fe content determined in the leaf was obtained with only 500 ppm Fe application, the second highest Fe content was obtained at the end of Fe+Zn application. On the other hand, low Fe values were measured in Fe+B and Fe+Zn+B mixtures. It was shown that the Fe concentration determined in the root increased in the application where Fe was together with Zn. Micro element concetrations in root varied between 132 and 335 ppm for Fe, 7.0 and 12.5 ppm for Mn, 36 and 52 ppm for Zn, 21.0 and 35.0 ppm for Cu and 14.3 and 19.8 ppm for B. The highest Fe concentrations was obtained with the combined applications of Fe and Zn (335 ppm) and followed by Fe applied and control (313 ppm) condition. While Fe+B and Fe+Zn+B combined treatments resulted in lower Fe concentrations the lowest Fe (132 ppm) were measured from the plants treated with Zn+B mixed appication. It was observed that Zn and B applications alone were among the applications that negatively affected the Fe conentration of the root. Foliar microelement applications either had no effect or had a negative effect on the Mn concentration of root. Comparing the control, while the applications of Zn, B, Fe+Zn, Fe+B and Fe+Zn+B negatively affected root Mn concentrations, Fe and Zn spreyings did not affect. Individual application of Zn and its combination with Fe (Zn+Fe) gave the highest Zn concentrations in root with the values of 52 and 51 ppm, respectively and followed by Fe+Zn+B with 47 ppm. The lowest Zn values were obtained in applications containing B alone and Zn+B and Fe+B combinations, and the values obtained from these applications were even below the control. Three of foliar fertilizations (Fe, B, Fe+B) did not effect root Cu concentration when compared the control and the lowes Cu (21 ppm) in root was measured from the plants treated with Fe+B. On the other hand Zn, Fe+Zn, Zn+N and Fe+Zn+B applications showed the positive impact on root Cu concentration. The highest Cu (35 ppm) was reached with the application Fe+Zn mixture. The highest B (19.8 ppm) was measured from the plants treated with solely B containg solution. The second highest B (19.1 ppm) was obtained with Fe application. The lowest root B concentration was obtained from the plants sprayed with Zn mixed with B. Compared to control, foliar application of Fe, Zn, B and their combinations showed a positive impact on leaf macronutrient concentrations generally. Leaf N, P K, Ca and Mg concentrations were found to be as 3.4%, 0.27%, 2.46%, 0.37% and 0.39% under control conditions. These values increased to 4.4 and 4.3% for N with Fe+B and Fe treatments, to 0.34% for P with Fe, Zn and Fe+Zn treatments, to 3.32% for K with Zn+B treatment, to 0.53% for Ca with Zn and Fe+Zn treatments and to 0.49% for Mg with Zn+B treatment. Leaf microelement applications either had no effect or had a negative effect on root N, P and Mg concentrations. On the other hand, root K and Ca concentrations were positively affected by Fe+Zn and Fe+Zn+B applications (Table 4).

Table 4. Effect of foliar micronutrient treatments on macroelement concentrations of sugar beet

Treatments	N	P	K	Ca	Mg
			Leaf		
Control	3.4 C	0.27 B	2.46 CD	0.37 B	0.39 B
Fe	4.3 A	0.34 A	2.60 C	0.43 AB	0.44 AB
Zn	3.7 BC	0.34 A	2.57 C	0.53 A	0.39 B
B	3.9 B	0.30 AB	2.56 C	0.46 AB	0.48 AB
Fe+Zn	3.7 BC	0.34 A	2.84 B	0.53 A	0.39 B
Zn+B	3.6 BC	0.27 B	3.32 A	0.50 AB	0.49 A
Fe+B	4.4 A	0.31 AB	2.30 D	0.36 B	0.47 AB
Fe+Zn+B	3.9 B	0.31 AB	2.42 CD	0.43 AB	0.42 AB
			Root		
Control	1.0 AB	0.42 AB	1.36 B	0.14 B	0.36 AB
Fe	1.0 AB	0.42 AB	1.36 B	0.14 B	0.36 AB
Zn	0.9 B	0.41 B	1.49 AB	0.15 AB	0.41 AB
B	0.9 B	0.38 B	1.64 AB	0.16 AB	0.37 AB
Fe+Zn	1.1 A	0.43 AB	2.12 A	0.21 A	0.45 A
Zn+B	1.0 AB	0.49 A	1.99 AB	0.19 AB	0.42 AB
Fe+B	1.0 AB	0.37 B	1.74 AB	0.17 AB	0.32 B
Fe+Zn+B	0.89 B	0.38 B	1.80 AB	0.18 AB	0.44 A

Fe: 500 ppm, Zn: 500 ppm, B:250 ppm, *means sharing the same latter are not significantly different (*P*>0.05)

Effect of treatments on some quality parameters sugar beet

The effects of foliar Fe, Zn and B spreying on some qulity parameters of root are presented in Table 5. Foliar applications of Zn, B, Zn+B, Fe+B and Fe+Zn+B significantly increased of α-amino N content up to 35% compared to control. On the other hand, Fe and Fe+Zn combination did not have significant affect. The most effective spreying on these increase was found to be sole B application. Foliar Zn and B individual treatments led to increase of reducing sugar content by up to 41 and 53%, respectively compared to control. The other treatments did not effect on reducing sugar contents in roots. Polar sugar content significantly increased with Fe+B (15.4%), Fe+Zn+B (15.0%) and Fe (15.0%) applications while it was around 14.0-14.6% under other treatments. Among the applications, only Fe+B mix treatments increased the TSS content, while Fe+Zn applications caused a decrease in TSS.

Table 5. Effect of foliar micronutrient treatments on α-amino N, reducing sugar, polar sugar and TSS values of root

Treatments	α-amino N (mmol 100 g^{-1})	Reducing sugar (%)	Polar sugar (%)	TSS (%)
Control	3.88 C	0.32 C	14.2 BC	20.0 BC
Fe	3.88 C	0.34 C	15.0 AB	21.1 AB
Zn	4.71 AB	0.45 AB	14.4 BC	19.7 BCD
B	5.22 A	0.49 A	14.6 ABC	19.2 CD
Fe+Zn	3.98 BC	0.39 BC	14.0 C	18.0 D
Zn+B	4.66 AB	0.37 C	14.4 BC	20.0 BC
Fe+B	4.76 A	0.38 BC	15.4 A	21.8 A
Fe+Zn+B	4.70 AB	0.39 BC	15.0 AB	19.5 BCD

Fe: 500 ppm, Zn: 500 ppm, B:250 ppm, TSS: total soluble solids, *means sharing the same latter are not significantly different (P>0.05)

Looking at the yield values, it was seen that the most effective individual application on both total yield and root yield was B. This situation shows that sugar beet responds more to B fertilization. This situation can be related to the low B content of the esperimental area. Again, it can be related to the fact that sugar beet is one of the plants with a high B requirement and therefore its need for B is higher than other micro elements. This can be explained with the specific effect of B on sugar beet growth. Draycott (2008) emphasized the crucial role of B as a trace element for sugar beet, as its inadequate supply can severely reduce root yield and quality. Also, other foliar spreyings containing B combinations with Fe and Zn showed positive impact on the yield of sugar beet. According to Masri & Hamza (2015), a higher concentration of micronutrients mixture resulted in a significant increase of 21.54% and 23.81% in sugar beet root weight, 28.00% and 24.40% in root yield, and 76.50% and 60.61% in sugar yield during the first and second growing seasons, respectively.

According to Mills & Jones (1996) micro nutrient sufficinecy ranges levels in sugar beet are between between 60-140 ppm for Fe, 26-600 ppm for Mn, 10-80 ppm for Zn and 30-200 for B. In other study (Haneklaus & Schnug 1998). it was raported that sufficiency ranges for Fe, Mn, Zn, Cu and B were between 80-200 ppm, 20-50 ppm, 40-60 ppm, 10-20 ppm and 24-40 ppm, respectively. Depending on the above sufficiency ranges, it can be said that leaf micronutrient concentrations for Fe and Zn under control conditions were around the criticial levels, and they increased up to sufficency levels with Fe, and Zn applications alone and their Fe+Zn and Zn+B combinations. However, triple combinations Fe, Zn and B did not affect positively leaf Fe and Zn concentrations. This may be due the triple competition of competition during leaf entry and transport stages (Adamec, 2002). On the other hand, non-favouarble properties such as pH and EC of multi-element solutions used for spraying may be effective on this subject (Erdal, 2023). Contrary to Fe and Zn, it has been observed that two or three microelement mixtures including

B are more effective on plant B nutrition than its individual effect. This may be positive effect of Fe and Zn on B nutrition of plant. Similarlly, in a study it was found that Fe, B and Zn foliar application increased their own concentrations and accompaning micro elements of cowpea

(Salih, 2013). Results showed that although Cu and Mn fertilization were not done, Fe, Zn and B spraying lead to increase their concentrations in leaves, althouh they were not sprayed. This situation can be explained by the fact that plants benefit more from other nutrients as a result of increased plant growth resulting from microelement fertilization. When a general evaluation is made, the results obtained show that the best application on leaf Fe content is the application of Fe alone, while the mixture of Fe with Zn also contributes to the plant's Fe nutrition. Similarly, in a study conducted by Fouda & Abd-Elhamied (2017), it was reported that both individual and combined application of Zn and Fe positively affected the Fe and Zn nutrition of cowpea. Niyigaba et al., (2019) and Pal et al. (2021) reported that Fe was slightly improved by an application of Zn in wheat and chickpea. It was observed that the amount of Zn in the root increased depending on the amount of Zn in the leaf. A possible reason for this increment is that foliar-applied can be readily translocated into other organs such as grain and root (Haslett et al., 2001; Erenoglu et al., 2011). An increase of Fe by Zn application was also reported by Wang et al. (2015), where the foliar application of Zn resulted in a significant increase in Fe concentration. Despite a strong negative correlation between Zn and Fe we did not observed strong negative effect of Zn on Fe nutrition of sugar beet especially for root. This may be related to concentrations of nutrient in the solution and source of nutrients etc. Increase in Fe concentration can olso be related improved plant metabolism and growth which encouruge more Fe uptake under Zn applied condition (Singh et al., 2013). Application of Zn together B was the most effective on the leaf Zn concentrations followed by the sole application of Zn. Having the same or higher effect of Zn+B with solely Zn treatment can be explained that B did not affect or increased Zn absorption of leaf. Similarly, Zn deficiency enhanced B concentration in wheat (*Triticum aestivum*) grown on Zn deficient soils (Singh et al, 1990). Sinha et al., (2000) noted a synergistic interaction between Zn and B in mustard (*Brassica nigra*) when both nutrients. Hosseini et al. (2007) reported that there was a significant B and Zn interaction on corn growth and tissue nutrient concentration which were rate dependent. They declare that in general, the effect was antagonistic in nature on nutrient concentration and synergistic on plant growth. As in Zn, the highest B was obtained when it was applied as mixture of Zn. The reasons of this can be explained with the similar approaches as mentioned for Zn. Although they did not applied as fertilizers, concentrations of Mn and Cu in the leaf and root increased with foliar Fe, Zn and B applications in most cases. This can be explained more nutrient uptake from the soil because of enhanced plant metabolism and growth with foliar fertilization. Foliar applications of Fe, Zn ad B lead to increase of these nutrient in root as well. While the highes B and Zn were translocated when the they were applied alone, the highest Fe translocation was determined from combined application of Fe and Zn. Sole application of Fe played a significant role on B translocation as much as sole B application. This can be explained by the fact that iron nutrition promotes the plant's B uptake from the soil and B translocation from leaf to root. Previous research has reported the synergistic effect of Zn and Cu (Aref, 2012; Xia *et al.* 2019). Zinc may have facilitated the uptake and translocation of Mn and B within the plant, either directly or indirectly through physiological processes. This resulted in increase in leaf Mn content with Zn application (Stewart et al., 2021). Further studies are required to elucidate these mechanisms and enhance our understanding. Results of leaf macronutrien cocentrations showed that the lowest values were determined from the control plants generally. Althought not for all treatments, individual applications of Zn, Fe and B and their mixtures increased the macro element concentrations of beet leaves on the macro elements determined in the leaf. These increases were approximately 20% for N, P and Ca and approximately 26% for potassium compared to the control. As in expressed by Marschner (2011), this may be due to the positive impact of foliar micronutrient fertilization on macronutrient concentrations are likely due to increased root growth induced by micronutrients (Srivastava et al., 2016). It was observed that foliar micro element fertilization had no effect on N and P, which are macro elements determined in the root, whereas Fe+Zn application had a positive effect on K, Ca and

Mg, and Fe+Zn+B application had a positive effect on Mg concentration in root. These increases macronutrients in the leaf and root of sugar beet with foliarly applied micronutrients can be explained with the increased plant growth and metabolisms resulting more nutrient uptakes from the soil it grown (Mengel & Kirkby, 1987; Kacar & Katkat, 2010; Marschner, 2011; Erdal, 2023). Results showed that B application had an specific effect on the of α-amino N concentratin of sugar beet. Similarlly, Nemeata Alla (2017) indicated that increasing levels of B foliar application increased α-amino N connet of sugar beet regularlly. As mentioned preivios studies, most of other quality parameters have been affected by foliar micronutrients especcially B and their combinations. These results can be explained with the privite role of B and other micrunutrients on sugar metabolism (de Oliveira Gondim et al., 2015; Rahımı et al., 2016; Rahimi et al., 2018; Pişkin, 2022).

It is thought that the high α-amino N content in B and Fe+B applications is related to the fact that these applications also significantly increase leaf N concentration. In fact, excessive N fertilization in sugar beet causes an increase in the α-amino N ratio. Excess N taken into the plant is accumulated as α-amino N in the beet top to be used later when needed. Excessive N addition was also reported to be responsible for high α-amino N concentrations in sugar beet roots (Hassan & Mostafa, 2018). Some researchers have also reported that B applications increase the amino N content in sugar beet (El- Kammash, 2007; Abbas et al., 2014). It has also been reported that foliar Fe and B applications increased the α-amino N content in sugar beet (Aghdam & Valilue, 2023). Sugar accumulates in the roots in the form of sucrose as a product of photosynthesis and sucrose is cleaved, mainly by sucrose synthase and converted into reducing sugars (glucose + fructose) for use in metabolic activity (El-Geddawy et al., 2024). The increase in the amount of reducing sugar with Zn and B applications in the study was probably due to the increase in metabolic activity in sugar beet. Boron through its role in cell wall synthesis, water uptake in plants; and Zn by activation of enzymes, strengthening of cell wall and cell division play an important role on yield and quality (Kuldip Kumar & Rajpaul Yadav, 2016). Increased nutrient uptake in different application of micronutrients might be responsible for the enhancement of sugar percentage and TSS because it has been established that there is a positive correlation between plant nutrient uptake and sugar content (Lehrsch et al., 2014; Zewail et al., 2020). Boron plays a significant role in vital activities of plants, e.g., the metabolism and translocation of sugars and hydrocarbon-containing compounds (Armin and Asgharipour, 2012). Thus, this may increase photosynthesis capacity, and the allocation of more assimilates to the metabolism of sugar synthesis in plants like sugar beets (Zewail et al., 2020; Nasar et al., 2021). The role of Fe in chloroplast ultra-structure, protein and lipid composition of thylakoid membranes, in addition Fe enhance electron transport capacity in thylakoidsand ATP formation (Arulanantham et al., 1990), consequently enhance growth, yields and quality of sugar beet (Abdelaal et al., 2015; Masri & Hamza, 2015 and Rassam et al., 2015). Amin et al. (2013) reported that the sugar yield increased from 8.64 tons per hectare in the control treatment to 8.79 and 9.17 tons per hectare with one and two foliar sprays of low consumption elements, respectively. Yarnia et al. (2008) also reported that the use of low consumption elements caused a 46% increase in sucrose yield compared to the control treatment. Abdelaal et al. (2015) revealed that spraying with B, Fe, Zn and Mn as mixture recorded the highest sucrose percent, root and sugar yields. Manal (2011) showed that spraying with solution of micronutrients mixture (B + Zn + Mn + Fe) significantly increased sucrose % and yields of root and sugar.

CONCLUSIONS

As conlusion, it can be said that foliar application of micronutrients positively affected the growth of sugar beet. While foliar Fe, Zn and B and their combination primarily increased the plant Fe, Zn and B concentrations, foliar sprayings also had a positive effect on some other nutritional elements which are not included nutrient solutions. In addition, micronutrient foliar spreyings espicially B and its combinations with Fe and Zn positivelly affected some root quality paramerets. Another noteworthy result is that there was no strict antogonism among the nutrients when applied from the leaves which we expected to have an antagonistic effects under soil conditions.

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HEREDITY OF MAIN EAR GRAIN WEIGHT IN F_1 OF SOFT WINTER WHEAT ACCORDING TO GENOTYPE OF INITIAL FORMS AND HYDROTHERMAL CONDITIONS OF THE YEAR

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Abstract

In 2020-2022, the types of inheritance of grain weight of the main ear in F_1 were studied during the hybridisation of short, medium, and tall varieties of soft winter wheat. The obtained hybrids formed a grain weight of the main ear ranging from 1.48 g to 3.01 g, while the parental forms ranged from 1.24 g to 2.05 g. The determined indices of the degree of phenotypic dominance (hp = -23.0 to 273.0) indicate significant differentiation. In most cases, the grain weight of the main spike was inherited with positive superdominance (hp = 1.3 to 273.0), accounting for 86.5% of hybrids. Negative dominance was observed in six hybrids (5.8%), partial positive dominance in four (3.8%), intermediate inheritance in three (2.9%), and partial negative inheritance in one hybrid. Over the three years, heredity by the type of positive superdominance was observed in 21 out of 36 crossing combinations, predominantly when the maternal form was one of the local varieties, Bilotserkivska semi-dwarf and Lisova Pisnya. The conducted studies indicate a significant influence of the parental components of hybridisation and the hydrothermal conditions of the year on the formation of grain weight of the main ear in F_1, as well as on the determined indices of the degree of phenotypic dominance.

***Key words**: hybrids, varieties, grain weight of the main ear, degree of phenotypic dominance.*

INTRODUCTION

Wheat, as one of the oldest and most valuable food crops, remains a key component of food security for a significant part of humanity under modern conditions and plays an important role in Ukraine's export potential (Lotysh, 2018). To meet the growing demand driven by the increasing global population, it is essential to find ways to enhance the production of winter soft wheat grain in the coming decades (Röder et al., 2014). As the productivity of modern varieties has increased, the problem of genetically enhancing their resistance to biotic and abiotic stress factors that can significantly reduce yields has become more actuality (Sarto et al., 2017).

The breeding of new high-yielding adapted winter wheat varieties is of primary importance for achieving food security in Ukraine, as it is at great risk due to the exhaustion of natural resources and climate change (Yurchenko & Voloshchuk, 2014). Wheat is affected by climatic conditions characterised by an increase in the frequency of extreme factors in recent years, which determine plant phenology, sowing dates, the duration of ontogenetic stages and, finally, grain yields (Raza et al., 2015; Wang Bin et al., 2019; Shiferaw et al., 2013; Yujie et al., 2021; Lozinskiy et al., 2023; Ustinova et al., 2024).

High temperatures affect crop productivity by accelerating phenological development. The most pronounced effect of high temperatures on wheat growth is a significantly shorter grain filling time (Tashiro & Wardlaw, 1989). It is therefore very important to closely monitor those climate changes that have the greatest impact on winter wheat yields in order to make timely adjustments to the technology of its cultivation (e.g. variety selection, sowing and harvesting dates). This close monitoring and rapid response will help to mitigate the potentially adverse effects of climate change on wheat production (Tao et al., 2008).

In recent years, meteorological conditions in Ukraine have become more diverse, with significant extremes that cause the actual climatic component to deviate from the optimum for wheat plant growth and development (Yarosh &

Ryabchun, 2021; Ustinova et al, 2024). Accordingly, the genetic potential of soft winter wheat varieties is realised only by 50-60% (Tao & Zhang, 2013). In order to realise the genetic potential of new varieties in production, it is necessary that their biological characteristics are best adapted to both climatic conditions and the technological capabilities of farms. Information on the dynamics of climate change allows breeders to adapt the model of the future variety and use it in the development of environmentally friendly varieties (Starichenko et al., 2014). However, recently, not only climate change, but also inappropriate use of available varietal resources has created obstacles to the realisation of the genetic potential of new winter wheat varieties (Ovezmyradova et al., 2020). One of the most common methods of creating breeding material and varieties of soft winter wheat is intraspecific hybridization with subsequent selection of breeding valuable recombinants (Burdeniuk-Tarasevych & Lozinsky, 2015; Vlasenko et al., 2015; Kolomiets et al., 2018). The crossing is not just a combination of traits of the parental components, but the basis of formation, which allows concentrating the desired economically valuable traits in one genotype. At the same time, the formation process is determined by both inheritance factors and environmental conditions (Pirych et al., 2021; Ustinova et al, 2024).

Knowledge of the patterns of variability of valuable economic traits and their inheritance by descendants during hybridisation makes it possible to select pairs for crossing more efficiently and obtain preliminary information about the possible end result from early hybrid generations (Khomenko, 2021; Luchna, 2013). Studies conducted to determine the degree of phenotypic dominance in hybrids confirm the importance of its use in the selection of parental forms of crossing and at the same time rapid evaluation of hybrid generations (Ustinova et al., 2022). However, the highest effect of heterosis is observed in the hybridisation of ecologically and geographically distant forms (Labroo et al., 2021).

In wheat breeding, the grain weight of the main spike has always been one of the most important indicators, because this trait, together with productive bushiness, determines the productivity of the plant (Lozinskyi & Filitska, 2023; Ustinova et al., 2024). Grain weight of the main ear of soft winter wheat is a genetically determined trait that is influenced by environmental conditions and is realised in the interaction ‘genotype - year conditions’ (Havryliuk & Kovalyshyna, 2024). The most common type of heredity of grain weight of the main ear in F_1 winter wheat is positive superdominance, which is found in 82.5% of hybrids (Lozinsky & Ustinova, 2021).

The study of the norms of response of different varieties of soft winter wheat to biotic and abiotic environmental factors, the nature of manifestation and interrelationships of quantitative traits is the basis for the targeted use of certain new varieties in adaptive breeding programmes (Litvinenko, 2010).

MATERIALS AND METHODS

In the experimental field of the Research and Production Centre of Bila Tserkva National Agrarian University (Ukraine), 36 crossing combinations obtained by hybridizing winter wheat varieties of different heights were studied in 2019-2022. The varieties were classified into height groups as follows:

- Short (Group II, 66-80 cm): Bilotserkivska semi-dwarf, Sonechko, Smuglyanka;
- Medium (Group I, 81-95 cm): Donska semi-dwarf, Lisova Pisnya;
- Medium (Group II, 96-110 cm): Stolichna, Pisanka, Vidrada, Albatros Odeskyi;
- Tall (Group I, 111-125 cm): Odeska 267, Lastivka Odeska, Pylypivka.

The F_1 seeds were sown using a manual seeder according to the following scheme: maternal form (♀), hybrid, and paternal form (♂), with three rows of each component, each one metre long and with an inter-row spacing of 15 cm. For all cross combinations and their parental forms, identical plot sizes were maintained throughout the years of research. The wheat hybrids studied in 2020, 2021, and 2022 were created in 2019, 2020, and 2021, respectively.

The hybrid generation was studied using the pedigree method. Throughout the growing season, phenological observations were conducted, and after full ripeness was reached, a biometric analysis was performed on an average sample of 25 plants in triplicate, following the methodology of Tkachyk et al. (2016).

Winter soft wheat was sown in the last days of the third decade of September to early October. The preceding crop was mustard for grain. The soil of the experimental field is classified as typical deep low-humus chernozem.
The degree of phenotypic dominance (**h_p**) for the length of the main ear in F_1 was determined according to B. Griffing (1950).

$$hp = \frac{(F1 - MP)}{(BP - MP)}$$

where: hp - degree of domination; F_1 - average arithmetic value of the hybrid; BP - arithmetic mean of the parental component with the stronger manifestation of the trait; MP - the average arithmetic value of the indicator of both parental forms.
The data obtained were grouped by classification G. M. Beil, R. E. Atkins (1965): positive dominance (heterosis) hp > +1; partial positive dominance $+0.5 < hp \leq +1$; intermediate inheritance $-0.5 \leq hp \leq +0.5$; partial negative inheritance $-1 \leq hp < -0.5$; negative dominance (depression) hp < –1.
To characterize the weather conditions of the years of soft winter wheat cultivation, the average monthly hydrothermal coefficient was calculated (HTC) (Pol'ovij et al., 2004). The following differentiation of the HTC indicators was adopted: < 0.4 - very strong drought; 0.4-0.5 - severe drought; 0.5-0.6 - medium drought; 0.7-0.9 - weak drought; 1.0-1.5 - sufficiently humid; > 1.5 - extremely humid.
The results of the experimental data were statistically processed using the Statistica 12.0 programme.
In September 2019-2021, the actual amount of precipitation was 19.2 mm, 26.7 mm, and 16.8 mm, which was 15.8 mm, 8.3 mm, and 18.2 mm less than the long-term average, respectively. Due to insufficient precipitation in these years, the wheat seedling stage was observed on 8 October in 2019 and 2020, and on 10 October in 2021.
The duration of the autumn vegetation period of winter wheat was 44 days in 2019, 38 days in 2020, and 49 days in 2021, with dormancy beginning on 21 November, 14 November, and 29 November, respectively. In general, autumn vegetation occurred at higher average air temperatures: 9.6°C (2019), 8.5°C (2020), and 5.7°C (2021), compared to the long-term average for this period of 4.7°C, 5.0°C, and 3.9°C, respectively (Table 1).

Table 1. Meteorological conditions in 2019-2022 (according to the Bila Tserkva meteorological station)

Month	Decade	Precipitation, mm					Air temperature, °C				
		Year				long-term average	Year				long-term average
		2019	2020	2021	2022		2019	2020	2021	2022	
September		19.2	26.7	16.8		35.0	15.3	17.3	12.7		13.8
October	I	5.7	27.3	0.0		11	10.3	16.0	7.3		10.1
	II	0.0	62.7	0.6		10	13.3	11.6	6.7		8.1
	III	0.4	6.8	0.6		12	8.2	10.4	7.6		5.4
November	I	6.7	12.5	7.2		13	9.5	7.0	6.7		3.4
	II	10.1	11.4	5.6		15	7.3	1.5	2.7		1.9
	III	6.6	3.3	7.3		13	-1.9	2.0	4.6		0.7
December		35.1	33.0	49.8		44.0	2.5	-0.5	-1.4		0.4
January			22.6	40.0	30.5	35.0		0.4	-2.6	-1.4	-5.9
February			38.4	47.7	10.2	33.0		2.2	-4.6	1.7	-4.4
March			17.2	21.2	16.0	30.0		5.9	1.8	1.7	0.3
April	I		0.0	8.6	14.0	14.0		7.9	5.9	7.0	7.0
	II		5.5	13.5	7.2	17.0		8.0	8.1	6.5	7.8
	III		7.7	6.8	18.6	16.0		11.7	8.3	10.8	10.4
May	I		30.8	24.9	0.0	16.0		12.8	12.0	12.8	13.5
	II		17.6	26.5	2.7	12.0		13.2	14.5	14.9	15.3
	III		53.9	47.9	32.4	18.0		11.5	15.4	15.6	15.8
June	I		7.1	6.3	2.8	23.0		18.5	16.1	20.4	17.3
	II		50.4	28.3	1.2	27.0		23.2	20.0	20.6	17.4
	III		3.2	0.7	14.6	23.0		22.0	23.6	21.3	18.7
July	I		36.6	11.3	0.8	35.0		21.3	22.6	21.8	18.5
	II		6.3	30.0	24.1	24.0		19.8	24.6	17.6	19.4

The amount of precipitation during the autumn growing season of wheat, with the exception of 2020 (115.3 mm), was significantly lower than the long-term average. Specifically, during the autumn growing season, the actual amount of precipitation was 22.9 mm in 2019 and 21.3 mm in 2021, compared to the long-term averages of 61.0 mm and 74.0 mm, respectively.

During the winter dormancy period, which lasted 99 days in 2019/2020, 135 days in 2020/2021, and 113 days in 2021/2022, the actual amount of precipitation was 102.7 mm, 150.6 mm, and 109.0 mm, respectively, which was 13.3 mm, 18.4 mm, and 12.0 mm less than the long-term average for the corresponding periods. The average air temperatures during the winter dormancy period were +1.4°C (2019/2020), -2.3°C (2020/2021), and -0.5°C (2021/2022), exceeding the long-term average by 1.5°C, 0.9°C, and 2.3°C, respectively. These conditions contributed to the successful overwintering of soft winter wheat.

The resumption of spring vegetation in 2020 occurred on February 28, with a gradual increase in the average monthly temperature regime in March and April. At the same time, the average monthly temperature in May was 2.4°C lower than the long-term average, while in June and the first two decades of July, it exceeded the long-term average by 3.4°C and 1.6°C, respectively. From the beginning of spring vegetation renewal to the first decade of May, 30.4 mm of precipitation fell, which accounted for only 39.5% of the long-term average. In May, 102.3 mm of precipitation was recorded, compared to the long-term average of 46.0 mm, significantly improving the moisture supply for wheat plants. The vegetation of winter wheat in June 2020 occurred under mild drought conditions (HTC = 0.92), while grain ripening in the second decade of July (HTC = 0.32) took place under extremely severe drought conditions.

The growth and development of winter wheat in 2021, from the resumption of spring vegetation (March 28), occurred under a gradual increase in temperature during April, May, and the first decade of June. However, in the second and third decades of June, as well as the first and second decades of July, the actual air temperature significantly exceeded the long-term average by 2.6°C, 4.9°C, 4.1°C, and 3.2°C, respectively. The amount of precipitation was lower than the long-term average in April, June, and the first two decades of July by 18.1 mm, 37.7 mm, and 17.7 mm, respectively, while in May, it was higher by 53.0 mm. The calculated hydrothermal coefficient for June and the first decade of July (HTC = 0.55) indicates moderate drought and unfavourable conditions during the grain formation and filling period of wheat.

During the resumption of spring vegetation in 2022 (March 22), a gradual increase in temperature was observed until the end of May. At the same time, the average monthly temperature in June (20.8°C) exceeded the long-term average by 3.0°C, while in the first decade of July, it was higher by 3.3°C. The actual amount of precipitation in April (39.8 mm), May (35.1 mm), June (18.6 mm), and the first two decades of July (24.9 mm) was lower than the long-term averages by 7.2 mm, 10.9 mm, 54.4 mm, and 34.1 mm, respectively.

Our research established that after the resumption of spring vegetation in each of the studied years, the growth and development of soft winter wheat plants lasted from 30 days (2022) to 40 days (2020) under insufficient precipitation. The most critical conditions for grain formation were observed in 2022 when wheat vegetation, from early June to the second decade of July, lasted 40 days under extreme drought conditions (HTC = 0.31), which significantly affected the grain weight per ear.

RESULTS AND DISCUSSIONS

When using the short-statured variety Bilotserkivska semi-dwarf as the maternal form in hybridisation during 2020-2022, F_1 hybrids formed a grain weight of the main ear ranging from 1.58 g to 2.54 g, exceeding the parental forms in 20 out of 24 hybrids. In 2021 (2.01 g) and 2020 (1.97 g), the obtained hybrids of winter wheat exhibited significantly higher average grain weight of the main ear compared to 2022, where the average was 1.76 g (Table 2). The grain weight in F_1 hybrids in 2020 exceeded the average for F_1 in the following combinations: Bilotserkivska semi-dwarf/ Albatross Odeskyi (2.19 g), Bilotserkivska semi-dwarf/Pylypivka (2.23 g), Bilotserkivska semi-dwarf / Vidrada (2.36 g).

Table 2. Grain weight of the main ear (g) and the degree of phenotypic dominance in F_1 hybrids obtained using the short variety Bilotserkivska semi-dwarf as the maternal form.

Variety, hybrid	2020		2021		2022	
	$\bar{x}\pm S\bar{x}$	h_p	$\bar{x}\pm S\bar{x}$	h_p	$\bar{x}\pm S\bar{x}$	h_p
♀ Short (Group II) / ♂ Short (Group II)						
Bilotserkivska semi-dwarf	1.52±0.04	-	1.55±0.03	-	1.47±0.05	-
Bilotserkivska semi-dwarf / Sonechko	1.93±0.14	14.7	1.98±0.11	4.3	1.39±0.09	-7.0
Sonechko	1.46±0.05	-	1.72±0.04	-	1.44±0.04	-
♀ Short (Group II) / ♂ Medium (Group I)						
Bilotserkivska semi-dwarf / Donska semi-dwarf	1.75±0.11	4.3	1.60±0.07	-0.6	1.69±0.08	0.8
Donska semi-dwarf	1.38±0.05	-	1.83±0.04	-	1.72±0.04	-
Bilotserkivska semi-dwarf / Lisova Pisnya	1.58±0.10	5.0	1.95±0.11	1.4	1.73±0.12	3.3
Lisova Pisnya	1.54±0.06	-	1.88±0.07	-	1.59±0.06	-
♀ Short (Group II) / ♂ Medium (Group II)						
Bilotserkivska semi-dwarf / Albatros Odeskyi	2.19±0.16	68.0	2.07±0.10	6.4	1.78±0.10	29.0
Albatros Odeskyi	1.49±0.04	-	1.69±0.06	-	1.50±0.05	-
Bilotserkivska semi-dwarf / Stolichna	1.92±0.15	4.3	1.83±0.12	0.1	1.71±0.13	5.0
Stolichna	1.28±0.05	-	2.04±0.07	-	1.55±0.06	-
Bilotserkivska semi-dwarf / Vidrada	2.36±0.14	19.8	2.34±0.11	3.2	2.54±0.25	7.9
Vidrada	1.61±0.05	-	1.93±0.05	-	1.71±0.05	-
♀ Short (Group II) / ♂ Tall (Group I)						
Bilotserkivska semi-dwarf / Odeska 267	1.81±0.14	3.1	2.17±0.15	5.6	1.62±0.14	2.0
Odeska 267	1.24±0.04	-	1.83±0.06	-	1.57±0.04	-
Bilotserkivska semi-dwarf / Pylypivka	2.23±0.22	143.0	2.16±0.11	3.0	1.62±0.09	2.5
Pylypivka	1.51±0.04	-	1.86±0.05	-	1.56±0.05	-
The average for F_1	1.97	-	2.01	-	1.76	-

In 2021, the following combinations exceeded the F_1 average: Bilotserkivska semi-dwarf / Albatros Odeskyi (2.07 g), Bilotserkivska semi-dwarf/Pylypivka (2.16 g), Bilotserkivska semi-dwarf/Odeska 267 (2.17 g), Bilotserkivska semi-dwarf / Vidrada (2.34 g). In 2022, the F_1 average grain weight was exceeded by two hybrids: Bilotserkivska semi-dwarf/Albatross Odeskyi (1.78 g) and Bilotserkivska semi-dwarf / Vidrada (2.54 g). The average grain weight of the main ear for 2020-2022 (1.91 g) was exceeded by 12 hybrids, six of which resulted from crosses with tall and medium-height varieties of group II.

The variation in the experiment ranged from 0.15 g to 0.61 g, with the least variability in grain weight of the main ear (from 0.15 g to 0.21 g) observed in the contrasting meteorological conditions of 2020-2022 for the following crossing combinations: Bilotserkivska semi-dwarf/Donska semi-dwarf, Bilotserkivska semi-dwarf/Vidrada, Bilotserkivska semi-dwarf/Stolichna. The average range of the trait (0.37 g to 0.41 g) was observed in the combinations Bilotserkivska semi-dwarf/Lisova Pisnya and Bilotserkivska semi-dwarf/Albatross Odeskyi. All other combinations showed significant variability, ranging from 0.55 g to 0.61 g.

In 2020–2022, positive superdominance was identified in 20 out of 24 hybrids. Partial positive dominance, intermediate inheritance, partial negative inheritance, and negative superdominance were observed in one hybrid for each type of grain weight inheritance of the main ear (Figure 1).

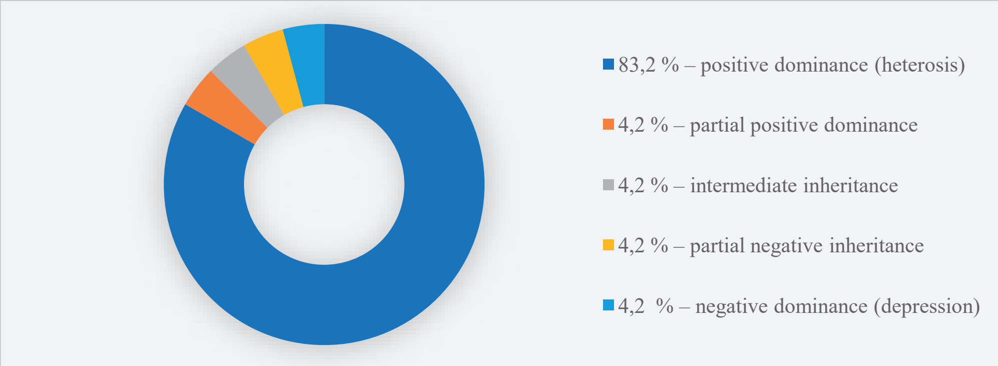

Figure 1. Types of heredity of grain weight per main ear in F_1 when using a stunted variety as a mother form Bilotserkivska semi-dwarf (2020-2022)

The degree of phenotypic dominance is a valuable genetic indicator that reflects the extent of inheritance of a particular quantitative trait in F1 and provides insights into its genetic control, as well as the potential to predict the effectiveness of selection in subsequent hybrid generations (Dubovyk et al., 2019). In hybrids exhibiting positive overdominance for productivity elements, the selection of transgressive forms in the second-generation population is possible, leading to individuals that surpass the parental forms (Samoylyk et al., 2023; Ustynova et al., 2024a; Ustynova et al., 2024b).

When using medium-height varieties of group I as the maternal form, the grain weight of the main ear in F_1 ranged from 1.58 g to 3.01 g, and in most cases exceeded the values observed in hybrids where the maternal component was the short-statured variety Bilotserkivska semi-dwarf**.** The parental forms for grain weight of the main ear exceeded the values of 19 out of 21 hybrids (Table 3).

Table 3. Grain weight per main ear (g) and the degree of phenotypic dominance of F_1 obtained by using the as a mother form varieties Medium Group I

Variety, hybrid	2020		2021		2022	
	x̄±Sx̄	h_p	x̄±Sx̄	h_p	x̄±Sx̄	h_p
♀ Medium (Group I)/ ♂ Short (Group II)						
Donska semi-dwarf / Sonechko	1.97±0.14	18.3	2.18±0.11	7.8	1.72±0.08	1.0
Lisova Pisnya / Smuglyanka	2.17±0.22	11.4	2.32±0.19	4.4	1.90±0.14	6.8
Smuglyanka	1.65±0.05	-	2.05±0.06	-	1.67±0.04	-
♀ Medium (Group I) / ♂ Medium (Group I)						
Donska semi-dwarf / Lisova Pisnya	2.11±0.25	8.1	2.35±0.12	24.5	1.72±0.09	1.0
♀ Medium (Group I)/ ♂ Medium (Group II)						
Donska semi-dwarf / Albatros Odeskyi	2.54±0.30	22.0	2.40±0.12	9.0	1.58±0.09	-0.3
Donska semi-dwarf / Stolichna	2.11±0.21	15.6	2.18±0.20	2.4	1.81±0.12	2.1
Donska semi-dwarf / Vidrada	2.45±0.27	8.6	2.53±0.13	16.0	1.60±0.12	-23.0
Lisova Pisnya / Albatros Odeskyi	2.13±0.14	30.5	2.51±0.15	8.0	1.67±0.09	3.0
Lisova Pisnya / Stolichna	2.50±0.19	8.4	2.87±0.22	11.4	1.86±0.22	14.5
Lisova Pisnya / Vidrada	1.86±0.14	9.3	2.04±0.08	6.5	1.82±0.12	2.8
♀ Medium (Group I)/ ♂ Tall (Group I)						
Donska semi-dwarf / Odeska 267	2.47±0.19	16.6	2.67±0.11	84.0	1.63±0.10	-0.3
Donska semi-dwarf / Pylypivka	2.53±0.33	18.0	2.46±0.09	61.0	2.08±0.17	5.5
Lisova Pisnya / Odeska 267	2.20±0.52	5.4	2.61±0.22	25.3	2.10±0.18	52.0
Lisova Pisnya / Pylypivka	3.01±0.66	148.0	2.55±0.18	68.0	1.84±0.09	26.0
The average for F_1	2.31	-	2.44	-	1.79	-

The most favourable conditions for the formation of grain weight in most F_1 hybrids (ranging from 2.18 g to 2.87 g) were observed in 2021, with the average value (2.44 g) being exceeded in seven out of 13 hybrids obtained by crossing medium-height varieties from group I with medium-height varieties from group II and tall varieties. In 2020, the average grain weight of the main ear in F_1 was slightly lower at 2.31 g, with significantly greater variability, ranging from 1.86 g to 3.01 g. A higher-than-average grain weight was observed in the following combinations: Donska semi-dwarf/Vidrada (2.45 g), Donska semi-dwarf/Odeska 267 (2.47 g), Lisova Pisnya/Stolichna (2.50 g), Donska semi-dwarf /Pylypivka (2.53 g), and Lisova Pisnya/ Pylypivka (3.01 g). The lowest grain weight in F_1 was recorded in 2022, with the average value (1.79 g) being exceeded in seven hybrids, and higher than the parental forms in eight out of 13 combinations.

With variability in grain weight of the main ear ranging from 0.22 g to 1.17 g in F_1, the least variability was observed in the following crossing combinations: Lisova Pisnya/ Vidrada, Donska semi-dwarf/Stolichna, Lisova Pisnya/ Smuglyanka, Donska semi-dwarf/Pylypivka, Donska semi-dwarf/ Sonechko, and Lisova Pisnya/Odeska 267. Average variability was recorded in the combinations Donska semi-dwarf/Lisova Pisnya (0.63 g) and Lisova Pisnya/Albatross Odeskyi (0.84 g), while significant variability was observed in other crossing combinations, ranging from 0.93 g to 1.17 g.

In all hybrids in 2020 and 2021, the determination of grain weight followed positive superdominance (hp = 4.4 to 148.0). In 2022, positive superdominance (hp = 2.1 to 52.0) was identified in eight hybrids, while two F_1 hybrids inherited the trait through partial positive dominance (hp = 1.0) and intermediate inheritance (hp = -0.3). The combination Donska semi-dwarf/Vidrada exhibited negative superdominance (hp = -23.0) (Figure 2).

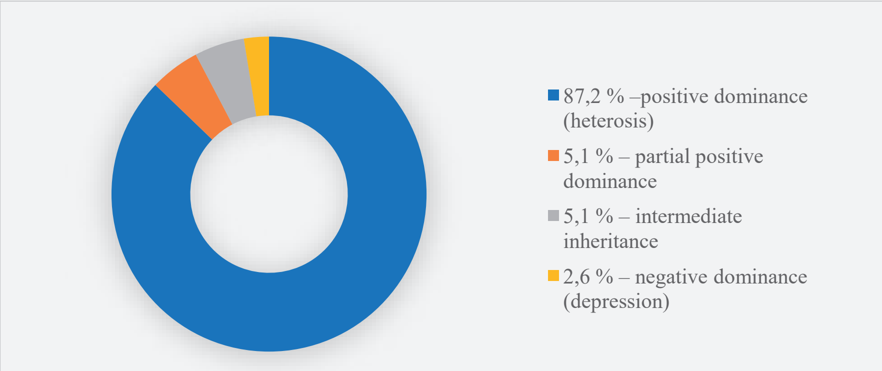

Figure 2. Types of heredity of grain weight from the main ear in F1 using the mother form of varieties of Medium Group I (2020-2022)

Hybrids obtained using medium-height varieties from group II as the maternal form formed grain weight of the main ear ranging from 1.48 g (Vidrada/Pylypivka) to 2.98 g (Pisanka/Vidrada) in the years of the study. Higher values, compared to the original components of the hybridisation, were observed in 29 out of 32 hybrids (Table 4).

Table 4. Grain weight per main spike (g) and the degree of phenotypic dominance in F1 obtained by using as a mother form varieties of medium group II

Variety, hybrid	2020 p.		2021 p.		2022 p.	
	$\bar{x}\pm S\bar{x}$	h_p	$\bar{x}\pm S\bar{x}$	h_p	$\bar{x}\pm S\bar{x}$	h_p
♀ Medium (Group II)/ ♂ Short (Group II)						
Albatros Odeskyi / Smuglyanka	2.02±0.29	5.6	2.26±0.13	2.2	1.69±0.11	1.3
♀ Medium (Group II)/ ♂ Medium (Group II)						
Albatros Odeskyi / Stolichna	2.49±0.23	11.0	2.24±0.10	2.2	2.09±0.21	28.0
Albatros Odeskyi / Vidrada	2.21±0.19	11.0	2.58±0.32	6.2	2.16±0.32	5.5
Stolichna / Pisanka	2.87±0.54	8.4	2.13±0.48	2.1	2.11±0.44	57.0
Pisanka	1.62±0.05	-	1.88±0.06	-	1.53±0.04	-
Stolichna / Vidrada	2.47±0.26	6.4	2.86±0.33	17.4	2.35±0.27	9.0
Pisanka / Vidrada	2.98±0.34	273.0	2.34±0.20	21.5	-	-
♀ Medium (Group II)/ ♂ Tall (Group I)						
Albatros Odeskyi / Odeska 267	2.19±0.19	6.4	2.53±0.23	12.8	1.60±0.18	2.0
Albatros Odeskyi / Pylypivka	2.38±0.27	44.0	2.40±0.43	7.8	2.13±0.18	20.0
Stolichna / Odeska 267	1.90±0.36	32.0	2.00±0.48	0.6	2.07±0.22	52.0
Stolichna / Pylypivka	2.74±0.39	12.2	2.34±0.25	4.3	-	-
Vidrada / Odeska 267	2.15±0.13	3.8	2.12±0.12	4.8	-	-
Vidrada / Pylypivka	1.48±0.20	-1.6	1.72±0.12	-6.0	-	-
The average for F_1	2.32	-	2.29	-	2.02	-

Consistent grain weight of the main ear was formed in the years of the study in the combinations Stolichna/Odeska 267, Albatross Odeskyi/Pylypivka, Albatross Odeskyi/Stolichna, Albatross Odeskyi/ Vidrada, with variability ranging from 0.17 g to 0.42 g. Average variability was observed in Stolichna/Vidrada (0.51 g) and Albatross Odeskyi/Smuglyanka (0.57 g). In the crossing combinations Stolichna/Pisanka and Albatross Odeskyi/Odeska 267, the variability of the trait was found to be 0.76 g and 0.93 g, respectively. In F_1, where medium-height varieties of group II were used as the maternal form, positive superdominance was observed in 29 out of 32 hybrids (90.6%) during the years of the study. At the same time, negative superdominance was identified in two hybrids (6.3%), and partial positive dominance in one hybrid (3.1%) (Figure 3).

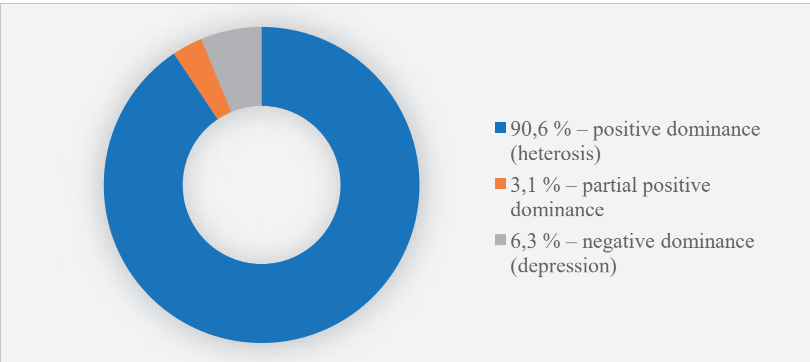

Figure 3. Types of inheritance of grain weight from the main ear in F_1 using medium-sized varieties of Medium Group II as a mother form (2020–2022)

In the hybridisation of tall varieties of group I, the grain weight of the main ear in F_1 ranged from 1.50 g to 2.81 g, with an increase over the parental forms in seven out of nine hybrids. The maximum average grain weight (2.31 g) was observed in 2020, with the highest value in the combination Odeska 267 / Lastivka Odeska – 2.81 g. Exceeding the average hybrid grain weight in 2021 was observed in Pylypivka/ Lastivka Odeska, and in 2022, in Odeska 267/Pylypivka and Pylypivka/Lastivka Odeska (Table 5).

Table 5. Grain weight per main ear (g) and the degree of phenotypic dominance in F1 obtained by hybridisation of tall varieties

Variety, hybrid	2020		2021		2022	
	x̄±Sx̄	h_p	x̄±Sx̄	h_p	x̄±Sx̄	h_p
Odeska 267 / Pylypivka	2.17±0.33	5.7	1.79±0.10	-2.5	1.83±0.21	53.0
Odeska 267 / Lastivka Odeska	2.81±0.50	77.5	1.96±0.10	3.2	1.50±0.13	-15.0
Lastivka Odeska	1.28±0.04	-	1.78±0.04	-	1.58±0.04	-
Pylypivka / Lastivka Odeska	1.96±0.14	5.1	2.35±0.17	13.3	1.82±0.43	25.0
The average for F_1	2.31	-	2.03	-	1.72	-

The variability in the grain weight of the main ear ranged from minimal in the crossing combinations Odeska 267 / Pylypivka (0.38 g) and Pylypivka / Lastivka Odeska (0.53 g) to high in Odeska 267 / Lastivka Odeska (1.31 g).

In seven out of nine hybrids, the inheritance of grain weight of the main ear occurred through positive superdominance, with the degree of phenotypic dominance ranging from 3.2 in 2021 to 77.5 (in 2020) in the combination Odeska 267/Lastivka Odeska (Figure 4).

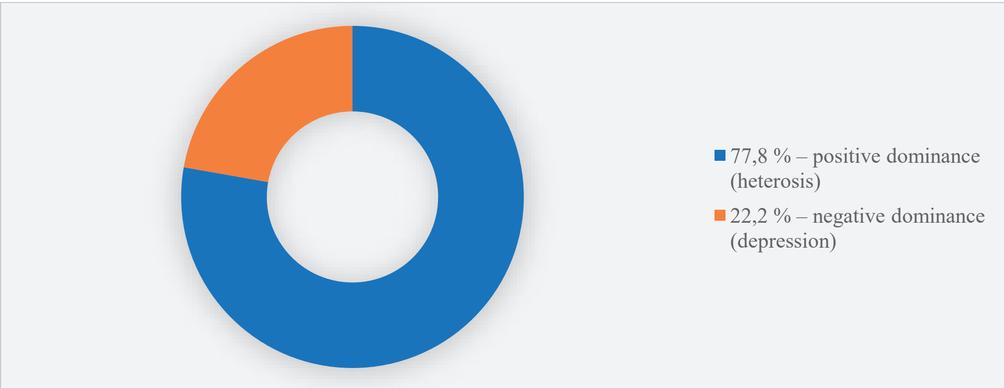

Figure 4. Types of inheritance of grain weight from the main spike in F1 at hybridisation of tall varieties (2020-2022)

Over the course of three years, the trait was inherited through positive superdominance only in the hybridisation of Pylypivka / Lastivka Odeska. Two hybrids determined the trait through negative superdominance.

The inheritance of grain weight from the main spike of winter wheat in reciprocal crosses of the forest-steppe, steppe, and Western European ecotypes (2022) followed a pattern of positive overdominance in 26 out of 27 hybrids. Depending on the parental pairs selected for hybridisation, the degree of phenotypic dominance varied within the range of hp = 2.1-83.0. In 18 F2 populations (2023), a positive degree of transgression was observed (Ts = 0.3-40.0%; Tch = 50-100%) (Samoylyk et al., 2023).

The methodology used in our study to determine the degree of phenotypic dominance for assessing first-generation hybrids has been widely applied in the early stages of winter wheat breeding (Yakymchuk, 2018; Bazaliy et al., 2020; Lozinskyi et al., 2021), as well as in the selection of winter rye (Huba, 2021), winter triticale (Tromsyuk & Bugayov, 2021), spring barley (Nyska & Petrynkova, 2018), and spring rapeseed (Kumanska, 2018).

CONCLUSIONS

The formation of grain weight of the main ear and the degree of phenotypic dominance in F_1 hybrids is determined by the selection of parental forms for crossing and is significantly modified by the meteorological conditions of the year. In most hybrids, the inheritance of grain weight followed positive superdominance, ranging from 77.8% in the hybridisation of tall varieties from group I to 90.6% with the use of medium-height varieties from group II as the maternal form. The most promising crossing combinations were identified as Albatros

Odeskyi/ Stolichna, Lisova Pisnya/Odeska 267, Albatros Odeskyi/Pylypivka, Albatros Odeskyi/Vidrada, Donska semi-dwarf/ Pylypivka, Stolichna/Pysanka, Bilotserkivska semi-dwarf/Vidrada, Lisova Pisnya/ Stolichna, Lisova Pisnya/Pylypivka, and Stolichna/Vidrada, which consistently produced high grain weight values for the main ear (2.27-2.56 g) over the three years, exceeding the average F_1 value. Further research will focus on the evaluation of hybrid populations of soft winter wheat for valuable selection traits and the selection of promising lines to create a new high-yielding parent material adapted to the conditions of the Forest-Steppe zone of Ukraine.

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RESEARCH REGARDING EFFICACY OF FENPICOXAMID TREATMENT IN THE CONTROL OF SOME PATHOGENS IN WINTER WHEAT

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Abstract

The aim of our research was to evaluate the performance of a new active ingredient, fenpicoxamid, from picolinamide group, in control of wheat pathogens. Trials were carried out in experimental fields located in Buzău county, with foreign wheat genotype and in Neamț county with Romanian wheat genotype. In both experimental fields was detected attack by Septoria spp. that causes wheat septoria, Puccinia recondita, pathogen responsible for brown rust, and Blumeria graminis, powdery mildew pathogen. The control scheme included treatments with a.i. from SDHI, Strobilurins, Triazoles, Picolinamides and their mixtures. Treatment with 50 g/l fenpicoxamid + 100 g/l prothioconazole performed the best with the highest efficacy in control of pathogens monitored in the experimental fields. Efficacy in septoria control was over 90% in both locations. Efficacy in control of brown rust was 89.6% in Buzau experimental field and 90.1% in Neamț experimental field and control of powdery mildew was 78.8% in Buzău county while in Neamț county was 83.1%.

Key words: *wheat, agent pathogen, treatment, fenpicoxamid, efficacy.*

INTRODUCTION

Wheat is one of the most cultivated and important cereal crops in the world. In 2024 at European level, also in Romania, wheat occupied the largest cultivated area, as shown in Figure 1. *Plant production for field crops in 2024 (provisional data)*. (n.d.). *INNSE. Retrieved from https://insse.ro/cms/sites/default/files/com_presa/com_pdf/prod_veg_r24.pdf.* However, wheat production faces significant challenges due to numerous fungal pathogens that can drastically reduce yields. The pathogens *Zymoseptoria tritici* (leaf septoria), *Puccinia recondita* (brown rust), and *Blumeria graminis* f. sp. *tritici* (wheat powdery mildew) are among the most destructive for wheat crops, causing substantial production losses globally. Leaf septoria frequently occurs in wheat crops in temperate regions and causes losses considered a major issue for wheat cultivation (Fones & Gurr, 2015). Also, brown rust or leaf rust is a fungal disease with high incidence, resulting in significant damage (Chaudhari & Chaudhari, 2013). Powdery mildew causes losses during severe attacks, affecting leaf area and yield (El Shamy et al., 2012; Costamilan, 2005). Efficient disease management is crucial for maintaining high wheat yields and ensuring food security. Controlling these pathogens, which have a major impact on plant health and wheat production, requires measures including development and cultivation of resistant varieties and integrated diseases management. The use of synthetic chemicals remains a necessary measure (Esmail & Draz, 2017). Traditionally, fungicides have been the primary method of control, utilizing substances from various chemical groups that target the pathogens. However, the development of resistance to certain fungicides has become a significant concern in integrated diseases management (IPM). *Wheat and barley disease management guide. (n.d.). AHDB cereals & oilseeds. Retrieved from https://projectblue.blob.core.windows.net/media/Default/Imported%20Publication%20Docs/AHDB%20Cereals%20&%20Oilseeds/Disease/Wheat%20and%20barley%20disease%20management%20guide%20(2024).pdf.*

Global efforts to discover and use new fungicide active ingredients for controlling foliar diseases in cereals have been successful with the development of fenpicoxamid (Inatreq active), a new fungicide from picolinamides group, which has shown the potential to control a wide range of fungal pathogens. In this study, we evaluated the efficacy of fenpicoxamid in combination with prothioconazole in managing wheat diseases caused by *Zymoseptoria tritici*, *Puccinia recondita*, and *Blumeria graminis* f. sp. *tritici.* The results of this study will provide valuable information on the role of fenpicoxamid in the sustainable management of wheat diseases.

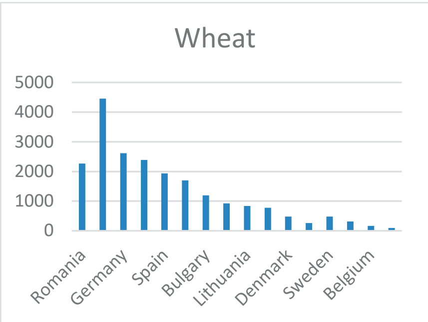


Figure 1. Wheat cultivated area in Romania and in some EU member states in 2024 (thousands of ha)

MATERIALS AND METHODS

Field trials were conducted in Dulbanu, Buzău County (foreign wheat genotype - Anapurna variety) and Zănești, Neamț County (Romanian wheat genotype - Glosa variety) during 2023/ 2024 growing season. Both locations were selected based on their historical vulnerability to *Septoria* spp., *Puccinia recondita*, and *Blumeria graminis*. The test fields were designed in randomized blocks with four replications per treatment, and a minimum plot size of 3 x 10 m. The fungicides tested included SDHIs, strobilurins, triazoles, picolinamides (fenpicoxamid), and their mixtures. Fenpicoxamid was applied at a rate of 1.5 L/ha (50 g/L fenpicoxamid + 100 g/L prothioconazole) as the treatment of interest. Table 1 shows the details of treatment applications at the two trial locations.
All treatments were applied according to trial protocol and following EPPO guidelines. Fungicides were applied using a hand sprayer calibrated for uniform coverage of each plot. The treatments were applied at BBCH45 wheat growth stage in accordance with fungicide labels and trial protocol to ensure optimal timing for pathogens control.

Table 1. List of treatments in the protocol and details of application

Trt. No.	Treatment name	Dose rate L/ha	Solution volume	Crop stage
1	Fenpicoxamid 50 g/l + Prothioconazole 100 g/l	1.5	200 l/ha	BBCH45
2	Bezovindiflupir 75 g/l + Prothioconazole 150 g/l	1.0	200 l/ha	BBCH45
3	Bixafen 75 g/l + Prothioconazole 150 g/l	1.0	200 l/ha	BBCH45
4	Fluxapiroxad 75 g/l+ Prothioconazole 150 g/l	1.0	200 l/ha	BBCH45
5	Azoxistrobin 250 g/l + Prothioconazole 250 g/l	0.8 +0.6	200 l/ha	BBCH45
6	Untreated	-	-	-

In Dulbanu, Buzău County, the treatments were applied on April 29, 2025, at 14:30. The application conditions were good, with an air temperature of 20°C, wind speed of 12 km/h, and air humidity of 52%. In Zănești, Neamț County, the treatments were applied on May 10, 2025, at 11:25. The application conditions were also good, with an air temperature of 19°C, wind speed of 9 km/h, and air humidity of 56%. After preparing the spraying solution for each treatment, we analyzed the treatments physically (miscibility, stability, sedimentation). All treatments were safe for the crop, with uniform distribution in the solution volume, without sedimentation. Immediately after application, we observed the coverage power of each treatment, where the treatment with fenpicoxamid and prothioconazole stood out for its excellent leaf coverage, due to the innovative IQ4 formulation. *Drip by drip: Delivering durable protection against fungal diseases. (n.d.). Corteva Agriscience. Retrieved from https://www.corteva.com/products-and-services/inatreq/Drip-by-drip-Delivering-durable-protection-against-fungal-diseases.html.* To determine the efficacy (E%)

of each treatment in the protocol at each location, a phytosanitary check (assessments) was conducted before application, as well as after application at 7 days, 14 days, and 28 days, identifying the pathogens present in the crop, and calculating the attack frequency (F%) and intensity (I%) to determine the severity of the attack. The following formulas were used:

- **F** = (n x N) / 100 (%), where: F = attack frequency, n = number of affected plants, N = total number of observed plants;
- **I (%)** = Σ(i x f) / n, where: I = intensity, i = attack percentage, f = number of plants with that percentage, n = total number of plants with the disease;
- **GA%** = F% x I% / 100.
- Based on the attack severity data, the efficacy of the treatments in the protocol was calculated using Abbott's formula: **E (%)** = [(Gam - Gav) / Gam] x 100, where:
  - Gam = attack severity of the untreated control;
  - Gav = attack severity of the treated variant.

RESULTS AND DISCUSSIONS

The 2023-2024 agricultural year was characterized by particularly significant climatic challenges, which greatly affected the development of crops and agricultural production. Extreme climatic factors, such as prolonged droughts, insufficient or irregularly distributed rainfall, and unusually high temperatures, put pressure on agricultural activities, necessitating the adoption of adaptive measures and efficient technologies to manage these difficult conditions. Even under these circumstances, the need for fungicide treatments in wheat cultivation arose. As shown in the graphs below (Figures 3, 4), the evolution of temperatures and precipitation in the test locations led us to apply the treatments before heading. *Historical weather data. (n.d.). Granular Link. (n.d.). Retrieved from https://link.granular.ag/map/farms/11423/fields* .These climatic conditions directly impacted the decisions regarding the timing of treatments, as the correct management of the application timing can significantly contribute to the effective control of pathogens and maximize crop yields.

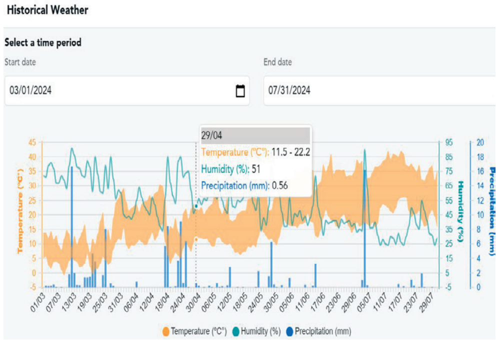


Figure 2. Evolution of temperatures and precipitation from March to July 2024 in Dulbanu location

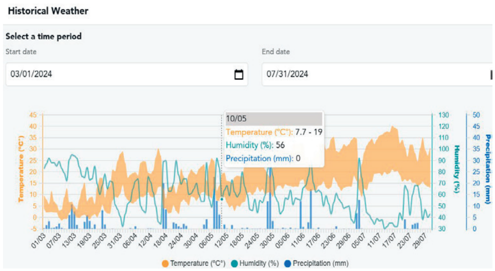


Figure 3. Evolution of temperatures and precipitation from March to July 2024 in Zănești location

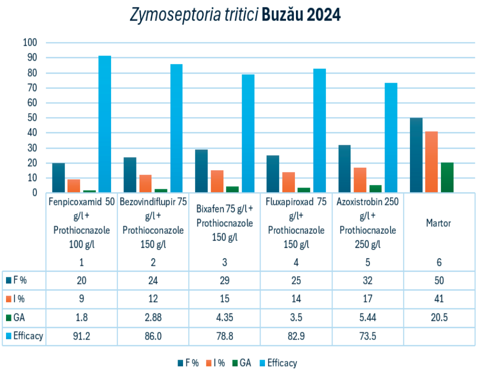
The treatment with 50 g/L fenpicoxamid + 100 g/L prothioconazole provided significant control over all the monitored pathogens in both Buzău and Neamț counties. The data show a high efficacy against the pathogen *Zymoseptoria tritici* (wheat septoria), *Puccinia recondita* (brown rust), and *Blumeria graminis* f. sp. *tritici* (wheat powdery mildew), with disease attack reductions of over 80%.

- Efficacy against *Zymoseptoria tritici* (wheat septoria)
  The combined treatment significantly reduced the severity of septoria in both counties, with an overall efficacy of 91.2% (Figure 4) in Buzău and 92.3% (Figure 5) in Neamț. These results demonstrate the robust protective activity of the fenpicoxamid-prothioconazole combination, which performed better than most of other treatments evaluated in the study.

- Efficacy against *Puccinia recondita* (brown rust**)**
  The treatment was also highly effective in controlling brown rust, showing disease severity reduction rates of 89.6% (Figure 6)

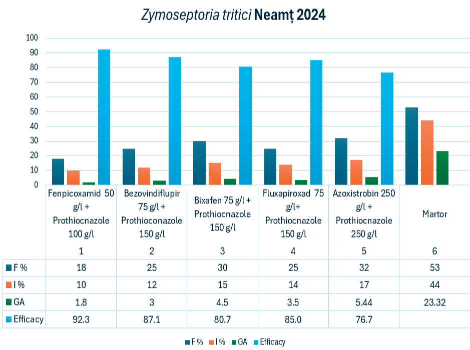
in Buzău and 90.1% (Figure 7) in Neamț. This suggests that fenpicoxamid has broad-spectrum activity, providing consistent control across different wheat genotypes.

- **Efficacy** against *Blumeria graminis* f. sp. *tritici* (wheat powdery mildew)
Although the combined treatment was somewhat less effective against wheat powdery mildew, it still provided a solid reduction in disease incidence. The efficacy rates were 78.8% (Figure 8) in Buzău and 83.1% (Figure 9) in Neamț, indicating that fenpicoxamid is a valuable tool for managing this pathogen.



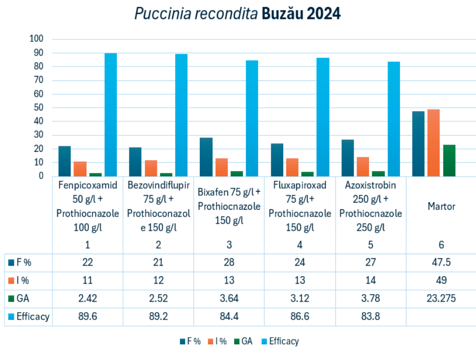
	Fenpicoxamid 50 g/l + Prothiocnazole 100 g/l	Bezovindiflupir 75 g/l + Prothiocnazole 150 g/l	Bixafen 75 g/l + Prothiocnazole 150 g/l	Fluxapiroxad 75 g/l + Prothiocnazole 150 g/l	Azoxistrobin 250 g/l + Prothiocnazole 250 g/l	Martor
	1	2	3	4	5	6
F %	20	24	29	25	32	50
I %	9	12	15	14	17	41
GA	1.8	2.88	4.35	3.5	5.44	20.5
Efficacy	91.2	86.0	78.8	82.9	73.5	

Figure 4. Efficacy of treatments in controlling *Zymoseptoria tritici* in Dulbanu, Buzău



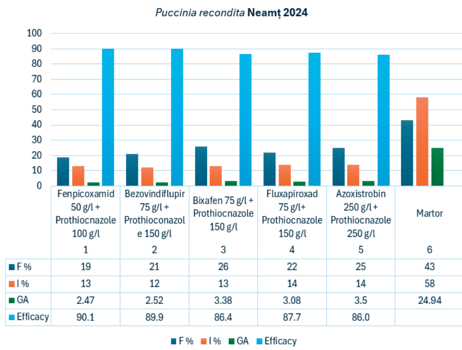
	Fenpicoxamid 50 g/l + Prothiocnazole 100 g/l	Bezovindiflupir 75 g/l + Prothiocnazole 150 g/l	Bixafen 75 g/l + Prothiocnazole 150 g/l	Fluxapiroxad 75 g/l + Prothiocnazole 150 g/l	Azoxistrobin 250 g/l + Prothiocnazole 250 g/l	Martor
	1	2	3	4	5	6
F %	18	25	30	25	32	53
I %	10	12	15	14	17	44
GA	1.8	3	4.5	3.5	5.44	23.32
Efficacy	92.3	87.1	80.7	85.0	76.7	

Figure 5. Efficacy of treatments in controlling *Zymoseptoria tritici* in Zănești, Neamț



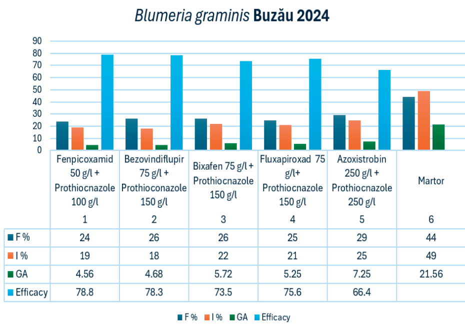
	Fenpicoxamid 50 g/l + Prothiocnazole 100 g/l	Bezovindiflupir 75 g/l + Prothiocnazole 150 g/l	Bixafen 75 g/l + Prothiocnazole 150 g/l	Fluxapiroxad 75 g/l + Prothiocnazole 150 g/l	Azoxistrobin 250 g/l + Prothiocnazole 250 g/l	Martor
	1	2	3	4	5	6
F %	22	21	28	24	27	47.5
I %	11	12	13	13	14	49
GA	2.42	2.52	3.64	3.12	3.78	23.275
Efficacy	89.6	89.2	84.4	86.6	83.8	

Figure 6. Efficacy of treatments in controlling *Puccinia recondita* in Dulbanu, Buzău



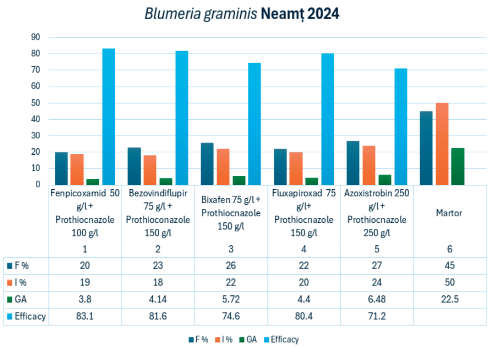
	Fenpicoxamid 50 g/l + Prothiocnazole 100 g/l	Bezovindiflupir 75 g/l + Prothiocnazole 150 g/l	Bixafen 75 g/l + Prothiocnazole 150 g/l	Fluxapiroxad 75 g/l + Prothiocnazole 150 g/l	Azoxistrobin 250 g/l + Prothiocnazole 250 g/l	Martor
	1	2	3	4	5	6
F %	19	21	26	22	25	43
I %	13	12	13	14	14	58
GA	2.47	2.52	3.38	3.08	3.5	24.94
Efficacy	90.1	89.9	86.4	87.7	86.0	

Figure 7. Efficacy of treatments in controlling *Puccinia recondita* in Zănești, Neamț



	Fenpicoxamid 50 g/l + Prothiocnazole 100 g/l	Bezovindiflupir 75 g/l + Prothiocnazole 150 g/l	Bixafen 75 g/l + Prothiocnazole 150 g/l	Fluxapiroxad 75 g/l + Prothiocnazole 150 g/l	Azoxistrobin 250 g/l + Prothiocnazole 250 g/l	Martor
	1	2	3	4	5	6
F %	24	26	26	25	29	44
I %	19	18	22	21	25	49
GA	4.56	4.68	5.72	5.25	7.25	21.56
Efficacy	78.8	78.3	73.5	75.6	66.4	

Figure 8. Efficacy of treatments in controlling *Blumeria graminis* in Dulbanu, Buzău



	Fenpicoxamid 50 g/l + Prothiocnazole 100 g/l	Bezovindiflupir 75 g/l + Prothioconazole 150 g/l	Bixafen 75 g/l + Prothiocnazole 150 g/l	Fluxapiroxad 75 g/l+ Prothiocnazole 150 g/l	Azoxistrobin 250 g/l + Prothiocnazole 250 g/l	Martor
	1	2	3	4	5	6
F %	20	23	26	22	27	45
I %	19	18	22	20	24	50
GA	3.8	4.14	5.72	4.4	6.48	22.5
Efficacy	83.1	81.6	74.6	80.4	71.2	

Figure 9. Efficacy of treatments in controlling *Blumeria graminis* in Zănești, Neamț

Research on the efficacy of treatments in controlling pathogens is a constant concern for establishing phytosanitary interventions and integrating them into plant integrated pest management schemes (Toth and Cristea, 2020; Chiriac and Cristea, 2021), as well as for wheat cultivation (Iosub et al., 2022). Iosub et al. (2022) determined the efficacy values of some molecules recommended for controlling wheat pathogens, including powdery mildew, brown rust, and septoria, ranging between 90% and 73%.

CONCLUSIONS

In conclusion, the novel mode of action of fenpicoxamid combined with the complementary and synergistic activity of the mixture partner, prothioconazole, demonstrated exceptional efficacy in controlling the pathogens present at the test locations, particularly *Zymoseptoria tritici* and *Puccinia recondita*, with a control efficacy of over 90%. The fungicidal action against *Blumeria graminis* ranged from 78.8% to 83.1%, within the standards of the fungicide market, thus providing an acceptable alternative as a control tool and preventing the development of cross-resistance. These results suggest that fenpicoxamid could be a valuable addition to fungicide management programs, especially for integrated pest management (IPM) strategies in wheat production. Further studies are needed to explore the long-term effects of this combination and its role in managing fungicide resistance.

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MANAGEMENT PRACTICES INFLUENCE THE NATURAL ARBUSCULAR MYCORRHIZAE COMMUNITY OF MAIZE

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Abstract

The productivity of the agroecosystems is affected by the presence and the biodiversity of arbuscular mycorrhizal fungi communities. Understanding how mycorrhizal communities respond to various factors is crucial for promoting sustainable agriculture. This study examines how four cropping systems affect the natural arbuscular mycorrhizal community associated with maize. The trial involved three corn hybrids: DKC4949 (FAO 390), P8523 (FAO 260), and P9537 (FAO 390). The experiment was conducted using a randomized complete block design with three replications in a plot size of 25 m^2. For the tested period the highest number of spores (348 in 2022 and 370 in 2023) has been recorded by the hybrid P8523 when cultivated according to the no-till technology combined with mulching. Hybrid P9537, the highest yielding for the region, also exhibits the greatest rate of mycorrhizal colonization. Although no-till and its combination with mulching enhanced the colonization potential of mycorrhiza by all hybrids, the positive result has no quantitative manifestation and the yields remain the lowest by those variants.

***Key words**: arbuscular mycorrhiza, colonization rate, cropping system, maize.*

INTRODUCTION

Nowadays, conventional intensive agriculture raises many concerns (Ujvári et al., 2023). Although inorganic fertilizers positively affect the productivity of crops, they may negatively impact soil health, fertility, and microbial biodiversity (Sun et al., 2015). The scientific community is trying to boost the transition toward sustainability (Foley et al., 2011) and focuses on beneficial traits, improved nutrient-use efficiency, soil health, and biodiversity of soil microbial communities (Menendez & Garcia-Fraile 2017; Ke et al., 2021). The utilization of plant growth-promoting bacteria or arbuscular mycorrhizal fungi is a successful strategy aiming to achieve sustainable crop production (Ujvári et al., 2023). Arbuscular mycorrhizal fungi (AMFs) are beneficial non-host-specific soil microbiomes and highly effective partners in our ecosystem. As plant symbionts, they colonize more than 80% of our land plants, including most cultivated plants such as maize. They support their development by promoting increased nutrient and water uptake, increased drought tolerance, and improved soil structure (Smith & Read 2008). Soil and crop management practices modulate biological soil fertility (Ortas, 2015). Establishing functional arbuscular mycorrhizal (AM) symbioses could improve soil nutrient availability (Ortas, 2015). Management strategies have a strong positive or negative influence on AM communities (Brito et al., 2012; Bedini et al., 2013). Additionally, low-input cropping systems impact population density and species composition (Harikumar, 2015). AM spore abundance is higher in organically maintained fields, compared to conventional ones (Gosling et al., 2010). AMFs are key factors in plant health and the productivity of agroecosystems is affected by their presence and biodiversity in soil (Lekberg & Koide, 2005). AMFs are obligated biotrophs and can infect various crop species, depending on soil conditions and soil fertility (Ortas, 2015). The knowledge of the influence of different factors on AMF's population is essential and supports sustainable agriculture (Brito et al., 2012). According to Dodd et al. (2000), *Glomus* spp. can survive upsets and predominate in disturbed agricultural systems. Jansa et al. (2003) suggested that variations in AMF community structure of maize roots could result from differences in AMF species tolerance to tillage-induced disruption of the hyphae. The properties of biofertilizers to improve the physicochemical properties of the soil, and stimulate root growth and vegetative mass, while supporting the resistance of plants to abiotic environmental factors, gives them key importance in the process of soil phytoremediation (Rasouli et al., 2022). Nowadays, arbuscular mycorrhiza is among the most widely used biological fertilizers that favour the resistance of plant and soil systems (Habeeb et al., 2020). Global demand for maize is increasing each year due to its versatile applications. (Ranum et al., 2017). In conventional farming systems maize productivity is supported and enhanced through higher inputs of mineral fertilizers (Tilman et al., 2002). Excessive application of these products reduces soil microbial biodiversity and causes loss of soil fertility (Thiele-Bruhn et al., 2012). Maize associates with native AMF communities (Alvarado-Herrejón et al., 2019). The common phenotypic reaction of maize to inoculation with AMF is growth promotion (Sawers et al., 2017). However, the strength of this expression depends on the genotype, crop rotation, tillage, and fertilization (Sarabia et al., 2017; López-Carmona et al., 2019). The current study investigates how four different cropping systems affect the density and species composition of native arbuscular mycorrhiza populations of three maize hybrids.

MATERIALS AND METHODS

Field experiment

A field trial was conducted in the village of Kapinovo (43.745003° n. w. 27.991992° e. l.), located in North-Eastern Bulgaria over two consecutive years (2022-2023). The following maize hybrids were included in the experiment: DKC4949 (FAO 390), P8523 (FAO 260), and P9537 (FAO 390). The study was set using a randomized complete block design in three replications with a plot size of 25 m^2.

The sowing was carried out in the period 1-10 of April with row spacing of 70 cm after wheat as a predecessor. The maize hybrids were planted using four different cropping systems: classical conventional tillage (T) with the removal of all residues; conventional tillage + mulching (T+M) crop residues remain on the soil surface as a cover; no-till (NT) performed with a double-disk opener assembly following a ripple coulter and no-till+mulching (NT+M) without removal of the crop residues.

Soil conditions

The dominant soil type in the region is represented by the chernozems. The area's soil is loamy, with physical clay constituting 50%. Chemical analysis revealed a significant amount of carbonates, and it has a neutral to alkaline pH of 7 to 7.2. The humus content is high, ranging from 5.2% to 6%. The total nitrogen content in the soil ranged from 55 to 58 mg per 1000 g, according to Gurov. Phosphorus levels were reported to be between 0.5 and 0.8 mg per 1000 g /Egner and Rheen/. Potassium content was between 26 to 30 g per 1000 g of soil. The presence of nitrates in larger quantities contributed positively to the physical and mechanical properties of the soil. Additionally, the humic content gradually decreased with soil depth. While the amounts of nitrogen, phosphorus, and potassium were significant, their available forms were limited. Soil nutritional regime was improved through fertilization. Phosphorus and nitrogen fertilizers were applied before sowing at rates of 50 kg per hectare of triple superphosphate and 30 kg per hectare of ammonium nitrate.

Weather conditions

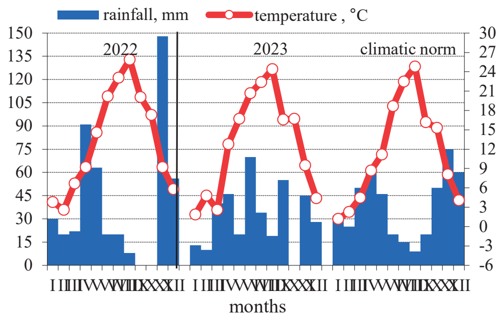

Figure 1. Meteorological data for the period

AMF colonization rate

To determine the impact of cropping systems on AMF population density, soil samples have been taken from the roots of 10 plants per plot. The samples were then mixed for statistical data processing. Sampling occurred at a depth of 0 to 27 cm at maize development stage R1. Fine roots were carefully separated from the soil, and 2 cm segments of these roots were used in the staining procedure to assess the density of the arbuscular mycorrhizal community. The plants were randomly selected. The cleaned root segments were placed in 15 ml falcon tubes filled with a 30% alcohol solution. The prepared root segments underwent a staining procedure. This process began with immersing the segments in a 10% potassium hydroxide (KOH) solution for one day to bleach them. Following this, the segments were boiled in a water bath at 90 °C for four minutes. After boiling, they were rinsed three times with tap water and stained using a 5% solution of ink and vinegar, following the method outlined by Vierheilig et al. (1998). Once stained, the roots were rinsed again with tap water and then stored in 30% ethanol. The colonization rate of the arbuscular mycorrhizal roots was determined using the gridline intersection method (Newman, 1966; Giovanetti & Mosse, 1980). The extent of root colonization was measured using the following formula:

$$Root\ Colonization\ Percentage = \frac{Number\ of\ AMF\ Positive\ Intersections}{Total\ Number\ of\ Intersections} x\ 100\%$$

Spores were counted using the wet sieving and decanting method developed by Gedemann and Nicolson (1963), followed by sucrose density centrifugation (Ianson & Allen, 1986). First, 100 g of soil from each variant was dispersed in 1 liter of water and centrifuged. The resulting suspension was then decanted through sieves with mesh diameters ranging from 500 to 40 µm, after which a 40% sucrose solution was added. All residues were filtered, and the intact spores were counted under a stereomicroscope.

Statistics

The results were statistically processed using analysis of variance (ANOVA), and differences between the groups were determined through the multi-rank LSD test.

RESULTS AND DISCUSSIONS

During the years of the investigation, the spore density in the soil varies depending mostly on the cropping system (Figure 2), as the values for the second year are slightly increased. For the tested period the highest number of spores (348 in 2022 and 370 in 2023) has been recorded by the hybrid P8523 when cultivated according to the no-till technology combined with mulching. On second place is the variant cultivated under no-till technology applied individually (between 320 and 340 spores, respectively), followed by the variant sown after classical tillage with mulching with a spore density of 107 in the first and 135 in the second year. By all hybrids, classical plowing significantly reduces the number of spores of the arbuscular mycorrhizal societies.

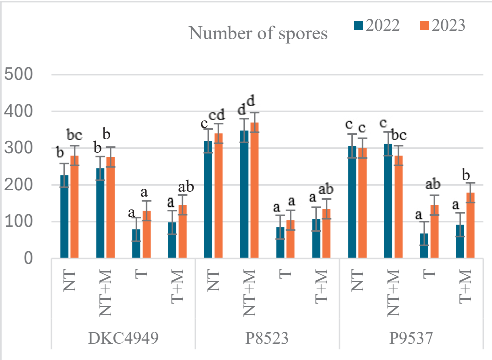

Figure 2. AMF spores in 10g[-1] soil

The differences between the variants grown using classical tillage technology and the combination of plowing with mulching by hybrid P9537 were statistically proven and significant in both years of the experiment, while by the other hybrids, the differences were proven only in the second year. By the hybrids DCC4949 and P9537, in the first year, the number of spores is highest in the variants sown according to the cropping system NT+M. In the second year, no-till farming produced slightly better results. Soil cultivation is a major factor in the breakage of the extraradical mycelium (Evans & Miller, 1990). Mixing surface residues with the soil profile stimulates or inhibits certain species of mycorrhizal societies (Klironomos & Hart, 2002). Brito et al. (2012) suggested that AMFs that depend mainly on extraradical mycelium to colonize the roots of newly sown plants would be more frequent in no-till systems, whereas those relying mostly on spores for colonization would be less affected by soil disturbance. Additionally, mulching improves the survival of AM fungal propagules by releasing water-soluble carbon, which enhances root colonization by AM fungi (Nyamwange et al., 2018).

During the study period fluctuations in the mycorrhizal colonization rate have been observed depending on the cropping system (Tables 1, 2). Although for the test period in all three hybrids, no-till and its combination with mulching contribute to increasing the colonization potential of mycorrhiza, the positive result has no quantitative manifestation and the yields remain the lowest by those variants.

Table 1. Mycorrhiza colonization rate depending on the cropping system during 2022

Variety	Cropping system	root colonization (%)	grain yield (t ha-1)
DKC4949	NT	25.2^{b}	8.4^{a}
	NT+Mulch	27.4^{b}	8.5^{a}
	Tillage	12.2^{a}	9.1^{a}
	Tillage+Mulch	15.6^{a}	8.9^{a}
P8523	NT	31.3^{c}	9.7^{b}
	NT+Mulch	30.1^{c}	9.5^{a}
	Tillage	16.2^{a}	10.2^{c}
	Tillage+Mulch	23.8^{b}	10^{c}
P9537	NT	38.5^{d}	10.2^{c}
	NT+Mulch	34.2^{c}	10^{c}
	Tillage	20.3^{b}	11.4^{e}
	Tillage+Mulch	25.8^{b}	11.3^{e}
LSD<5%		*7.6*	*0.65*

[a]Means within columns followed by different lowercase letters are significantly different (P<0.05)

Table 2. Mycorrhiza colonization rate depending on the cropping system during 2023

Variety	Cropping system	root colonization (%)	grain yield (t ha-1)
DKC4949	NT	38.2^{c}	8.6^{c}
	NT+Mulch	32.7^{b}	8.5^{b}
	Tillage	19.5^{a}	9.0^{c}
	Tillage+Mulch	20.2^{a}	9.0^{c}
P8523	NT	43.5^{d}	7.7^{a}
	NT+Mulch	40.2^{d}	7.5^{a}
	Tillage	28.8^{b}	8.2^{b}
	Tillage+Mulch	29.2^{b}	8.8^{c}
P9537	NT	40.1^{d}	8.1^{a}
	NT+Mulch	42.8^{d}	8.4^{b}
	Tillage	33.5^{c}	9.2^{c}
	Tillage+Mulch	36.7^{c}	9.0^{c}
LSD<5%		*6.7*	*0.52*

[a]Means within columns followed by different lowercase letters are significantly different (P<0.05)

However, the results make it possible to establish genotypic differences between hybrids, expressed as differences in the percentage of mycorrhizal colonization. The trend that is found is that the P9537 hybrid, which is the highest yielding for the region, also has the highest rate of mycorrhizal colonization. By hybrid DKC4949 yields are the lowest and, accordingly, the degree of mycorrhization is also at the lowest value. Hybrid P8523 occupies an intermediate position in terms of test performance. The productivity of agricultural plants depends on many factors, among which are the genotypic capabilities and potential of the respective hybrid. Although for the tested period the hybrid DKC4949 distinguished as the lowest yielding for the region, its yields are not affected by the cultivation technology, which is evident from the absence of statistically significant differences between the different variants.

Differences in climatic terms do not lead to sharp variations in productivity, from which it can be concluded that the hybrid exhibits great ecological plasticity. For the other two hybrids, classical plowing and its combination with mulching lead to the highest productivity, and the differences between the two technologies are statistically insignificant and unproven. Opinions about the impact of tillage on yield are contradictory. While Mathers et al. (2023) and Gaudin et al. (2015) found a positive effect of no-till or reduced tillage on maize productivity, Daigh et al. (2018) did not observe any effect on yield.

During the study, 9 mycorrhizal species were identified, reflected in Table 3.

Table 3. Frequency of identified AMF communities

Identified species	Cropping system NT	NT+M	T	T+M
Funneliformis geosporum	C	D	R	
Funneliformis mosseae	D	D	R	R
Glomus macrocarpum	D	D	C	C
Glomus glomerulatum	C	D	C	C
Glomus ambisporum	C	C	C	C
Glomus pansihalos	D	D	C	C
Rhizophagus irregularis	D	D	D	D
Rhizophagus claris	C	C		R
Rhiizaphagus fasciculatus	D	D		

*D-dominant (FO>66%), C-common (33%≤FO≤66%), R-rare (FO≤33%)

Concerning the composition of the mycorrhizal society, no differences were found between the individual hybrids, but only between the applied cropping systems. Our results align with those of Brito et al. (2012) and Mathimaran et al.

(2005), who found that *R. irregulare* spores were predominant in a soil community associated with cereals as the host plant. Several studies have reported a reduction in the diversity of AM fungal communities in roots from different agroecosystems specifically caused by conventional tillage (Alguacil et al., 2008; Schnoor et al., 2011).

Reduced tillage can enhance the diversity of arbuscular mycorrhizal (AM) fungi and lead to higher colonization rates compared to conventionally maintained soils (Bowles et al., 2017). Understanding how cropping systems affect various soil chemical, biological, and agronomic aspects is crucial for effective management and decision-making in farming (Mhlanga et al., 2022) Even more essential is grasping the causal relationships within cropping systems, maize production, and other related variables, as this knowledge can enhance our understanding of the multifunctionality of agroecosystems (Mhlanga et al., 2022). The choice of appropriate tillage practices is of great importance for the sustainability of the AMF communities.

CONCLUSIONS

Soil cropping system influences the population density and richness of the natural arbuscular communities of the tested maize hybrids. Genotypic differences between hybrids, expressed as differences in the percentage of mycorrhizal colonization. Hybrid P9537, which is the highest yielding for the region, also has the highest rate of mycorrhizal colonization. For the tested period hybrid DKC4949 exhibits the greatest ecological plasticity because its yields are neither affected by the cultivation technology nor by the climatic conditions of the year. No-till had a positive impact on natural mycorrhizal societies but not on the productivity of corn hybrids.

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RESEARCH ON MAIZE YIELD POTENTIAL BY MATURITY GROUP UNDER CONDITIONS AT ARDS BRĂILA

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Abstract

In the current conditions of changing climatic conditions, especially in South-East Romania, it is necessary that the choice of maize hybrids is made correctly, depending on the maturity group, in order to obtain satisfactory yields and thus counteract the effect of climate change. The aim of this research was to study the yield and productive capacity of a set of maize hybrids of different maturity groups in the context of climate change. The field experiment was conducted in the 2023 and 2024 growing seasons on a vermic soil of chernozem with an average humus content of 2.4-3.1% in the upper horizons and only 1.6% in the transition horizon, total nitrogen content of 0.14-0.25% at the test site of the Agricultural Research and Development Station (ARDS) Brăila - Chiscani Experimental Center where a large number of maize hybrids from different maturity groups are tested annually.

Key words*: maize, FAO maturity groups, grain yields hybrids.*

INTRODUCTION

Maize (*Zea mays* L.) is, along with rice and wheat, one of the most widespread cereal crops in the world.In the European Union, maize is the second-most cultivated crop after wheat. In 2024, maize was cultivated on 8.8 million ha for grain maize and in Romania the cultivated area was 2.2 million ha. (EUROSTAT, 2024).

Maize is a major global agricultural crop of strategic importance in international agri-food systems, both directly for human consumption and indirectly as an essential source of animal feed. In recent decades, world production of this cereal has increased significantly, driven by growing global demand, as well as by improvements in productivity per unit area and the expansion of cultivated areas. Maize currently ranks first among cereals in terms of total volume produced and is expected to become the most widely grown agricultural crop in terms of area in the next ten years (Erenstein et al., 2022).

Climate change has led to lower yields for the main agricultural crops. A number of effects of climate change on crop yields have been observed in Eastern Europe, with generalized yield losses of -24.5% in maize (Ray et al., 2019).

The climatic changes continue to differ under different scenarios until 2100 and beyond. Thus under the RCP 4.5, RCP 6.0 and RCP 8.5 scenarios global temperature change is projected to exceed 1.5°C-2°C (IPCC, 2014).

Climate change and maize crop management practices have had an effect on cropping systems in South-Eastern Europe, including Bulgaria, Croatia, Hungary, Romania and Serbia, allowing earlier planting and the cultivation of early maturing hybrids to avoid unfavorable weather conditions, especially during flowering (Buhiniček et al., 2021).

Choosing the right maize maturity for a particular region to achieve higher yields in the future is not straightforward and is not just a matter of prevailing expectations of climate change. Also, management effects might play a more important role in maize phenology and yield formation compared to climate change, not only under sub-optimal growing conditions often found in South-Eastern Europe (Zdunić et al., 2022).

In a 10-year analysis it was concluded that the correlation between the length of the growing season and grain moisture at harvest is positive and significant. The highest grain yield was in the medium-early FAO 500-600 maturity group but the best combination of high grain yield and low moisture at harvest was in the early FAO 300-400 maturity group (Vulchinkov et al., 2013). Under climate change, there are shifts in maize maturity groups, particularly over the last 10 years, from extremely early and early-maturing maize to more hybrids in the intermediate and late-maturing groups, as rising average temperatures and warmer autumns have meant yield increases can be achieved and early frosts have been pushed later, from late September to early to mid-October (Haraga and Ion, 2022).

The objective of this research is to highlight the productive capacities, adaptation potential and grain moisture at harvest of maize hybrids from different FAO groups tested in the trials conducted at the Agricultural Research and Development Station Braila.

MATERIALS AND METHODS

The study was conducted during 2023 and 2024 to evaluate the adaptability to local conditions of 12 maize hybrids of different maturity groups, FAO 300-340 (DKC4109, DKC4391), FAO 350-390 (DKC4728, DKC4709, DKC4712, DKC4897, DKC4908, DKC5092), FAO 400-440 (DKC5016, DKC5404) and FAO 450-490 (DKC5810, DKC5812).

The study and field experiments were organized in the Experimental Center Chiscani of ARSD Braila on a carbonate vermic chernozem soil with an apparent density ranging from 1.19 g/cm^3 in the worked horizon (Ap) to 1.44 g/cm^3 in the other soil horizons.

Regarding the chemical characteristics of the soil profile in the experimental perimeter of SCDA Brăila, the content of mobile phosphorus, with values ranging from 41 ppm to 62 ppm, the soil falls into the medium and is well supplied with phosphate. Mobile potassium supply is medium, with values ranging from 98 ppm to 108 ppm, and the humus content in the worked layer is 3.04%. (Trifan et al., 2021).

The pre-planting crop was fall wheat. The applied technological elements included ploughing, one disc tillage, base fertilization with complex fertilizers 200 kg/ha NPK 20.20.20.0 and urea phase fertilization 100 kg/ha. Sowing was carried out on 24.04.2023 and 16.04.2024, respectively. Crop maintenance was carried out by applying treatments in vegetation to control weeds and pests and applying two irrigation rules totalling 800 mc/ha.

The production capacity and quality elements such as hectoliter weight HM (kg/hl) and thousand kernel weight TKW (g) were followed. Analysis of variance (ANOVA) was performed to examine differences and a Fisher's protected least significant difference (LSD) test was used to determine the significance of the differences among the variants results and control (p-values 0.05, 0.01, and 0.001).

RESULTS AND DISCUSSIONS

The analysis of the rainfall regime of the agricultural year 2022-2023 allows us to state thatoverall, the analyzed period can be characterized in terms of normal rainfall, but with uneven rainfall distribution during the agricultural year, alternating dry periods with periods better supplied with rainfall. Rainfall totaled 439 mm with a deficit of 3 mm compared to the multi-year monthly average of 442 mm. Thermally, the agricultural year was warmer than the multi-year average by 2.1°C. From October 2022 through September 2023, there were positive deviations from the multi-year monthly average in every month except April and May which were cooler than the multi-year monthly average by 0.8°C and 0.1°C respectively (Table 1).

Table 1. The main climatic elements of agricultural year 2022-2023

Climatic elements		Month values												Total/ Average
		X	XI	XII	I	II	III	IV	V	VI	VII	VII	IX	
Precipitation (mm)	Agricultural year 2022-2023	6	31	20	64	7	13	66	40	26	106	55	5	**439**
	Normal	30	33	36	28	27	26	35	48	62	46	39	32	**442**
	Deviation ±	**-24**	**-2**	**-16**	**36**	**-20**	**-13**	**31**	**-8**	**-36**	**60**	**16**	**-27**	**-3**
Air Temperature (^{0}C)	Agricultural year 2022-2023	13	8.1	2.9	4.4	1.4	7.9	10.4	16.6	21.6	24.7	24.7	20.9	**13.1**
	Normal	11.5	5.6	0.6	-2.1	-0.2	4.7	11.2	16.7	20.9	22.9	22.1	17.3	**10.9**
	Deviation ±	**1.5**	**2.5**	**2.3**	**6.5**	**1.6**	**3.2**	**-0.8**	**-0.1**	**0.7**	**1.8**	**2.6**	**3.6**	**2.1**

Source: Meteorological Station of Braila

The analysis of the rainfall regime of the agricultural year 2023-2024 allows to state that overall, the analyzed period can be characterized from the normal rainfall point of view, the rainfall totaled 471 mm with a positive deviation of 29 mm compared to the multi-year monthly average of 442 mm (Figure 1).

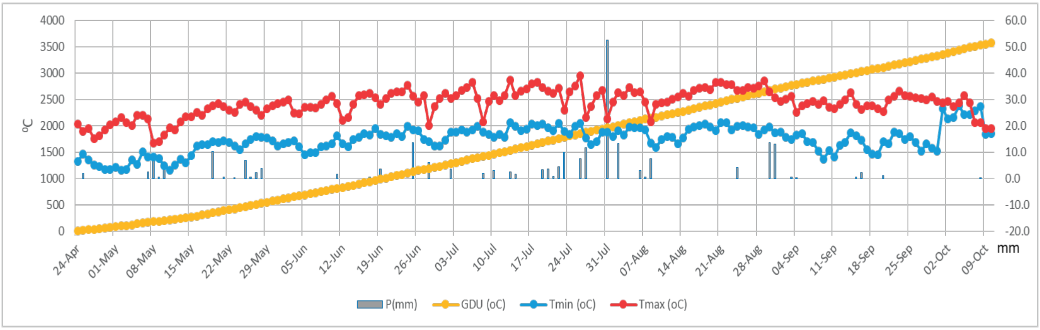

Figure 1. Presentation of climatic conditions and the sum of degrees of useful temperature during the vegetation period for maize, at ARDS Braila, in 2023

From a thermal point of view, the agricultural year was very warm compared to the multiannual average by 3.5°C (Table 2). On the whole, the analyzed period can be characterized as normal in terms of rainfall, but with an uneven rainfall distribution during the agricultural year, alternating dry periods with periods better supplied with rainfall.

Table 2. The main climatic elements of agricultural year 2023-2024

Climatic elements		Month values												Total/ Average
		X	XI	XII	I	II	III	IV	V	VI	VII	VII	IX	
Precipitation (mm)	Agricultural year 2023-2024	3	124	23	16	0	40	26	35	47	33	17	107	471
	Normal	30	33	36	28	27	26	35	48	62	46	39	32	442
	Deviation ±	-27	91	-13	-12	-27	14	-9	-13	-15	-13	-22	75	29
Air Temperature (^{0}C)	Agricultural year 2023-2024	15.9	8.1	3.6	0.4	7.4	8.2	16.8	16.6	24.3	26.5	25.2	20.0	14.4
	Normal	11.5	5.6	0.6	-2.1	-0.2	4.7	11.2	16.7	20.9	22.9	22.1	17.3	10.9
	Deviation ±	4.4	2.5	3	2.5	7.6	3.5	5.6	-0.1	3.4	3.6	3.1	2.7	3.5

Source: Meteorological Station of Brăila.

In the 2023 agricultural year, the maize crop experienced a vegetation period of 169 days, from sowing to harvest. During this period, the total accumulation of Growing Degree Units (GDU) reached 3567 °C, a value considered favorable for the full development of even semi-late and late hybrids. In contrast to the favorable thermal regime, the rainfall regime proved to be below the physiological requirements of the crop. The total precipitation accumulated over the entire vegetation period was only 233 mm, with an uneven distribution and extended periods of both atmospheric and soil drought. This water deficit is particularly critical during pollination and grain filling, potentially leading to a reduction in the number of kernels per ear and the weight of 1000 kernels.

Although the thermal conditions in 2023 provided excellent premises for a high-performing maize crop, the significant precipitation deficit represents a major limiting factor for yield, highlighting the importance of adopting adaptation measures such as the use of drought-tolerant hybrids and the application of irrigation.

In 2024, the maize crop underwent a vegetation period of 177 days from sowing to harvest, characterized by a high thermal regime and a slight improvement in rainfall compared to the previous year (Figure 2). The total GDU accumulated during this interval was 3741 °C, exceeding the value recorded in 2023, indicating an increased thermal accumulation. The rainfall regime recorded a total of 320 mm of precipitation, significantly higher than the previous year (+87 mm), yet still below the optimal requirement for maize under non-irrigated conditions.

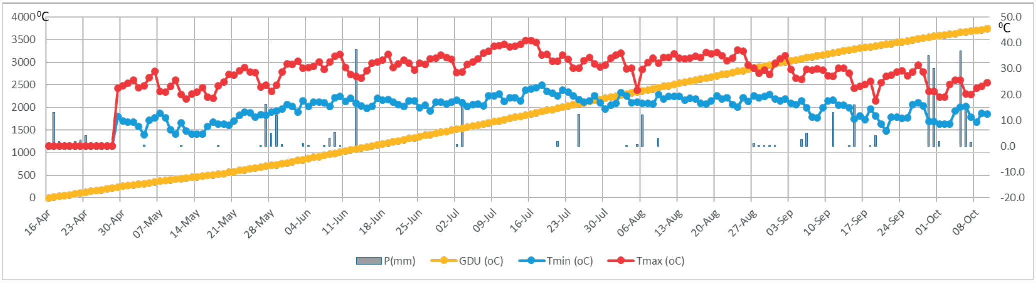

Figure 2. Presentation of climatic conditions and the sum of degrees of useful temperature during the vegetation period for maize, at ARDS Brăila, in 2024

Grain yield

The climatic conditions in 2023 were favorable especially for late maturing hybrids in the FAO 450-490 group allowing very good yields (Table 3).

Table 3. Production results of maize hybrids in 2023

Maize hybrids	Yield 2023 kg/ha	%	Diff. by control	Significance
FAO hybrids 300-340				
DKC 4109	10984	92.2	-935	-
DKC 4391	11980	100.5	60	-
FAO hybrids 350-390				
DKC 4728	9603	80.6	-2316	ooo
DKC 4709	7877	66.1	-4042	ooo
DKC 4712	9236	77.5	-2683	ooo
DKC 4897	12253	102.8	333	-
DKC 4908	9644	80.9	-2275	ooo
DKC 5092	17004	142.7	5084	***
FAO hybrids 400-440				
DKC 5016	11666	97.9	-253	-
DKC 5404	9826	82.4	-2093	oo
FAO hybrids 450-490				
DKC 5810	15206	127.6	3286	***
DKC 5812	17758	149.0	5838	***
Average (control)	**11919**	100.0	Mt.	
LSD 5%=1227.82kg/ha; LSD 1%=1672.69 kg/ha; LSD0.1%=2248.05 kg/ha				

The results confirm the need to choose hybrids according to their adaptability to climatic variations and their capacity to efficiently capitalize on available resources. The late hybrids (FAO 450-490), DKC 5812 and DKC 5810 benefited significantly from the high rainfall (106 mm in July and 55 mm in August) and high temperatures in July and August, obtaining yields of 15206 kg/ha and 17758 kg/ha, respectively.

The hybrid DKC 5092 of the FAO 350-390 group made efficient use of climatic and technological resources and obtained a very good yield and a significant difference compared to the average of the experiment.

The poorer results of early and intermediate hybrids can be explained by the drier period in May and June during generative development.

The climatic conditions of 2023-2024, with consistently high temperatures and extremely high deviations in June-August ranging from 3.1-3.6°C and precipitation deficits in April-August, put pressure on late hybrids (especially DKC 5810 and DKC 5812), significantly reducing their yields compared to the first year of testing. The climatic conditions were favorable to the intermediate hybrids FAO 350-390, DKC 4728, and DKC 4897, which obtained the highest yields of 12506 kg/ha respectively 12546 kg/ha, statistically assured results compared to the average yield of the experiment (Table 4).

Table 4. Production results of maize hybrids in 2024

Maize hybrids	Yield 2024 kg/ha	%	Diff. by control	Significance
FAO hybrids 300-340				
DKC 4109	11088	109.8	991	**
DKC 4391	9133	90.5	-964	o
FAO hybrids 350-390				
DKC 4728	12506	123.9	2408	***
DKC 4709	8004	79.3	-2093	ooo
DKC 4712	7883	78.1	-2214	ooo
DKC 4897	12546	124.2	2248	***
DKC 4908	9926	98.3	-171	-
DKC 5092	9767	96.7	-330	-
FAO hybrids 400-440				
DKC 5016	9703	96.1	-394	-
DKC 5404	12003	118.9	1906	***
FAO hybrids 450-490				
DKC 5810	9533	94.4	-564	-
DKC 5812	9075	89.9	-1023	oo
Average (control)	**10097**	100.00	Mt.	
LSD 5%=725.04 kg/ha; LSD 1%=987.74 kg/ha; LSD0.1%=1327.49 kg/ha				

From the FAO 400-440 group, the hybrid DKC 5404 stood out with a yield of 12003 kg/ha with a positive difference that was very semi-significant compared to the average of the experiment.

The choice of hybrids for drought-prone areas should focus on those with better water and heat stress adaptability, such as DKC 5404, DKC 4728, and DKC 4897, which have demonstrated stability and performance even under extreme climatic conditions.

Values of **the hectolitre mass (HM)** for the analyzed maize hybrids are presented in Table 5. Results confirm that 2023 was generally favorable for maize development, allowing most hybrids to achieve high hectolitre mass values. The best hectolitre mass results were achieved by the intermediate hybrids in the FAO 350-390 group, in particular DKC 4728 (75.46 kg/hl) and DKC 4709 (76.03 kg/hl). The above-average hectoliter mass of most hybrids indicates favorable climatic conditions during the ripening and grain-filling period in 2023**.**

Table 5. Results of hectolitre mass (HM) 2023-2024

Maize hybrids	MH 2023 kg/hl	MH 2024 kg/hl	Average MH hl/kg	Diff. by control kg/hl
FAO hybrids 300-340				
DKC 4109	74.40	69.9	72.15	0.14
DKC 4391	72.83	67.7	70.27	-1.75
FAO hybrids 350-390				
DKC 4728	75.46	71.7	73.58	1.57
DKC 4709	76.03	70.4	73.22	1.21
DKC 4712	71.86	65.30	68.58	-3.43
DKC 4897	72.06	69.3	70.68	-1.33
DKC 4908	74.26	65.2	69.73	-2.28
DKC 5092	72.83	68.00	70.42	-1.60
FAO hybrids 400-440				
DKC 5016	73.9	66.8	70.35	-1.66
DKC 5404	74.36	70.8	72.58	0.57
FAO hybrids 450-490				
DKC 5810	73.23	69.4	71.32	-0.70
DKC 5812	73.6	71.8	72.70	0.69
Average (control)	**73.42**	**70.60**	**72.01**	**Mt.**

The average hectolitre weight for 2024 was 70.60 kg/hl, down on the previous year 73.42 kg/hl. This decrease can be directly correlated to the more stressful climatic conditions in the 2024 season, extremely high temperatures and a pronounced water deficit during specific growing periods.

The hybrids with the best overall stability and performance, demonstrated by the high average MH values over the two years, are: DKC 4728 (73.58 kg/hl) and DKC 4709 (73.22 kg/hl) from FAO 350-390; DKC 5404 (72.58 kg/hl) from FAO 400-440; DKC 5812 (72.70 kg/hl) from FAO 450-490.

In year 2023 the average **thousand-kernel weight** (TKW)was 378.83 g, a very high value, indicating very favorable conditions for the development and filling of the corn grain (Table 6). The semi-late hybrids DKC 5016 with 409.63 g and DKC 5404 with 407.94 g were highlighted. Very good results were also obtained by the late hybrids of the FAO 450-490 group, above the average of the experience, showing the capacity of late hybrids to capitalize on the growing conditions.

Table 6. Thousand-kernel weight (TKW) results 2023-2024

Maize hybrids	TKW 2023 g	TKW 2024 g	Average g	Diff. by control g
FAO hybrids 300-340				
DKC 4109	338.06	218.43	278.46	-31.30
DKC 4391	344.32	245.66	294.99	-14.55
FAO hybrids 350-390				
DKC 4728	327.34	220.52	273.93	-35.61
DKC 4709	317.92	251.16	284.54	-25.00
DKC 4712	301.12	241.34	271.23	-38.31
DKC 4897	328.30	274.26	301.28	-8.26
DKC 4908	339.37	211.40	275.39	-34.16
DKC 5092	381.20	232.62	306.91	-2.63
FAO hybrids 400-440				
DKC 5016	409.63	249.45	329.54	20
DKC 5404	307.94	251.87	279.91	-29.64
FAO hybrids 450-490				
DKC 5810	397.10	253.90	325.50	15.96
DKC 5812	360.56	226.60	293.58	15.96
Average (control)	**378.83**	**240.25**	**309.54**	**Mt.**

In 2024 which was climatically more difficult, the average TKW was 240.25 g, significantly lower compared to 2023, indicating stress conditions such as dry spells, extreme temperatures, which reduced dry matter accumulation in the grain. The best results were obtained by the semi-late hybrids of FAO group 400-440, DKC 5404 with 251.87 g and hybrid DKC 5016 with 249.45 g.

The hybrids with the most stable TKW results for the two test years were the FAO 400-440, DKC 5404 and DKC 5016 hybrids that showed the best adaptability, performance and overall stability under contrasting climatic conditions in the two test years.

CONCLUSIONS

Concerning the yields obtained by the hybrids studied, the year 2023 was particularly favorable for late hybrids, group FAO 450-490, with DKC 5812 and DKC 5810 being the most favorable. The year 2024, with more unfavorable periods for maize crop especially in the generative periods, led to significant yield decreases especially for late hybrids and was more favorable for the semi-late hybrids of the FAO 350-390 group.

The stability of hectolitre mass under variable conditions emphasizes hybrids with good stress tolerance, in particular DKC 4728, DKC 5404, DKC 5812, which should be preferred to ensure consistent yield quality. Concerning TKW values, DKC 5404 and DKC 5016 hybrids provide good quality stability regardless of climatic conditions.

In the context of current climate change and increased variability of annual climatic conditions, the recommendation is to prioritize the choice of hybrids that combine good yields with stability of physical quality of production (MH and TKW), such as DKC 5404 and DKC 4728, to reduce the risk of economic losses under difficult climatic conditions.

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GLUTEN AND PROTEIN CONTENT IN WHEAT GENOTYPES – COMPARATIVE ANALYSIS

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Abstract

Gluten and protein content were analyzed in 25 wheat genotypes (Wg5026 to Wg5050). The study was conducted at ARDS Lovrin, during the agricultural year 2023 – 2024. The comparative crops were organized in randomized replicates. The gluten content (Glt) varied between Glt = 46.00±0.48% (Wg5027, Wg5029) and Glt = 54.00±0.48% (Wg5042). The protein content (Pro, %) varied between Pro = 24.20±0.56% (Wg5041) and Pro = 35.90±0.56% (Wg5035). A comparative analysis was used to find out the differences between genotypes in relation to the quality indices studied. The gluten increase (ΔGlt) was between ΔGlt = 0.92% and ΔGlt = 3.92% (Wg5036, Wg5042), and eight genotypes showed statistical safety. The protein increase (ΔPro) ranged from ΔPro = 0.04% to ΔPro = 7.24% (Wg5035), and nine genotypes showed statistical safety. According to PCA, PC1 explained 53.466% of variance, and PC2 explained 46.534% of variance. Cluster analysis grouped the genotypes based on similarity, and genotypes ranking was done, based on the quality indices considered. The results are valuable for genotype selection in the wheat breeding program, as well as for agricultural practice.

Key words: *breeding program, genetic potential, gluten, protein, wheat genotypes.*

INTRODUCTION

Gluten and protein in wheat grains are quality indices of high importance for the quality of flour, for the food industry, and the quality of finished products (Zilić et al., 2011; Schopf et al., 2021; Schuster et al., 2023).

Improving the nutritional properties of wheat grains is a major objective in wheat breeding programs (Guzman et al., 2016; Fradgley et al., 2023; Petrović et al., 2024).

The performance of wheat genotypes for yield, for the main quality indices, essential in relation to the finished products, but also the "genotype x environment" interaction proven for different cultivation areas, are important criteria for selecting valuable genotypes (Tanin et al., 2022; Petrović et al., 2024).

Testing wheat genotypes in multiple locations, with varying climate and soil conditions, is important to understand and explain the "genotype x environment" interaction, and for selecting performing genotypes for specific locations (Tanin et al., 2022; Petrović et al., 2024; Temizgul et al., 2024).

The need to identify wheat genotypes adaptable to environmental conditions and climate change has been analyzed and communicated in various studies (Gebrewahid et al., 2020; Takač et al., 2021; Javed et al., 2022; Dimitrov et al., 2023).

For certain "key traits" differentiated variability was recorded in wheat, in relation to genotype, crop location, and "genotype x location" interactions (Gebrewahid et al., 2020).

Agronomic traits, yield and quality indices were studied in different collections of wheat genotypes, and valuable genotypes, or groups of genotypes, were identified (Amiri et al., 2018; Thungo et al., 2020; Alemu et al., 2021; Mahdavi et al., 2022; Gheorghe and Nicolae, 2023; Temizgul et al., 2024).

In response to environmental and technological conditions, variations in quality indices and wheat yield have been recorded, in relation to water, nutrient supply, crop rotation or different inputs (Sala et al., 2016; Attafy et al., 2023; Hao et al., 2023; Ceclan et al., 2024; Yordanova et al., 2024). Associated with environmental conditions, interactions between

morphological parameters of wheat grains and certain quality indices, e.g. starch and protein, have also been recorded (Mahdavi et al., 2022).
Under current crop and technological conditions, comparative studies have been conducted between old and modern wheat genotypes (De Santis et al., 2017; Brouns et al., 2022). In relation to the quality indices considered, similarities and differences were recorded in wheat genotypes (e.g. in gluten index, protein content), and in relation to the quality of the finished products and certain dietary diets, interest was shown, and different genotypes were selected (De Santis et al., 2017; Abdelaleem and Al-Azab, 2021; Brouns et al., 2022)
Comparative studies have facilitated the selection of appropriate wheat genotypes in relation to the cultivation location (climate and soil conditions), the agricultural system (conventional, organic) and the quality of the finished products (Takač et al., 2021).
Various data analysis methods have been used to differentiate valuable wheat genotypes, producers, or other associated elements, in relation to specific quality and yield objectives (Alemu et al., 2021; Schopf et al., 2021; Javed et al., 2022; Tanin et al., 2022).
This study analyzed the gluten and protein content of wheat grains, in a collection of 25 wheat genotypes, to identify performing genotypes for the considered quality indices, with utility for the wheat breeding program, and for their recommendation in agricultural practice.

MATERIALS AND METHODS

The study, research, and field experiments were organized and conducted within the ARDS Lovrin. The field experiments were organized in the agricultural year 2023-2024. The climatic conditions during the study period are presented in Figure 1.

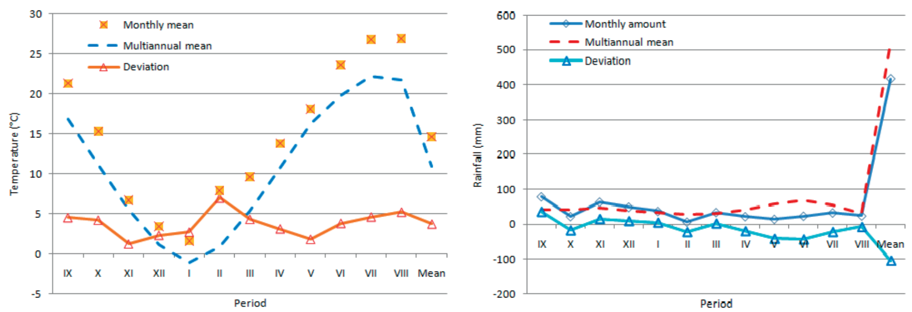

Figure 1. Climatic conditions in experimental area; temperature and precipitation values

Twenty-five wheat genotypes were considered and tested in comparative crops. The genotype names were in the format Wg5025 to Wg5050. Each genotype was cultivated in three replicates.
The field experiments were located in flat terrain conditions, medium fertility soil, chernozem type. The previous crop was field peas. The land was prepared for sowing by plowing, disking (two works), and combinator (two works). Sowing was done in the second decade of October 2023. Emergence was recorded in the first decade of November 2023.
Fertilization was done in the fall with complex fertilizer 15N:15P:15K at a dose of 180 kg/ha. In the spring, fertilization was completed with urea at a dose of 118 kg/ha.
Treatments were made with Omnera at a dose of 1 L/ha for weed control, and Inazuma for phytosanitary control in wheat crops.
Harvesting was done on each experimental variant (genotype and repetition) at maturity (Meier, 2001).
Subsamples were taken from the grain production of each genotype to determine the gluten (Glt, %) and protein (Pro, %). The analyses were made in the Wheat Breeding Laboratory, ARDS Lovrin.
According to the purpose of the study, the experimental data were analyzed in order to compare the genotypes tested for the two quality indices. The Anova Test (EXCEL),

Descriptive Statistical Analysis, t Test, Wilcoxon tests, Multivariate analysis, and Ranking were applied (Hammer et al., 2001).

RESULTS AND DISCUSSIONS

The mean values of the quality indices (Glt, Pro), and the calculated ratios (Glt/Pro, Pro/Glt) for the 25 studied wheat genotypes were analyzed by Anova Test (Alpha 0.05) and Descriptive Statistical Analysis for the general characterization of the experimental data. The presence of variance and statistical safety of the data were confirmed (Table 1).

Table 1. Anova Test results

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	42238.15	3	14079.38	4073.143	5E-101	2.6994
Within Groups	331.8373	96	3.456638			
Total	42569.99	99				

In the case of gluten content, values between Glt = 46.00±0.48% were recorded for the Wg5027 genotype, and Glt = 54.00±0.48% for the Wg5029 and Wg5042 genotypes. In the case of protein content, values between Pro = 24.20±0.56% for the Wg5041 genotype, and Pro = 35.90±0.56% for the Wg5035 genotype were recorded (Table 2). In the case of the Glt/Pro ratio, the recorded values were between Glt/Pro = 1.31±0.04 in the Wg5035 genotype, and Glt/Pro = 2.07±0.04 in the Wg5041 genotype. In the case of the Pro/Glt ratio, the recorded values were between Pro/Glt = 0.48±0.01 in the Wg5041 genotype, and Pro/Glt = 0.76±0.01 in the Wg5035 genotype (Table 2). The graphical distribution in box-plot format of the recorded values for the indices and the calculated ratios is presented in Figure 2.

Table 2. Descriptive statistical analysis values for quality indices and calculated ratios for the studied wheat genotypes

Statistical Parameters	Glt	Pro	Glt/Pro	Pro/Glt
N	25	25	25	25
Min	46.00	24.20	1.31	0.48
Max	54.00	35.90	2.07	0.76
Sum	1252.00	716.60	44.08	14.34
Mean	50.08	28.66	1.76	0.57
Std. error	0.48	0.56	0.04	0.01
Variance	5.8267	7.9591	0.0363	0.0044
Stand. dev	2.41	2.82	0.19	0.07
Median	50.00	28.70	1.74	0.58
25 prcntil	48.00	26.15	1.64	0.52
75 prcntil	52.00	30.30	1.94	0.61
Skewness	-0.14031	0.53459	-0.31474	0.85446
Kurtosis	-0.98621	0.29252	-0.18694	1.07475
Coeff. var	4.82	9.84	10.80	11.63

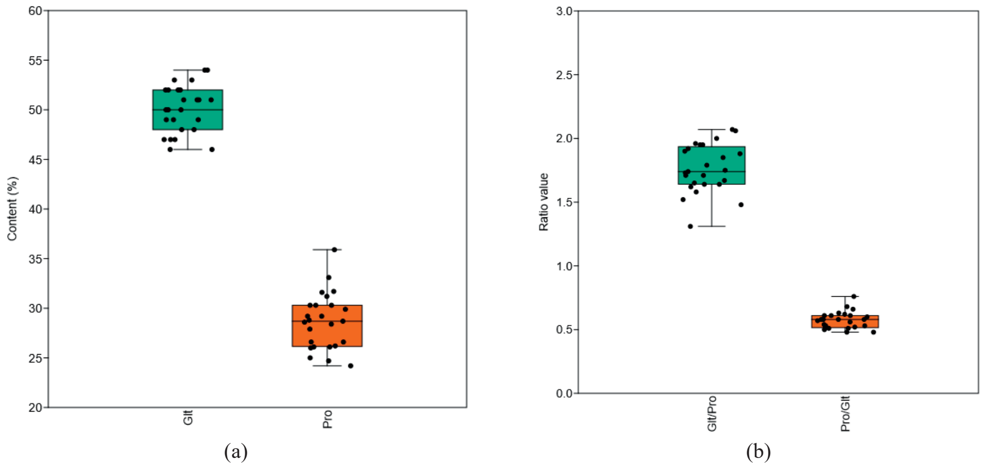

Figure 2. Distribution of data series for quality indices (a), and calculated ratios (b), for the studied wheat genotypes

The results regarding gluten content showed variability at the level of CV = 4.82, and protein content showed variability at the level of CV = 9.84. In the case of calculated ratios, the variability was at the level of CV = 10.80 for the Glu/Pro ratio, and CV = 11.63 for the Pro/Glu ratio respectively.

To compare the performance of wheat genotypes for grain gluten and protein content, the mean value at the experiment level, for each index, was calculated, and the results of each genotype were compared against the mean value. In the case of gluten content, the mean value at the experiment level was $\overline{Glt}$ = 50.08±0.48%. Compared to the mean value, a number of 12 genotypes showed positive differences, and 13 genotypes showed negative differences (Table 3).

Table 3. Gluten content of the studied wheat genotypes

Wheat genotypes	Gluten content (Glt)	Differences compared to the mean value	Percentage expression of differences (100%)	Sig
Wg5026	51	0.92	101.84	ns
Wg5027	46	-4.08	91.85	ooo
Wg5028	47	-3.08	93.85	ooo
Wg5029	46	-4.08	91.85	ooo
Wg5030	49	-1.08	97.84	o
Wg5031	51	0.92	101.84	ns
Wg5032	50	-0.08	99.84	ns
Wg5033	53	2.92	105.83	***
Wg5034	52	1.92	103.83	***
Wg5035	47	-3.08	93.85	ooo
Wg5036	54	3.92	107.83	***
Wg5037	52	1.92	103.83	***
Wg5038	51	0.92	101.84	ns
Wg5039	51	0.92	101.84	ns
Wg5040	50	-0.08	99.84	ns
Wg5041	50	-0.08	99.84	ns
Wg5042	54	3.92	107.83	***
Wg5043	48	-2.08	95.85	ooo
Wg5044	53	2.92	105.83	***
Wg5045	48	-2.08	95.85	ooo
Wg5046	52	1.92	103.83	***
Wg5047	52	1.92	103.83	***
Wg5048	49	-1.08	97.84	o
Wg5049	47	-3.08	93.85	ooo
Wg5050	49	-1.08	97.84	o
Mean	50.08	-	100.00	-
SE	±0.48	-	-	-

In the case of genotypes with values above the mean, the increase in gluten content (ΔGlt) was between ΔGlt = 0.92% (four genotypes), and ΔGlt = 3.92% (two genotypes). In the case of genotypes with values above the mean gluten content, in eight genotypes the increase in gluten content (ΔGlt) presented statistical safety, at the p<0.001 level (Table 3).

In the case of genotypes with values below the mean of the experiment, the differences in gluten content (ΔGlt) ranged between ΔGlt = - 4.08% (two genotypes), and ΔGlt = - 0.08% (three genotypes).

In the case of genotypes with gluten content values lower than the mean of the experiment, in ten genotypes the negative increase in gluten content (ΔGlt) presented statistical safety, at the p<0.05 level (three genotypes), and at the p<0.001 level (seven genotypes) (Table 3). The differences in gluten content, in relation to the mean value, recorded in the wheat genotypes studied, are presented in Figure 3.

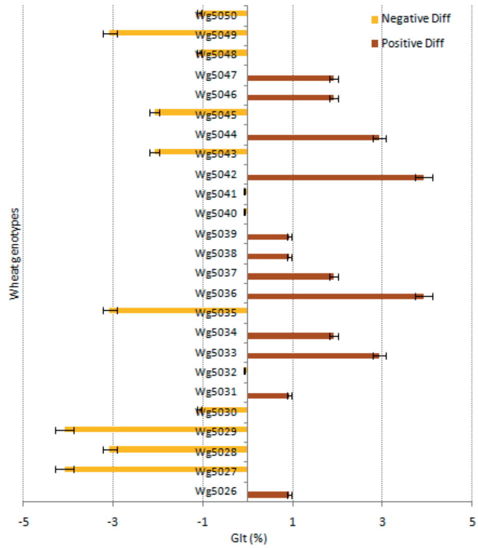

Figure 3. Gluten content differences in wheat genotypes

In the case of protein content, the mean value at the experiment level was $\overline{Pro}$ = 28.66±0.56%. Compared to the mean value, a number of 13 genotypes showed positive differences, and 12 genotypes showed negative differences (Table 4). In the case of genotypes with above-mean values, the increase in protein content (ΔPro) was between ΔPro = 0.04% (genotype Wg5049), and ΔPro = 7.24% (genotype Wg5035).

In the case of genotypes with above-mean protein content values, in nine genotypes the protein increase (ΔPro) presented statistical safety, at $p<0.05$ level (one genotype), at $p<0.01$ level (three genotypes), and at $p<0.001$ level (five genotypes) (Table 4).
In the case of genotypes with lower values compared to the mean, the negative increase in protein content (ΔPro) was between ΔPro = -4.46% (genotype Wg5041), and ΔPro = -0.06% (genotype Wg5050). In the case of genotypes with below-mean protein content values, in nine genotypes the protein increase (ΔPro) presented statistical safety, at the $p<0.01$ level (two genotypes), and at the $p<0.001$ level (seven genotypes) (Table 4).

Table 4. Protein content of the studied wheat genotypes

Wheat genotypes	Protein content (Pro)	Differences compared to the mean value	Percentage expression of differences (100%)	Sig
Wg5026	26.10	-2.56	91.07	ooo
Wg5027	30.30	1.64	105.72	**
Wg5028	26.20	-2.46	91.42	ooo
Wg5029	28.40	-0.26	99.09	ns
Wg5030	26.10	-2.56	91.07	ooo
Wg5031	26.00	-2.66	90.72	ooo
Wg5032	29.20	0.54	101.88	ns
Wg5033	27.90	-0.76	97.35	ns
Wg5034	29.90	1.24	104.33	*
Wg5035	35.90	7.24	125.26	***
Wg5036	29.20	0.54	101.88	ns
Wg5037	26.60	-2.06	92.81	oo
Wg5038	26.60	-2.06	92.81	oo
Wg5039	24.70	-3.96	86.18	ooo
Wg5040	25.00	-3.66	87.23	ooo
Wg5041	24.20	-4.46	84.44	ooo
Wg5042	31.20	2.54	108.86	***
Wg5043	28.80	0.14	100.49	ns
Wg5044	30.30	1.64	105.72	**
Wg5045	30.30	1.64	105.72	**
Wg5046	31.60	2.94	110.26	***
Wg5047	31.70	3.04	110.61	***
Wg5048	33.10	4.44	115.49	***
Wg5049	28.70	0.04	100.14	ns
Wg5050	28.60	-0.06	99.79	ns
Mean	28.66	-	100.00	-
SE	±0.56			

The differences in protein content compared to the mean value, recorded by the studied wheat genotypes, are presented in Figure 4.

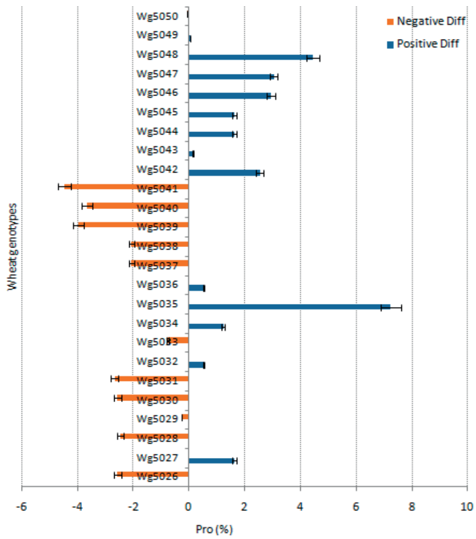

Figure 4. Protein content differences in wheat genotypes

The ratio between the quality indices considered (gluten, protein) was calculated as a result of the importance of these indices in wheat quality and the way of capitalizing on wheat production.
In the case of the gluten to protein ratio, the values varied between Glt/Pro = 1.31±0.04 in the Wg5035 genotype, and Glt/Pro = 2.07±0.04 in the Wg5041 genotype (Table 5). In the case of the protein to gluten ratio, values between Pro/Glt = 0.48±0.01 in the Wg5041 genotype, and Pro/Glt = 0.76±0.01 in the Wg5035 genotype were recorded (Table 5).
In the case of the gluten to protein ratio, the value GLT/PRO = 2.00 indicated a double gluten content relative to the protein content. Higher values of the ratio indicated a higher proportion of gluten relative to the protein content in the wheat grain.
Values lower than the identified threshold indicated a higher share of protein content in the grains, in relation to gluten. These values can be useful for the direction of grain production valorization, depending on the final products, or industrialization processes.
In the case of the Wg5040 genotype, Glt/Pro = 2.00 was recorded. In the wheat genotypes

Wg5039 and Wg5041, Glt/Pro>2.00 was recorded, and in all other genotypes studied, Glt/Pro<2.00 was recorded. The lowest value of the Glt/Pro ratio was recorded in the Wg5035 genotype, which recorded the highest protein content.

Table 5. The values of the calculated ratios between quality indices in wheat genotypes

Wheat genotype	Glt/Pro	Pro/Glt	Wheat genotype	Glt/Pro	Pro/Glt
Wg5026	1.954	0.512	Wg5039	2.065	0.484
Wg5027	1.518	0.659	Wg5040	2.000	0.500
Wg5028	1.794	0.557	Wg5041	2.066	0.484
Wg5029	1.620	0.617	Wg5042	1.731	0.578
Wg5030	1.877	0.533	Wg5043	1.667	0.600
Wg5031	1.962	0.510	Wg5044	1.749	0.572
Wg5032	1.712	0.584	Wg5045	1.584	0.631
Wg5033	1.900	0.526	Wg5046	1.646	0.608
Wg5034	1.739	0.575	Wg5047	1.640	0.610
Wg5035	1.309	0.764	Wg5048	1.480	0.676
Wg5036	1.849	0.541	Wg5049	1.638	0.611
Wg5037	1.955	0.512	Wg5050	1.713	0.584
Wg5038	1.917	0.522			
SE	±0.04	±0.01	SE	±0.04	±0.01

The multivariate analysis generated the diagram in Figure 5, in which the wheat genotypes were distributed in relation to the quality indices and the calculated ratios, as biplot. PC1 explained 76.124% of variance, and PC2 explained 23.582% of variance. Correlated with gluten was the Wg5036 genotype, which presented the highest gluten content. The Wg5035 genotype, which recorded the highest protein content, was associated with the Pro/Glt ratio.

The parameter loadings, as factors, were analyzed in relation to the principal components within the PCA (Table 6).

In relation to PC1, gluten showed a value of r = -0.500, protein showed a value of r = 0.897, the Glt/Pro ratio showed a value of r = -0.996, and the Pro/Glt ratio showed a value of r = 0.999. In relation to PC2, gluten showed a value of r = 0.866, protein showed a value of r = 0.440, the Glt/Pro ratio showed a value of -0.025, and the Pro/Glt ratio showed a value of r = 0.014.

In relation to PC3 and PC4, the correlation values recorded for the parameters considered were insignificant (Table 6).

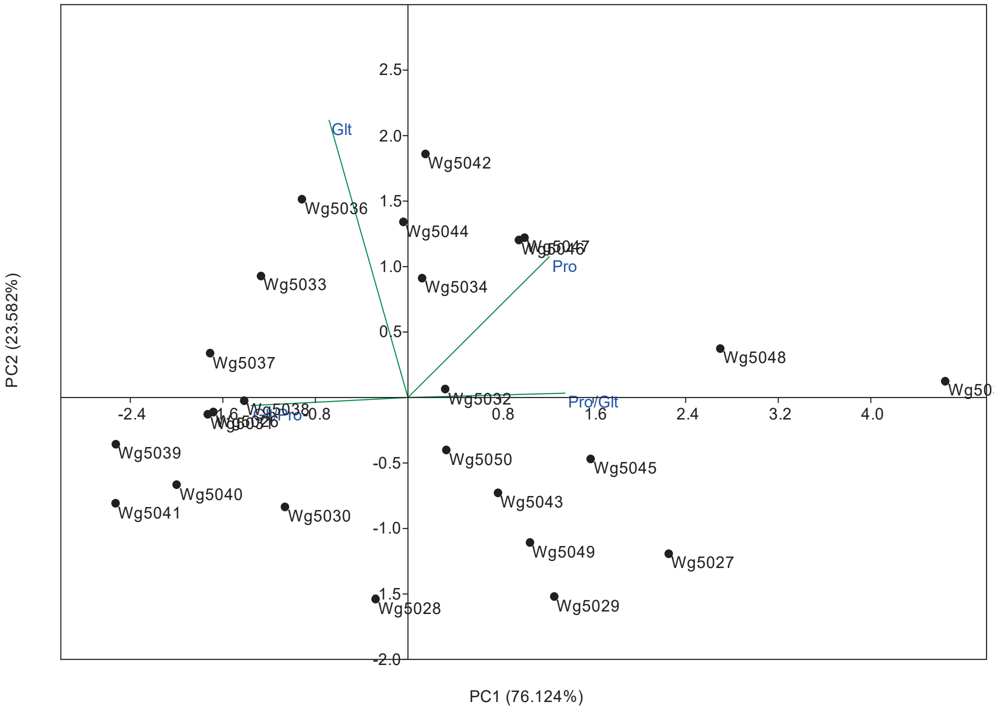

Figure 5. PCA diagram of the distribution of wheat genotypes in relation to quality indices and calculated ratios

Tabelul 6. Loadings values of the parameters in the PCA analysis

Parameters	Components			
	PC 1	PC 2	PC 3	PC 4
Glt	-0.500	0.866	-0.015	0.010
Pro	0.897	0.440	0.034	-0.020
Glt/Pro	-0.996	-0.025	0.085	-0.002
Pro/Glt	0.999	0.014	0.047	0.021

Cluster analysis generated cluster dendrograms, based on similarity in relation to gluten content values, Coph.corr. = 0.752 (Figure 6) and protein, Coph.corr. = 0.797 (Figure 7).
In relation to the Glt parameter (Figure 6), the wheat genotypes were grouped into two distinct clusters, with several subclusters each. Cluster C1 included the genotypes with low gluten content values, and cluster C2 included the genotypes with high and medium gluten content. Within cluster C2, subcluster C2-1 grouped the genotypes with the highest gluten content (Wg5036,Wg5038) and (Wg5033, Wg5044).

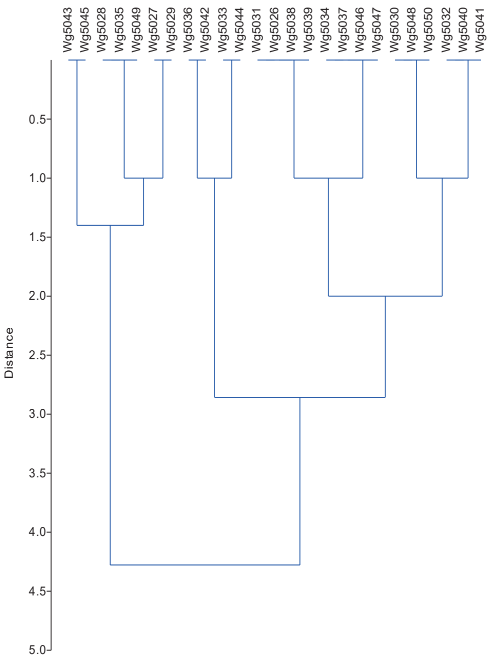

Figure 6. Cluster dendrogram of wheat genotypes based on gluten content

In relation to protein content (Figure 7), two clusters emerged, with three major subclusters.
Cluster C2 included two genotypes, with the highest protein content values (Wg5035, Wg5048).
Cluster C1 included the other genotypes, grouped into two major clusters, with several subclusters.

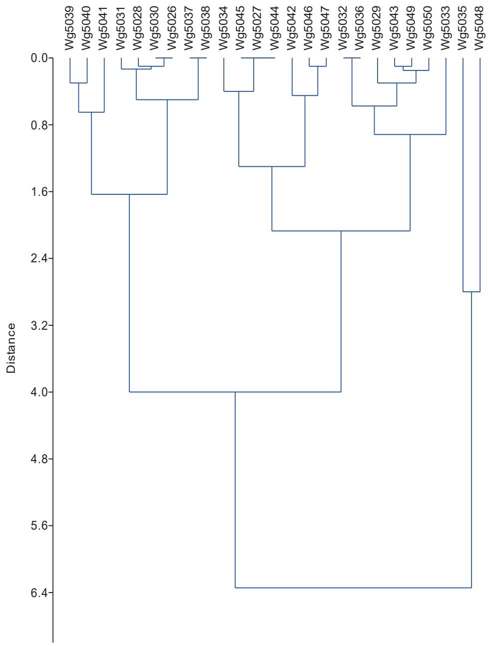

Figure 7. Cluster dendrogram of wheat genotypes based on protein content

Based on the two dendrograms, valuable wheat genotypes can be selected based on similarity, in relation to the genetic potential for gluten and protein production, respectively.
They can be used in the breeding process, as a source of germplasm. At the same time, they can be recommended for the economic sector, for farmers, in order to promote the level of agricultural crops in vegetable farms.
In relation to the two quality parameters (Glt, Pro) a value hierarchy of wheat genotypes was made (Figure 8).
The genotypes were ranked in descending order, from the top to the bottom of the diagram. This ranking facilitates the selection of genotypes in relation to their potential for the two quality indices considered.
The quality of wheat production is important for farmers, for the food industry and the quality of finished products, and for consumers

(Schopf et al., 2021; Schuster et al., 2023; Hoang et al., 2024).

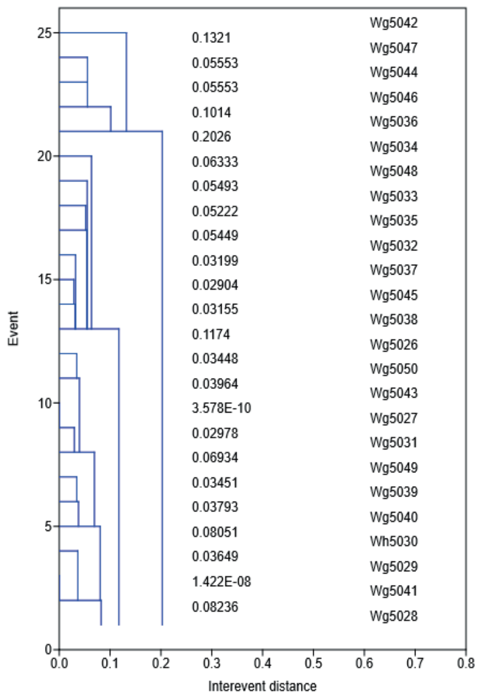

Figure 8. Ranking of wheat genotypes based on Glt and Pro indices

Wheat breeding programs place high importance on quality indices, in order to promote high-performing genotypes (De Santis et al., 2017; Abdelaleem and Al-Azab, 2021; Alemu et al., 2021).

Regarding protein and gluten, various values of content in wheat grains have been reported, in relation to the genotypes tested, environmental conditions, agricultural technologies, stress factors (Alemu et al., 2021; Temizgul et al., 2024).

In the case of the 25 genotypes tested in the present study, seven genotypes were identified with gluten content above the mean value of the experiment, with a gluten increase (ΔGlt) in conditions of statistical safety ($p<0.001$).

Regarding protein content, eight genotypes recorded values above the mean, in conditions of statistical safety, at different safety level ($p<0.05$ level – one genotype; $p<0.01$ level – three genotypes; $p<0.001$ level – five genotypes).

Parallel analysis of wheat genotypes, based on the two quality indices, showed that five genotypes ranked above the mean at the experiment level, in the case of both quality indices considered (Wg5034, Wh5042, Wg5044, Wg5046, and Wg5047). These genotypes can be considered as a valuable genetic source for the wheat breeding program. At the same time, they can be promoted for the crop, to the attention of farmers.

CONCLUSIONS

The wheat genotypes tested generated different gluten and protein content, under the study conditions, in relation to the specific genetic potential of each genotype for the quality indices considered.

Seven genotypes provided a gluten content higher than the mean of the experiment, with differences ranging between 0.92% and 3.92% (Wg5036 – the best tested genotype).

Nine genotypes provided a protein content above mean, with differences ranging between 0.04% and 2.54% (Wg5042 – the best tested genotype).

Multivariate analysis generated a PCA plot of genotypes with a scatter plot relative to the values recorded for the quality indices. The principal components fully explained the variation in the data set.

Grouping based on genotype similarity was obtained through cluster analysis, which facilitated the identification of groups of genotypes with similar results for each quality index considered.

The selection of wheat genotypes with high gluten and protein performance was possible based on the results recorded. Five genotypes were identified with above-mean values for both, gluten and protein. They will be important for the wheat breeding program, but also in the recommendation for farmers.

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STUDY OF THE COTTON VARIETY IZABELL WITH NATURAL COLORED FIBER UNDER NITROGEN FERTILIZATION AND IRRIGATION

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Abstract

Optimizing nitrogen (N) fertilization in cotton is essential for balancing yield, fiber quality, and environmental sustainability. This study examines the response of the Bulgarian cotton cultivar Izabell to varying nitrogen rates (N0, N80, N160, and N240) under irrigated and non-irrigated conditions over a three-year period (2018-2020) in Central Southern Bulgaria. The results indicate that nitrogen application significantly affects plant height, boll number, and cotton yield, with the optimal rate determined at N160 level. Irrigation was the dominant factor influencing productivity, increasing cotton yield by 33.2% compared to non-irrigated conditions. Yield variation was strongly influenced by meteorological conditions, with drought stress during flowering leading to significant yield reductions. Excessive nitrogen fertilization (N240) resulted in delayed maturity and did not further improve yield. The findings highlight the importance of site-specific nitrogen management and irrigation practices in optimizing cotton production.

***Key words**: cotton, fertilization, fiber, nitrogen, yield.*

INTRODUCTION

In many areas, both domestically and abroad, nitrogen fertilization with a positive balance is commonly applied. Excessive nitrogen doses promote excessive vegetative growth and delay maturity (Hodges, 2002). When high nitrogen rates are used, plants are unable to absorb the entire surplus of nitrogen in the soil. These additional nitrogen levels are gradually leached from the soil through water edema, resulting in the contamination of groundwater and drinking water with nitrates (Clawson, 2008; Stavrinos & Georgiev, 2006). In relation to improving nitrogen use efficiency in cotton cultivation, recent years have seen a critical reassessment of the application of high fertilizer rates.

Researchers (Silvertooth & Norton, 2011) have confirmed that nitrogen exerts a greater influence on cotton yield than any other nutrient element. Nitrogen deficiency limits plant growth and can lead to premature shedding of floral buds in cotton, ultimately resulting in reduced yields.

Managing nitrogen (N) fertilization in cotton is challenging due to issues with low or excessively high levels, the influence of other agronomic practices, and abiotic stress factors such as drought and salinity (Luo et al., 2019). Raphael et al. (2019) report that plant density and nitrogen fertilization ranging from 0 to 180 kg/ha strongly affect the yield of raw cotton and its earliness. At higher plant densities, the number of fertilized bolls in the early positions decreases by 33% and 40%, respectively, while earliness is reduced at higher nitrogen levels due to later-maturing fertilized bolls in the upper positions. Nitrogen deficiency during the growing season leads to inadequate vegetative growth, a reduction in the number of fertilized bolls, and premature aging (Zhang et al., 2017). Furthermore, nitrogen deficiency results in lower fiber quality (Hu et al., 2006; Mohsen & Rashidi, 2011; Zhang and Zhang, 2010).

According to Szilvay (2020), high nutrient application rates may lead to luxury consumption or delayed maturity, while too little nitrogen results in plant stress or suboptimal yields. Sawan et al. (2006) report that the maximum raw cotton yield (3,310 kg ha^{-1}) was achieved with the application of 160 kg N ha^{-1}, whereas the lowest yield (1,016 kg ha^{-1}) was observed in the control treatment. Gormus et al. (2016) indicate that among the tested nitrogen rates, the best results were obtained with 180 kg N ha^{-1} combined with 150 kg K ha^{-1}, leading to the highest raw cotton yield and the highest fiber turnout.

Varietal specificity in nutrient uptake has been established by Zhang et al. (2018) in an evaluation of 100 cotton genotypes based on total dry mass, total accumulated nitrogen, nitrogen uptake efficiency, and other parameters. The authors emphasize that the development of cotton varieties with higher nitrogen use efficiency, suitable for cultivation in low-nitrogen conditions, is crucial for sustainable production.

Under the influence of specific conditions and predominantly cultivation irrigation cotton in Bulgaria develops less biomass and has a shorter vegetation period compared to Greece and Spain (Saldjiev et al., 2008; Shah et al., 2021). Constable and Bange (2015) indicate that low water reserves and nitrogen concentrations in the soil are increasingly becoming a serious problem, especially in arid regions. The impact of meteorological conditions on yield, structural elements, and fiber quality is of utmost importance, as Bulgaria is at the northern limit for cotton cultivation.

The objective of this study was to determine the effect of drip irrigation and nitrogen fertilization at different rates on the productivity and quality of the Bulgarian cotton variety Izabell under the conditions of Central Southern Bulgaria.

MATERIALS AND METHODS

The cotton cultivar *Izabell* represents a significant achievement in Bulgarian cotton breeding, marking the beginning of a new generation of varieties with naturally colored brown fiber, offering both ecological and economic benefits. The fiber is short, medium-fine, with high uniformity, good elongation, and strength. In terms of fiber length and some related characteristics, *Izabell* falls slightly behind the standard cultivars *Chirpan-539* (recognized for earliness and yield potential) and *Avangard-264* (a benchmark for fiber quality).

The study examined the following parameters: meteorological conditions for the period 2018–2020; soil characteristics; plant height and dry matter accumulation rates at the stages of bud formation, flowering, and boll maturation; total yield of raw (un-ginned) cotton and fiber (t ha^{-1}); yield components, including the number of mature harvested bolls per plant and the mass of a single boll (g); fiber lint percentage, calculated as the ratio of fiber yield to total raw cotton yield (%); fiber length (mm); and the weight of 100 seeds (g).

Figure 1. A view from an experimental field with Bulgarian cotton variety *Isabell*

The study was conducted during the period 2018-2020 at Trakia University, Stara Zagora, under field conditions with both irrigated and non-irrigated treatments. The preceding crop was common wheat. The field experiment was arranged in a randomized block design with four replications, and the harvested plot size was 15 m^2 (1.80 × 8.34 m). Four nitrogen fertilization rates were tested: N0, N80, N160, and N240. Nitrogen in the form of NH_4NO_3 was applied as a single pre-sowing dose. Phosphorus in the form of triple superphosphate at a rate of P80 was applied post-harvest and incorporated into the soil through deep plowing. Drip irrigation was applied with an irrigation rate of 15 mm when soil moisture dropped below 75% of field capacity in the 0–50 cm soil layer.

The soil is well supplied with mineral nitrogen and experimental area is classified as a typical meadow-cinnamon soil. In terms of mechanical composition, it is a medium sandy-clay loam. The soil exhibits a slightly alkaline to alkaline reaction, with pH(H_2O) values averaging 7.7 in the plow layer and 7.9 in the subsoil layer.

Sowing was carried out between April 25 and April 30 at an inter-row spacing of 60 cm and an intra-row spacing of 5 cm, with a seeding depth of 3–5 cm. The plant density ranged between 120,000 and 160,000 plants per hectare. The experimental area was treated post-sowing but before emergence with the herbicide Stomp 33 EC (4 L ha^{-1}), which is effective against both grass and broadleaf weeds. Pest control measures were implemented in response to aphid and thrips infestations at the early vegetative stage. The cotton harvest was performed manually.

RESULTS AND DISCUSSIONS

The average plant height of the *Isabell* cotton variety at the flower bud formation stage over the study period was 28.4 cm, with irrigation exerting a significant effect-increasing from 25.9 cm under non-irrigated conditions to 30.9 cm under irrigation (Table 1). As the nitrogen application rate increased, plant height also increased; however, under non-irrigated conditions, no significant differences were observed between N160 and N240.

The growth rate of cotton plants was considerably more intense during the flower bud formation–flowering period (0.46 cm day^{-1}) compared to the flowering–maturity period. At the flowering stage, the average plant height reached 42.7 cm, with both studied factors (irrigation and nitrogen application) having a similar effect.

Table 1. Plant height (cm) of the Isabell cotton cultivar at the square initiation and flowering stages (2018–2020)

Version			Buttoning phase				Flowering phase			
№	Irrigation method	N	2018	2019	2020	Averag	2018	2019	2020	Average
1.	Non-irrigation	N_0	28.2	21.4	18.5	22.7	51.4	24.0	28.0	34.5
2.		N_{80}	29.5	22.7	22.5*	24.9	55.0	27.1	34.0	38.7
3.		N_{160}	29.7	24.8*	28.0***	27.5	53.2	35.5*	40.0**	42.9
4.		N_{240}	29.5	27.1**	26.0**	27.5	58.7*	34.5*	41.0**	44.7
		Average	**29.2**	**24.0**	**23.8**	**25.9**	**54.1**	**30.3**	**35.8**	**40.1**
5.	Irrigation	N_0	33.9**	23.2	23.0*	26.7	47.7	29.7*	41.0**	39.5
6.		N_{80}	35.3***	24.6**	31.5***	30.5	54.8	28.6*	40.5**	41.3
7.		N_{160}	35.3***	26.9**	34.5***	32.2	59.4*	39.6***	45.5***	48.2
8.		N_{240}	35.2***	28.1***	39.0***	34.1	62.4**	38.8***	48.0***	49.7
		Average	**34.9**	**25.7**	**32.0**	**30.9**	**57.8**	**34.2**	**43.8**	**45.3**
Average			**32.05**	**24.85**	**27.9**	**28.4**	**55.95**	**32.25**	**39.8**	**42.7**

* - P≤0.05; ** - P≤0.01; *** - P≤0.001

In ripening with increasing nitrogen rate the average plant height over the study period increased from 51.2 cm in the unfertilized control to 67.7 cm under the highest nitrogen application rate (N240) (Table 2). Under non-irrigated conditions, the application of N240 resulted in a 19.4% increase in plant height compared to the unfertilized control, whereas, in combination with irrigation, this increase reached 44.8%. Irrigation led to an average increase in plant height of 13.0% compared to non-irrigated conditions. The meteorological conditions had a significant impact on plant height, with the most favorable year, 2018, producing the tallest plants an average of 74.2 cm representing an increase of 52.0% and 30.3% compared to years 2019 and 2020.

The average total yield of unginned cotton for the Isabella variety over the study period was notably high - 1.70 t ha^{-1} (Table 3). The combined effects of irrigation and nitrogen fertilization significantly increased total yield across all years, as well as the overall mean for the period. A well-documented interaction between nitrogen fertilization, irrigation, and meteorological conditions was observed throughout the three years of the study. A comparative analysis of cotton yield under irrigated and rainfed conditions revealed that the prevailing climatic conditions had the strongest impact on yield variability, accounting for 51.27% of the total factor influence under irrigated conditions and 85.33% under rainfed conditions.

In 2018, the highest mean yield was recorded - 1.89 t ha^{-1}, exceeding the yields of 2019 and 2020 by 14.5% and 22.2%, respectively. The substantial interannual variation in yield underscores the pronounced influence of environmental factors. The meteorological conditions of 2018 were particularly favorable for the full realization of the genetic potential and timely ripening of the variety, which incorporates germplasm from *Gossypium* *barbadense*. The primary reason for the high yields in 2018 was the optimal combination of temperature and precipitation during the growing season. The rainfall during May–July 2018, totalizing 259.7 mm, facilitated timely germination, ensured good stand establishment, and stimulated the retention and development of a high number of fertile bolls.

Table 2. The height of cultivar cotton Izabell in ripening phase (cm), 2018-2020

Version			Year of experiment			Average		
№	Irrigation method	N	2018	2019	2020	cm	% into no-irrigation	% into N_0
1.	No-Irrigation	N_0	63.5	43.7	43.0	50.1	100.0	100.0
2.		N_{80}	74.5**	47.8	43.0	55.1	100.0	110.0
3.		N_{160}	80.0***	49.7*	51.0	60.2	100.0	120.2
4.		N_{240}	75.0**	50.3*	54.0*	59.8	100.0	119.4
Average no-irrigation			**73.3**	**47.9**	**47.8**	**56.3**	-	-
5.	Irrigation	N_0	61.5	42.2	53.0*	52.2	104.2	100.0
6.		N_{80}	63.5	46.5	62.5*	57.5	104.4	110.2
7.		N_{160}	84.5***	53.0**	70.0*	69.2**	115.0	132.6
8.		N_{240}	91.0***	56.8***	79.0*	75.6***	126.4	144.8
Average irrigation			**75.1**	**49.6**	**66.1**	**63.6**	-	-
Average			**74.2**	**48.75**	**56.95**	**59.95**	-	-

* - P≤0.05; ** - P≤0.01; *** - P≤0.001

In contrast, the lower yields in 2020 were attributed to elevated temperatures during the growing season and prolonged summer drought, which led to premature shedding of buds and flowers and hindered proper boll development. Khan et al. (2017) emphasize that nitrogen fertilization in cotton, particularly under water-deficit conditions, plays a crucial role in mitigating drought-induced stress and promoting growth and development.

The total yield reached its maximum value of 2.42 t ha⁻¹ under N240 fertilization in irrigated conditions in 2020, representing an increase of 214.8% compared to the unfertilized control in the same year, with statistical significance at p ≤ 0.1%.

The results for the total yield of non-defoliated cotton under both irrigation regimes indicate high efficiency of the applied nitrogen fertilization rates. Nutrient deficiency or insufficient fertilizer supply fails to provide adequate assimilates, thereby limiting cotton plant development, reducing the number and size of bolls, and ultimately decreasing both total cotton yield and fiber yield. Yield increased from 1.52 t ha⁻¹ under unfertilized conditions to 1.81 t ha⁻¹ under N160, which represents a 19.3% increase compared to the control. However, at N240, a slight decline in yield was observed (by 2.8% compared to N160). On average over the study period, N160 proved to be the optimal nitrogen rate. The strong interaction between irrigation and nitrogen fertilization led to a significant increase in yield, confirmed across years and for the entire period. The results also demonstrate that the application of nitrogen at a rate of 80 kg ha⁻¹ had a positive impact, increasing yield by 17.2% compared to the unfertilized control under non-irrigated conditions and by 8.3% under irrigation. No significant differences were detected between the yields obtained under N80 and N240. The high nitrogen rate was associated with delayed maturity due to excessive vegetative growth. Excessive nitrogen application can lead to a reduction in total yield, does not result in a proven increase in productivity, and is not an effective agronomic practice.

Our findings align with those of Karademir et al. (2006), who reported a decline in cotton yield at higher nitrogen application rates. Data from Saleem et al. (2010) on seed cotton yield under nitrogen fertilization indicate that nitrogen has a significant effect on cotton yield, with the highest yield (3002 kg ha⁻¹) recorded at a nitrogen rate of 120 kg ha⁻¹, while the lowest yield was obtained under the unfertilized control. Silvertooth and Norton (2011) confirmed that nitrogen deficiency reduces plant growth and can cause premature shedding of fruiting structures, leading to lower yields, whereas excessive nitrogen promotes excessive vegetative growth, delays fruit development, prolongs maturity, complicates defoliation, and increases pest and disease susceptibility. Geng et al. (2015) also reported that high nitrogen rates during boll filling can negatively impact seed cotton yield by stimulating excessive vegetative growth. Szilvay (2020) noted that plant responses to environmental conditions vary considerably. Therefore, the optimal nitrogen rate for cotton must be determined on a case-by-case basis and adjusted according to environmental conditions and crop status.

Irrigation conditions tense the strongest influence on cotton productivity. The *Isabel* cultivar exhibited a highly positive response to irrigation, with an average yield of 1.94 t ha^{-1} under irrigated conditions, exceeding the yield under non-irrigated conditions by 33.2%. Yield variations across years were clearly expressed, ranging from 1.78 t ha^{-1} in 2019 to 2.21 t ha^{-1} in 2020. Under non-irrigated conditions, plant growth, development, and yield formation in 2020 were constrained by high temperatures and minimal summer precipitation, resulting in an average yield of 0.895 t ha^{-1}, ranging from 0.77 t ha^{-1} under N0 to 0.989 t ha^{-1} under N160.

Table 3. Effect of the interaction between irrigation and nitrogen fertilization on the total yield (t ha^{-1}) of cotton cultivar Izabell., 2018-2020

Irrigation method	N	Year of experiment			Average		
		2018	2019	2020	t ha^{-1}	% into non-irrigation	% into N_0
Non-irrigation	N_0	1.548	1.456	0.769	1.257	100,0	100.0
	N_{80}	2.030^{***}	1.551	0.839	1.474	100.0	117.2
	N_{160}	2.127^{***}	1.611^{**}	0.989^{***}	1.576	100.0	125.3
	N_{240}	2.115^{***}	1.491	0.984^{***}	1.530	100.0	121.7
	Average	**1.955**	**1.527**	**0.895**	**1.459**	-	-
Irrigation	N_0	1.741	1.686^{***}	1.948^{***}	1.792*	142.52	100.0
	N_{80}	1.888^{*}	1.784^{***}	2.150^{***}	1.941^{**}	131.72	108.3
	N_{160}	1.956^{**}	1.880^{***}	2.320^{***}	2.052^{***}	130.23	114.5
	N_{240}	1.764	1.784^{***}	2.421^{***}	1.990^{***}	130.05	111.0
	Average	**1.837**	**1.784**	**2.210**	**1.944**	-	-
Average		**1.896**	**1.656**	**1.552**	**1.702**	-	-

* - P≤0.05; ** - P≤0.01; *** - P≤0.001

The average number of ripening bolls per plant is 4.42, reaching a maximum of 5.77 bolls per plant under N160 fertilization with irrigation (Table 4). Fertilization with 80, 160, and 240 kg N ha^{-1} increased the number of ripening bolls by 9.4%, 20.0%, and 19.3%, respectively, compared to the non-fertilized control (3.94 bolls per plant). The application of drip irrigation significantly increased the number of matured bolls by an average of 44.2% compared to non-irrigated conditions.

Table 4. The number of ripened bolls per plant under irrigation conditions and nitrogen fertilization in cotton cultivar Isabel, average for the 2018-2020

№	Irrigation method	N	2018	2019	2020	Average	% into non-irrigation	% intoN_0
1.	Non-irrigation	N_0	3.75	3.10	2.70	3.18	100.0	100.0
2.		N_{80}	4.40***	3.53	2.95	3.63	100.0	114.2
3.		N_{160}	4.50***	3.42	3.16	3.69	100.0	116.0
4.		N_{240}	4.65***	3.93	3.40*	3.99	100.0	125.5
Average non-irrigation			**4.33**	**3.50**	**3.04**	**3.62**	-	-
5.	Irrigation	N_0	4.25***	4.03	5.83***	4.70	147.8	100.0
6.		N_{80}	4.6***	4.48	5.90***	4.99	137.5	106.2
7.		N_{160}	4.7***	6.60***	6.00***	5.77	156.4	122.8
8.		N_{240}	4.8***	4.93*	6.50***	5.41	135.6	115.1
Average irrigation			**4.59**	**5.01**	**6.06**	**5.22**	**144.2**	-
Average			**4.46**	**4.26**	**4.55**	**4.42**	-	-

* - P≤0.05; ** - P≤0.01; *** - P≤0.001

On average over the study period, the *Isabell* variety exhibited a trend towards a very good boll weight, reaching 4.68 g per boll. Without fertilization, the average boll weight was 4.47 g, whereas with the application of 80, 160, and 240 kg N ha^{-1}, it increased to 4.66, 4.72, and 4.86 g, respectively, representing a 4.2–8.7% increase compared to the unfertilized control (Table 5). Under irrigation, the average boll weight reached 4.78 g, which was 4.6% higher than under non-irrigated conditions.

Table 5. One of weight boll (g) under irrigation and nitrogen strategies, 2018-2020

№	Irrigation method	Fertilization	2018	2019	2020	Average	% into non-irrigated	% into N_0
1.	Non-irrigation	N_0	4.36	4.90	4.01	4.42	100.0	100.0
2.		N_{80}	4.62***	4.95	4.56*	4.71	100.0	106.6
3.		N_{160}	4.98***	4.97	4.35	4.77	100.0	107.9
4.		N_{240}	5.19***	4.53	4.23	4.37	100.0	98.9
Average non-irrigation			**4.79**	**4.84**	**4.29**	**4.57**	**-**	**-**
5.	Irrigation	N_0	4.58***	4.60	4.94***	4.71	106.6	100.0
6.		N_{80}	4.63***	4.63	5.02***	4.76	101.1	101.1
7.		N_{160}	4.65***	4.41	5.18***	4.75	99.6	100.8
8.		N_{240}	4.80***	4.73	5.21***	4.91*	112.4	104.2
Average irrigation			**4.67**	**4.59**	**5.09**	**4.78**	**104.6**	**-**
Average			**4.73**	**4.72**	**4.69**	**4.68**	**-**	**-**

* - P≤0.05; ** - P≤0.01; *** - P≤0.001

CONCLUSIONS

A pronounced effect of variable meteorological conditions on cotton yield and fiber quality has been substantiated, with drought stress during the flowering phase being particularly detrimental. A statistically significant interaction between nitrogen fertilization, irrigation, and meteorological factors has been established over the three-year study period.

The irrigation regime exerts the most significant influence on cotton productivity. The Isabel cultivar responds exceptionally well to irrigation, with an average yield under irrigated conditions of 1.94 t ha^{-1}, exceeding the yield under non-irrigated conditions by 33.2%.

The number of mature bolls averages 4.42 per plant, reaching a maximum of 5.77 bolls per plant with N160 fertilization under irrigation.

A strong impact of fluctuating meteorological conditions on cotton yield and quality has been confirmed, particularly the adverse effects of drought during the flowering phase. A well-established interaction between nitrogen fertilization, irrigation, and meteorological conditions has been observed over the three-year study period.

The characterization of the Isabel cultivar regarding productivity and nitrogen use efficiency has identified the effects of the studied factors - irrigation regime, nitrogen fertilization rates, and cultivar performance.

The evaluation of the Isabel cultivar in terms of productivity and nitrogen use efficiency has elucidated the effects of the examined factors - irrigation regime, nitrogen fertilization rates, and cultivar-specific characteristics.

The cultivation of the naturally pigmented brown cotton cultivar Isabel is strongly recommended. Given its potential to enhance environmental sustainability and minimize chemical inputs in the fabric manufacturing.

Considering the enhanced environmental benefits and the reduced use of chemicals in the textile industry, the cultivation of the naturally pigmented brown cotton cultivar Isabel is recommended.

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EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF *Origanum vulgare* L. ESSENTIAL OIL AND ITS POTENTIAL APPLICATIONS IN PLANT PROTECTION

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Abstract

In the current context of promoting sustainable agriculture, it is important to find alternative solutions to synthetic pesticides in the fight against phytopathogenic microorganisms. Volatile oils in general, and those of plants from the Lamiaceae family in particular, through their chemical profile, have proven their antifungal and bactericidal efficacy, offering an ecological solution for the protection of agricultural crops, without affecting the environment. The antimicrobial activity of oregano oil on plant pathogens is closely related to the oil concentration, chemotype, as well as the synergy of biologically active compounds. The scientific paper is a review and aims to present the comparative effectiveness of the essential oil obtained from species and subspecies of Origanum vulgare L. on the main pathogenic bacteria and fungi, in correlation with the chemical profile.

Key words*: antifungal effect, antibacterial effect, oregano.*

INTRODUCTION

Plant pathogens, such as fungi, bacteria, phytoplasmas and viruses, threaten agricultural and forestry production, causing diseases with significant economic and environmental impact (Camele et al., 2012).

Plant protection products, including herbicides, fungicides, and insecticides, are widely used to combat plant diseases, weeds, and pests. Excessive use of plant protection products can lead to microorganisms, weeds and insect resistance. Even though plant protection products are effective, they are not exclusive to target pathogens and can negatively affect soil, human and animal commensal and beneficial microorganisms, leaving toxic residues and contributing to environmental pollution (Onaran et al., 2014). According to the research of Ashraf & Zuhaib (2013), only 0.1% of synthetic chemicals reach the targeted pathogens, and the remaining 99.9% contaminate the environment. Globalisation and climate change have exacerbated these problems by facilitating emerging diseases' rapid emergence and spread. A trend exists towards reducing dependence on conventional pesticides and implementing integrated pest management (IPM) (Bonaterra et al., 2022). Modern agriculture is increasingly replacing synthetic chemicals with environmentally friendly biocontrol solutions, such as botanicals and industrial by-products used as organic fertilisers, which can inhibit pathogens and improve soil and crop quality (Greff et al., 2023).

Essential oils (EO), respectively, their chemical compounds, have become promising substitutes for traditional plant protection products due to their recognised antimicrobial properties, single or in combination duet synergistic reactions between constituents (Álvarez-García et al., 2023; Košćak et al., 2023). However, the composition of essential oils obtained from the same plant species can vary significantly depending on numerous factors, including chemotype**,** plant growing conditions, and genetic variability of plant species (Gruľová et al., 2020; Vasinauskienė et al., 2006; Gurita et al., 2019; Beicu et al., 2023). Fungi and bacteria cause various complex plant diseases. In the juvenile stage of plants, they can suffer from rot and wilting, and in adulthood, from moulds (Kosakowska et al., 2024).

Phytopathogenic fungi are responsible for about 30% of all diseases affecting crops and can significantly impact both during cultivation and, after harvesting, during the storage period (Raveau et al., 2020).

The antimicrobial activity of essential oils on bacteria is based on several effective and complex mechanisms. They degrade the bacterial cell wall and cytoplasmic membrane structure, increasing permeability by modifying fatty acids, polysaccharides, and phospholipid layers. These lead to reduced membrane potential, leakage of ions and cellular contents, reduction of ATP, and eventually cell lysis (Sotelo et al., 2023; Bălășoiu (Jigău) et al., 2024). The main mechanisms of action of essential oils on fungi are given by inhibition of cell wall formation, destruction of the cell membrane by inhibition of ergosterol synthesis, dysfunction of fungal mitochondria by inhibition of proton pumps and mitochondrial electron transport, inhibition of efflux pumps, cell division and protein or RNA/DNA synthesis (Raveau et al., 2020; Nazzaro et al., 2017; Taheri et al., 2023).

The Lamiaceae family comprises about 7200 plants organised into 236 genera, most of which are aromatic species containing essential oils, which can be easily cultivated and multiplied. The genus *Origanum* includes 10 sections, comprising 43 plant species, six subspecies, three botanical varieties, and 18 natural hybrids (Ietswaart, 1980; Kokkini, 1997; Mutu, 2020; Maithani et al., 2023). Due to its biological activities, *Origanum vulgare* L. (elderberry, oregano) is a medicinal species among the most commercially important plants (Morshedloo et al., 2017; Lukas et al., 2015). From the taxonomy point of view, Ietswaart described six subspecies of *Origanum vulgare*, such as ssp. *vulgare* L., ssp. *glandulosum* (Desf.) Ietsw., ssp. *gracile* (K. Koch) Ietsw., ssp. *hirtum* (Link) Ietsw., ssp. *virens* (Hoffmanns. & Link) Ietsw., ssp. *viridulum* (Martin-Donos) Nyman (Ietswaart, 1980; Kokkini, 1997; Lotti et al., 2019; Lukas et al., 2015; Mutu, 2020; Kaouther et al., 2017; Mechergui et al., 2016). Oregano (*Origanum vulgare* L.) grows wild in Mediterranean areas and is widely distributed naturally in Europe and North Africa (Kokkini, 1997; Lotti et al., 2019; Skoufogianni et al., 2019; Kaouther et al., 2017; Marrelli et al., 2018; Mechergui et al., 2016).

This paper aims to highlight the mode of oregano essential oils action (O.E.O.s) on phytopathogenic bacteria and fungi affecting cereals. The paper will also highlight the benefits of using oregano essential oil in grain protection, highlighting their potential in sustainable agriculture.

CHEMICAL COMPOSITION OF *Origanum vulgare* L. ESSENTIAL OIL

Essential oils are oily liquids extracted by hydrodistillation or solvent extraction from different parts of plants (leaves, stems, roots, seeds, fruit, flowers, resins/bark), containing over 300 compounds (Dhifi et al., 2016; Raveau et al., 2020; Taheri et al., 2023). Essential oils are soluble in organic solvents (alcohol, ether) and insoluble in inorganic solvents (water) (Dhifi et al., 2016), contain 90-95% volatile components and 1-10% non-volatile components (Malik, 2019). Oils are vital components of many plants, located in secretory trichomes or mucous canals, and protect plants from insects, bacteria, and fungi attack (Harčárová et al., 2021; Horablaga et al., 2023).

The quantitative and qualitative profile of essential oils is influenced by environmental factors, soil pollution, water stress, salinity (Morshedloo et al., 2017), genotype, cultivation conditions and geographical locations (Lotti et al., 2019; Gurita et al., 2019; Skoufogianni et al., 2019; Raveau et al., 2020), leading to notable variation between populations of the genus *Origanum*.

Skoufogianni et al. (2019), observed significant seasonal variation in the quantitative profile of essential oil from the Crete and Amorogos Islands (Greece). In the autumn, the plant essential oil content varied between 1.0-3.1%, while in the summer it varied between 4.8-8.2%.

From a chemical point of view, the main constituents of essential oils are terpenes, terpenoids, phenylpropanoids and other constituents (lipids, sulphur derivatives, amino acids: alanine, isoleucine, leucine, valine and methionine) (Masyita et al., 2022; Butta et al., 2023).

Terpenes (terpenoids, isoprenoids) are the main compounds of essential oils and constitute the most diverse class of chemical compounds among the plant's secondary metabolites. They are classified according to the number of isoprene contain: hemiterpenes (C_5H_8), monoterpenes ($C_{10}H_{16}$), sesquiterpenes ($C_{15}H_{24}$), diterpenes ($C_{20}H_{32}$), norisoprenoids (C_{13}); depending on the biochemical structure: acyclic, cyclic, phenolic monoterpenes (Mutu, 2020; Marrelli et al., 2018; Morshedloo et al., 2017; Taheri et al., 2023; Butta et al., 2023; Oliva et al., 2015; Nazzaro et al., 2017). Terpenoids are terpenes with oxygen molecules added or with modified methyl groups, the most known being: thymol, carvacrol, linalool and menthol (Nazzaro et al., 2013). Moreover, essential oils also contain terpene-free compounds, such as eugenol, cinnamaldehyde, and safrole, produced via the phenylpropanoid pathway. Biogenetically, terpenoids and phenylpropanoids have different precursors and biosynthesis pathways: terpenoids are formed via the mevalonate and the deoxyxylulose phosphate pathway, and phenylpropanoids are generated via the shikimate pathway (Dhifi et al., 2016).

Numerous studies have been carried out on the *Origanum vulgare* L. essential oil, highlighting the biochemical variability of the six subspecies: ssp. *vulgaris* contains carvacrol and thymol; ssp. *hirtum* is rich in carvacrol, thymol, *p*-cymene and γ-terpinene; ssp. *glandulosum* is rich in monoterpene compounds (carvacrol, thymol and their derivatives); ssp. *gracile* contains acyclic compounds, carvacrol or sesquiterpenoids; ssp. *virens* is rich in acyclic compounds and sesquiterpenes; ssp. *viridulum* contains a high amount of sabinene (Mutu, 2020).

On the other hand, the results obtained by studying essential oils from Lithuania (Vilnius) have shown that the main constituents in ssp. *glandulosum* is carvacrol, ssp. *gracile* contains thymol and sabinen-germacren D., ssp. *virens* has increased amounts of germacrene D-sabinene, γ-terpinene, and ssp. *viride* is rich in linalyl acetate, β-caryophyllene and sabinene (Mockute et al., 2001).

The oils also have remarkable antimicrobial properties and can be found in all parts of aromatic plants (Imbrea et al., 2016; Álvarez-García et al., 2023; Maithani et al., 2023). Volatile oils allow plants to regulate the environment, acting as a chemical signal to attract pollinators, inhibit seed germination, communicate between plants, and repel predators (Taheri et al., 2023).

ANTIBACTERIAL ACTIVITY OF OREGANO ESSENTIAL OIL AGAINST PHYTOPATHOGENIC BACTERIA

The soil may contain important bacterial pathogens, including *Agrobacterium***,** *Pectobacterium***,** *Pseudomonas***,** *Ralstonia* and *Xanthomonas*. However, these microorganisms are conditionally pathogenic; the disease occurrence is influenced by the presence of an open wound or natural holes to invade the host plant. The bacteria determine morphological changes in the host plant's root system, invade the vessels of the xylem and cause wilting symptoms, eventually leading to the death of the plants (Greff et al., 2023).

Chemical composition of *Origanum vulgare* ssp. *hirtum* essential oil (Greek oregano), is characterised by the predominance of monoterpenes, respectively of phenolic monoterpenes such as carvacrol (35.79%), of monoterpene hydrocarbons (*p*-cymene - 17.01% and γ-terpinene - 13.76%), and oxygenated monoterpenes (6.65%). On the other hand, the essential oil of *Origanum vulgare* ssp. *vulgare* (common oregano) is dominated by oxygenated sesquiterpenes (35.80%), mainly caryophyllene oxide (18.89%), but it also contains sesquiterpene hydrocarbons and oxygenated monoterpenes in considerable quantities. Due to these compounds, Greek oregano essential oil presents antimicrobial activity against *Pseudomonas syringae* (see Table 1) and *Xanthomonas hortorum.* According to the study, Greek oregano essential oil has more antimicrobial activity against *Xanthomonas hortorum* than common oregano essential oil (Kosakowska et al., 2024).

Similarly, Gruľová et al. (2020), demonstrated significant antibacterial activity of thymol (76%) chemotype oregano essential oil against *Pseudomonas savastanoi* and *Xanthomonas campestris* (see Table 2). Moreover, Sotelo et al. (2023), observed that oregano essential oils inhibited all phytopathogenic *Pseudomonas syringae* strains, with MIC values between 11.56 mg·mL^{-1} to 92.5 mg·mL^{-1}.

Other studies demonstrated the effect of oregano essential oils against pathogenic factors of bacteria. Carezzano et al. (2017), investigated the impact of *Origanum vulgare* L. essential oils on some pathogenic factors of *Pseudomonas syringae*, including anti-toxin and anti-biofilm activities. The results showed that oregano essential oil strongly inhibited biofilm formation and the production of coronatin, syringomycin, and tabtoxin at MIC values between 5.8 and 11.6 mg·mL^{-1}. Moreover, the essential oil also demonstrated complete inhibition (100%) of syringomycin production in all *Pseudomonas syringae* strains at concentrations between 0.003 and 0.11 mg·mL^{-1}.

Table 1. Antibacterial effect of oregano essential oil against *Pseudomonas* ssp.

O.E.O.	Bacterial strain	Methods	Results	Major essential oil compounds identified by GC-MS / GC-FID	References
Origanum vulgare	*Pseudomonas savastanoi* pv. *glycinea*	Disk-diffusion	MIC = 1600 ppm MBC = 3200 ppm Inhibition of bacteria growth by 2.0±0.8 mm	Carvacrol 62.37± 0.01%, γ-terpynen 4.85± 0.002%, thymol 3.15± 0.055%	Tarakanov & Dzhalilov, 2022
	Pseudomonas savastanoi	Disk-diffusion	Inhibited bacterial growth at 10.000 ppm	Thymol 76.0%, *p*-cymene 5.7%, carvacrol 3.2%, linalool 2.6%	Gruľová et al., 2020
	Pseudomonas syringae	Broth microdilution method	MIC = 5.8 - 46.3 mg·mL^{-1} MBC = 0.022 - 0.36 mg·mL^{-1} O.E.O. inhibited the production of coronatine at concentrations between 0.045 mg·mL^{-1} to 0.09 mg·mL^{-1}	Carvacrol, *p*-cymene, c-terpinene	Carezzano et al., 2017
	Pseudomonas syringae pv. *atropurpurea*		MIC = 11.9 mg·mL^{-1} MBC = 0.012 mg·mL^{-1} O.E.O. inhibited tatoxin production at 0.006 mg·mL^{-1}		
	Pseudomonas savastanoi pv. *glycinea*		MIC = 5.8 mg·mL^{-1} MBC = 0.22 mg·mL^{-1}		
	Pseudomonas syringae pv *syringae*	Broth microdilution method	MIC = 23.1 - 46.3 mg·mL^{-1}	γ-terpinene 22.7%, carvacrol 19.7%, cis-sabinene hydrate 19.7%, *p*- cymene 11.5%	Oliva et al., 2015
	Pseudomonas syringae	Broth microdilution method	MIC = 11.56 - 46.26 mg·mL^{-1}	Cis-sabinene hydrate 20.99%, thymol 12.03%, carvacrol 11.39%	Sotelo et al., 2023
Origanum vulgare* spp. *vulgare	*Pseudomonas syringae*	Broth microdilution method	MIC = 4 μL·mL^{-1} MBC = 4 μL·mL^{-1}	Caryophyllene oxide 18.89%, β-caryophyllene 5.64%, β-cubebene 5.51%, carvacrol 3.97%	Kosakowska et al., 2024
Origanum vulgare* spp. *Hirtum			MIC = 0.125 μL·mL^{-1} MBC = 0.250 μL·mL^{-1}	Carvacrol 35.79%, *p*-cymene 17.01%, γ-terpinene 13.76%	

*O.E.O. (Oregano Essential Oil), MIC (Minimum Inhibitory Concentration), MBC (Minimum Bactericidal Concentration), GC-MS (Gas Chromatography-Mass Spectrometry), GC-FID (Flame Isomerism Detector for Gas Chromatography.

Table 2. Antibacterial effect of oregano essential oil against *Xanthomonas* ssp.

O.E.O.	Bacterial strain	Methods	Results	Major Essential Oil Components determined by GC-MS / GC-FID	References
Origanum vulgare	*Xanthomonas campestris*	Disk - diffusion	Inhibited growth at 10.000 ppm	Thymol 76.0%, *p*-cymene 5.7%, carvacrol 3.2%, linalool 2.6%	Gruľová et al., 2020
Origanum vulgare* spp. *vulgare	*Xanthomonas hortorum*	Broth microdilution	MIC = 2 $\mu L \cdot mL^{-1}$ MBC = 2 $\mu L \cdot mL^{-1}$	Caryophyllene oxide 18.89%, β-caryophyllene 5.64%, β-cubebene 5.51%, carvacrol 3.97%	Kosakowska et al., 2024
Origanum vulgare* spp. *hirtum			MIC = 0.125 $\mu L \cdot mL^{-1}$ MBC = 0.250 $\mu L \cdot mL^{-1}$	Carvacrol 35.79%, *p*-cymene 17.01%, γ-terpinene 13.76%	

*O.E.O. (Oregano Essential Oil), MIC (Minimum Inhibitory Concentration), MBC (Minimum Bactericidal Concentration), GC-MS (Gas Chromatography-Mass Spectrometry), GC-FID (Flame Isomerism Detector for Gas Chromatography).

ANTIFUNGAL EFFICACY OF OREGANO ESSENTIAL OIL AGAINST PHYTOPATHOGENIC FUNGI

Infections caused by fungal pathogens can be transmitted through agricultural and horticultural products, affecting the soil. Species such as *Fusarium*, *Verticillium*, *Rhizoctonia*, *Sclerotinia*, *Phytophthora* and *Pythium* can survive in soil and plant debris for long periods, forming resistant structures (sclerotia, microsclerotia, oospores, chlamydospores or hyphae). Some species can invade the host plant through roots and stems or spread rapidly among seedlings, causing root necrosis, vascular disease, rot, gall, and root and tuber proliferation (Greff et al., 2023).

The antifungal components of plant-derived essential oils have been intensively studied and can be classified in the following order: phenols-alcohols-aldehydes-ketones-ethers-hydrocarbons (Xiang et al., 2020; López et al., 2004). The active antifungal compounds are monoterpenes, sesquiterpenes, phenols, aldehydes and ketones. Constituents such as terpenoids, phenolic terpenes, and alcohols significantly increase the antifungal activity of volatile oils (Butta et al., 2023).

The inhibitory activity of oregano essential oil was demonstrated against *Aspergillus* spp., *Fusarium* spp. and *Penicillium ochrochloron*. After 13 and 10 days of incubation, in the presence of essential oil, *Aspergillus niger* and *Aspergillus flavus* reached the complete growth of the mycelium (4.75 cm). Instead, *Aspergillus* spp., *Penicillium ochrochloron* did not reach maximum growth, highlighting that this strain is less invasive than those studied (Střelková et al., 2021).

Similarly, Duan et al. (2024), highlighted that the carvacrol (66.01%) chemotype of the oregano essential oil inhibited the proliferation of *Aspergillus flavus* in wheat grain. The carvacrol disrupts the cell membrane and cell wall structure, causing mitochondrial dysfunction and preventing DNA replication. Studies conducted by Ji et al. (2022), demonstrated the efficacy of fumigation treatment with oregano essential oil (carvacrol ≥88.3%) on *Aspergillus flavus*, by inducing oxidative stress. The treatment resulted in a significant increase in the production of superoxide anions (from 1.57 to 9.14 times) and the degree of lipid peroxidation (from 2.89 to 11.06 times) in the fumigation interval of 2 to 8 hours at 45°C, resulting in decreased mitochondrial activity (by 50.17%, 64.21%, and 89.41%) and ATP production (by 21.55%, 60.34%, and 70.30%) over the 4 to 8-hour fumigation interval. In addition, oregano essential oil vapour inhibited metabolic activities related to protein and methylglyoxal synthesis and the production of aflatoxin AFB1 (AFB1) during 5 days of incubation.

Numerous studies have shown that the antifungal activity of volatile oils can be amplified by combining different essential oils to achieve a synergistic effect. For example, Xiang et al. (2020), demonstrated the synergistic activity of cinnamon, oregano and lemon oils (COL-CEO) against the aflatoxin B1 production of (AFB1) *Aspergillus flavus*. The production of this toxin is reduced by 67.53% at a concentration of 0.6 µL/disc and by 72.68% at a concentration of 1.0 µL/disc (see Table 3). COL-CEOs also have synergistic effects when the three oils are combined in a ratio of 1:5:48.

Most essential oils of the *Origanum* subspecies (*Origanum vulgare* spp. *virens*, *Origanum vulgare* spp. *vulgare*) have demonstrated significant antifungal activity against *Fusarium verticillioides* due to their high content of phenolic compounds (see Table 4). These phenolic compounds are essential for fighting pathogens, as they affect the permeability of the cell membrane of fungi, leading to the loss of cellular components and inhibition of cellular metabolism (Pizzolitto et al., 2020).

Also**,** oregano essential oil inhibited (100%) mycelial growth of *Fusarium graminearum***,** at concentrations of 1000 µg/mL and 500 µg/mL. A lower concentration of essential oil inhibited a lower percentage of mycelial growth. A concentration of 100 µg/mL inhibited the mycelium growth of *Fusarium graminearum* strain by 37.4% and 40.7% for the *Fusarium graminearum* (Harčárová et al., 2021).

Oregano essential oil composed of trans-caryophyllene 30.729%, sabinene 18.16%, caryophyllene oxide 8.635%, germacrene-D 8.59% (see Table 5), inhibited the growth of *Penicillium aurantiogriseum* by 2 mm in diameter, 9 days after inoculation on a medium with a concentration of 0.25 $mg \cdot L^{-1}$ (Rus et al., 2016).

Table 3. Antifungal effect of oregano essential oil against *Aspergillus* ssp.

O.E.O.	Fungal strain	Methods	Results	Major essential oil compounds identified by GC-MS / GC-FID	References
Origanum vulgare	*Aspergillus flavus*	Agar well diffusion	MIC of carvacrol = 0.18 µL/mL	Carvacrol 66.01%, linalool 5.17%, thymol 3.51%, o-cymene 3.03%, *y*-terpinene 2.54%	Duan et al., 2024
	Aspergillus flavus	Disk volatilisation	MIC = 2.0 µl/L	Thymol 54%, carvacrol%	Paster et al., 1995
	Aspergillus flavus	Broth microdilution	MFC = 312.5 mg/L	Carvacrol ≥88.3%	Ji et al., 2022
	Aspergillus flavus	Disk - diffusion	5.0 µl O.E.O. produces a 40.93% inhibition of fungal growth; Volumes of 10 µl, 20 µl, 50 µl, and 100 µl showed inhibitory effects on the production of aflatoxin B1 in soybeans by 54.4%, 88.68%, 86.94% and 88.16%	4-terpineol 44.11%, linalool 15.22%, α-terpineol 5.96%, *y*-terpinene 5.02%	Esper et al., 2014
***Origanum vulgare* in vapor phase**	*Aspergillus flavus*	Disk-diffusion	Inhibits growth at 2.50 µL/full disc	Carvacrol 84.96%, thymol 13.26%	Xiang et al., 2020
	Aspergillus flavus	Disk-diffusion	MIC = 62.5 µL/L	Carvacrol 70%, *p*-cymene 11%, thymol 3%	Střelková et al., 2021

Table 3. Antifungal effect of oregano essential oil against *Aspergillus* ssp. - continuation

O.E.O.	Fungal strain	Methods	Results	Major essential oil compounds identified by GC-MS / GC-FID	References
Origanum vulgare* ssp. *hirtum	*Aspergillus flavus*	Broth microdilution	MIC = 450 ppm MFC = 600 ppm	Z-sabinene hydrate 23.03%, thymol 18.66%, γ-terpinene 7.12 %, terpinen-4-ol 6.20%	Camiletti et al., 2014
	Aspergillus flavus		MIC = 650 ppm MFC = 800 ppm		
Origanum vulgare* ssp. *vulgare	*Aspergillus flavus*		MIC = 550 ppm MFC = 1100 ppm	o-cymene 14.33%, terpinen-4-ol 12.55%, E-*β*-terpineol 10.46%, thymol 9.44	
	Aspergillus flavus		MIC = 550 ppm MFC = 700 ppm		
Origanum vulgare	*Aspergillus niger*	Disk volatilisation	MIC = 62.5 µL/L	Carvacrol 70%, *p*-cymene 11%, thymol 3%	Střelková et al., 2021
	Aspergillus niger	Disk - diffusion	The highest inhibition activity was observed at 1000 and 500 ppm	Thymol 76.0%, *p*-cymene 5.7%, carvacrol 3.2%, linalool 2.6%	Gruľová et al., 2020
	Aspergillus niger	Disk volatilisation	MIC = 2.0 µl/L	Thymol 54%, carvacrol%	Paster et al., 1995
	Aspergillus ochraceus				

*O.E.O. (Oregano Essential Oil), MIC (Minimum Inhibitory Concentration), MFC (Minimum Fungicidal Concentration), GC-MS (Gas Chromatography-Mass Spectrometry), GC-FID (Flame Isomerism Detector for Gas Chromatography)

Table 4. Antifungal activity of oregano essential oil against *Fusarium* ssp.

O.E.O.	Fungal strain	Methods	Results	Major essential oil compounds identified by GC-MS / GC-FID	References
Origanum vulgare	*Fusarium avenaceum*	Disk-diffusion	Inhibition of fungal growth by 77.4%	Thymol 29.60%, *p*-cymene 29.40%, β-terpinene 26.80%	Hanana et al., 2017
	Fusarium culmorum		Inhibition of fungal growth by 90.3%		
	Fusarium culmorum	Disk-diffusion	MIC< 0.8 µL/cm3 At 20% O.E.O. concentration: ERG has been reduced by 99.98%, ZEA has been reduced by 99.08%, and DON has been reduced by 99.26%		Perczak et al., 2019
			The concentration of ERG was reduced by 90.31%, ZEA was reduced by 62.79% and DON by 100.00%	Carvacrol ≤ 80%, thymol 2%	Perczak et al., 2020
Origanum vulgare* spp. *vulgare	*Fusarium culmorum*	Broth microdilution	MIC = 1 µL/mL MFC = 1 µL/mL	Caryophyllene oxide 18.89%, β-caryophyllene 5.64%, β-cubebene 5.51%, carvacrol 3.97%	Kosakowska et al., 2024

Table 4. Antifungal activity of oregano essential oil against *Fusarium* ssp. - continuation

O.E.O.	Fungal strain	Methods	Results	Major essential oil compounds identified by GC-MS / GC-FID	References
Origanum vulgare* spp. *hirtum	*Fusarium culmorum*	Broth microdilution	MIC = 0.032 µL/mL MFC = 0.250 µL/mL	Carvacrol 35.79%, *p*- cymene 17.01%, γ-terpinene 13.76%	Kosakowska et al., 2024
Origanum vulgare	*Fusarium graminearum*	Disk-diffusion	MIC< 0.8 µL/cm3 At 20% O.E.O. concentration: ERG has been reduced by 99.97%, ZEA has been reduced by 100.00%, and DON has been reduced by 99.70%.		Perczak et al., 2019
	Fusarium graminearum		The concentration of ERG was reduced by 83.24%, ZEA was reduced by 26.05% and DON by 100.00%	Carvacrol ≤ 80%, thymol 2%	Perczak et al., 2020
	Fusarium graminearum	Broth microdilution	MIC = 0.4 mg·mL^{-1} IE = 47.8%	Carvacrol 85±3%	Harčárová et al., 2021
	Fusarium graminearum		MIC = 0.4 mg·mL^{-1}		
	Fusarium graminearum	Disk volatilisation	MIC = 0.02 µl/mL MFC = 0.02 µl/mL		Rekanović et al., 2012
	Fusarium graminearum		MIC = 0.08 µl/mL MFC = 0.08 µl/mL		
	Fusarium sporotrichioides	Disk volatilisation	MIC = 62.5 µL/L MFC = 125 µL/L	Carvacrol 70%, *p*-cymene 11%, thymol 3%	Střelková et al., 2021
	Fusarium solani				
	Fusarium subglutinans	Disk - diffusion	Inhibition of fungal growth by 94.4%	Thymol 29.60%, *p*-cymene 29.40%, β-terpinene 26.80%	Hanana et al., 2017
	Fusarium verticillioides		Inhibition of fungal growth by 67.3%		
	Fusarium verticillioides	Disk - diffusion	40, 50, 100, 200 µl/l O.E.O. reduced mycelium growth by 75%. On day 20, oregano essential oil (30 ppm) decreased the production of fumonizin B1 (FB1)	Thymol 21.2%, terpineol 21.1%, γ-terpinene 9.1%	López et al., 2004
Origanum vulgare* spp. *vulgare	*Fusarium verticillioides*	Mathematical model using arbitrary descriptors based on EO components	MIC = 250 µL/L	Thymol 29.950%, cis-sabinene hydrat 24.654%, γ-terpinene 12.045%, terpinen-4-ol 7.764%	Pizzolitto et al., 2020
Origanum vulgare* spp. *virens	*Fusarium verticillioides*		MIC = 250 µL/L	Thymol 34.045, cis-sabinene hydrat 19.094%, γ-terpinene 6.811%, terpinen-4-ol 6.660%	

*O.E.O. (Oregano Essential Oil), MIC (Minimum Inhibitory Concentration), MFC (Minimum Fungicidal Concentration), IE = inhibitory effect, ERG (ergosterol), ZEA (zearalenone), DON (deoxynivalenol), GC-MS (Gas Chromatography-Mass Spectrometry), GC-FID (Flame Isomerism Detector for Gas Chromatography)

Table 5. Antifungal effect of oregano essential oil against *Penicillium* spp.

O.E.O.	Fungal strain	Methods	Results	Major essential oil compounds identified by GC-MS / GC-FID	References
Origanum vulgare	*Penicillium aurantiogriseum*	Poisoned medium method	MIC = 0.5 $mg \cdot L^{-1}$ MFC = 5 $mg \cdot L^{-1}$	Trans-caryophyllene 30.729%, sabinene 18.16%, caryophyllene oxide 8.635%, germacrene-D 8.159%	Rus et al., 2016
	Penicillium ochrochloron	Disk volatilisation	MIC = 62.5 μL/L	Carvacrol 70%, *p*-cymene 11%, thymol 3%	Střelková et al., 2021
	Penicillium expansum	Disk - diffusion	The highest inhibition activity was in the case of 1000 and 500 ppm	Thymol 76.0%, *p*-cymene 5.7%, carvacrol 3.2%, linalool 2.6%	Gruľová et al., 2020
Origanum vulgare* ssp. *hirtum	*Penicillium oxalicum*	Poisoned medium method	MIC = 350 ppm MFC = 500 ppm	Z-sabinene hydrat 23.03%, thymol 18.66%, γ-terpinene 7.12 %, terpinen-4-ol 6.20%	Camiletti et al., 2014
	Penicillium minioluteum		MIC = 400 ppm MFC = 600 ppm		
Origanum vulgare* ssp. *vulgare	*Penicillium oxalicum*		MIC = 400 ppm MFC = 700 ppm	o-cymene 14.33%, terpinen-4-ol 12.55%, E-*β*-terpineol 10.46%, thymol 9.44%	
	Penicillium minioluteum		MIC = 500 ppm MFC = 600 ppm		

*O.E.O. (Oregano Essential Oil), MIC (Minimum Inhibitory Concentration), MFC (Minimum Fungicidal Concentration), GC-MS (Gas Chromatography-Mass Spectrometry), GC-FID (Flame Isomerism Detector for Gas Chromatography)

CONCLUSIONS

Plants and botanicals play a crucial role in promoting a sustainable and balanced agricultural system, which is important in environmental protection, biodiversity promotion, pest management, soil improvement, and reduction of dependence on synthetic products and toxicity. Sustainable agriculture aims to reduce the use of chemicals because most pathogens become resistant; thus, they are replaced by plant-derived products. Botanical substances, especially essential oils, are among the most widely used treatments against soil plant pathogens, having antibacterial and antifungal properties. Using less toxic essential oils than synthetic products represents an adequate alternative to controlling aflatoxins. Thus, oregano essential oil could protect against aflatoxin B1 in soybean and corn crops. This scientific research aimed to carry out an analysis of oregano essential oil, highlighting the effects and mode of antibacterial and antifungal action, as well as the mode of action of the chemical components identified in their composition, on the bacteria and fungi present in cereals.

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ASSESSMENT OF WET GLUTEN CONTENT BASED ON THE INTERACTION BETWEEN NITROGEN LEVEL OF FERTILIZATION AND WINTER WHEAT VARIETY CULTIVATED AT DUDEȘTII NOI, AN IMPORTANT AGRICULTURAL AREA OF ROMANIA

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Abstract

The objective of this study was to analyse the effect of variety, nitric, amoniacal nitrogen and level of fertilization for values of wet gluten content on winter wheat. The research was carried out in 2021-2023 and the method of planting was carried out in subdivided randomized blocks with three repetitions. The subject of the experiment consisted in testing twenty-seven modern winter wheat varieties with the experimental variants: 120, 150, 170 kg ha^{-1} a.s. nitric and amammoniacal N. Compared with the mean of the experience of 32.83% the highest value was obtained of Ciprian variety – 35.64%. The application of treatments on a chernozem soil weakly acidic with nitric N positively influenced this index were the highest values of 34.54%, 33.86% and 34.73% were obtained. In those fertilizated with ammonium N, the values obtained were below the experience. The results were discussed in view of classical statistics using ANOVA and The Student`s t-tests.

Key words: *wheat quality, fertilization levels, nitrogen types, wet gluten content.*

INTRODUCTION

The history of wheat cultivation is as long as the history of civilization. Historical data tells us that wheat is the oldest cultivated plant. It is believed that its domestication took place in the Fertile Crescent about 10,000 years ago and was part of the Neolithic Revolution, when sapiens acquired enough knowledge of the surrounding world and discovered this plant in the spontaneous flora that could serve as food, a fact which represented "*a radical change*", "*full of revolutionary consequences for whole species*", a plant that then spread to all parts of the world through the first farmers, who adapted local populations to different climates (Venske et al., 2019; Dubcovsky et al., 2007).
Therefore, it has transformed from a simple wild herbaceous plant, apparently insignificant, to a ubiquitous one and continues to be the staple food, like a *Panis caelestis* for humanity. The first ancient forms of wheat, einkorn, emmer and spelt, not only played an important role as a food source, but became the ancestors of the modern species currently cultivated around the world, with *Triticum aestivum* L. which now accounts for about 95% of world production. The success of this plant is inextricably linked to the ability of the gluten protein fraction that allows flour to be processed to produce bread, other pastries, noodles and pasta (Ozkan et al., 2002).
For wheat, maximum nitrogen (N) uptake occurs at the elongation of the first internode and until the heading period. N accumulated during these growth stages is used primarily for production elements. N accumulated after tillering has little effect on yield, but increases protein content (Tabak et al., 2020).
Increased levels of nitrogen before the emergence of the first internode, at BBCH 30-31 growth stage, lead to increased number of spikelet's/ear and fertile flowers/spike (Guarda et al., 2004). Increasing nitrogen levels also has a positive effect on the number of fertile tillers/m^2 (Abedi et al., 2011). Applications at sowing lead to increased grain dry matter. Nitrogen uptake during grain filling is closely related to the amount of N applied and has no

substantial effect on filling duration (Brennan et al., 2014; Foulkes et al., 2009). Excess nitrogen applications, above the optimum level, can delay the growth and development of the ear and lead to drop because the internodes are greatly elongated and the leaf mass has a lush but useless development, also the ripening time of the wheat is delayed and leads to grain germination in the ear (Kadar et al., 2018). The application of high doses of N fertilizer does not always imply an increase in production yields; but on the contrary, it even leads to a decrease (Yin et al., 2019; Liu et al., 2021).

Nitrogen is absorbed from the soil mainly in two forms of ions, ammoniacal nitrogen (NH_4^+) and nitric nitrogen (NO_3^-), and their absorption constitutes approximately 70% of the total of cations and anions. Most of the nitrogen uptake is in the form of NO_3^- which moves from the soil solution into the plant root cells along with the water absorbed by it. NO_3^- is then either stored in vacuoles or reduced in the cytosol and plastids as NH_4^+ by nitrate and nitrite reductase activity. NH_4^+ can then be assimilated to produce more complex nitrogen-containing compounds. These compounds include chlorophyll which captures light during the photosynthesis process. Nitric nitrogen is first converted to ammonium because it is the only reduced form of N that plants can use to assimilate it into N-bearing amino acids. The assimilation process consists of two steps to convert nitrates to NH_4^+; in the first step, nitrate reductase converts NO_3^- to nitrite in the cytoplasm, and in the second step, nitrite is converted to NH_4^+ by nitrite reductase in the plastids (Chen et al., 2024; Wang et al., 2024). Nitric nitrogen is absorbed by plants and then becomes mobile in the xylem vessels. Xylem is the main element for long-distance transport of N from roots to N-metabolizing organs (Liu et al., 2021).

Xylem therefore transports NO_3^- from roots to leaves, and the phloem is the main transport pathway for N stored or assimilated in leaves and transported to other parts of the plant (eg from leaf to caryopses) (Lemaire et al., 2008). Nitrate is the substance that is mostly absorbed by wheat plants through transporters located on the plasma membrane of the root cells. The rate of this process is influenced by several factors, such as soil nitrogen availability, pH, and ion competition. N-assimilating enzymes that are closely related to the process of N uptake in roots include nitrate reductase (NR), nitrite reductase (NRi), glutamine synthetase (GS) and glutamate aminotransferase (GA). NO_3^- entering the cell is reduced by the process of NR to NO_2. NO_2 is toxic to cells and is rapidly transported into the plastid for further re-duction to ammonium by NRi. Ammoniacal nitrogen bypasses the process of conversion into amino acids and enters directly into the plant's metabolism (Chauhan et al., 2022).

Wheat is one of the most used crops in human nutrition. The quality wheat is deter-mined by a combination of several parameters. To determine the final quality of the product must be evaluated multiple characteristics of the grain, flour and the dough. The future of wheat production and quality must be coordinated by a considerable reduction of nitrogen losses. Reducing the use of nitrogen-based fertilizers without loss of quality and quantity of production might be achievable. Only one direction will provide the significant increase in productions and sustainability, is the effective management of nutrients applied to the wheat crop. Nitrogen in particular is a factor that has generated a lot of interest also due to the increase in its production cost in recent years. In addition, having a negative impact on the environment when the application is not carried out correctly and efficiently. Improving the efficient use of nitrogen, maintaining high wheat yields and im-proving baking indices are global aspirations, aspirations that are also the purpose of this research.

MATERIALS AND METHODS

The study was conducted in 2021-2023 in the Dudeștii Noi experimental fields (45°50′51″N 21°06′30″E, 87 m altitude, located in the Western Plain of Romania. The experimental field was located on a cambic chernozem soil, wet phreatic, slightly levigated, medium clay loam, developed on fluvial, carbonate, medium fine materials with a well-defined profile and with insignificant differences regarding the physical, hydric and chemical properties (Roman et al., 2011). The soil profile is characterized by the sequence of the following horizons: Ap (bioaccumulative mineral horizon

- anthropogenically disturbed), Am (bioacumultive mineral horizon – mollic), Bv (subsurface horizon - with accumulation of clay formed in situ and through weathering processes, Ck (mineral horizon not significantly affected by pedogenetic processes and not consolidated - with calcium carbonate accumulation). Ap horizon: 0-15 cm, gradual transition, dark gray brown clayey texture, small glomerular structure, plastic, weak adhesive, small pores, frequent roots, dry. Am horizon: 15-35 cm, gradual transition, dark brown loamy texture, medium glomerular structure, well developed, plastic, adhesive, small pores, weak compact, thin roots frequent. Bv horizon: 35-60 cm, gradual transition, dark gray-brown loamy texture, medium subangular polyhedral structure, plastic, adhesive, fine porous, moderately compact, sparse thin roots, dry. Ck horizon: 60-100 cm, gradual transition, yellowish brown light gray loamy texture, subangular and medium angular polyhedral structure well developed, plastic, adhesive, dry (Sala, 2008).

Physical and hydraulic properties: the loamy-clay texture - dusty in the first 70 cm and loamy-dusty in the rest, makes the apparent density (AD) medium throughout the profile of the soil, with 1.46 g/cm3 in the Ap horizon and 1.58 g/cm3 in Am. Total porosity (TP) is medium in Ap (48.8%) and high in Am (58.2%). Air porosity (AP) is medium in Ap (15.2%), high in Am (23.8%), medium in Bv (21.7%) and low in Ck (12.2%). The ranges of variation for clay are between 33.4-40.3%. The processed Ap horizon has a clay content of 36% compared to the Am horizon with a content of 40.3%. In horizons Bv and Ck, the amount of clay shows lower values (33.8%) and 33.4%, respectively. The soil is weakly to moderately compacted on the horizons below the tilled horizon. The values of the hydro-physical indices show different values, correlated with the physical properties. A medium value of the withering coefficient is noted in the Ap and Am horizons (11.7%), high in Bv (13.2%) and medium in Ck (12%) (Imbrea, 2014).

The field capacity (FC) is medium over the entire soil profile, with values of 23.3% in Ap, Am, Bv and 22.4% in Ck. Chemical properties were determined using the Kjeldahl and Olsen et al. methods (Dumitru et al., 2011) The soil has a humus content of 3.04% in the Ap horizon and 1.78% in the Am horizon, decreasing by 1.24% in the Bv horizon. It is medium supplied in total nitrogen, with 0.172% in Ap, very well supplied in Am with mobile phosphorus (168 ppm) and well supplied with mobile potassium (248 ppm). The pH was tested using the distilled glass electrode method, 1:2.5 soil and water. The reaction of the soil on the profile is weakly acid in Ap horizon 5,58, weakly alkaline 7,66. The amount of exchangeable bases is medium (23.2 in Ap, 24.8 in Am), and the degree of saturation in bases shows values of 92.62% which gradually increases on the Bv horizon. Trace element content is high for zinc (176 ppm) in the Ap and extremely low in the Bv horizon (0.40 ppm).

Climatic data during the period of the experiment were collected from the meteorological station of Dudeștii Noi and are represented in Figure 1. The climate of this area is temperate - continental with slight Mediterranean influences. The average annual temperature is 10.8°C and the warmest month was July. Temperatures were also extremely high for September and October. As can be seen, during the entire experimental period and especially during the vegetation months, the average monthly temperatures exceeded the multiannual averages, with the highest values during the vegetation period being recorded between June and September in all two experimental years.

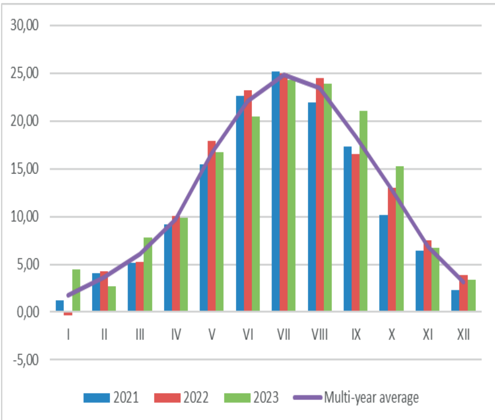

Figure 1. The average monthly temperatures (°C) recorded in Dudeștii Noi (2021-2023)

The average annual precipitation was frequently around 540 mm. In May and July, as a rule, the maximum pluviometric occurs (Figure 2). Sowing was carried out late, after successful application of a watering to help the suitable preparation of the field. The months from August until October were dry. These were registered against the background of acute lack of precipitation. The last three years, especially, have positioned the western area of Romania in an unprecedented position. The Western Plain of Romania, an important region of the country for wheat cultivation, has recently had to deal with long periods of very hot and dry weather and very wet and rainy periods with heavy rainfall in a short period of time. These climate events often exceed the relevant meteorological observations, some of which vary strongly from the multiyear mean values recorded to date.

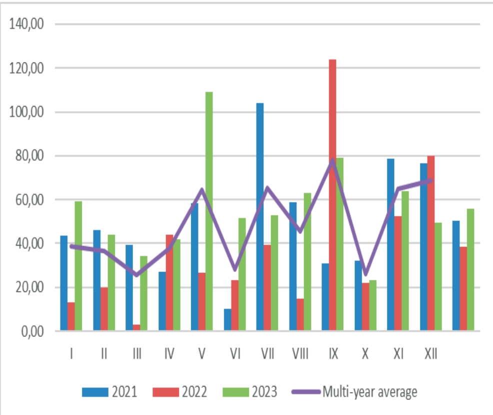

Figure 2. The average monthly rainfall (mm) recorded in Dudeștii Noi (2021-2023)

The layout of the experimental plan was done using the stratified randomization block method. Cultivars were factorially combined and arranged in completely randomized blocks. This experimental method was chosen in order to avoid the interfering effects of various environmental factors and to adequately and accurately estimate nitrogen utilization. The size of each plot was 1.2 m × 7 m, the distance between plots and adjacent blocks was kept at 0.5 m and 1.5 m, respectively, and the buffer strip was 2.5 m. Each block consisted of 27 plots with three replicates. The study is based on a trifactorial experiment, in subdivided plots, on the 27 × 3 type, with the following grading of the experimental factors:

FACTOR A - cultivated wheat variety:

a1 - Dacic, a2 - Miranda, a3 - Alex, a4 - Litera, a5 - Ciprian, a6 - Crişana, a7 - Biharia, a8 - Glosa, a9 - Bohemia, a10 - Sothys, a11 - Sacramento, a12 - Rubisko, a13 - Certiva, a14 - Aurelius, a15 - Aspect, a16 - Papillon, a17 - Activus, a18 - Centurion, a19 - Tika Taka, a20 - Chevignon, a21 - Sosthene, a22 - Vivendo, a23 - Sophie, a24 - Solindo, a25 - Tiberius, a26 - Arrezo and a27 - Apexus.

FACTOR B - types of fertilizers:

b1 - Ammoniacal nitrogen, b2 - Nitric nitrogen.

FACTOR C - fertilization level:

c1 - 120 kg/ha N a.s., c2 - 150 kg/ha N a.s., c3 - 170 kg/h N a.s.

The fertilizer consisted of two types of nitrogen (nitric and ammoniacal) that were applied in the form of ammonium sulfate and calcium nitrate, in three contrasting fertilization doses and administered to the plants in - a single dose, unfractionated, at the stage of growth BBCH 30-31. In both years of study, the sowing rate for each variety was established considering aspects related to the sowing rate such as purity, germination and the 1,000 kernel weight, and the density at sowing was 550 germinating grains/m^2. Wheat was sown at a depth of 4 centimeters with a row spacing of 12.5 cm. The treatments applied were V1 - nitric N dose of 120 kg/ha N a.s., V2 - nitric N dose of 150 kg/ha N a.s, V3 - nitric N dose of 170 kg/ha N a.s, V4 - ammoniacal N dose of 120 kg/ha N a.s., V5 - ammoniacal N dose of 150 kg/ha N a.s, V6 - ammoniacal N dose of 170 kg/ha N a.s. and the Cv - control variant was the average of the experimental field.

Plots were harvested mechanically at ripening, after reaching full grain maturity at 12% moisture content and the production for each plot was calculated automatically by the harvest machine by weighing the amount of grain yield. Harvesting was a complex process that was carried out in the shortest possible time without loss of product. The experimental lots were harvested mechanically, after reaching full maturity (BBCH 89-92), in which on the one hand the losses were the lowest, and on the other hand there were no difficulties in terms of keeping until performing quality tests. More specifically, it was not necessary to consume energy to bring the storage moisture. The harvest quality indices were monitored throughout the process and referred to grain losses, grain breakage and grain purity.

Wet gluten content was measured using the Perten Glutomatic System ac-cording to ISO 21415-2:2015, an apparatus system consisting of the Perten Glutomatic 2000 gluten washer, the Gluten Index 2010 centrifuge and the Glutork 2020 gluten dryer. The samples were grounded with a laboratory steel hammer mill fitted with a 200 μm sieve to produce a flour of the appropriate particle size for testing, and the flour was al-lowed to cool for one hour before being analyzed.

Principle of the method for obtaining wet gluten content: for each sample, 10 g of flour were grounded with a laboratory mill with a precision of 0.01 g. Then a dough was formed from the flour mass and 4.8 ml of NaCl solution which was mixed with the help of the aluminium paddles of the device for 20 seconds in the washing vessel equipped with a sieve with 88μ holes. The separation of protein substances in the form of wet gluten con-sists in washing with sodium chloride solution 20 g/l (2%), at a temperature of 22°C, used as a reagent of the formed dough. For the preparation of NaCl, distilled water was used so that the results would not suffer changes due to minerals or other substances with which the tap water could have been contaminated. The formed dough is placed in the washing vessel equipped with a fine sieve with holes of 88 microns and washed for 5 minutes with sodium chloride solution with a flow rate of 50-56 ml/minute. The gluten obtained in this way is placed in specially designed boxes in the centrifuge for whipping. The samples were processed for one minute, the centrifuge operating at 6,000 revolutions/ minute. The dough is removed with the help of a spatula and weighed with an accuracy of 0.01g and then the wet gluten is calculated with the formula *Wet gluten = mass of gluten × 10* (Hellemans, 2018).

In parallel, two determinations were performed for each analyzed sample.

Based on the results of two years' field experiment, wet gluten content parameter was calculated for optimal nitrogen dose and maximum wet gluten content of winter grain for that dose. First of all, we used the Analysis of variance (ANOVA), Student t tests which is an analysis tool used in statistics that splits an observed aggregate variability found inside a data set into two parts: systematic factors and random factors (Boldea et al., 2010).

RESULTS AND DISCUSSIONS

Gluten consists of 90% protein, 8% fat and 2% carbohydrate (Pronin et al., 2020). The gluten content is directly correlated with the protein content which is strongly influenced by pedoclimatic conditions and nitrogen fertilization. Wheat genotype is considered the most important factor influencing gluten quality characteristics (Simic et al., 2006). Total protein content of wheat correlates positively with gluten content (Shewry et al., 2002).

Gluten can be defined as the "*cohesive, visco-elastic protein material*" resulting as a by-product obtained by isolating starch from wheat flour. The gluten content of a flour depends on the climatic and technological conditions of the culture (especially the ad-ministration of nitrogen fertilizers during the vegetation period), as well as on the degree of extraction of the flour. A higher degree of extraction means a higher gluten content, because gliadins and glutelins are located in the endosperm, and through extraction the seed coats are removed. From a qualitative point of view, the gluten must be agglomerated, quite resistant and elastic. In no case should it deform, be soft, stringy or sticky. The quality of gluten is genetically determined, but it can be affected by excess moisture or the presence of pests. The minimum content of wheat gluten's flour should be about 24% - wet and 9% - dry (Singh et al., 2006). F test (Table 1, p column), shows that: A factor (variety), B factor (level of fertilization) and their interaction A × B had very significant action, meaning: wet gluten content differences between varieties are very significant; there are very significant differences between the 6 fertilization systems; A × B interaction has very significant action. Hence, the 27 winter wheat varieties reacted differently; within the 6 agrofunds, in terms of wet gluten content, the values obtained differ significantly between them.

Table 1. Variation analysis

Variation source	SSP (SP)	Degree of Freedom	Weighed Least of Squares WSL (s^2)	F test for s^2 error		
				Value	P	Signification
A (variety)	851.56	26	32.8	9.2	0.000000	***
B (agrofund)	1254.28	5	250.9	70.3	0.000000	***
A×B	773.15	130	5.9	1.7	0.000152	***
Error	1155.89	324	3.6			
Total	4034.89					

*, **, or *** indicate statistically significant differences between sample means and Mt based on t-test, at p ≤ 0.05, p ≤ 0.01, or p ≤ 0.001, respectively. NS (not significant) indicates the t-test difference between sample means was p > 0.05.
0, 00 or 000 also indicate significant differences between sample means and Mt based on t-test at p ≤ 0.05, p ≤ 0.01, or p ≤ 0.001, respectively, but with negative values.

Table 2. Student test for factor A (variety) - witness (Cv), average of the field

Variety	Wet gluten content (%)	Difference (%)	Signification
a1 - Dacic	34.42	1.60	*
a2 - Miranda	32.78	-0.04	
a3 - Alex	33.33	0.50	
a4 - Litera	33.64	0.81	
a5 - Ciprian	35.64	2.81	***
a6 - Crișana	34.20	1.37	*
a7 - Biharia	33.06	0.24	
a8 - Glossa	33.38	0.55	
a9 - Boema	33.91	1.09	
a10 - Sothys	31.39	-0.43	0
a11 - Sacramento	31.94	-0.88	
a12 - Rubisko	30.63	-2.20	000
a13 - Certiva	31.71	-1.12	
a14 - Aurelius	34.48	1.66	**
a15 - Aspekt	31.60	-1.23	
a16 - Papillon	30.94	-1.89	00
a17 - Activus	32.32	-0.51	
a18 - Centurion	30.47	-2.36	000
a19 - Tika Taka	34.69	1.86	**
a20 - Chevignon	33.48	0.65	
a21 - Sosthene	31.50	-1.33	0
a22 - Vivendo	32.14	-0.69	
a23 - Sophie	32.58	-0.25	
a24 - Solindo	32.55	-0.27	
a25 - Tiberius	34.21	1.38	*
a26 - Arrezo	33.59	0.76	
a27 - Apexus	31.72	-1.10	
Average	32.83	Cv	
LSD 5% = 1.240; LSD 1% = 1.632; LSD 0.1% = 2.081			

*, **, or *** indicate statistically significant differences between sample means and Mt based on t-test, at p ≤ 0.05, p ≤ 0.01, or p ≤ 0.001, respectively. NS (not significant) indicates the t-test difference between sample means was p > 0.05.
0, 00 or 000 also indicate significant differences between sample means and Mt based on t-test at p ≤ 0.05, p ≤ 0.01, or p ≤ 0.001, respectively, but with negative values.

In conclusion, the null hypothesis H_0 is rejected for factor A (variety), factor B (level of fertilization) and the A × B interaction. To examine the differences between the different varieties, the differences between the agrofunds, as well as the interaction A × B (variety × agrofund) we apply the t-test and we will use as the error variant: $s^2 = 3.6$, degrees of freedom = 324.

The wet gluten content achieved using the 27 winter wheat varieties is shown in Table 2. Compared to the control - the average of the experience, the following values were obtained: significant in the varieties: Dacic, Crișana and Tiberius; distinctly significant in Aurelius and Tika Taka varieties; very significant in the Ciprian variety. It is worth noting that there were also varieties that obtained negative increases, that is, the values of the wet gluten content obtained in these varieties were lower than the average of the experience, the varieties Sothys and Sosthene having significant differences, Papillon with a distinctly significant difference and Rubisko and Centurion with very significant differences. The other varieties did not register differences. The highest values of this index were obtained by the varieties Ciprian - 35.64% and Tika Taka - 34.69%. Below the experience average, with the lowest values, the Rubisko - 30.63% and Centurion - 30.47% varieties were ranked.

Table 3. Student test for factor B (level of fertilizer) – witness (Cv), average of the field

Variant	Wet gluten content (%)	Difference (%)	Signification
V1 - 120 kg/ha N a.s nitric N	34.54	1.72	***
V2 - 150 kg/ha N a.s nitric N	34.73	1.91	***
V3 - 170 kg/ha N a.s nitric N	33.86	1.04	***
V4 - 120 kg/ha N a.s ammoniacal N	30.74	-2.09	000
V5 - 150 kg/ha N a.s ammoniacal N	31.26	-1.56	000
V6 - 170 kg/ha N a.s ammoniacal N	31.81	-1.02	000
Average	32.83	Cv	
LSD 5% = 0.584; LSD 1% = 0.769; LSD 0.1% = 0.981			

*, **, or *** indicate statistically significant differences between sample means and Mt based on t-test, at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively. NS (not significant) indicates the t-test difference between sample means was $p > 0.05$.
0, 00 or 000 also indicate significant differences between sample means and Mt based on t-test at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively, but with negative values.

Table 4. Student test for A × B interaction (variety × level of fertilization) - witness (Cv), average of the field

Variety	V 1		V 2		V 3		V 4		V 5		V 6	
	WG	Diff	WG	Diff	WG	Diff	WG	Diff	WG	Diff	WG	Diff
a1 - Dacic	36.32	3.49*	35.85	3.02	35.59	2.76	31.94	-0.89	31.84	-0.99	35.02	2.19
a2 - Miranda	36.01	3.18*	36.02	3.19*	32.70	-0.13	30.04	-2.79	31.62	-1.21	30.31	-2.52
a3 - Alex	35.48	2.65	36.00	3.17*	35.44	2.61	27.36	-5.47	31.98	-0.85	33.72	0.89
a4 - Litera	35.19	2.36	34.26	1.43	35.29	2.47	29.03	-3.80^{0}	35.89	3.06*	32.16	-0.67
a5 - Ciprian	38.32	5.49***	36.62	3.79*	36.40	3.58*	32.44	-0.39	34.92	2.09	35.12	2.29
a6 - Crișana	36.03	3.20*	34.40	1.57	36.34	3.52*	33.43	0.60	31.07	-1.76	33.93	1.10
a7 - Biharia	36.49	3.66*	37.41	4.58**	33.42	0.60	30.89	-1.94	30.06	-2.77	30.10	-2.73
a8 - Glossa	37.57	4.74**	34.96	2.13	35.47	2.64	29.94	-2.89	31.56	-1.26	30.77	-2.06
a9 - Boema	36.12	3.29*	35.73	2.90	35.81	2.98	32.30	-0.53	30.98	-1.85	32.56	-0.27
a10 - Sothys	34.04	1.21	33.95	1.12	34.09	1.27	29.67	-3.16^{0}	27.77	-5.06^{00}	28.83	-4.00^{00}
a11 - Sacramento	32.83	0.00	33.37	0.54	33.45	0.62	30.33	-2.50	30.63	-2.20	31.05	-1.78
a12 - Rubisko	31.58	-1.25	33.96	1.13	32.04	-0.79	29.62	-3.21^{0}	28.21	-4.62^{00}	28.38	-4.45^{00}
a13 - Certiva	33.40	0.57	35.39	2.56	30.19	-2.63	29.65	-3.18^{0}	30.60	-2.23	31.03	-1.80
a14 - Aurelius	34.68	1.85	36.45	3.62*	36.77	3.94*	32.56	-0.27	33.04	0.21	33.42	0.59
a15 - Aspekt	32.05	-0.78	32.62	-0.21	32.48	-0.34	30.27	-2.56	30.48	-2.35	31.71	-1.12
a16 - Papilon	33.75	0.92	33.16	0.33	32.05	-0.78	29.44	-3.39^{0}	29.28	-3.55^{0}	27.97	-4.86^{00}
a17 - Activus	34.47	1.64	32.88	0.05	33.06	0.23	31.43	-1.40	30.39	-2.44	31.71	-1.12
a18 - Centurion	31.79	-1.04	34.15	1.32	29.57	-3.25^{0}	28.28	-4.55^{00}	28.25	-4.58^{00}	30.77	-2.06
a19 - Tika Taka	36.18	3.35*	35.29	2.46	32.83	0.00	34.91	2.08	33.37	0.54	35.55	2.72
a20 - Chevignon	36.80	3.97*	34.65	1.82	34.00	1.17	32.11	-0.72	32.61	-0.22	30.73	-2.10
a21 - Sosthene	31.89	-0.94	32.90	0.07	35.74	2.91	28.33	-4.50^{00}	28.08	-4.75^{00}	32.08	-0.75
a22 - Vivendo	33.47	0.64	33.52	0.69	30.73	-2.09	32.95	0.12	31.02	-1.81	31.16	-1.67
a23 - Sophie	30.28	-2.55	34.31	1.48	34.12	1.29	32.23	-0.60	32.71	-0.12	31.84	-0.99
a24 - Solindo	33.44	0.61	35.14	2.31	32.65	-0.18	29.33	-3.50^{0}	32.68	-0.15	32.09	-0.74
a25 - Tiberius	37.34	4.51**	36.19	3.36*	36.79	3.96*	31.82	-1.01	31.49	-1.34	31.64	-1.19
a26 - Arrezo	34.49	1.66	35.16	2.33	34.15	1.32	31.43	-1.40	32.42	-0.41	33.89	1.06
a27 - Apexus	32.67	-0.16	33.51	0.68	33.20	0.38	28.30	-4.53^{00}	31.24	-1.59	31.41	-1.42
Average	32.83											
LSD 5% = 3.036; LSD 1% = 3.997; LSD 0.1% = 5.097												

*, **, or *** indicate statistically significant differences between sample means and Mt based on t-test, at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively. NS (not significant) indicates the t-test difference between sample means was $p > 0.05$.
0, 00 or 000 also indicate significant differences between sample means and Mt based on t-test at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively, but with negative values.

The wet gluten content achieved using the nitric and ammoniacal nitrogen fertilization is shown in Table 3. Compared to the control variant (Cv)- the average of the experience, very significant increases were obtained at all of the treatments, regardless of the level or type of N fertilizer. The highest value of this index was obtained at V2 - 34.73, followed by V1 -

34.54% and V3 - 33.86%. It should be noted, however, that in the treatments applied with nitric nitrogen, the wet gluten content values were ranked above the experience mean, and in those performed with ammonium nitrogen, the values obtained are below the experience average of the two years of study.
From Table 4 it can be seen that compared to the control – the average of the experiment, the statistically assured wet gluten content values were both positive and negative, i.e. the values were both below and above the average of the experiment. The varieties whose wet gluten content values are higher than the average of the experiment are classified as follows:
• V1: Dacic, Miranda, Ciprian, Crișana, Biharia, Glossa, Boema, Tika Taka, Chevignon, Tiberius;
• V2: Miranda, Alex, Ciprian, Biharia, Aurelius, Tiberius;
• V3: Ciprian, Crisana, Aurelius, Tiberius;
• V5: Litera.
Influence of variety on wet gluten content: The wet gluten content values vary between 30.7% (Centurion variety) and 35.7% (Ciprian variety). The wet gluten content values for all other 25 varieties vary between 30.8 and 34.8%. The differences between varieties are highly significant ($p<0.001$), according to the F-test value.
Influence of agrofund on wet gluten content: The wet gluten content values vary between 30.8% (120 kg/ha a.s. ammoniacal nitrogen) and 34.7% (150 kg/ha a.s. nitric nitrogen). The differences between agrofunds are highly significant ($p<0.001$), according to the F-test value. The wet gluten content value has an upward trend from the first fertilization level of 120 kg/ha a.s. nitric nitrogen, at the second of 150 kg/ha a.s. nitric nitrogen, after which the trend is downward until the fertilization level of 170 kg/ha a.s. nitric nitrogen. In the case of fertilization with ammoniacal nitrogen, the trend is upward until the last fertilization level with ammoniacal nitrogen of 170 kg/ha a.s.
Influence of the A × B interaction on the wet gluten content: The highest value of this index was recorded for the Ciprian variety - 34.7% at the fertilization level of 150 kg/ha a.s. nitric nitrogen. And the lowest value was obtained by the Sosthene variety - 27.9% at the fertilization level of 150 kg/ha a.s. ammoniacal nitrogen.

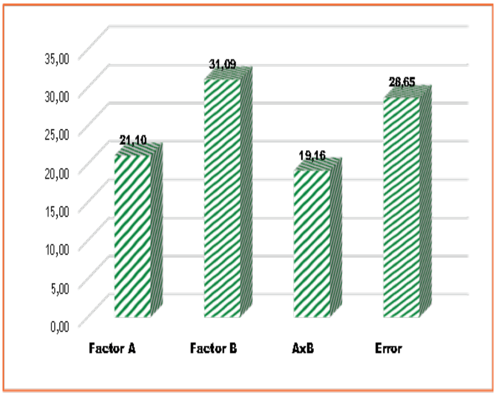

Figure 3. The contribution of factors and the interaction of factors on wet gluten content

Factor A - variety contributes to the formation of the wet gluten content index by 21.10%, factor B - fertilization system by 31.09%, and the interaction of the two mentioned factors A×B by 19.16%. The greatest contribution to the formation of the wet gluten content is made by factor B - fertilization system, followed by other factors that were not taken into account in this study, then by factor A - variety and by the interaction of factors A × B and with a very small difference.

CONCLUSIONS

The average of the two years of study for the wet gluten content is 32.83%. The highest values of this index were obtained by the varieties Ciprian - 35.64% and Tika Taka - 34.69%. Below the average of the experience, with the lowest values, the varieties Rubisko - 30.63% and Centurion - 30.47% were ranked. Regarding the fertilization levels, the highest value of 34.73 was obtained at agrofond 2, followed by agrofond 1 - 34.54% and agrofond 3 - 33.86%. The agrofonds in which fertilization was carried out with ammoniacal nitrogen were ranked below the average of the experience. Influence of the A × B interaction on the wet gluten content: The highest value of this index was recorded at the Ciprian variety - 34.7% at the fertilization level of 150 kg/ha a.s. nitric nitrogen. And the lowest value was obtained by the Sosthene variety - 27.9% at the fertilization level of 150 kg/ha of ammoniacal nitrogen.

Nitrogen which is essential in the formation of chlorophyll, which is stressed when the plant is exposed to excess ammonium (Kong et al., 2022). However, ammonium toxicity can be ameliorated to some extent by preventive strategies. Studies have revealed how ammonium uptake could be mitigated when nitric nitrogen is included in the fertilizer formulation. Wheat plants innately have the mechanism to store unused nitrate in vacuoles, where it is used for plant development, providing a way to mitigate the effect of ammonium toxicity (Wang et al., 2022). High levels of ammoniacal nitrogen in plants have the effect of significantly altering the oxidative metabolism of wheat roots, as well as most of their enzymatic activities. Research has shown that wheat root activity is negatively affected by ammonia nitrogen through reduced activity of key enzymes such as ascorbic acid, phosphoenolpyruvate carboxylase and mannose pyrophosphorylase (Lyu et al., 2022). In addition, NH_4^+, through its means of disrupting the electron transport chain, has been shown to negatively influence membrane-bound oxidase and thereby increase cellular production of reactive oxygen, damaging photosynthesis, and organs that enhance uptake electrons inhibits the photosynthesis process (Wang et al., 2020).

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PERFORMANCE OF WINTER TRITICALE (*Triticosecale* Wittm.) IN TRANSYLVANIAN PLAIN

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Abstract

The aim of this study is to determine an appropriate genotype for the Transylvanian Plain by analysing the responses of 17 winter triticale genotypes created at NARDI Fundulea to different weather patterns and additional nitrogen fertilisation. Field experiments were conducted at ARDS Turda over three growing seasons (2020/2021, 2021/2022, and 2022/2023). A randomized block design in six replications was used to conduct the experiments, with the first 3 replications additionally fertilized (N_{100} kg ha^{-1}). Grain yield from triticale varied significantly based on nitrogen rates, weather conditions, and genotype. According to biplot analysis, the genotypes Vifor, Cordial, Utrifun, Zaraza, Zori, and Cascador consistently produced the highest yields across the three years of the study, demonstrating a lack of sensitivity to environmental interactions. With notable yield increases (more than 700 kg ha^{-1}) over N_{50}, the genotypes Titan, Tulnic, Zaraza, Zvelt, Zori, Stil, Vultur, and Utrifun have stood out in response to extra nitrogen fertilisation (N_{100}). According to the evaluation of the experimental elements' interactions, the varieties Vifor, Utrifun, and Cordial were determined to be the most appropriate for the Transylvanian region.

Key words: *additional nitrogen fertilization, winter triticale, yield.*

INTRODUCTION

Common wheat with rye was crossed to create a cereal species known as Triticale (*Triticosecale* Wittm.), which has a strong ability to adapt to different agro-ecological conditions (Đekić et al., 2014). Scientists combined the best qualities of rye (good resistance to diseases, pests, cold, and drought; a large number of spikelets per spike; etc.) with the best qualities of wheat (high and stable yield and early maturity) in an effort to create a species with great adaptability and stable yields (Mălinaș et al., 2020). It can be grown on more marginal soil (desert, acidic, etc.) and adapt to lower tillage techniques (Ciftci and Eleroglu, 2003). Triticale is the crop that adapts to waterlogged and alkaline soils the best. It can also withstand low pH soils, thrive on sodic soils, and resist high boron, high aluminium, and saline soils. Additionally, it is valued for its ability to withstand drought during the growing season (https://grdc.com.au/). This is because it develops a fairly deep root system early on, which makes it easier to use water from deeper soil layers (Ittu et al., 2007). Triticale's advantages are also used in regions where the production of other cereals is limited (Đekić et al., 2014).

Triticale is known as a cereal species with a hight protein content in the grain and a beneficial content of essential amino acids compared with other cereal. Because of its great nutritional content and ability to substitute maize and wheat in the diet, breeders and experts in animal nutrition advise feeding it to all animal species (Đekić et al., 2014, Pintilie et al., 2023).

It is known that triticale genotypes can be classified into three basic types: winter, spring, and facultative (Abdelaal et al., 2019). Globally, triticale production areas are expanding annually due to its biological qualities and improved selection. According to Maričević et al. (2021), in recent years, more than 4 mil ha of triticale have been sawn worldwide. In Romania, triticale is grown on less than 100,000 hectares of agricultural land, despite the fact that it is recommended for animal nutrition. This is due to a small population of cattle. FAOSTAT (2024) reports that the average output of triticale in Romania ranged from 2789 to 4272 kg ha^{-1}, while the area under cultivation varied from 48019 to 82660 ha (Table 1). Transylvania falls under the first development macro-region, which is in the middle and northwest of the country. The area sown to triticale in this macro-region averaged between 25,000 and 28,500 hectares over the 2021-2023 study period, with yields ranging from 3400 to 3800 kg ha^{-1} (Brodeală et al., 2023).

Table 1. Romania's triticale area (ha) and average yield (kg ha^{-1}) fluctuations since 2012

Year	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
ha	48019	72529	76713	75722	82660	80115	78990	78770	73790	64550	56990	59462
kg ha^{-1}	2789.1	3378.3	3587.6	3500.8	3480.0	4138.6	4272.1	3986.3	3196.6	4015.6	3376.2	3315

Every agricultural crop system attempts to produce high and consistent yields. The implementation of appropriate in-field technology, careful selection of seed genotypes, and suitable agroclimatic conditions can all help achieve these goals (Biberdžić et al., 2012; Madić et al., 2018).
The interconnections between genotype and environment define its adaptation. Plants are influenced by both genetic and environmental factors during the vegetative cycle, which ultimately determines the genotype's real genetic potential (Kucukozdemir et al., 2021): The more ecological conditions satisfy its agrobiological requirements, the higher its level (Postolati et al., 2019).
Genotype, environmental factors, and their interactions all affect winter triticale yield, which can be increased with more N fertilisation. Like other cereals, winter triticale requires nitrogen fertilisation above all other agrotechnical measures in order to produce high quality (Alaru et al., 2004; Rajičić V. et al., 2023) and large yields (Alazmani, 2015; Gerdzhikova et al., 2017; Bielski et al., 2020; Stefanova-Dobreva and Muhova, 2023). Based on their studies, several researchers have recommended 120 kg ha^{-1} N rates for achieving these objectives (yield and quality), including Oral (2018), Rajičić et al. (2020), Mălinaș et al. (2020), Stefanova-Dobreva and Muhova (2023), and Rajičić et al. (2023). As a solution to enhancing nutrient deficits during the growing season, foliar fertilisation was proposed by Szpunar-Krok et al. (2021) for managing inadequate plant nutrition caused by drought, intensive plant growth, or agrotechnical errors.
The study's objectives were to determine a suitable genotype for the Transylvanian Plain by analysing the behaviour of 17 winter triticale genotypes developed at NARDI Fundulea in response to various weather conditions and supplementary nitrogen fertilisation.

MATERIALS AND METHODS

Research Methods

Field experiments were conducted at ARDS Turda over three growing seasons (2020/2021, 2021/2022, and 2022/2023). Seventeen winter triticale genotypes from NARDI Fundulea were tested as biological material, specifically: TF2, Plai, Titan, Stil, Haiduc, Negoiu, Odă FD, Pisc, Tulnic, Cascador, Utrifun, Vifor, Vultur, Zori, Zvelt, Zaraza and Cordial. The first eight genotypes were registered prior to 2015, TF2 being the oldest cultivar (since 1984).
The type of soil was a Phaeozem soil with a loamy-clay texture, a neutral pH of 6.8-7.2, clay content between 51.8% and 55.5%, humus content of 2.20-3.12%, total nitrogen of 0.162-0.124%, phosphorus levels of 0.9-5 ppm, and potassium levels well supplied at 126-140 ppm. Agrochemical analyzes were conducted on soil samples collected from the arable layer (0-20 cm). The experimental layout was a randomized block design with six replications, with the first 3 replications additionally fertilized. We utilized a Wintersteiger Plot Seed Drill for precise sowing at a seeding rate of 500 germinating grains m^{-2}, with a sowing depth of 4 cm and a row spacing of 12.5 cm. Each harvestable plot covered an area of 6.25 m^{-2}. Unfertilised peas were the preceding harvest.
Additional fertilisation was conducted in two stages: in autumn (before sowing) with $N_{50}P_{92}K_0$ kg ha^{-1} active substance (N_{50}) and in spring, at head swollen sheath, with N_{50} kg ha^{-1} a.s. more (N_{100}).

Sekator Progress 0.150 l/ha, Amino 600 SL 0.6 l/ha, Apis 0.2 l/ha, Falcon 0.6 l/ha, and Vital 0.2 l/ha were the sole phytosanitary treatments used in the spring. Each year of the trial was conducted with this kind of treatment.

Harvesting was performed using a Wintersteiger Plot Combine equipped with a 1.4 m working width.

Weather conditions

Weather conditions (rainfall, temperatures) at the experimental site were monitored by the Turda Meteorological Station and are shown in Figure 1.

Normal fall temperatures were only measured in 2021; the other two years showed positive deviations from the 65-years average. Winter temperatures were warm across the three research years, with January 2023 showing a significant deviation of 6.1°C from the long-term average. Additionally, April's average temperatures were colder than the 65-years average by 1.2°C (2022, 2023) to 2.1°C (2021). While 2021 and 2022 experienced regular rainfall, 2023 was considered extremely rainy having a deviation of 138 mm, of which 88.8 mm occurred during the triticale growing season (January: 21 mm, February: 7.9 mm, June: 59.9 mm). It's important to note that in 2023, there was a severe dearth of precipitation during the most critical months for triticale plant growth and development (March, April, May, and the first 10 days of June). This was reflected in the grain harvest.

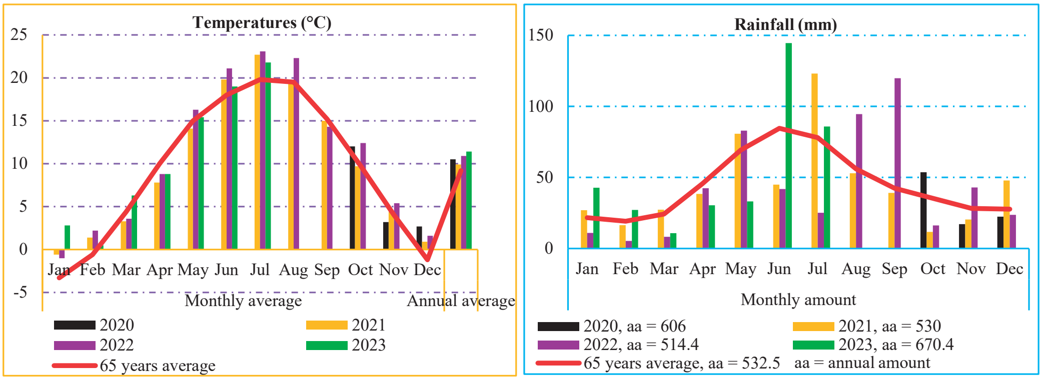

Figure 1. Weather conditions of the experimental site, Turda Meteorological Station

Statistical Analysis

The Poly Fact Software Version 2020 and Microsoft Excel software 2012 were used to statistically analyse the collected data using the standard analysis of variance (ANOVA). The Poly Fact program allowed the execution of Least Significant Difference (LSD) tests at significance levels of 5%, 1%, and 0.1%. Biplot analysis in Past 4.03 was used to identify a suitable genotype for the Transylvanian Plain (to determine which winter triticale genotypes achieve high yields under different environmental conditions to an optimal nitrogen dose).

RESULTS AND DISCUSSIONS

Over the three years of study, grain yield varied on average from 7880 to 8508 kg ha^{-1}. In 2021, the triticale crop experienced the most favourable growth and development conditions reaching an average yield of 8500 kg ha^{-1} that significantly exceeded the mean of the experimental results, as shown in Figure 2. Due to the drought that occurred during the spring months of March, April, and May, as well as the first decade of June, the average yield in 2023 did not exceed 8000 kg ha^{-1}. In accordance with Mălinaș et al. (2020), who conducted an 8-year study (2012-2019) on six winter triticale genotypes, the lowest yields were achieved in dry years, such as 2012. Similar to wheat, drought during the boot stage had the greatest impact on decreasing grain output, our results being in line with those obtained from studies conducted in Mexico by Perez et al. (2007) on drought challenges in different growing stages in triticale.

Alaru et al. (2004) reported that the highest grain output occurred in 2002, when the weather was warm and dry from anthesis to harvest, and the lowest in 2003, when the spring was cold and the summer was humid. According to research by Bielski et al. (2020), winter triticale yields varied greatly throughout the course of the three-year trial, averaging 4.56 t ha^{-1} in 2013, 4.62 t ha^{-1} in 2014, and 3.82 t ha^{-1} in 2015.

In winter triticale, Kirchenv and Georgieva (2017) showed that the growing season significantly affected grain yield; however, Lalević et al. (2022) found no discernible impact from the year.

The analysis of variance in the Rajičić et al. (2020) study shows that the year had a significant effect on grain yield. In accordance with Đekić et al. (2014), an increase in the winter triticale grain production is additional proof of the year's highly significant effects.

As demonstrated by Rajičić et al. (2023) the influence of meteorological conditions on the yield components and quality of triticale grains was also quite substantial. Triticale grains produced a very high yield and quality for the majority of the 2015 - 16 production year due to good weather conditions.

Nitrogen fertilisation is a key agronomic component of winter triticale grain yield production (Abdelaal et al., 2019; Bielski et al., 2020). The nitrogen dose and plant stage at which it is applied have an important effect on obtaining higher triticale yields. Nitrogen fertilisation, usually applied from the tillering to the booting stage, is meant to make up for nutritional deficiencies that occur during the growing season. This is done in order to rectify poor plant nutrition that may have been brought on by agrotechnical errors, harsh plant growth, or drought (like we experienced in 2023). According to Alaru et al. (2004), higher amounts of N applied in the spring during the triticale's EC30 stage enhanced grain production, but when applied later, at the EC47 stage, significantly increased test weight and protein content and decreased stem height.

As demonstrated by our research, output significantly increased when higher nitrogen doses were administered during the booting stage. The application of increased fertilisation (N_{100}) was significant throughout the three years of the study, particularly in the final year, as evidenced by the differences from the control (N_{50}) of 600 kg ha^{-1} in 2021, 557 kg ha^{-1} in 2022, and 785 kg ha^{-1} in 2023 (Figure 2).

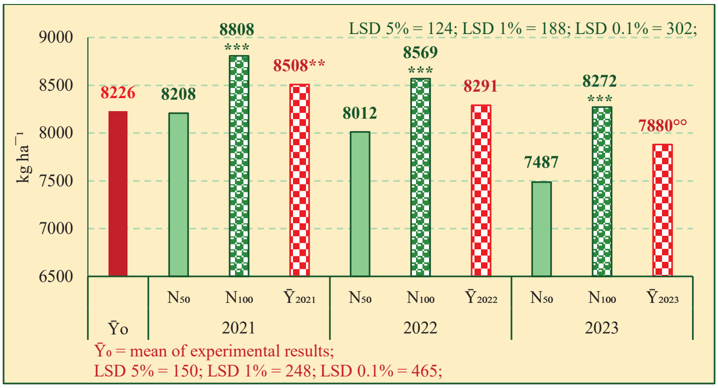

Figure 2. The effects of weather conditions and additional nitrogen fertilization on winter triticale yield

Significant differences between genotype performances were noted, as illustrated in Figure 3. Thus, analysing the influence of the genotype factor, it's observed that the Vifor, Cordial and Utrifun genotypes outperformed the control (Go – mean of the genotypes) by 864 kg ha^{-1}, 773 kg ha^{-1} and 688 kg ha^{-1}, respectively. The Cascador, Zori, and Zaraza genotypes are the next highest yielding cultivars, according to the Duncan test. In contrast, the TF2, Pisc and Titan genotypes showed yield decreases of 1012 $kgha^{-1}$, 532 kg ha^{-1} and 517 kg ha^{-1}, respectively, compared to the control's mean yield (Go = 8226 kg ha^{-1}). According to our results, the most productive varieties in the Transylvania area were Vifor, Cordial, and Utrifun, which produced average yields of 9090 kg ha^{-1}, 9000 kg ha^{-1}, and 8914 kg ha^{-1}, respectively. Mălinaş et al. (2020) claimed that the most productive variety was Haiduc, with an 8-year average yield of 6933.14 kg ha^{-1}. Thus, it emphasises the good adaptability and, at the same time, high yield capacity of the newest genotypes.

The PC 1 (principal component) analysis of the genotype x weather conditions interaction (Figure 4) shows that 79.87% of the variance is explained, suggesting that winter triticale genotypes located closer to the PC 2 line of the biplot are less susceptible to environmental interactions. Conversely, triticale genotypes located further from the biplot's origin exhibit notable interaction effects and are more vulnerable (Erdemci, 2018; Wodebo et al., 2023; Chețan et al., 2024). The genotype x weather conditions interaction results in distinct responses among genotypes: Vifor, Plai, Stil and TF2 yielded higher in the 2022 conditions, whereas the other 13 genotypes performed better in 2021. Accordingly, biplot analysis indicates that the Vifor, Cordial, Utrifun, Zaraza, Zori and Cascador genotypes exhibit a lack of sensitivity to environmental interactions, consistently achieving the highest yields over the three years of the study. Notably, TF2, Pisc, Stil and Titan produced the lowest yields in all three experimental years. The Vultur genotype proved to regularly achieve yields that were comparable to the average of experience.

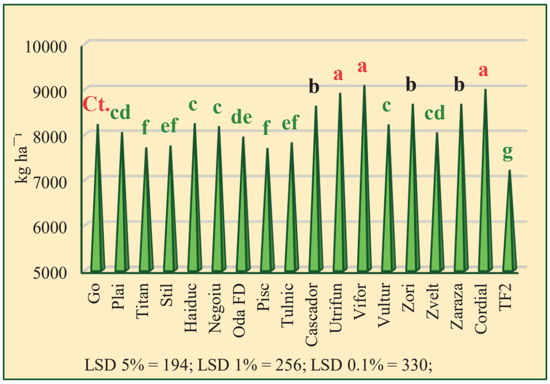

Figure 3. Duncan classification of winter triticale genotypes according to average yield obtained

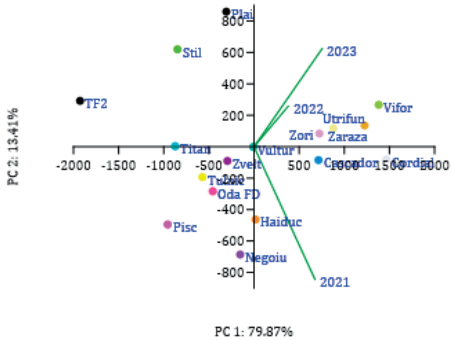

Figure 4. Graphics of Past4.03.exe biplot analysis on winter triticale genotypes' grain yield to determine the interaction between genotype and weather

Following the study, all 17 winter triticale genotypes under observation showed increases in grain yield with N dose, with gains ranging from 426 kg ha^{-1} (Odă FD) to 871 kg ha^{-1} (Titan), as depicted in Figure 5. Different triticale genotypes responded differently to nitrogen supplementation, emphasising that genotypes differ in N uptake, and it depends on the environment (Belete et al., 2018).

In Past 4.03, as illustrated in Figure 6, we employed biplot analysis to identify the genotypes that provide high yields when additional N fertiliser is applied. To additional nitrogen fertilisation (N_{100}), the genotypes Titan, Tulnic, Zaraza, Zvelt, Zori, Stil, Vultur, and Utrifun have distinguished themselves with significant yield increases (over 700 kg ha^{-1}) compared to N_{50}, these results justifying the need for its use. Genotypes that generated comparable yields at both nitrogen doses were identified, the outcome suggesting that the additional rate of N does not necessarily justify its use. Thus, the supplementary nitrogen dose (N_{100}) had minimal impact on the Odă FD, Pisc, and Negoiu genotypes, showing the slightest increases in grain yield. Vifor, Utrifun, and Cordial delivered the highest yields for both nitrogen rates used (N_{50}, N_{100}), confirming the genetic determinism of their yield potential.

We advise using the N_{100} fertilisation treatment when the prior plant was a pea, based on the results. It is safe to say that our findings are comparable to those of other researchers. Oral (2018) reported that the experimental plots fertilised with 120 kg N ha^{-1} produced the highest results for majority components and grain yield. According to Mălinaş et al. (2020), fertilising with N_{100} increased the yields of all six of the winter triticale varieties studied. Stefanova-Dobreva and Muhova (2023) concluded that mineral fertilisation at a rate of 120 kg N ha^{-1} significantly affected all of the characteristics of the Colorit, Attila, and Boomerang genotypes under study, including number of spiklets per spike, number of grains per spike, plant height, grain weight, and grain yield. The treatment with 120 kg ha^{-1} nitrogen fertilisation delivered the highest grain yield of triticale during the three-year trial (4.80 t ha^{-1}), while the control treatment without nitrogen fertilisation gave the lowest (3.17 t ha^{-1}), as determined by Bielski et al. (2020). Lalević et al. (2022) noted that the nitrogen fertilisation variant of 120 kg ha^{-1} generated the highest average yield (6.60 t ha^{-1} in the 1st and 6.37 t ha^{-1} in the 2nd year) among all the fertilisation variants studied in both testing years. Rajičić et al. (2023) found that applying fertilisers that contained NPK, lime, and manure (120 kg N ha^{-1}, 80 kg P_2O_5 ha^{-1}, 60 kg K_2O ha^{-1} + 5.0 t ha^{-1} lime + 20 t ha^{-1} manure) had a significant impact on winter triticale yield.

After applying nitrogen fertiliser at a rate of 120 kg ha^{-1} to spring triticale varieties, Katarzyna et al. (2015) and Abdelaal et al. (2019) obtained comparable results.

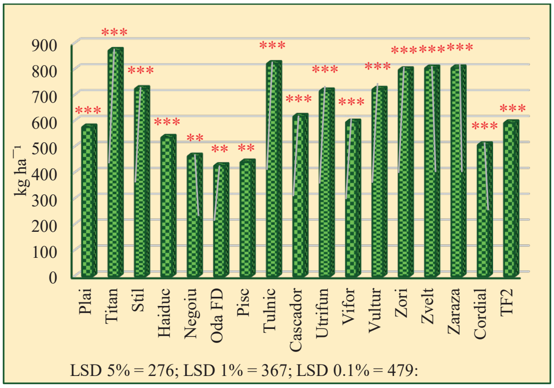

Figure 5. The gains in grain yield attained following additional fertiliser was applied

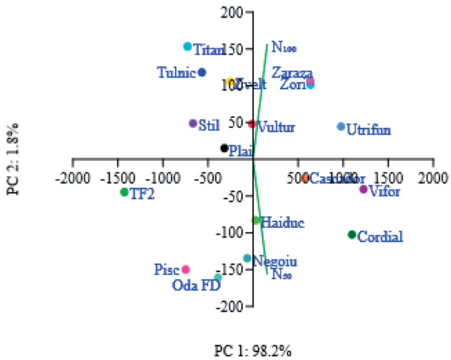

Figure 6. Graphics of Past4.03.exe biplot analysis of winter triticale genotypes' grain yield to assess how fertilisation and genotype interact

The crop's requirement for nitrogen to produce high yields is mostly determined by the genotype, soil type, fertility, and moisture content, as well as the prior plant, growing region, and year-round weather (Muntean et al., 2014). The soil's characteristics, along with the yearly variations in the climate, lead to notable variations in crop yields (Pintilie et al., 2023).

The analysis of variance and F-test results in our study showed that the experimental factors (weather conditions, fertilisation, and genotype) and the weather conditions x genotype interaction had a significant ($p < 0.1\%$) effect on the winter triticale yield. Therefore, our findings are consistent with those of Alaru et al. (2004), who concluded that weather had the most significant influence on winter triticale production and quality, followed by nitrogen rates and genotype. Mălinaș et al. (2020) found that the yield of triticale grain varied significantly ($p < 0.001$) based on the variety, experimental year, and nitrogen treatments. In their analysis of the consequences of different fertiliser types and growing seasons on triticale grain yield, yield components, and protein content over three production years, Rajičić et al. (2023) found that the results varied between the years researched and between fertilisation types.

Yields in the first year of the trial varied from 6860 kg ha^{-1} (TF2, N_{50}) to 9544 kg ha^{-1} (Cordial, N_{100}). The yield variance in 2022 ranged from 7327 kg ha^{-1} (Tulnic, N_{50}) to 9680 kg ha^{-1} (Vifor, N_{100}). Similar to 2021, TF2 and Cordial genotypes achieved the lowest yields (6092 kg ha^{-1}, N_{50}) and highest yields (9529 kg ha^{-1}, N_{100}) in the last year of research. In all experimental settings, the genotypes Vultur and Haiduc exhibit yields comparable to the control (Go), with the reported differences being negligible, according to an analysis of the impact of the interaction between experimental factors on yield (Table 2). The genotypes that can more efficiently absorb nitrogen, producing constantly higher yields (even under less favourable circumstances, as was the case in 2023), are clearly depicted in Figure 7. These include Vifor, Utrifun, Cordial, Zaraza, Zori, and Cascador, some of the most recent genotypes (registered after 2015).

Table 2. Significance of interactions between experimental factors on triticale yield (kg ha^{-1})

Years	2021		2022		2023	
Genotypes	N_{50}	N_{100}	N_{50}	N_{100}	N_{50}	N_{100}
Go – control	8208	8808	8012	8569	7487	8272
Plai	°°	°°°	ns	ns	ns	ns
Titan	°°	ns	°	ns	°°	ns
Stil	°°°	°°°	ns	ns	ns	ns
Haiduc	ns	ns	ns	ns	ns	ns
Negoiu	*	ns	ns	ns	°	°°
Oda FD	ns	ns	ns	ns	ns	°°
Pisc	ns	ns	ns	°	°°°	°°°
Tulnic	°	ns	°°	°°	ns	ns
Cascador	*	ns	ns	ns	ns	ns
Utrifun	*	**	*	ns	***	***
Vifor	***	ns	***	***	**	***
Vultur	ns	ns	ns	ns	ns	ns
Zori	ns	**	ns	ns	*	*
Zvelt	ns	ns	°	ns	ns	ns
Zaraza	ns	ns	ns	ns	**	***
Cordial	***	**	ns	ns	***	***
TF2	°°°	°°°	ns	ns	°°°	°°°
LSD	5%	476	1%	628	0.1%	808

ns = not significant; *, °, **, °°, ***, °°°- significant at the 5%, 1% and 0.1% positive and negative probability levels respectively.

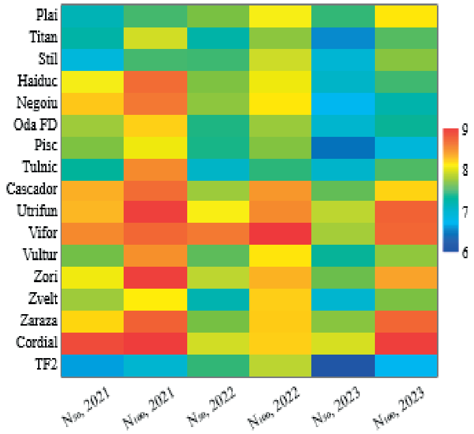

Figure 7. Graphic illustration of the interactions between experimental factors on triticale yield (t ha^{-1})

CONCLUSIONS

Winter triticale behaves well in Transylvanian circumstances, according to the results. The output from the N_{100} fertilisation was suitable, despite the fact that the years had different climates. Yields varied from 6092 kg ha^{-1} (TF2, N_{50}, 2023) to 9680 kg ha^{-1} (Vifor, N_{100}, 2022), considering the interactions of experimental conditions. Due to their excellent average yields, the genotypes Vifor, Utrifun, Cordial, Zaraza, Zori, and Cascador are the most suitable for the Transylvania region.

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RESEARCH ON THE RESPONSE TO PROLONGED DROUGHT OF AN ASSORTMENT OF WHEAT VARIETIES, THROUGH THE RATE OF WATER LOSS FROM THE FLAG LEAF, ON THE CHERNOZEM OF CARACAL

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Abstract

A collection composed of 220 wheat varieties of various origins was studied in terms of the water loss rate at 4 h and 24 h from the initial weighing of 6 flag leaves and through the relationship between the determinations on 15 ears of each variant of the analyzed assortment (ear length, number of spikelets/ear, ear density, number of grains/ear, weight of grains/ear, mass of 1000 grains, ear harvest index) on the one hand and yield, on the other. The lowest water loss at 4 hours was manifested by the Pajura variety (0.019) and at 24 h by the Izvor variety (0.343). The correlations between yield and each of the studied elements suggested that it is strongly correlated with the ear harvest index (r=0.208) and correlated with the thousand weight grains (r=0.150) in a positive sense. There wasn't correlation between yield and the rate of water loss in 24 h, but the results obtained suggest possibilities to direct the behavior of wheat in drought conditions, if genes with a favorable effect are accumulated.

Key words*: wheat, drought, flag leaf, rate of water loss, yield.*

INTRODUCTION

It is necessary to increase wheat yield through breeding or agronomic management to cope with the continuous population growth and global food demand (Liu et al., 2024).

Drought is a severe environmental constraint, which significantly affects plant growth, productivity, and quality. Plants have developed specific mechanisms that perceive the stress signals and respond to external environmental changes via different mitigation strategies (Ali et al., 2020). One of the main objective of researchers is to maintain high productivity of crops under climate changes condition. In support of this objective comes genetic engineering, which is revolutionising agriculture, increasing resilience and improving crop adaptation (Bonciu et al., 2021; De Souza and Bonciu, 2022).

Winter wheat is characterized by high water requirements and a transpiration coefficient of 400-500 L/kg dry matter (Kus, 2016). This species is particularly sensitive to the lack of water in the period from tillering to the end of the shooting stage. The size and stability of winter wheat yields are also affected by the optimal sowing date (Shah et al., 2020).

Among biotic constrainers of wheat, fungal pathogens are the most important yield and quality limiting factors leading to yield losses by 15-10% and in favorable climatic conditions even by 60-70% (Cristea et al., 2015; Cotuna et al., 2021, 2022; Paraschivu et al., 2021). The management of wheat fungal diseases complex is based on technological measures and also on alternative organic products with fungicide effects (Paraschivu et al., 2024).

Excised leaf water loss has been suggested as a technique to identify cereal genotypes that loose less water through cuticle and incompletely closed stomata, mainly during the night. Environmental conditions have a large influence on the water content of freshly harvested leaves and on water loss from excised leaves (David, 2010).

Assessment of water loss from excised leaves has shown promise for characterizing drought resistance and thermo tolerance in wheat genotypes (Kaur et al., 2016; Mir et al., 2012).

According to Petcu (2005), the quantity of water lost through cuticle is up to 10-20 time lower than water loss by stomata. Nevertheless, under water stress conditions, when the stomata are closed, it represents the main way of water loss. The analysis of variance regarding water loss by cuticular transpiration showed a very significant influence of the treatment, genotype and their interaction, but the variance of treatment was higher than the variance due to genotypes (Petcu, 2005).

The current task of wheat breeding is to obtain productive cultivars with improved quality characteristics, high nutritional value, and resistance to adverse environmental conditions (Juzoń-Sikora et al., 2024). Drought stress affects wheat plants on many levels: growth inhibition and developmental disorders (Vassileva et al., 2023; Yasir et al., 2019; Yu et al., 2024).

A pot experiment was conducted by Shi et al. (2014) to investigate the physiological and morphological mechanisms of water use in two cultivars of winter wheat via partial root-zone drying (PRD). The results indicated that the weight of 1000 grains was higher under PRD conditions for both cultivars. Total water consumption per plant significantly decreased by 11.6 and 17.3%, and water-use efficiency significantly increased by 17.2 and 20.3% under PRD. Stomatal density increased under PRD, but stomatal width and area declined. Overall, the two types of winter wheat had both common and individual means of improving WUE under PRD conditions (Shi et al., 2014).

Saeidi and Abdoli (2015), reported that post anthesis water stress caused 34 and 27% reduction in grain yield and 1,000 grain weight in average, respectively. Also, post anthesis water stress significantly decreased harvest index in most cultivars. Under post-anthesis water stress, a positive correlation was found between grain weight and harvest index (Saeidi and Abdoli, 2015).

MATERIALS AND METHODS

Among the methods for identifying wheat (*Triticum* spp.) cultivars with better drought tolerance, the rate of water loss (RWL) from detached leaves was used, a method proposed by Clarke and McCaig (1982) and Clarke et al. (1989).

Six flag leaves were detached from the 220 wheat cultivars tested from each of the 3 replicates in the experimental field plots. The detached leaves were transported to the laboratory no later than 30 minutes and weighed to obtain the initial water content (IWC). Then, the leaves were dried for 4 hours under laboratory conditions in the PANASONIC climate chamber (20°C, in the dark) and weighed to obtain W_{4h} (weighing after 4 hours). The water lost after 4 hours by drying was calculated using the formula:

$WL_{4h} = (IWC-W_{4h})/DW$

where: IWC is the initial weight, W_{4h} – the weight after 4 hours and DW – the dry weight of the leaves.

Next, the leaves were dried for another 20 hours at 20°C in the same climate chamber and reweighed to obtain W_{24h} (weighing after 20 hours). After this, the leaves were oven-dried at 70°C to obtain the dry weight (DW).

Throughout the experimental period, the leaves were dried in controlled environments, in air-conditioned rooms, the parameters (temperature, light and humidity) being uniform throughout the experimental period.

Water loss in the period between 4 hours and 24 hours was estimated using the formula:

$WL_{4h\text{-}24h} = (W_{4h}-W_{24h})/DW$.

The differentiation was made according to the average of all tested varieties. The varieties that recorded values of the amount of water lost after 24 h above this average by 25% minus were highlighted – the maximum limit difference that is perceived as correct in statistical calculation. In our case: 0.580.

Determinations were made on 15 ears of each variant of the analyzed assortment regarding ear length, number of spikelets/ear, ear density, number of grains/ear, grain weight/ear, mass of 1000 grains, ear harvest index.

RESULTS AND DISCUSSIONS

Food security has a fundamental importance for human existence, and increasing wheat production under climate change is, from this point of view, one of the permanent challenges for farmers (Bonciu et al., 2021; Dihoru et al., 2023; Paunescu et al., 2021; 2023; Rădoi et al., 2022; Rosculete et al., 2023). Also, wheat contribute to economic stability and growth in rural communities (Bonciu, 2023).

Water scarcity in agricultural systems can occur slowly or suddenly. Plant responses to water stress differ depending on the degree of manifestation and its duration. The level of sensitivity of plants under water scarcity conditions depends primarily on the management of its content and the adjustments of their own metabolism (Juzoń-Sikora, 2024).

The rate of water loss from detached leaves, determined using the initial water content of the leaves, fresh biomass or dry biomass, has been proposed by many authors as a criterion for the selection of drought-tolerant plants (David, 2010). Numerous national and global studies have highlighted significant and positive correlations between water content in cut leaves and wheat production under drought conditions (Bhutto et al., 2023; Geravandi et al., 2011; Hussein et al., 2023), but there have also been studies that have not highlighted this aspect (Clarke et al., 1989; Dabiry et al., 2015).

Lower values of water loss indicate better tolerance of plants to water deficits, and significant negative correlations between water loss and yield were found in doubled haploid wheat lines (Czyczylo-Mysza et al., 2018). The results of the above study indicated that genotypes with the lowest values of water loss are characterized by efficient water management and can perform better under water deficit conditions, unlike those with high values of this parameter.

In our study, genetic variability was identified for the wheat genotypes analyzed for the analyzed indicator. The results showed that the dynamics of water loss differed significantly between varieties, the coefficient of variability being 18.1%. The highest initial water content was recorded by the varieties Exsal, Lemmy, Agilis, Klima, Tika Taka, Unic, all above the average values (1.930 g).

In the first 4 hours, on average, the lowest water losses were suffered by the varieties Pajura, Ekonom, Izvor, Tarroca, Papillon, Thalamus, Dacic, Evident, Litera, Biharia, Bezostaia, Foxil Gruia, Capo and Nikifor, all with values below 0.3 g. The results coincide with the information we have about the tested varieties, but it should be noted that most of them are Romanian varieties, much better adapted to the area conditions.

It is known that the Izvor variety is a drought-resistant variety, an aspect confirmed by Păunescu R.A. and Păunescu G. (2019) who determined the water loss rate of an assortment of 50 varieties on the luvosol from Șimnic. Also, the Biharia variety achieved some of the highest productions in the area despite the drought.

Regarding water loss after another 20 hours, on average, the Izvor, Izalco and Carom varieties stood out, with very low losses, below 0.5 g. The results are confirmed by the fact that the Carom wheat variety, the newest creation of the University of Craiova, was obtained through the improvement process under the conditions of Caracal, where the testing was done (Table 1).

In addition, based on the results, the varieties Absint, Tarroca, Solveig, Atuan, Pibrac, Cazimir, Mobile, Novic and Palmeo stood out with water loss in 24 hours above the average of all varieties reduced by 25%. In conclusion, the rate of water loss from the flag leaf can be an indicator based on which to make an assessment of drought tolerance, at least for the type of drought that occurred in Caracal.

The correlation between production and water loss after 24 hours is not evident for the 220 wheat varieties tested (r=-0.05). The cloud of points placed in the form of a ball next to the right of the equation visually indicates this finding. However, the result suggests that the Concret and Stromboli varieties that recorded very high yields (over 9000 kg/ha) lost little water after 24 hours, and they can be recommended as tolerant to the drought manifested in Caracal (Figure 1).

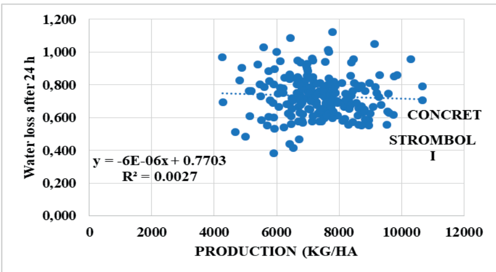

Figure 1. Production - water loss relationship after 24 hours

Table 1. Results regarding water loss from flag leaves of the tested variety

VARIETIES	WEIGHINGS			LOSSES after		dry weight	WL_{4h}	WL_{24h}
	initial weight	after 4 h	after 20 h	4 h (W_{4h})	24 h (W_{24h})			
IZVOR	2.312	2.204	1.961	0.108	0.351	0.709	0.152	0.343
IZALCO	2.565	2.178	1.846	0.387	0.719	0.765	0.506	0.434
CAROM	2.312	1.953	1.639	0.359	0.673	0.720	0.498	0.436
ABSINT	2.06	1.832	1.534	0.228	0.526	0.588	0.388	0.507
TARROCA	2.512	2.347	1.947	0.165	0.565	0.745	0.221	0.537
SOLVEIG	1.437	1.285	1.018	0.152	0.419	0.492	0.309	0.543
ATUAN	1.982	1.687	1.39	0.295	0.592	0.542	0.544	0.548
PIBRAC	1.984	1.606	1.265	0.378	0.719	0.620	0.610	0.550
CAZIMIR	1.902	1.618	1.291	0.284	0.611	0.592	0.480	0.552
MOBILE	1.696	1.409	1.081	0.287	0.615	0.588	0.488	0.558
NOVIC	1.693	1.421	1.128	0.272	0.565	0.525	0.518	0.558
RGT PALMEO	1.44	1.234	0.953	0.206	0.487	0.492	0.419	0.571
AMANDUS	1.836	1.519	1.151	0.317	0.685	0.630	0.503	0.584
LENNOX	2.602	2.045	1.551	0.557	1.051	0.844	0.660	0.585
MOSCHUS	2.122	1.793	1.442	0.329	0.68	0.599	0.549	0.586
TRUBLION	1.846	1.527	1.103	0.319	0.743	0.722	0.442	0.587
KWS FLEXUM	1.858	1.534	1.114	0.324	0.744	0.714	0.454	0.588
JOKER	2.396	2.051	1.628	0.345	0.768	0.712	0.485	0.594
BASALTIC	1.447	1.282	0.988	0.165	0.459	0.490	0.337	0.600
EPILOG	2.086	1.758	1.368	0.328	0.718	0.650	0.505	0.600
SOMTUOSO	1.86	1.595	1.242	0.265	0.618	0.588	0.451	0.600
ATHLON	1.599	1.123	0.854	0.476	0.745	0.448	1.063	0.600
KWS USUEL	2.088	1.755	1.341	0.333	0.747	0.683	0.488	0.606
SORRIAL	2.036	1.81	1.445	0.226	0.591	0.602	0.375	0.606
RGT VENEZIO	1.885	1.568	1.217	0.317	0.668	0.575	0.551	0.610
SOSTHENE	2.11	1.844	1.482	0.266	0.628	0.592	0.449	0.611
KWS ULTIM	1.346	1.145	0.905	0.201	0.441	0.392	0.513	0.612
HY FI	2.334	1.421	1	0.913	1.334	0.682	1.339	0.617
RGT CESARIO	1.751	1.526	1.183	0.225	0.568	0.548	0.411	0.626
KWS STROMBOLI	2.137	1.884	1.514	0.253	0.623	0.590	0.429	0.627
PROMITOR	1.795	1.516	1.129	0.279	0.666	0.616	0.453	0.628
AURELIUS	1.651	1.438	1.138	0.213	0.513	0.475	0.448	0.632
SY ROCINANTE	1.844	1.671	1.341	0.173	0.503	0.522	0.331	0.632
ASTERION	1.248	1.119	0.88	0.129	0.368	0.377	0.342	0.634
BC OPSESIJA	1.401	1.177	0.893	0.224	0.508	0.445	0.503	0.638
THALAMUS	1.331	1.217	0.964	0.114	0.367	0.390	0.292	0.649
ADELINA	1.192	0.884	0.666	0.308	0.526	0.336	0.917	0.649
PG 102	2.12	1.734	1.354	0.386	0.766	0.585	0.660	0.650
ATTRAKTION	1.714	1.427	1.08	0.287	0.634	0.534	0.537	0.650
MAXENCE	2.16	1.813	1.42	0.347	0.74	0.603	0.575	0.652
MUTIC	1.355	1.186	0.922	0.169	0.433	0.405	0.417	0.652
AVENUE	1.48	1.246	0.964	0.234	0.516	0.432	0.542	0.653
OMERSSON	1.714	1.484	1.128	0.23	0.586	0.544	0.423	0.654

COLUMNA	2.397	2.077	1.626	0.32	0.771	0.689	0.464	0.655
CONCRET	1.511	1.24	0.95	0.271	0.561	0.442	0.613	0.656
PYTHON	2.001	1.668	1.27	0.333	0.731	0.605	0.550	0.658
KWS PEPLUM	2.096	1.835	1.449	0.261	0.647	0.585	0.446	0.660
KWS RHUM	2.18	1.807	1.411	0.373	0.769	0.599	0.623	0.661
EMBLEM	1.765	1.457	1.092	0.308	0.673	0.550	0.560	0.664
PERKUSSIO	1.425	1.27	0.942	0.155	0.483	0.493	0.314	0.665
KWS USUELLUM	1.309	1.13	0.881	0.179	0.428	0.374	0.479	0.666
CORDIAL	1.457	1.241	0.908	0.216	0.549	0.498	0.434	0.669
RGT LETSGO	1.415	1.16	0.873	0.255	0.542	0.429	0.594	0.669
FLAVOR	2.434	1.538	1.073	0.896	1.361	0.694	1.291	0.670
BOGDANA	1.558	1.308	0.99	0.25	0.568	0.470	0.532	0.677
AIRBUS	2.042	1.799	1.401	0.243	0.641	0.587	0.414	0.678
SOPHIE	1.736	1.55	1.201	0.186	0.535	0.511	0.364	0.683
LG ABSALON	1.286	1.131	0.9	0.155	0.386	0.338	0.459	0.683
AGX	1.805	1.482	1.109	0.323	0.696	0.544	0.594	0.686
SACRAMENTO	1.872	1.577	1.166	0.295	0.706	0.597	0.494	0.688
CONCURENT	1.906	1.605	1.273	0.301	0.633	0.482	0.624	0.689
RUBISKO	1.146	0.974	0.726	0.172	0.42	0.358	0.480	0.693
ASCONA	1.217	1.02	0.771	0.197	0.446	0.358	0.550	0.696
FAGUR	1.741	1.473	1.061	0.268	0.68	0.592	0.453	0.696
EVERY	2.376	1.949	1.431	0.427	0.945	0.744	0.574	0.696
FOXIL	1.909	1.754	1.365	0.155	0.544	0.557	0.278	0.698
RGT PACTEO	1.611	1.47	1.142	0.141	0.469	0.469	0.301	0.699
SILAS	1.594	1.389	1.078	0.205	0.516	0.444	0.462	0.700
PROVIDENCE	1.517	1.243	0.917	0.274	0.6	0.464	0.591	0.703
CONSORTIUM	1.808	1.614	1.239	0.194	0.569	0.533	0.364	0.704
BASILIO	1.714	1.372	0.983	0.342	0.731	0.552	0.620	0.705
SIMNIC 60	1.902	1.677	1.245	0.225	0.657	0.612	0.368	0.706
SOTHYS	1.734	1.507	1.147	0.227	0.587	0.509	0.446	0.707
AMBURGO	1.762	1.481	1.091	0.281	0.671	0.550	0.511	0.709
TARASCON	1.914	1.672	1.261	0.242	0.653	0.579	0.418	0.710
CSIKO	2.601	2.181	1.627	0.42	0.974	0.780	0.538	0.710
OTILIA	2.239	1.551	1.079	0.688	1.16	0.664	1.036	0.711
BOLOGNA	1.731	1.453	1.066	0.278	0.665	0.544	0.511	0.711
LG APILCO	1.711	1.172	0.875	0.539	0.836	0.416	1.296	0.714
RGT VIVENDO	1.463	1.285	0.945	0.178	0.518	0.475	0.375	0.716
CENTURION	2.096	1.741	1.295	0.355	0.801	0.622	0.571	0.717
SY EXALTATION	1.602	1.391	1.101	0.211	0.501	0.402	0.525	0.721
COMBIN	1.554	1.295	0.96	0.259	0.594	0.464	0.558	0.722
SOLENZARA	1.659	1.482	1.156	0.177	0.503	0.451	0.392	0.723
MIX CEREALE	2.28	1.881	1.368	0.399	0.912	0.709	0.563	0.724
SY SANLUCA	2.236	1.981	1.488	0.255	0.748	0.680	0.375	0.725
ANDINO	1.492	1.283	0.927	0.209	0.565	0.491	0.426	0.725
SONATHINE	2.24	1.968	1.474	0.272	0.766	0.680	0.400	0.726
BRIA	2.325	1.868	1.316	0.457	1.009	0.757	0.604	0.729
LUXEO	1.535	1.314	0.99	0.221	0.545	0.444	0.498	0.730

GABRIO	1.779	1.483	1.101	0.296	0.678	0.522	0.567	0.732
ALGORITMO	2.005	1.618	1.13	0.387	0.875	0.662	0.585	0.737
PG 101	1.758	1.454	1.049	0.304	0.709	0.549	0.553	0.737
EXSAL	3.068	2.697	2.034	0.371	1.034	0.897	0.414	0.739
SOFRU	1.606	1.421	1.109	0.185	0.497	0.420	0.440	0.743
DALLARA	2.252	1.946	1.457	0.306	0.795	0.658	0.465	0.743
APEXUS	1.777	1.469	1.048	0.308	0.729	0.566	0.544	0.744
BARBA	2.119	1.819	1.392	0.3	0.727	0.572	0.524	0.747
VOLTEO	1.706	1.438	1.039	0.268	0.667	0.533	0.503	0.748
BASMATI	1.442	1.193	0.871	0.249	0.571	0.429	0.580	0.751
IRUN	2.028	1.823	1.373	0.205	0.655	0.599	0.342	0.751
LOVRIN 9Z	1.865	1.593	1.222	0.272	0.643	0.492	0.553	0.754
DARNIC	2.326	1.905	1.369	0.421	0.957	0.710	0.593	0.755
CARACAL 1	2.011	1.666	1.221	0.345	0.79	0.588	0.587	0.757
SILVERIO	1.672	1.385	1.032	0.287	0.64	0.466	0.616	0.758
OBIWAN	1.95	1.679	1.251	0.271	0.699	0.564	0.480	0.759
SY EXCEPTION	1.934	1.679	1.26	0.255	0.674	0.552	0.462	0.759
LOVRIN 90	1.914	1.62	1.215	0.294	0.699	0.533	0.552	0.760
ANAPURNA	1.426	1.183	0.857	0.243	0.569	0.429	0.566	0.760
CEZAR	1.639	1.397	1.038	0.242	0.601	0.472	0.513	0.761
GARAVUSA	1.914	1.547	1.01	0.367	0.904	0.702	0.523	0.765
SY LIRICO	1.565	1.385	1.086	0.18	0.479	0.390	0.462	0.767
RGT BORSALINO	2.006	1.713	1.21	0.293	0.796	0.652	0.449	0.771
SOCADE_CS	1.277	1.104	0.827	0.173	0.45	0.358	0.483	0.774
GK ARATO	2.369	1.922	1.348	0.447	1.021	0.740	0.604	0.775
VICTORAS	2.136	1.722	1.206	0.414	0.93	0.664	0.623	0.777
BALTAG	1.435	1.225	0.891	0.21	0.544	0.429	0.490	0.779
TOCAYO	1.713	1.423	0.989	0.29	0.724	0.555	0.523	0.782
SOLINDO	1.738	1.549	1.207	0.189	0.531	0.437	0.432	0.783
AIDA	1.98	1.687	1.235	0.293	0.745	0.577	0.508	0.783
MOISSON	1.694	1.465	1.096	0.229	0.598	0.469	0.488	0.787
GUIDO	2.51	1.951	1.341	0.559	1.169	0.774	0.722	0.788
WINDO	2.256	1.95	1.415	0.306	0.841	0.675	0.453	0.793
SY TRANSITION	2.368	2.05	1.533	0.318	0.835	0.652	0.488	0.793
PITAR	2.049	1.799	1.32	0.25	0.729	0.601	0.416	0.797
MUSIK	1.635	1.385	1.027	0.25	0.608	0.448	0.558	0.799
WINNER	1.44	1.187	0.837	0.253	0.603	0.437	0.579	0.801
SORELA	1.933	1.661	1.18	0.272	0.753	0.600	0.453	0.802
ARNOLD	1.079	0.898	0.642	0.181	0.437	0.319	0.567	0.803
ACHIM	2.543	2.198	1.602	0.345	0.941	0.740	0.466	0.805
UNIC	2.882	2.406	1.697	0.476	1.185	0.877	0.543	0.808
KWS LAZULI	1.795	1.523	1.104	0.272	0.691	0.518	0.525	0.809
MIRANDA FDL	2.392	1.95	1.345	0.442	1.047	0.748	0.591	0.809
KWS MILANUM	1.776	1.443	0.967	0.333	0.809	0.588	0.566	0.810
FOXX	1.956	1.547	1.068	0.409	0.888	0.591	0.692	0.810
KWS ETERNEL	1.73	1.5	1.046	0.23	0.684	0.559	0.411	0.812

ZENDALEE	1.519	1.258	0.915	0.261	0.604	0.422	0.618	0.813
ARMURA	2.029	1.781	1.303	0.248	0.726	0.588	0.422	0.813
SCRAMBLER	1.543	1.281	0.922	0.262	0.621	0.440	0.595	0.816
MONTE CARLO	1.898	1.645	1.249	0.253	0.649	0.485	0.522	0.816
SY PASSION	1.644	1.448	1.091	0.196	0.553	0.437	0.449	0.817
SY STARLORD	2.089	1.817	1.331	0.272	0.758	0.592	0.459	0.821
EXOTIC	2.331	2.088	1.502	0.243	0.829	0.711	0.342	0.824
TIBERIUS	1.845	1.631	1.155	0.214	0.69	0.577	0.371	0.826
LEXIO	1.799	1.549	1.108	0.25	0.691	0.532	0.470	0.829
KAPITOL	1.815	1.506	1.051	0.309	0.764	0.548	0.564	0.830
SY OLEN	1.664	1.456	1.061	0.208	0.603	0.475	0.438	0.832
CARACAL	2.847	2.178	1.437	0.669	1.41	0.890	0.752	0.833
KRALJICA	2.171	1.683	1.006	0.488	1.165	0.811	0.602	0.835
DJANGO	1.87	1.554	1.093	0.316	0.777	0.550	0.575	0.838
FALADO	2.188	1.814	1.262	0.374	0.926	0.657	0.569	0.840
KWS CRITERIUM	1.845	1.469	1.055	0.376	0.79	0.492	0.764	0.841
Mv Nador	2.504	2.159	1.515	0.345	0.989	0.765	0.451	0.842
VLADIMIR	1.828	1.485	1.003	0.343	0.825	0.571	0.600	0.844
SY MILTEO	1.892	1.674	1.209	0.218	0.683	0.550	0.396	0.845
ALCANTARA	1.651	1.476	1.127	0.175	0.524	0.412	0.425	0.847
BORSALINO	1.541	1.31	0.93	0.231	0.611	0.448	0.516	0.848
SIMNIC 1412	1.99	1.556	0.96	0.434	1.03	0.702	0.618	0.849
PROPULSO	1.996	1.724	1.229	0.272	0.767	0.583	0.467	0.849
FILON	1.598	1.342	0.97	0.256	0.628	0.438	0.584	0.849
BOEMA	1.742	1.474	1.011	0.268	0.731	0.545	0.492	0.850
SOLIFLOR	1.923	1.644	1.176	0.279	0.747	0.550	0.507	0.851
TIKA TAKA	2.978	2.586	1.878	0.392	1.1	0.831	0.472	0.852
PAPILLON	2.318	2.123	1.517	0.195	0.801	0.710	0.275	0.854
SOESKI	1.678	1.479	1.118	0.199	0.56	0.422	0.472	0.855
GLOSA	1.69	1.388	1.027	0.302	0.663	0.422	0.716	0.855
ABUND	1.615	1.273	0.917	0.342	0.698	0.416	0.822	0.856
DACIC	2.274	2.092	1.499	0.182	0.775	0.689	0.264	0.861
ORTOLAN	2.767	2.306	1.605	0.461	1.162	0.812	0.568	0.863
AMICUS	1.331	1.151	0.828	0.18	0.503	0.374	0.481	0.864
KWS ENCLUM	1.634	1.354	0.98	0.28	0.654	0.433	0.647	0.864
VIKTORIA	1.518	1.303	0.933	0.215	0.585	0.428	0.502	0.864
ACTIVUS	1.79	1.442	0.995	0.348	0.795	0.516	0.674	0.866
INGENIO	2.536	2.017	1.368	0.519	1.168	0.745	0.697	0.871
AXUM	1.8	1.553	1.106	0.247	0.694	0.512	0.482	0.873
AMURG	2.812	2.388	1.702	0.424	1.11	0.785	0.540	0.874
MODERN	1.877	1.624	1.216	0.253	0.661	0.466	0.543	0.876
IRIS 12	2.053	1.691	1.172	0.362	0.881	0.591	0.613	0.878
LEMMY	3.285	2.789	2.066	0.496	1.219	0.822	0.603	0.880
EUCLIDE	2.31	1.884	1.249	0.426	1.061	0.720	0.592	0.882
TATA MATA	2.014	1.713	1.181	0.301	0.833	0.602	0.500	0.884
COMPLICE	1.668	1.438	1.053	0.23	0.615	0.435	0.529	0.885

KORELI	2.103	1.655	1.117	0.448	0.986	0.603	0.743	0.892
KWS EXTREM	1.636	1.491	1.085	0.145	0.551	0.455	0.319	0.892
EKONOM	2.264	2.231	1.653	0.033	0.611	0.645	0.051	0.896
OROLOGE	2.603	2.116	1.387	0.487	1.216	0.813	0.599	0.896
MONTECRISTO	2.409	2.051	1.408	0.358	1.001	0.711	0.504	0.904
KWS SPHERE	1.608	1.408	1.031	0.2	0.577	0.415	0.482	0.908
RULER	1.655	1.44	1.054	0.215	0.601	0.423	0.508	0.913
ELENUS	1.75	1.441	0.982	0.309	0.768	0.502	0.616	0.914
TOSCADOU	1.901	1.538	1.003	0.363	0.898	0.584	0.622	0.916
KAROQUE	2.079	1.743	1.19	0.336	0.889	0.600	0.560	0.922
EMISAR	2.484	1.935	1.314	0.549	1.17	0.672	0.817	0.924
EVIDENT	1.844	1.761	1.217	0.083	0.627	0.586	0.142	0.928
KWS MARVEL	1.786	1.592	1.161	0.194	0.625	0.464	0.418	0.929
FRENETIC	1.859	1.664	1.185	0.195	0.674	0.512	0.381	0.936
REGINA	2.548	2.061	1.312	0.487	1.236	0.797	0.611	0.940
SOLEHIO	1.652	1.425	1.038	0.227	0.614	0.411	0.552	0.942
SEMNAL	2.198	1.892	1.299	0.306	0.899	0.628	0.487	0.944
ASPEKT	1.793	1.552	1.059	0.241	0.734	0.522	0.462	0.944
LITERA	2.133	1.997	1.437	0.136	0.696	0.585	0.232	0.957
TOMCAT	1.5	1.341	0.967	0.159	0.533	0.388	0.410	0.964
LEONIDUS	2.736	2.371	1.639	0.365	1.097	0.755	0.483	0.970
SIMNIC 1619	2.638	2.113	1.335	0.525	1.303	0.802	0.655	0.970
VOINIC	2.209	1.678	1.098	0.531	1.111	0.596	0.891	0.973
ANGELICA	1.642	1.405	0.995	0.237	0.647	0.415	0.571	0.988
GERRY	1.979	1.751	1.172	0.228	0.807	0.578	0.394	1.002
URSITA	1.641	1.358	0.946	0.283	0.695	0.411	0.689	1.002
BIHARIA	2.733	2.533	1.744	0.2	0.989	0.780	0.256	1.012
TORONTO	1.714	1.416	0.855	0.298	0.859	0.552	0.540	1.016
PAJURA	2.051	2.04	1.44	0.011	0.611	0.585	0.019	1.026
KLIMA	2.934	2.356	1.517	0.578	1.417	0.817	0.707	1.027
CONSECVENT	1.731	1.524	0.986	0.207	0.745	0.522	0.397	1.031
KATARINA	2.529	2.235	1.436	0.294	1.093	0.749	0.393	1.067
BEZOSTAIA	1.283	1.217	0.792	0.066	0.491	0.393	0.168	1.081
FELIX	2.12	1.922	1.289	0.198	0.831	0.585	0.338	1.082
CHEVIGNON	1.888	1.65	1.045	0.238	0.843	0.550	0.433	1.100
ALAHAMBRA	2.155	1.795	1.16	0.36	0.995	0.555	0.649	1.144
GATINEL	2.269	1.871	1.077	0.398	1.192	0.664	0.599	1.196
IS AGILIS	3.283	2.659	1.547	0.624	1.736	0.832	0.750	1.337
AVERAGE								0.773

In contrast, the correlation between the initial water quantity and water loss after 24 hours is a strongly positive correlation (r=0.216) (>P 1%). Thus, a variety with a high initial quantity has an equally high water loss. At the opposite pole, the Izvor, Carom, Tarroca and Izalco varieties were differentiated, which, although they presented high initial quantities, had low water losses after 24 hours (Figure 2).

The variability of the characters was quite pronounced. The limits of variation were as follows: for spike length between 7.1 cm for the Musik variety and 12.8 cm for the Yhalamus variety; for the number of spikelets/spike from 14.5 for the SY Olen variety to 26.8 for the Tomcat variety; for spike density from 15.66 for the Promitor variety to 26.17 for the Cordial variety; in the number of grains/spike from 28 grains/sp. in Emisar to 74 grains/spike in Tarroca; in the weight of grains/spike from 1.14 g/spike in Emisar to 3.10 g/spike in Tarroca; in the mass of 1000 grains from 29.83 g in the Kapitol variety to 53.56 g in the Ingenio variety and in the harvest index of the spike from 0.41 in Emisar to 1.27 in the KWS Peplum variety.

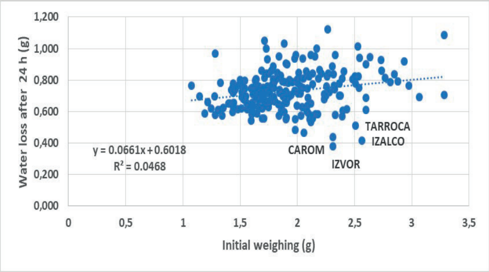

Figure 2. Relationship between initial weight and water loss after 24 hours

In general, long spikes are lax. The correlation, although strong (r=-0.233) (>P 1%) is negative in the sense that varieties that presented long spikes recorded low yields. The only variety that stood out as having high production – 9220 kg/ha at a spike length of approximately 10 cm was Lazuli. The variability of spike length explains 5% of the variability of production for the studied variety, in terms of the coefficient of determination (Figure 3).

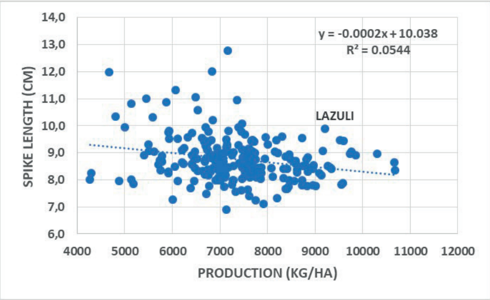

Figure 3. Production - ear length relationship

The correlation between production and the harvest index of the ears is a strong correlation (r=0.208) (>P 1%) (Figure 4).

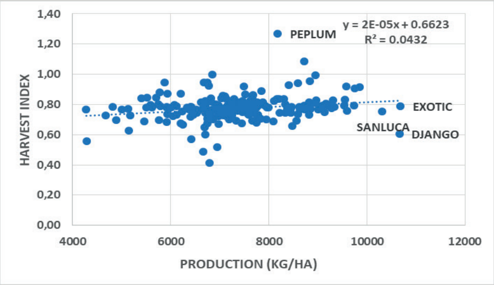

Figure 4. Production - harvest index relationship

CONCLUSIONS

The results showed that the dynamics of water loss differed greatly between varieties, the coefficient of variability being quite high (18.1%) but still average.

The highest initial water content was recorded by the varieties Exsal, Lemmy, Agilis, Klima, Tika Taka, Unic, all above the average values (1.930 g).

In the first 4 hours, on average, the lowest water losses were suffered by the varieties Pajura, Ekonom, Izvor, Tarroca, Papillon, Thalamus, Dacic, Evident, Litera, Biharia, Bezostaia, Foxil Gruia, Capo and Nikifor, all with values below 0.3 g. Most of these varieties are Romanian varieties, much better adapted to the conditions in the area.

Regarding water loss after another 20 hours, on average, the Izvor varieties (confirmed result), Izalco and Carom stood out, with very low losses, below 0.5 g.

The results are confirmed by the fact that the Carom wheat variety, the newest creation of the University of Craiova, was obtained through the improvement process under the conditions of Caracal, where the testing was done. Also, the correlation between the initial amount of water and the water loss after 24 hours, which is a strongly positive correlation, highlighted as deviations the Izvor, Carom, Tarroca and Izalco varieties, which, although they presented high initial amounts, had low water losses after 24 hours.

In addition, based on the results, the varieties Absint, Tarroca, Solveig, Atuan, Pibrac, Cazimir, Mobile, Novic and Palmeo stood out with water losses in 24 hours above the average of all varieties reduced by 25%.

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RESEARCH ON THE ADAPTATION OF TRITICALE VARIETIES TO DIFFERENT FERTILIZATION SYSTEMS IN THE CONTEXT OF CLIMATE CHANGE IN CENTRAL MOLDOVA

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Abstract

This paper presents the results obtained at A.R.D.S. Secuieni regarding the adaptability of the triticale species, under different fertilization systems, in the context of climate change and has as a main purpose to evaluate the efficiency of the cultivation of this species under increasingly variable pedoclimatic conditions. Ten triticale genotypes were studied in two systems under fertilized and unfertilized cropping regimes during 2020/2023. The average yields realized during the analyzed period ranged from 4224 kg/ha to 6503 kg/ha. The most productive variety was Zaraza, in the fertilized system. The fertilized system yielded a 2.3% higher protein content than the unfertilized system (average of 15.5% in the fertilized system and 13.2% in the unfertilized system), which shows the importance of fertilization in increasing the nutritive value of triticale. The Zaraza variety had a maximum protein content of 18%. The unfertilized system's thousand kernel weight is higher than the fertilized system's. Based on the results obtained, the non-fertilized variants had equal or even higher yields and thousand kernel weight than the fertilized variants, but the average protein percentage was higher in the fertilized variants.

***Key words**: fertilization, production, protein, triticale, variet.*

INTRODUCTION

Triticale (×*Triticosecale* Wittmack.) is a crop that was created by humans around 1875 by crossing rye (*Secale cereale* L.) and wheat (*Triticum durum* L. or *Triticum aestivum* L.). The purpose of growing triticale was to create a species that could combine the quality and grain yield potential of wheat with the resistance to disease and drought tolerance of rye (Jańczak-Pieniążek, 2023). Developing crop genotypes that meet specific food and industrial needs is the main purpose of plant breeding (Stoyanov, 2023).

This species has high yield potential, good grain quality for fodder use and high resistance to abiotic and biotic stresses (Jańczak-Pieniążek, 2023). Triticale is a good drought-tolerant crop (Stoyanov, 2022).

Interest in cultivating triticale is increasing due to its high yield potential, suitable agronomic properties and high nutritional value of the grain. This species is mainly cultivated for fodder purposes, but several studies suggest its potential for human consumption, including the production of bread (Jańczak-Pieniążek, & Kaszuba, 2024).

Triticale's characteristics include high productivity with low initial investment, better adaptation to moist, acidic and alkaline soils with nutrient deficiencies compared to other cereals, good grain quality and high protein content (Woldeyohannes et al., 2019, Cionca et al., 2024).

A balanced mineral nutrition is essential for grain yield and quality. Soil type, climate and other agro-ecological factors influence mineral nutrition (Rajičić et al., 2023). An important role for high yields in triticale crop is occupied by nitrogen fertilization (Alenicheva et al., 2023). One of the essential minerals for plants is nitrogen (N). It is the main component of protein (Wang et al., 2024).

The world's constantly growing population requires an increase in agricultural production. This is supported by agro-chemical products, such as fertilizers and pesticides, but the harmful effect on the environment has to be taken into account. Therefore, non-chemical and environmentally friendly strategies are increasingly supported in recent times (Jastrzębska et al., 2023). It is widely grown in a large number of countries, with Poland, Belarus, Germany, France, Germany and Spain among the top producers (Jańczak-Pieniążek, 2024).

Climate change has had a significant impact on the temperature and precipitation regime in the Moldovan region in recent years. This has a direct impact on agriculture and consequently on crop productivity. Under these circumstances, breeding triticale varieties that are able to adapt and produce high yields is essential for maintaining and improving agricultural profitability (Isticioaia et al., 2020). Considering the need to reduce these harmful effects on the environment, the aim of this research is to compare the impact of genotypes and different fertilization systems in the context of thermal and hydric stress in central Moldova on the quantitative and qualitative parameters of triticale species.

MATERIALS AND METHODS

The research was carried out at A.R.D.S. Secuieni to evaluate the influence of genotypes and cropping system on production and quality of ×*Triticosecale* Wittmack.

The experiments were implemented at A.R.D.S. Secuieni, located at 26°5' east longitude and 46°5' north latitude, in the period 2021/2023. The soil on which the studies were realised is a typical cambic chernozemic (Pintilie et al., 2023). It is characterized by a medium supply of nitrogen (IN: 2.7), a very good supply of phosphorus (P_{AL}: 106.7 mg/kg soil) and potassium (K_{AL}: 208.3 mg/kg soil), a good supply of magnesium (assimilable magnesium: 47.2 mg/kg soil), sulfur (S: 20.5 mg/kg soil), iron (Fe: 50.7 mg/kg soil) and copper (Cu: 3.1 mg/kg soil), a medium supply of manganese (Mn: 18.4 mg/kg soil), zinc (Zn: 2.5 mg/kg soil), organic carbon (Organic C: 1.62%) and humus (2.79%) and a poor supply of boron (B: 19.5 µg/kg soil) and molybdenum (Mo: 6.2 mg/kg), with a neutral pH of 7.20. The soil is non saline (CE in H_20: 144.8 µS/cm; 50.7 mg/100 g soil).

In the fertlized system, 150 kg/ha NPK 18:46:0 was applied in the autumn and 200 kg/ha NH_4NO_3 was applied in the spring at straw elongation. No plant protection products were applied during the growing season.

According to Figure 1, the deviation from the multi-annual average ranged between +0.4°C in May and +5°C in February. Over the three years of the study, an increase in monthly average temperatures of 2.4°C above the multi-year average was noticed. The month with the lowest temperatures was January, with an average over the three years of study of 0.4˚C. The highest temperatures were recorded in August, with an average of 23.3˚C in the experimental years 2020-2023.

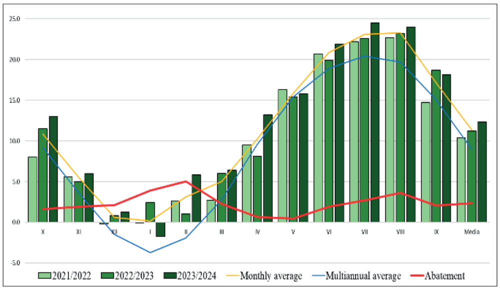

Figure 1. Thermal regime and multiannual average (1962/2023) recorded at A.R.D.S. Secuieni in the period 2020-2023

From the climatic data in Figure 2, the most significant precipitation deficit was recorded in July, -38.7 mm. The average monthly precipitation was higher than the multiannual average in September, with an increase of 17.4 mm.

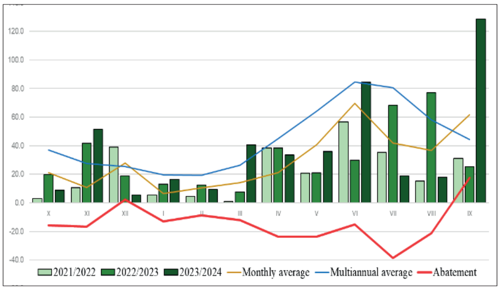

Figure 2. Pluviometric regime and multiannual average (1962/2023) recorded at A.R.D.S. Secuieni in the period 2020-2023

From the above, the three experimental years were characterized as dry and very hot. The average temperature deviation in the three experimental years was +2.4˚C. Regarding precipitation, the sum of deviations was -169.8 mm. The climatic conditions recorded in the period 2020-2023 were atypical for field crops, with the technological work of establishing and maintaining the crop being realized in a dry and poorly prepared soil, due to the lack of precipitations and high temperatures recorded in the last period.

RESULTS AND DISCUSSIONS

In the studies on triticale production, the average values obtained in 2021 in the fertilized system ranged from 4086 kg/ha (Negoiu) to 7118 kg/ha (Zaraza). For statistical calculation, the control considered was the average yield of the experiment, 5945 kg/ha (Figure 3).

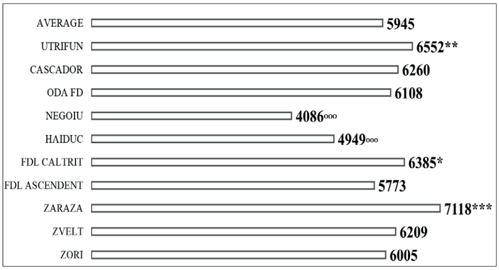

Figure 3. Average triticale yields (kg/ha) obtained in the fertilised system in 2021

In 2021, the yield increases statistically interpreted as highly significant were obtained for the variants sown with the variety Zaraza, with a difference from the control of +1173 kg/ha. Distinctly significant was the variety seeded with Utrifun, with a difference from the control of +607 kg/ha. Significant was the FDL Caltrit variety with a yield of 6385 kg/ha. The varieties Cascador (6260 kg/ha), Oda FD (6108 kg/ha), FDL Ascendent (5773 kg/ha), Zvelt (6209 kg/ha) and Zori (6005 kg/ha) had no statistically significant differences from the control. Results statistically interpreted as negative highly significant were obtained for the variants seeded with the varieties Negoiu (4086 kg/ha) and Haiduc (4949 kg/ha) (Figure 3).

In the next year, the experience average was 245 kg/ha less than the 2021 average. The control variant considered this year was the same as last year, the average of the variants of 5700 kg/ha. Three of the variants sown in the fertilized system in 2022 had significant yield increases, namely the variants seeded with Utrifun, FDL Ascendent and Zvelt. Five of the variants had no statistically significant differences from the control, these being the variants sown with Cascador (5608 kg/ha), Oda FD (5455 kg/ha), FDL Caltrit (5668 kg/ha), Zaraza (5685 kg/ha) and Zori (5760 kg/ha). The yield increases interpreted as negatively distinctly significant were obtained for the variants sown with the variety Negoiu and Haiduc. The variety seeded with Negoiu had a difference from the control of -908 kg/ha, while the variety seeded with Haiduc had a difference from the control of -820 kg/ha (Figure 4).

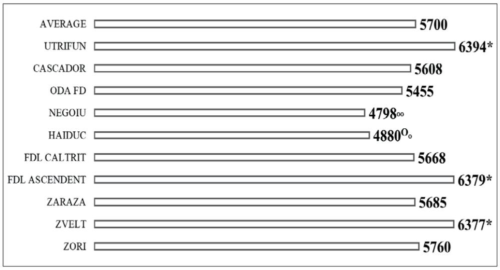

Figure 4. Average triticale yields (kg/ha) obtained in the fertilised system in 2022

In the third experimental year, hydric and thermal stress influenced negatively the yield of triticale grains. The average of the experiment was 4765 kg/ha, the lowest average of all the studied years. This was also the control experiment. Very significant yield increases were recorded in the variants sown with the varieties Utrifun (5527 kg/ha), Cascador (5589 kg/ha), Oda FD (5366 kg/ha) and Zaraza (5366 kg/ha). The highest difference from the control was +824 kg/ha in the variety sown with Cascador. There were no statistically significant differences from the control in the variants seeded with FDL Caltrit and FDL Ascendent, with yields of 4769 kg/ha and 4784 kg/ha. The variants sown with Negoiu (3786 kg/ha), Haiduc (3863 kg/ha), Zvelt (4258 kg/ha) and Zori (4340 kg/ha) were interpreted as highly significant negatives (Figure 5).

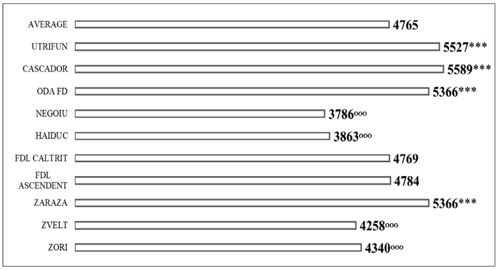

Figure 5. Average triticale yields (kg/ha) obtained in the fertilised system in 2023

Analyzing Figure 6, we can see that the average of the unfertilized experiment is slightly lower than the average of the fertilized experiment in 2021, respectively 5774 kg/ha. This yield was also considered as the experiment control. This year, yields ranged between 3809 kg/ha (Negoiu) and 6844 kg/ha (Utrifun). The yield increases were interpreted as very significant in the variants sown with Utrifun and Zvelt, which had yields of 6844 kg/ha and 6648 kg/ha. Distinctly significant is the yield increase obtained for the Cascador variety. The variant seeded with Zaraza variety, which recorded a yield of 6303 kg/ha is interpreted statistically significant. The varieties Oda FD (5313 kg/ha), FDL Caltrit (5956 kg/ha), FDL Ascendent (6189 kg/ha) and Zori (5591 kg/ha) have no statistically significant differences from the control. The only variants that were interpreted as negative highly significant are the ones sown with Negoiu (3809 kg/ha) and Haiduc (4638 kg/ha).

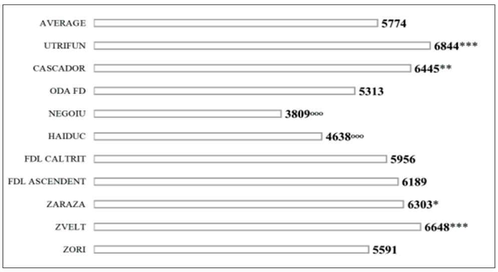

Figure 6. Average triticale yields (kg/ha) obtained in the unfertilised system in 2021

In the next crop year, 2022, the experience average, which was the control, was 6014 kg/ha. The only variety that recorded significant yield increases was Utrifun with a yield of 6919 kg/ha, and a difference from the control of +905 kg/ha. Eight of the ten varieties tested did not register statistically significant differences, including Cascador (6050 kg/ha), Oda FD (6081 kg/ha), Haiduc (5425 kg/ha), FDL Caltrit (6498 kg/ha), FDL Ascendent (6355 kg/ha), Zaraza (5821 kg/ha), Zvelt (5659 kg/ha) and Zori (6379 kg/ha). The Negoiu variety, with a yield of 4955 kg/ha, and a difference from the control of -1059 kg/ha, is interpreted statistically as a negative distinctly significant yield increase (Figure 7).

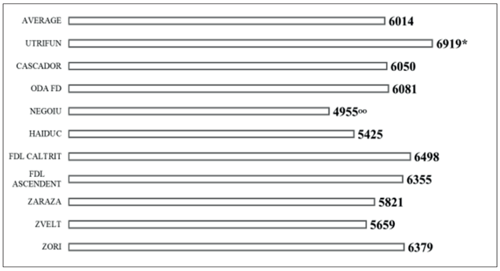

Figure 7. Average triticale yields (kg/ha) obtained in the unfertilised system in 2022

In 2023, the yields in the unfertilized system were higher than in the fertilized system, due to thermal and hydric stress, averaging 587 kg/ha higher than in the variants on which chemical fertilizers were applied. Very significant yield increases were recorded in the variants seeded with Utrifun, with a difference of +395 kg/ha compared to the control; FDL Caltrit, with an increase of 559 kg/ha compared to the control; FDL Ascendent, with an increase of 636 kg/ha compared to the control and Zaraza, which recorded the highest yield in the unfertilized system, 6089 kg/ha, with a difference of +737 kg/ha compared to the control. Significant yield increases were registered in the Zori variety with a yield of 5545 kg/ha. The variants seeded with Cascador (5468 kg/ha) and Zvelt (5222 kg/ha) have no statistically significant differences from the control. The variants sown with the variety Oda FD (4827 kg/ha), Negoiu (4287 kg/ha) and Haiduc (4437 kg/ha) were interpreted negative highly significant. Negoiu variety recorded the lowest yield (4287 kg/ha) in this experimental year in the unfertilized system (Figure 8).

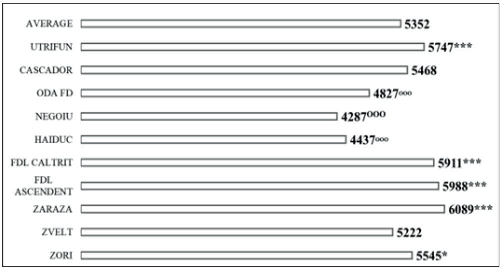

Figure 8. Average triticale yields (kg/ha) obtained in the unfertilised system in 2023

During the research on triticale yield, the average values obtained in the fertilised system ranged from 4224 kg/ha (Negoiu) to 6158 kg/ha (Utrifun). The average yield in the fertilized system during the study years is 5470 kg/ha. It can be noticed that the variants sown with Utrifun and Zaraza varieties have very significant yield increases, with a difference from the control of +688 kg/ha, respectively +587 kg/ha. With an average yield of 5819 kg/ha, the Cascador variant is interpreted as distinctly significant. The varieties Oda FD (5643 kg/ha), FDL Caltrit (5607 kg/ha), FDL Ascendent (5645 kg/ha), Zvelt (5615 kg/ha) and Zaraza (6057 kg/ha) have no statistically significant differences from the control. The average yield increases obtained in the variants seeded with the varieties Negoiu (4224 kg/ha) and Haiduc (4564 kg/ha) were recorded as negative highly significant (Figure 9).

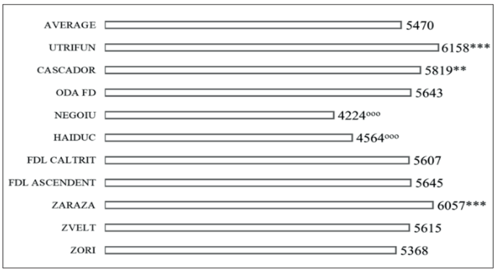

Figure 9. Average triticale yields (kg/ha) obtained in the fertilised system in 2021-2023

Comparing the average yields in the fertilized and unfertilized systems, we can see that for nine out of the ten varieties tested yields were slightly higher in the unfertilized system, proving that a variety's productivity is closely related to its adaptability to environmental conditions, even though fertilization can increase the yield. The average of the unfertilized system over the three years was 5713 kg/ha, 243 kg/ha higher than the average of the variants on which chemical fertilizers were applied. As in the fertilized system, the variants sown with the Utrifun variety stood out with a high yield and a very significant yield increase. The difference from the average is +790 kg/ha. Distinctly significant are the variants sown with FDL Caltrit (6121 kg/ha) and FDL Ascendent (6177 kg/ha). With a yield of 6071 kg/ha, the variants seeded with the variety Zaraza recorded a significant yield increase. Without statistically significant differences were the varieties sown with Cascador (5988 kg/ha), Oda FD (5407 kg/ha), Zvelt (5843 kg/ha) and Zori (5838 kg/ha). Negative highly significant statistical interpretation is obtained for the variants seeded with the varieties Negoiu and Haiduc, with a difference from the control of -1363 kg/ha, respectively -880 kg/ha (Figure 10).

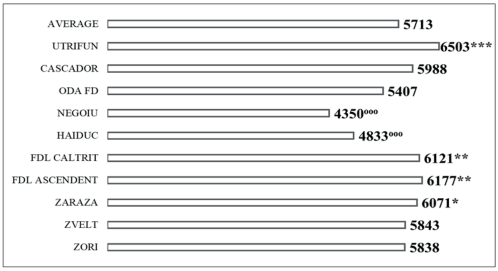

Figure 10. Average triticale yields (kg/ha) obtained in the unfertilised system in 2021-2023

Analyzing the protein content of triticale grains, the average of the fertilized system in the three years studied is 15.5%, 2.3% higher than the average of the unfertilized system. In 2022, the average protein content of triticale grains was the highest in the three experimental years. A difference between the mean of the fertilized system and the mean of the unfertilized system can be observed, thus observing the positive influence of chemical fertilizer application to triticale crop on protein content. Zaraza variety stands out with the highest protein content of 18% in fertilized system in 2022 and 2023. However, in 2021 it recorded an average protein content of only 14%. The variety that recorded the lowest values was FDL Caltrit (13.8%). In the unfertilized system, the average protein content of triticale grains was 13.2%. The experimental year 2021 recorded the highest percentages, with a maximum of 17% (Zvelt, Haiduc), and a minimum of 15.2% (Zaraza). The next year recorded the lowest values, ranging from 10.1% (Utrifun) to 11.6% (Cascador). In 2023, the Zvelt soil stood out with the highest recorded value of 13.4%, while the FDL varieties Caltrit and Haiduc recorded the lowest values of 11.8% (Figure 11).

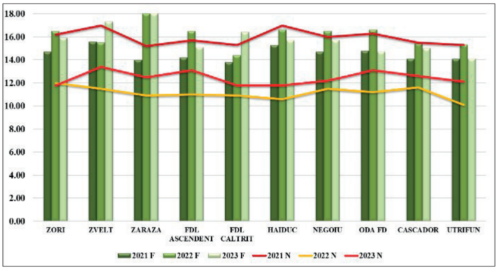

Figure 11. Protein content (%) of triticale varieties from A.R.D.S. Secuieni in the period 2021/2023 in the fertilised and unfertilised system

According to figure 12, an increase in the thousand-kernel weight (TKW) can be observed in the unfertilized system in almost all the varieties studied. The difference between the average of the variants on which fertilizer was applied and those on which it was not is 4.2 g. In the fertilized system in 2021, the highest weight was obtained on the variants sown with the variety Negoiu, of 53.47 g. The lowest value was recorded in the variety Cascador (36.38 g). The highest TKW values were obtained in this crop year from the three years studied (2021). In the following year, values ranged from 32.52 g (Zori) to 41.58 g (Zvelt). The values recorded in the year 2023 in the fertilized system had a maximum of 42.40 g (Zaraza) and a minimum of 30.20 g (FDL Ascendent). Analyzing the unfertilized experiments, we can observe that the TKW values are higher than the values of the experiments on which chemical fertilizers were applied. The variants seeded with Haiduc variety recorded the maximum of 50.17 g in 2022. The minimum was obtained by variety Oda FD (35.6 g).

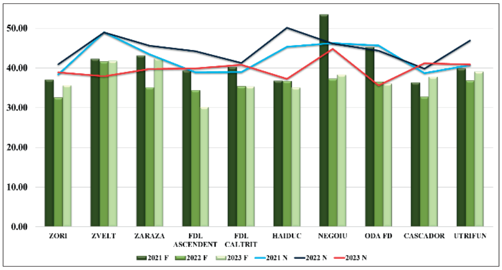

Figure 12. Thousand-kernel weight (grams) recorded for triticale varieties within A.R.D.S. Secuieni in the period 2021/2023 in the fertilised and unfertilised system

CONCLUSIONS

Yields ranged from 3786 kg/ha for variants sown with the variety Negoiu in 2023, fertilized, to 7118 kg/ha for variants seeded with the variety Zaraza in 2021, fertilized. In the three years of the study, the Negoiu variety was the least productive, and the Utrifun variety had the highest yields.

Looking at the average of the experiments, we can say that the yields in the unfertilized system were higher than in the fertilized system. The average of the most productive variety, Utrifun, on the non-fertilized variants was 6503 kg/ha, 345 kg/ha more than the average of the fertilized system. The lowest average values recorded were 4350 kg/ha in the unfertilized system (Negoiu) and 4224 kg/ha in the fertilized system, also for the variants sown with Negoiu.

The fertilization with chemical fertilizers positively influenced the quality of triticale grains. The average of the fertilized system is 15.5% and that of the unfertilized system is only 13.2%. The highest value in the fertilized system was 18% (Zaraza), the same value in the unfertilized system was 17% (Zvelt, Haiduc).

While in general, fertilization leads to increased grain mass, in this study, the thousand kernel weight of the unfertilized system is higher than that of the fertilized system. The highest values were recorded for the Negoiu variety, 53.47 g on the fertilized variants and for the Haiduc variety, 50.17 g on the unfertilized variants. FDL Ascendent obtained the lowest values in the fertilized system (30.20 g) and Oda FD in the unfertilized system (35.60 g). The difference between the two systems average was 4.4 g.

AKNOWLEDGEMENTS

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PARASITIC NEMATOFAUNA IN PEA CROPS (*Pisum sativum* L.) UNDER THE IMPACT OF THE UNSTABLE ENVIRONMENTAL CONDITIONS OF THE REPUBLIC OF MOLDOVA

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Abstract

The results of the present research estimated the efficiency of the phytosanitary helmintological control in peas grown in open field and elucidated the helmintological parasitic impact, establishing the range of the invasive nematofauna, the frequency and the abundance of the associations. As a result of the phytosanitary research and the analysis of the helmintological impact on pea plants, it was found that the parasitic nematode complexes consisted of 14 species included in 4 families: Aphelenchidae, Hoplolaimidae, Tylenchidae, Heteroderidae, of the order Tylenchida, class Nematoda, distributed according to the investigated areas and classified according to the spectrum of trophic specialization in 5 groups. A larger number of species was detected in the Center area (14 species), as compared to the North area (8 species). It was found that the values varied from 7 to 30%, the damage being caused mainly by invasive associations of parasite nematodes of the genera: Ditylenchus, Pratylenchus, Heterodera, Meloidogyne, Helicotylenchus, Aphelenchus. The results of the phytosanitary monitoring contributed to elucidating the degree of nematological damage and brought new evidence in favor of applying sustainable pest control measures suitable for fabaceae agrocenoses.

***Key words**: nematodes, helmintological control, pea sectors, parasitic impact, trophic specialization.*

INTRODUCTION

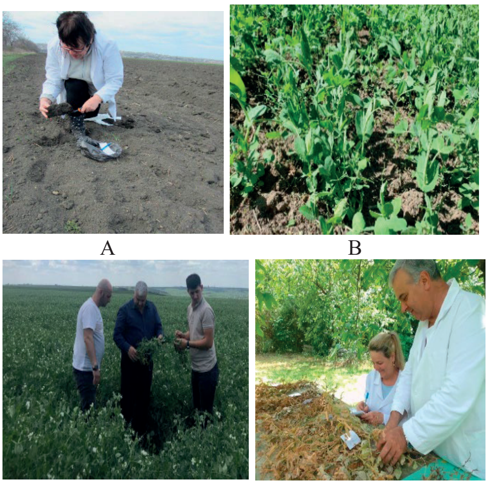
Cultivating peas for grains, seed material and fodder solves three strategic problems: increasing seed production, vegetable protein production and improving soil fertility, being 2-3 times richer in vegetable protein and having the advantage that it can be sown and harvested extra early (Starodub & Gheorghiev, 2013; Starodub et al., 2015). They are sown as a first priority under the environmental conditions of the Republic of Moldova, when the soil has sufficient moisture and allows sowing, usually in early March, but delayed sowing leads to significant production losses. Pea plantations for grain and fodder represent 8% of the arable land in the Republic of Moldova. They are annually invaded by over 40 harmful species, which are also associated with invasive nematode complexes that trigger helminthiasis, which motivates specific annual monitoring to control the populations and their parasitic impact in the cultivation process of this crop (Nesterov, 1997; Poiras et al., 2016; Iurcu-Străistaru et al., 2019). Annually, according to the new institutional project 2024-2027, we have conducted nematological research, including biodiversity estimates, morpho-ecological assessments and analyses of the structure of complexes of parasitic fauna, affecting various field crops, including peas.

In the Republic of Moldova, helminthological research and monitoring of the biodiversity and structure of parasitic nematode complexes, detected in the agrocenoses of field crops cultivated in state-owned and private enterprises, were initiated and conducted by specialists in phytohelminthology and parasitology, renowned at the national level and recognized internationally, such as: Petru Nesterov (1979-1980); Maria Melnic, Dumitru Erhan, Ștefan Rusu (2014); Larisa Poirasa (1996-2016).

Currently, taking into account the specifics of areas with contrasting and unstable climates, we aim at investigating invasive nematode complexes from the families *Heteroderidae Hoplolaimidae, Tylenchidae*, affecting pea plants (*Pisum sativum* L.), in the context of applying new modern cultivation technologies, comparatively by areas, production associations, plantations and purpose of production. Based on current events and the estimated purpose, the goal of our research has been to establish the structure and diversity of invasive helminth species from the families: *Heteroderidae*, *Hoplolaimidae* and *Tylenchidae,* associated with parasitic forms affecting pea (*Pisum sativum* L.), determining the parasitic impact by comparative analyses of frequency and abundance indices, in various production and experimental sectors, in the dynamics of phenological stages.

MATERIALS AND METHODS

The proposed program included specific, helminthological investigations in field crop plantations (legumes, winter cereals, technical crops), where samples of soil and plants affected by helminths were taken and surveys and periodic records were made comparatively across various investigated sectors, mainly in the Central area as compared with the Northern area.

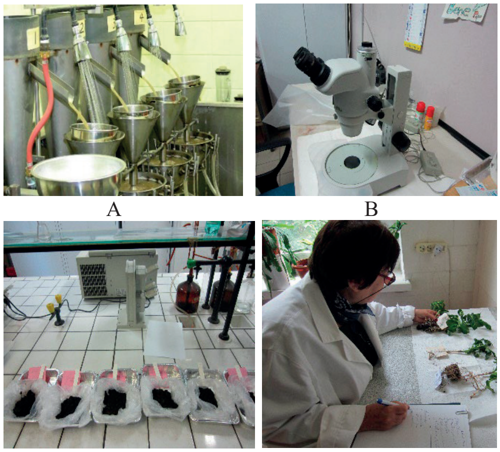


A B C D

Figure 1. Surveys with soil sampling and analysis of plants collected during the growing season (Criuleni district, March-June 2023-2024). A - Taking samples before sowing; B - Pea - vegetative stage; C - Field surveys - flowering stage;
D - Plant analysis - ripening stage

To establish the areas affected by helminthiasis, over 6 field trips, in 10 localities, 12 sectors, from 4 administrative districts in the Central area, were undertaken, where over 200 samples of soil and diseased plant organs were collected and analyzed (Figure 1).

Subsequently, indices of parasitic impact were determined, such as: species diversity, population density, frequency of attack (F%), intensity of attack (I%), estimation scales were used following phytosanitary control, field and laboratory records were made.



A B C D

Figure 2. Logistics and helminthological extraction analyses and microscopic establishment of morpho-taxonomic indices of nematodes extracted from analyzed samples: A - instalația Baermann funnel;
B - binocularul fotonic; C - Analysis of soil samples;
D - visual analysis of samples

The collected samples were subsequently analyzed in the laboratory, according to the classical and new methods, with some modifications depending on the specifics of the nematode genera. Through specific techniques, nematodes were extracted from the soil and affected organs, applying the classic flotation-decantation method (Baermann funnel), with some methodological adjustments, with subsequent fixation in 4% formalin, at temperature of 60°C, for morpho-taxonomic studies, reflected in figure 2. The fixed material was analyzed under a microscope, establishing the population size, trophic groups, as well as other parasitic indices in the investigated soil and plants. Methodological and logistical support was offered by the Parasitology and Helminthology Laboratory, with methodology according to Парамонов, 1970; Nesterov P., 1978-1988; Melnic M., Erhan D., Rusu Ș., 2014.; Poiras L. et al., 2016; Iurcu-Straistaru E. et al., 2018-2023. The taxonomic units were determined using nematological identification guidelines according to: Decker, 1972; Baldwin & Moens, 2004; Perry & Moens, 2006; Decramer & Hunt, 2006; Siddiqi, 2010; Sasanelli et al., 2018.

Based on phytopathological research, the frequency of attack **(F %)** and the intensity of development **(I%)** were established. The frequency of attack **(F%)** represents the relative proportion of attacked plants or plant organs **(n)** compared to the total number of analyzed plants or organs **(N)** and is calculated using a standard formula. The intensity of attack **(I %)** represents the extent, as a percentage, to which a plant or organ is affected. The following formula is used to calculate the intensity of attack:

$$I\% = \frac{(n_1 . 1) + (n_2 . 2) + (n_3 . 3) + (n_4 . 4)}{N \times 4},$$

where: *n1, n2, n3, n4* - the number of plants or organs attacked at the respective grade; N - the total number of plants or organs examined; 4 - the maximum grade on the scale used (Îndrumări metodice pentru testarea produselor chimice şi biologice de protecţie a plantelor în Republica Moldova, 2012).

RESULTS AND DISCUSSIONS

Biological control of pea plantations affected by helminths was verified in various private households and production associations in the North and Center of the Republic of Moldova and in the administrative districts of Criuleni, Anenii Noi, Nisporeni, located in the South-East and South-West, where winter cereals, corn, sunflower, fodder crops, etc. are also intensively cultivated. Peas are cultivated on large and significant areas, in the sectors of the Central area, but in the North the areas are limited, instead, soybean plantations for grains and fodder predominate. For this reason, our research was focused mainly on sectors of the Central area, making detailed surveys and analyses.

Phytosanitary surveys were conducted monthly, in the period March-June, 2023-2024, which were characterized by relatively warm and humid weather conditions, which had an advantageous influence on the development of plants including but also of parasitic nematode complexes. The average frequency and intensity of helminthic attack were established, reaching values up to 25-30%. Abundant precipitation and higher temperatures have determined the severity of helminthiasis in association with various disastrous diseases such as: ascochytosis, fusariosis, which appeared early on the roots and stems during the vegetative phase. Simultaneously, on the preventively marked samples the presence of helminthic diseases was detected, the values of intensity and frequency of attack were analyzed comparatively, and the structure and predominance of certain genera and species with specific pathogenic invasive impact were highlighted. In all the investigated sectors, helminthic diseases and infestations were reported through local outbreaks, with retarded plants, in association with wilted, partially necrotic ones in variegated colors, presented in Figure 3.

Figure 3. Plants affected by mixed helminthic diseases associated with downy mildew and powdery mildew, on all plant organs, in the pod formation phase; A - plant samples affected by multiple pests productive sector May-June 2024 Criuleni district; B - sector affected locally by helminths in a productive field; C - plants affected severely by multiple pests; D - visual analysis of plants affected by helminths on roots; experimental sector of "Al. Ciubotaru" NBGI, 2024

The periods with abundant rainfall and unstable daytime temperatures, in the months of April-June, caused an increase in the damage severity and in the numerical density of the pest populations, reaching average values in the Central area - 15-250 individuals/100 g/soil. It was found that, in all the investigated sectors, there were extensive nematode complexes that produce helminthiasis, in certain sectors affecting the plants severely. This is due to the adaptive resistance capacities in the soil. Meanwhile, nematodes form specialized associations in these crops, caused by non-compliance with maintenance techniques. Such conditions favored the increase in the degree of parasitic helminthological impact, affecting plants in the stages of leaf formation, until the formation of pods. During this period, the reported helminth symptoms remain clearly accentuated and extensive, the visual symptoms including chlorosis, a reduced number of mature leaves, dwarf leaves, roots seriously affected by necrosis and rot caused by helminths. The species of pathogenic fungi in the soil estimated in ascending comparative values, by phenological stages, vary from 5% in the stage of 2-5 leaves up to 30% in the fruit development stage, presented in Table 1.

Table 1. The estimation of phytosanitary indices by comparative values of helminthic parasitic impact in various phenological stages of pea plants grown in the districts of the Central area of RM (March-June, 2024)

Central area and pilot districts	26 March, stage of 2-5 developed leaves			25 April, stage of 10-15 developed leaves			27 May, flowering stage			26 June, fruit development - ripening stage		
	D. (100 g soil)	F (%)	I (%)	D (100 g soil)	F (%)	I (%)	D (100 g soil)	F (%)	I (%)	D (100 g soil)	F (%)	I (%)
Nisporeni district, Vărzărești village	5-17	7-10	5-7	17-23	9-12	7-10	25-35	12-15	8-11	180	25-28	22-25
Criuleni district, Pașcani village	7-15	5-9	3-5	14-18	8-9	6-8	20-28	10-13	9-12	250	27-30	24-27
Criuleni district, Bălăbănești village	8-16	8-12	5-7	10-15	7-10	6-9	28-33	13-18	10-13	220	25-28	22-25
Anenii Noi district, Mereni village	6-14	5-8	2-5	13-16	10-14	8-12	23-30	9-14	7-9	190	20-25	18-22

Legend: D. – density of nematode populations per 100 grams of soil; F (%) – frequency of attack; I (%) – intensity of attack.

A C

Figure 4. development stages, poly-invasively affected by helminthiasis and other diseases associated with specific pathogens, May - June 2024, Nisporeni district. A - productive sector affected by helminths; B - pea plantations in the budding-fruit

Such investigations were carried out for the first time in the Republic of Moldova on legume crops, including peas, as this research was conducted in another technologically intensive format and in the new private system, related to establishing the diversity and structure of nematode complexes, aspects of parasitic impact, trophic specialization, strategically adapted specialization, with the estimation of the most invasive forms in this early-spring crop. The investigations carried out consistently reflect in the seasonal dynamics, during growth stages, of the parasitic helminthic impact associated with specific diseases affecting peas, such as: downy mildew - pathogen *Peronospora pisi* Syd., powdery mildew - pathogen *Erysiphe pisi* Diet., blight - pathogen *Ascochyta pisi* Sacc., wilt - pathogen *Fusarium* spp., triggered by some problems in the cultivation techniques and environmental factors. Pea plantations affected by the above-mentioned diseases, in the budding-fruit development stages, are presented in Figure 4 A, B. These analyses and results are useful for adjusting short and long-term forecasts and are useful for applying an efficient integrated protection management for peas, namely by reducing the damage caused by pest invasions, the degree of infestation, the resistance in critical phases etc., aimed at production associations, experimental research sectors and private households. In this regard, by analyzing the samples collected from soil and affected plants in the laboratory, the structure and taxonomic diversity of invasive nematode complexes was determined in the dynamics of plant growth and development stages, in the presence of helminth associations accumulated in the soil planted with peas, the growing season March-June 2024, presented in Table 2. The analyses presented in Table 2 estimate the structure, diversity and density of the nematode populations, established at genus level, and the level of parasitic impact and infestation, up to 3 points (250 individuals per 100 grams/soil), detected from the first stages of plant development and increasing each month, according to the biological development cycle. In the detected complexes, the individuals belonged to 8 genera, namely: *Ditylenchus* spp., *Helycotilechus* spp., *Tylenchus* spp., *Fylenchus* spp, *Nothotylenchus* spp., *Heterodera* spp., of the order Tylenchida, with diverse trophic specialization (endo- ecto- and semi-endoparasites), migratory and sedentary.

Table 2. Diversity and structure of nematode complexes, accumulated in the soil planted with peas (March-June, 2024) in the dynamics of phenological stages

Detected genera	Trophic group	Date of collecting and analyzing soil samples (100 g) from the plant's rhizosphere			
		25.03	25.04	25.05	30.06
1. *Ditylenchus* sp.,	Migratory endoparasites	+	+	++	++
2. *Prathylenchus* sp.,		-	+	++	+++
3. *Helycotilechus* sp.,	Ectoparasites	-	+	+	++
4. *Tylenchus* sp.,		-	+	+	++
5. *Fylenchus* sp.,		-	+	++	++
6. *Nothotylenchus* sp.		-		+	
7. *Meloidogyne* sp.,	Cyst and gall-inducing semi-endoparasites	+	+	++	+++
8. *Heterodera* sp.,		-	+	++	++

Legend: - no individuals; + from 30 to 100 individuals are present; ++ from 100 to 200 individuals are present; +++ over 200 individuals are present.

All the estimated genera were found practically in all the investigated sectors, but the most invasive, with high frequency and abundance, were the forms belonging to the genera *Meloidogyne* and *Prathylenchus*, from the families Tylenchidae and Heteroderidae. These highlighted complexes represent a significant danger to pea plants, because they are associated with various species of insects and diseases, and are also vectors of microbial infections in the soil.

CONCLUSIONS

As a result of the phytosanitary and helminthological monitoring, carried out in 4 production associations from 4 districts, mainly belonging to the Central area of the Republic of Moldova, in plantations of peas grown for grains (*Pisum sativum* L.), the diversity and structure of invasive nematode complexes in the dynamics of phenological stages were established for the first time, detecting the presence of the most dangerous forms associated in 8 genera, such as: *Ditylenchus* spp., *Pratylenchus* spp., *Meloidogyne* spp., *Helycotilechus* spp., *Tylenchus* spp., *Fylenchus* spp., *Nothotylenchus* spp., *Heterodera* spp., which belong to the families Heteroderydae, Aphelenchidae Tylenchidae, Hoplolaimidae, Neotylenchidae, order Tylenchida, with diverse trophic specialization (endo- ecto- and semi-endoparasites), migratory and sedentary.

The more abundant and frequent pests were the forms of migratory endoparasitic nematodes of the Tylenchidae family and the gall-inducing forms belonging to the Heteroderidae family, class Nematoda.

After establishing the indices of the population density and frequency and intensity of attack, it was found that the nematodes that appear early, at the seed germination stage, the complexes belonging to the genera *Pratylenchus* and *Meloidogyne*, detected in the rhizosphere of young pea plants, had an abundance of 100 to

250 individuals (adult forms, eggs, invasive larvae).

Helminthic diseases were reported as local outbreaks, with a frequency of 5 to 30% with an average attack intensity of 7-25%. These highlighted complexes represent a significant danger to pea plants, because they are also associated with species of lepidopteran and coleopteran insects in the soil, various diseases, causing unfavorable conditions for obtaining biological and agricultural harvests.

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THE IMPACT OF NITROGEN FERTILIZATION ON THE PRODUCTIVITY OF SOYBEAN CROPS IN SOUTHERN AND CENTRAL ROMANIA IN THE CONTEXT OF CLIMATE CHANGE

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Abstract

This study investigates the effects of nitrogen fertilization on the productivity of soybean crops in Southern and Central Romania under changing climatic conditions. The research focuses on two soybean varieties, Amiata and Orakel, cultivated in the regions of Caracal and Crişcior, which differ in soil characteristics and environmental stressors. Field experiments were conducted using three nitrogen application rates, and key agronomic traits such as plant height, number of pods, grain yield, and seed quality parameters were measured. Although statistical analysis did not reveal significant differences across treatments, strong positive correlations between nitrogen levels and several performance indicators were observed. The findings underscore the importance of adaptive nitrogen management strategies for maintaining soybean productivity and resilience in climate-sensitive agricultural regions.

***Key words**: climate, nitrogen, productivity, soybean, sustainability.*

INTRODUCTION

The global challenges posed by climate change have significantly impacted agricultural systems worldwide, compelling researchers and practitioners to seek innovative solutions to ensure food security and sustainability. Soybean (*Glycine max* L.) is a crop of paramount economic and nutritional importance, particularly in regions like Southern and Central Romania, where agricultural productivity is increasingly constrained by environmental variability. This study investigates the critical role of nitrogen fertilization in enhancing soybean productivity, focusing on the interplay between nitrogen application and climatic stressors such as drought, temperature fluctuations, and irregular precipitation patterns. Understanding these interactions is essential for developing resilient and adaptive agricultural practices tailored to climate-sensitive regions.

Nitrogen is a vital nutrient that profoundly influences plant growth and productivity (Zhang et al., 2021). In the context of soybean cultivation, nitrogen management is particularly complex due to the crop's ability to fix atmospheric nitrogen through symbiotic relationships with rhizobia. However, under stressful climatic conditions, the efficiency of biological nitrogen fixation may decline, necessitating the application of nitrogen fertilizers to maintain yield levels (López-Bellido et al., 2020). This dual reliance on biological and synthetic nitrogen sources underscores the need for precise management strategies that optimize nitrogen use while minimizing environmental impacts.

Recent studies highlight the exacerbating effects of climate change on nitrogen dynamics in agricultural soils. For instance, drought conditions can impair nitrogen availability and uptake, while excessive rainfall can lead to nitrogen leaching and loss (Schlesinger & Bernhardt, 2020). Such challenges are particularly pronounced in Romania, where climate projections indicate increased variability in precipitation and rising temperatures (Ion et al., 2021). These climatic shifts demand a reevaluation of fertilization practices to ensure both productivity and environmental sustainability.

Field-based research provides invaluable insights into the complex interactions between nitrogen application, crop physiology, and environmental factors. Experiments conducted in similar agro-climatic regions have

demonstrated that tailored nitrogen management can significantly enhance soybean yields, even under adverse conditions (Singh et al., 2021). For example, split application of nitrogen fertilizers has been shown to improve nitrogen use efficiency and reduce losses, thereby supporting sustainable production systems (Chen et al., 2020). Furthermore, integrating organic amendments such as compost or biochar with synthetic fertilizers can enhance soil health and mitigate the ecological footprint of fertilization practices (Gao et al., 2021).
The environmental implications of nitrogen fertilization extend beyond soil health to encompass broader ecological concerns. Excessive nitrogen application is a major contributor to greenhouse gas emissions, particularly nitrous oxide, a potent greenhouse gas (Davidson et al., 2021). Additionally, nitrogen runoff into water bodies can lead to eutrophication, disrupting aquatic ecosystems (Vitousek et al., 2021). These challenges necessitate the adoption of integrated nutrient management approaches that balance productivity goals with ecological stewardship.
In this context, the concept of sustainable intensification emerges as a guiding principle for modern agriculture. Sustainable intensification seeks to increase crop yields on existing farmland while minimizing negative environmental impacts (Pretty et al., 2020). For soybean cultivation, this approach involves leveraging advanced technologies such as precision agriculture to optimize nitrogen application rates and timings. Moreover, fostering farmer awareness and capacity building is crucial for the successful implementation of these practices (Tilman et al., 2020).
The findings of this study contribute to the growing body of evidence supporting the strategic management of nitrogen in soybean cultivation. By elucidating the interactions between nitrogen application, climatic factors, and crop performance, this research aims to empower farmers with actionable insights to navigate the challenges of a changing climate. Furthermore, the study underscores the importance of integrating agronomic, environmental, and socio-economic considerations into fertilization strategies, paving the way for resilient and sustainable agricultural systems in Romania and beyond.

MATERIALS AND METHODS

Experimental period and locations

In the spring of 2024, both locations Caracal and Crişcior experienced difficult climate conditions. As shown in Figure 1, the temperatures during the growing season were higher than usual, which resulted in delays in soybean planting. Consequently, the soybean growing season in these areas was restricted to a narrower timeframe, specifically between the second half of April and the second half of September. During this period, the precipitation levels, presented in Figure 2, were also insufficient to support optimal soybean growth.

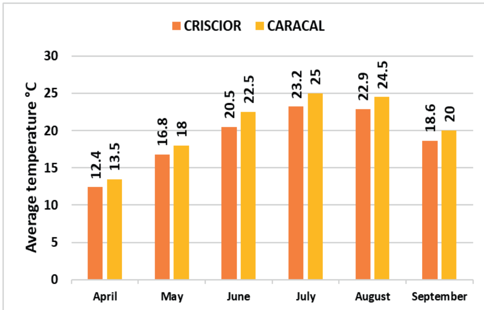

Figure 1. The average temperature during the growing season of 2024 (https://freemeteo.com/frame.asp?ifrid=236788_cazare-ranca.ro&pid)

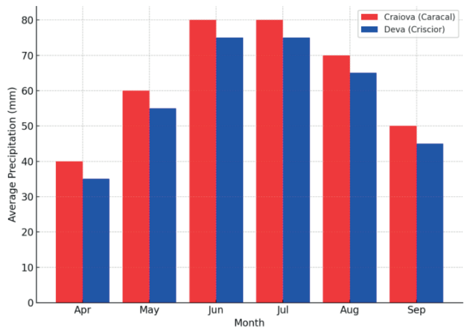

Figure 2. Average precipitation during the growing season of 2024 (https://www.meteoblue.com/ro/%20vreme/historyclimate/weatherarchive/romania)

As shown in Table 1, the soil in Caracal presents significantly more favorable conditions for soybean cultivation than that in Crişcior. With a slightly alkaline pH, higher levels of nitrogen, phosphorus, and potassium, and its classify-cation as a clay-illuvial Chernozem, the Caracal soil offers optimal conditions for soybean growth, supporting good root development and nutrient uptake. In contrast, the podzolic soil in Crişcior, characterized by higher acidity and lower nutrient availability, is less suitable for soybeans unless properly amended.

Table 1. Soil Properties in CARACAL and CRIŞCIOR – 2024

Location	CARACAL	CRIŞCIOR
Year	2024	2024
pH (water)	7.6	6.2
Humus (%)	2,58	2.51
Total N (%)	0,189	0.15
P (ppm)	93	33
K (ppm)	235	166
Soil type	Clay-illuvial Chernozem	Podzolic

This limited growing window impacted the development of the crop, shortening the overall vegetation period and potentially affecting yields due to the reduced time available for the plants to grow and mature.

Soybean varieties

This study examines the performance of different soybean varieties in various regions of Romania. Below, we present the four varieties selected for our experiment.

The **Orakel PZO** soybean variety is a moderate type with strong production potential, classified in maturity group 00, similar to wheat in its growth cycle.

Yields typically range from 3000-3500 kg/ha but can exceed 4500 kg/ha in favorable conditions(https://ig-pflanzenzucht.de/wp-content/plugins/igp-filter/tpl/saatgut-generate-pdf.php).

For sowing, it is recommended to follow the optimal period based on local conditions, use a seeding density of 60-65 viable seeds per square meter, and adjust the seeding depth according to soil type. Orakel PZO is well-suited for farmers seeking reliable, high-quality harvests in a variety of climates.

Amiata is an early soybean variety in maturity group 00, highly adaptable to various cultivation conditions and known for its outstanding traits.

It has indeterminate growth, early flowering and harvesting, medium to tall plants, and violet-colored flowers.

The beans weigh 170-210 grams, with high fat content (over 20%) and protein levels (over 40%), along with an open hilum (https://binealegibineculegi.ro/pdf/?id=9296)

Amiata demonstrates strong vigor, resistance to shattering and lodging, and high yield potential. It is resilient, showing good tolerance to diseases and challenging environmental conditions.

The ideal sowing period is between late April and mid-May, with recommended row spacing of 15-24 cm. Thanks to its early maturity, harvesting can be done in a short timeframe.

Used techniques

This study examines the impact of climate variations and fertilizer application on soybean cultivation in southern and central Romania. Experimental plots were established in these regions, applying different fertilizer types and quantities.

Plant development and yields were monitored throughout the growing season, with data analyzed to identify trends and correlations between climate, fertilization, and crop performance.

Key measurements included plant height, the height of the first pod, and the number of pods per plant. Additionally, analyses were conducted to determine moisture levels, hectoliter weight, and oil and protein content, providing a comprehensive evaluation of crop quality and productivity.

The results offer valuable insights for improving agricultural techniques and optimizing soybean production in Romania.

The collected data and graphical representtations, created using Excel from the Office 365 suite, serve as valuable tools for enhancing agricultural practices and optimizing future production.

The correlations were also performed using Excel, while the ANOVA analysis was conducted with the help of a Python program, which can be accessed at the following link-: https://replit.com/@raulcristian19/ThreeWayAnova.

RESULTS AND DISCUSSIONS

Results

Soybeans, as leguminous plants, has a unique ability to fix atmospheric nitrogen through symbiosis with rhizobia bacteria. This allows them to partially meet their nitrogen needs. However, soil nitrogen levels still influence their growth and yield, requiring careful management to maintain a balance between nitrogen fixation and soil nutrient availability. (Rymuza et al., 2020).

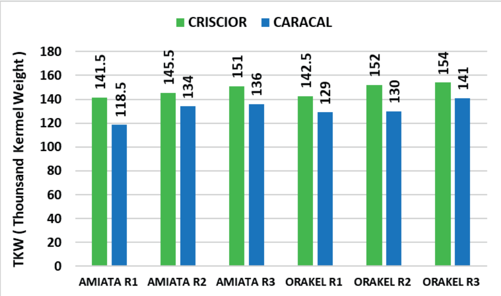

Figure 3. Thousand kernel weight across all two regions

In Figure 3 we illustrated the effects of nitrogen application rates on the Thousand Kernel Weight of two soybean varieties, Amiata and Orakel. The nitrogen rates were applied in three quantities: R1 (75 kg N/ha), R2 (125 kg N/ha), and R3 (150 kg N/ha). The Thousand Kernel Weight was higher for Amiata compared to Orakel in all nitrogen treatments. At the lowest nitrogen level (R1), Amiata reached a TKW of 141.5 g, while Orakel had 118.5 g. This pattern continued at higher nitrogen doses. At R2 (125 kg N/ha), Amiata's TKW increased to 145.5 g, while Orakel reached 134 g. At the highest nitrogen level (R3, 150 kg N/ha), Amiata had its highest TKW of 151 g, and Orakel reached 136 g.

Table 2. Thousand kernel weight correlation

AMIATA				
Region	**Repetiton**	**TKW**	**TKW Correlation**	**N applied**
Criscior	R1	141.5	0.960768923	75
	R2	145.5		125
	R3	151		150
Caracal	R1	118.5	0.973920338	75
	R2	134		125
	R3	136		150
ORAKEL				
Region	**Repetiton**	**TKW**	**TKW Correlation**	**N applied**
Criscior	R1	142.5	0.985586763	75
	R2	152		125
	R3	154		150
Caracal	R1	129	0.802955069	75
	R2	130		125
	R3	141		150

Even though these differences were not statistically significant according to the ANOVA analysis shown in Table 3 ($p > 0.05$), the results still show a clear trend as it can be seen in Table 3.

Table 3. Thousand kernel weight ANOVA analysis

Variation source	Sum of squares	Degrees of freedom	F - statistic	p-value
Intercept	140185.6	1	20.176	0.00002
Genotype	2668.05	1	0.384	0.53677432
Region	684.45	1	0.0985	0.75423022
Nitrogen	1861.26	2	0.1339	0.87478547
Genotype x Region x Nitrogen	1804.51	2	0.1299	0.87835657
Residual	750385	108		

The correlation between TKW and nitrogen amount presented in Table 2 was very strong, with $r > 0.96$ for Amiata and $r = 0.80–0.98$ for Orakel as it can be seen in Table 2. This suggests a positive relationship between nitrogen application and kernel weight for both genotypes. Amiata showed a steady and stronger increase in TKW as nitrogen levels rose, suggesting it is better at using nitrogen efficiently.

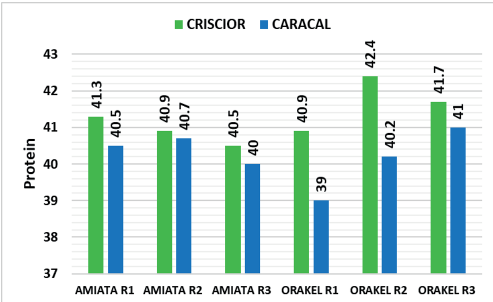

Figure 4. Protein content of soybeans across all two regions

The analysis of protein content presented in Figure 4, for the Amiata and Orakel varieties grown in Crişcior and Caracal, reveals distinct regional and varietal trends. In Crişcior, Amiata shows consistent protein levels across replicates (R1: 41.3%, R2: 40.9%, R3: 40.5%), suggesting high stability. Orakel, although more variable, reaches the highest protein value of 42.4% in R2, indicating strong potential under favorable conditions.

In Caracal, both varieties show lower protein content compared to Crişcior. Amiata's values slightly decline (R1: 40.5%, R2: 40.7%, R3: 40%), maintaining overall stability. Orakel displays greater sensitivity, with R1 dropping to 39%, R2 at 40.2%, and R3 improving to 41%. This suggests that Crişcior offers a more favorable environment, especially for Orakel, which is more responsive to regional conditions.

Table 4. Protein correlation

AMIATA				
Region	**Repetiton**	**Protein**	**Protein correlation**	**N applied**
Criscior	R1	41.3	-0.981980506	75
	R2	40.9		125
	R3	40.5		150
Caracal	R1	40.5	-0.544704779	75
	R2	40.7		125
	R3	40		150
ORAKEL				
Region	**Repetiton**	**Protein**	**Protein correlation**	**N applied**
Criscior	R1	40.9	0.68324347	75
	R2	42.4		125
	R3	41.7		150
Caracal	R1	39	0.997176465	75
	R2	40.2		125
	R3	41		150

Despite these trends, the ANOVA analysis presented in Table 5 revealed no statistically significant differences in protein content by genotype ($F = 0.008$, $p = 0.9288$), region ($F = 0.019$, $p = 0.8894$), nitrogen levels ($F = 0.0014$, $p = 0.9986$), or their interaction ($F = 0.032$, $p = 0.9968$).

Table 5. Protein ANOVA analysis

Variation source	Sum of squares	Degrees of freedom	F - statistic	p-value
Intercept	16321.6	1	32.3491	0.0000001
Genotype	4.05	1	0.008	0.9288
Region	9.8	1	0.0194	0.8894
Nitrogen	1.4	2	0.0014	0.9986
Genotype x Region x Nitrogen	3.2167	2	0.0032	0.9968
Residual	54490.9	108		

However, correlation analyses between nitrogen application and protein content from Table 4 revealed notable patterns. For Amiata, strong negative correlations were observed in both Crişcior ($r = –0.98$) and Caracal ($r = –0.54$), suggesting a decrease in protein with increasing nitrogen. Orakel showed a positive correlation in Crişcior ($r = 0.68$) but a strong negative one in Caracal ($r = –0.99$), indicating location-dependent variability.

The data presented in Figure 5 illustrate variations in oil content for the two soybean genotypes, Amiata and Orakel, cultivated in the Crişcior and Caracal regions. In Crişcior, Amiata displayed moderate variability among replicates: R1 recorded 20.8%, R2 reached 21.5%, and R3 showed the lowest value at 20.3%. Orakel exhibited both higher oil content and slightly greater variability. Orakel R1 achieved the highest oil value in Crişcior (21.9%), R2 dropped to 20.6%, and R3 balanced between the two extremes at 21.4%. In Caracal, both genotypes showed improved oil content compared to Crişcior. Amiata recorded consistent gains, with R1 and R2 at 21.4%, and R3 reaching 21.8%. Orakel also improved, with R1 at 22%, R2 at 22.1% (the highest value across all samples), and R3 at 21.6%.

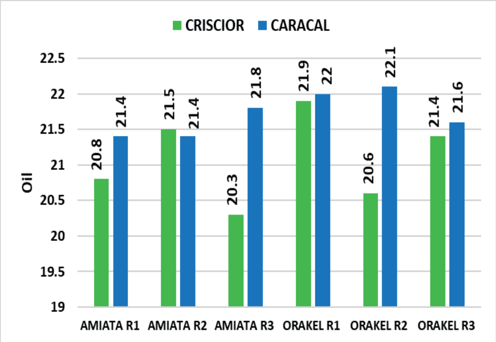

Figure 5. Oil content of soybeans across all two regions

However, correlation analyses between nitrogen levels and oil content suggest nuanced patterns. For Amiata, a slightly negative correlation was observed in Criscior ($r = –0.253$), while a positive correlation emerged in Caracal ($r = 0.756$). Orakel showed negative correlations in both regions: $r = –0.549$ in Criscior and $r = –0.616$ in Caracal (Table 6).

Table 6. Oil content correlation

AMIATA				
Region	**Repetiton**	**Oil**	**Oil Correlation**	**N applied**
Criscior	R1	20.8	-0.2353158	75
	R2	21.5		125
	R3	20.3		150
Caracal	R1	21.4	0.755928946	75
	R2	21.4		125
	R3	21.8		150
ORAKEL				
Region	**Repetiton**	**Oil**	**Oil Correlation**	**N applied**
Criscior	R1	21.9	-0.549085619	75
	R2	20.6		125
	R3	21.4		150
Caracal	R1	22	-0.618589574	75
	R2	22.1		125
	R3	21.6		150

Despite these observed trends in Table 7, the ANOVA analysis revealed no statistically significant differences in oil content based on genotype, location, or nitrogen level. All p-values were well above the 0.05 threshold: genotype (F = 0.0149, p = 0.9029), location (F = 0.0149, p = 0.9029), nitrogen (F = 0.0051, p = 0.9949), and the three-way interaction (F = 0.0381, p = 0.9626).

Table 7. Oil content ANOVA analysis

Variation source	Sum of squares	Degrees of freedom	F - statistic	p-value
Intercept	4579.6	1	27.9143	0.0000006
Genotype	2.45	1	0.0149	0.903
Region	2.45	1	0.0149	0.903
Nitrogen	16.667	2	0.0051	0.9949
Genotype x Region x Nitrogen	12.5167	2	0.0381	0.9626
Residual	17718.4	108		

Figure 6 presents the test weight data for the soybean genotypes Amiata and Orakel cultivated in the regions of Crişcior and Caracal, offering insights into varietal performance and environmental responses. In Criscior, Amiata exhibited moderate variability across its three replicates, with R1 recording 69.12 kg/hL, R2 peaking at 71.88 kg/hL, and R3 reaching the lowest value at 68.96 kg/hL. Orakel demonstrated slightly less results and generally higher test weight values: 70.24 kg/hL (R1), 71.55 kg/hL (R2), and 70.55 kg/hL (R3), consistently outperforming Amiata in this region.

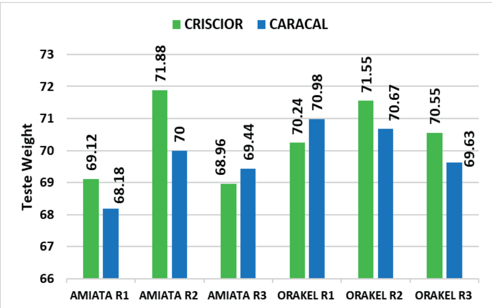

Figure 6. Test weight of soybeans across all two regions

In Caracal, both genotypes recorded slightly lower test weights compared to Crişcior. Amiata R1 measured 68.18 kg/hL, R2 achieved 70.00 kg/hL, and R3 reached 69.44 kg/hL, reflecting a minor decline. Similarly, Orakel maintained its superior performance, with R1 at 70.98 kg/hL - the highest among all Caracal replicates - R2 at 70.67 kg/hL, and R3 at 69.63 kg/hL

Table 8. Test weight correlation

AMIATA				
Region	**Repetiton**	**Test weight**	**Test weight Correlation**	**N applied**
Criscior	R1	69.12	0.140903638	75
	R2	71.88		125
	R3	68.96		150
Caracal	R1	68.18	0.802955069	75
	R2	70		125
	R3	69.44		150
ORAKEL				
Region	**Repetiton**	**Test weight**	**Test weight Correlation**	**N applied**
Criscior	R1	70.24	0.406399402	75
	R2	71.55		125
	R3	70.55		150
Caracal	R1	70.98	-0.881042761	75
	R2	70.67		125
	R3	69.63		150

Statistical analysis through ANOVA revealed no significant effects of genotype as it can be senn o Table 9, region, or nitrogen level on test weight. The p-values were well above the 0.05 threshold: genotype (F = 0.0023, p = 0.9622), region (F = 0.0203, p = 0.887), nitrogen (F = 0.0054, p = 0.9946), and the interaction between all three factors (F = 0.0018, p = 0.9982). These results confirm the absence of statistically significant influences on test weight from the experimental factors.

Table 9. Test weight ANOVA analysis

Variation source	Sum of squares	Degrees of freedom	F - statistic	p-value
Intercept	46512.4	1	25.8812	0.0000002
Genotype	4.05	1	0.0023	0.9622
Region	36.45	1	0.0203	0.887
Nitrogen	194.667	2	0.0054	0.9946
Genotype x Region x Nitrogen	6.4667	2	0.0018	0.9982
Residual	194092.5	108		

Yet, correlation analysis from Table 8 suggests subtle trends. For Amiata, a weak positive correlation was observed in Crişcior (r = 0.14), and a moderate one in Caracal (r = 0.80), implying a potential increase in test weight with higher nitrogen doses, particularly under Caracal conditions.

Orakel, on the other hand, displayed a positive correlation in Crişcior (r = 0.40) and a strong negative correlation in Caracal (r = –0.88), indicating contrasting responses depending on location. These findings suggest a possible interaction between nitrogen, genotype, and environment, though not supported by statistically significant differences.

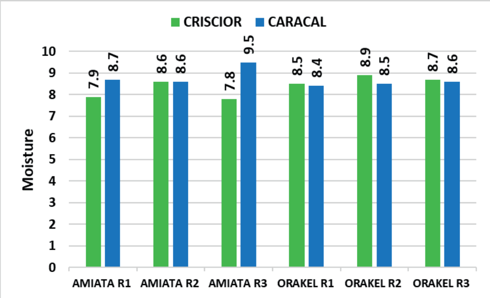

Figure 7. Moisture of soybeans across all two regions

Figure 7 presents the analysis of moisture content in the soybean varieties Amiata and Orakel, cultivated in the regions of Crişcior and Caracal. Although the descriptive statistics reveal noticeable variation between the two varieties and across the two locations, inferential statistical analysis indicates that these differences are not statistically significant.

Table 10. Moisture correlation

AMIATA				
Region	Repetiton	Moisture	Moisture Correlation	N applied
Criscior	R1	7.9	0.97622104	75
	R2	8.6		125
	R3	8.7		150
Caracal	R1	8.7	0.685679631	75
	R2	8.6		125
	R3	9.5		150
ORAKEL				
Region	Repetiton	Moisture	Moisture Correlation	N applied
Criscior	R1	8.5	0.654653671	75
	R2	8.9		125
	R3	8.7		150
Caracal	R1	8.4	0.981980506	75
	R2	8.5		125
	R3	8.6		150

The ANOVA results from Table 11 reveal no statistically significant differences in moisture content between genotypes, locations, nitrogen levels, or their interactions. The p-values were high across all factors: genotype ($F = 0.067$, $p = 0.9622$), location ($F = 0.186$, $p = 0.887$), nitrogen treatment ($F = 0.0645$, $p = 0.9946$), and the three-way interaction ($F = 0.0645$, $p = 0.9982$). These results suggest that none of the examined variables had a statistically measurable impact on moisture content at the chosen significance level.

However, correlation analysis from Table 10 between applied nitrogen levels and moisture content reveals consistent positive trends, indicating a possible physiological influence not captured through ANOVA. For the Amiata genotype, very strong and positive correlations were observed: $r = 0.9762$ in Criscior and $r = 0.6857$ in Caracal. Similarly, Orakel showed strong correlations: $r = 0.6547$ in Criscior and $r = 0.9820$ in Caracal. These values suggest that increased nitrogen application tends to elevate moisture content, likely due to enhanced water retention in the seeds.

Table 11. Moisture ANOVA analysis

Variation source	Sum of squares	Degrees of freedom	F - statistic	p-value
Intercept	774.4	1	28.8192	0.0000002
Genotype	1.8	1	0.067	0.9622
Region	5	1	0.186	0.887
Nitrogen	3.4667	2	0.645	0.9946
Genotype x Region x Nitrogen	3.4667	2	0.0645	0.9982
Residual	2902.7	108		

Although not statistically significant in a strict sense, the consistently strong correlation coefficients support the hypothesis of a physiological response of soybean seeds to nitrogen fertilization in terms of moisture accumulation. This trend is especially evident for both genotypes in Caracal, where environmental conditions may further accentuate nitrogen's effect on seed hydration.

The bar chart from Figure 8 illustrates the average plant height of two soybean varieties, Amiata and Orakel, across three replications (R1-R3) in the regions of Crişcior and Caracal. Overall, higher plant heights are recorded in Caracal compared to Crişcior, suggesting that the environmental and soil conditions in Caracal may have a more favorable effect on the vegetative growth of soybean plants.

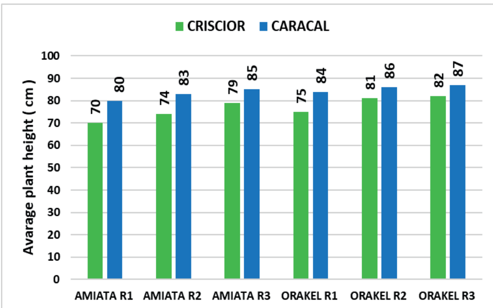

Figure 8. The average height of soybean plants across all two regions

The bar chart from Figure 8 illustrates the average plant height of two soybean varieties, Amiata and Orakel, across three replications (R1-R3) in the regions of Crişcior and Caracal. Overall, higher plant heights are recorded in Caracal compared to Crişcior, suggesting that the environmental and soil conditions in Caracal may have a more favorable effect on the vegetative growth of soybean plants.

The analysis of variance (ANOVA) for average plant height, Table12, revealed no statistically significant effects among the tested factors. The p-values were notably high across all variables: region ($F = 0.166$, $p = 0.6843$), fertilization type ($F = 0.021$, $p = 0.9788$), genotype ($F = 0.0258$, $p = 0.8727$), and the three-way interaction ($F = 0.0026$, $p = 0.9974$).

These results indicate that neither genotype, region, nor nitrogen application levels had a statistically significant impact on plant height.

Table 12. The average height of soybean plants ANOVA analysis

Variation source	Sum of squares	Degrees of freedom	F - statistic	p-value
Intercept	64000	1	21.7026	0.0000009
Genotype	76.05	1	0.0258	0.8727
Region	490.05	1	0.1662	0.6843
Nitrogen	126.6667	2	0.0215	0.9788
Genotype x Region x Nitrogen	15.5167	2	0.0026	0.9974
Residual	318487.4	108		

Table 13. The average height of soybean plants correlation

AMIATA				
Region	Repetiton	Avarage height	Avarage height Correlation	N applied
Criscior	R1	70	0.967867837	75
	R2	74		125
	R3	79		150
Caracal	R1	80	0.997176465	75
	R2	83		125
	R3	85		150
ORAKEL				
Region	Repetiton	Avarage height	Avarage height Correlation	N applied
Criscior	R1	75	0.97986371	75
	R2	81		125
	R3	82		150
Caracal	R1	84	1	75
	R2	86		125
	R3	87		150

Despite the lack of statistical significance in the ANOVA results, the correlation analysis presented in Table 13 revealed strong and consistent positive relationships between nitrogen application and plant height. For the Amiata genotype, correlation coefficients were $r = 0.9678$ in Crişcior and $r = 0.9971$ in Caracal, suggesting a near-linear increase in height with increasing nitrogen levels. Similarly, the Orakel genotype showed extremely strong correlations: $r = 0.9798$ in Criscior and $r = 1.0000$ in Caracal.

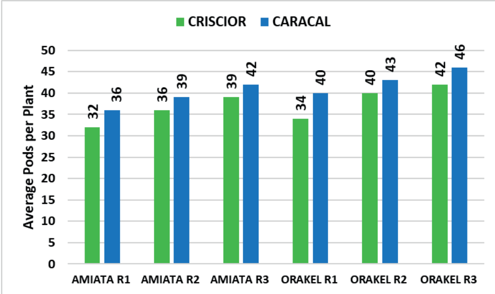

Figure 9. Avarage pods per plant across all two regions

The average number of pods per plant for the soybean varieties Amiata and Orakel, across three replications (R1-R3), is presented in the Figure 9. A consistent increase in pod number is observed in Caracal compared to Crişcior, indicating that the environmental and soil conditions in Caracal are more conducive to the reproductive performance of soybean plants.

Table14. Average pods per plant correlation

AMIATA				
Region	Repetiton	Avarage pods	Avarage pods Correlation	N applied
Criscior	R1	32	0.994191626	75
	R2	36		125
	R3	39		150
Caracal	R1	36	0.981980506	75
	R2	39		125
	R3	42		150
ORAKEL				
Region	Repetiton	Avarage pods	Avarage pods Correlation	N applied
Criscior	R1	34	0.995870595	75
	R2	40		125
	R3	42		150
Caracal	R1	40	0.981980506	75
	R2	43		125
	R3	46		150

Table 15. Average pods per plant ANOVA analysis

Variation source	Sum of squares	Degrees of freedom	F - statistic	p-value
Intercept	13032.1	1	22.8377	0.0000006
Genotype	84.05	1	0.1473	0.7019
Region	174.2	1	0.1526	0.8586
Nitrogen	72.2	2	0.1265	0.7228
Genotype x Region x Nitrogen	4.05	2	0.0035	0.9965
Residual	61629.1	108		

The analysis of variance (ANOVA) for the average number of pods per plant, as shown in Table 15, did not reveal statistically significant differences among the analyzed factors. High p-values were recorded for all variables: genotype (F = 0.147, p = 0.7019), region (F = 0.152, p = 0.8586), nitrogen application rate (F = 0.126, p = 0.7228), and the three-way interaction (F = 0.0035, p = 0.9965).

On the same time, correlation analysis between the average number of pods and the nitrogen application rate revealed a strong positive relationship in all cases as it can be seen in Table14. For the Amiata genotype, extremely high correlation coefficients were found: r = 0.994 in Crişcior and r = 0.981 in Caracal, suggesting a significant increase in pod production with higher nitrogen doses. Similarly, the Orakel genotype showed very high correlations as well: r = 0.995 in Crişcior and r = 0.981 in Caracal.

Despite the ANOVA results did not indicate statistical significance, the consistently strong positive correlations point to a clear physiological effect of nitrogen fertilization on pod development.

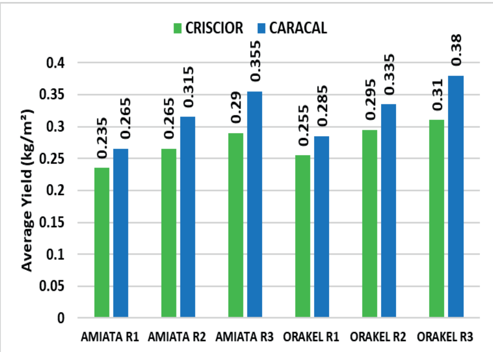

Figure 10. The average grain yield across all two regions

In the 2024 growing season, both Amiata and Orakel soybean varieties demonstrated significant yield improvements with increasing nitrogen application in the regions of Crişcior and Caracal, as shown in Figure 10. In Crişcior, Amiata's yield increased progressively with higher fertilizer rates, from 0.235 kg/m² at 75 kg/ha to 0.29 kg/m² at 150 kg/ha. Orakel outperformed Amiata, achieving 0.31 kg/m² at 150 kg/ha.

In Caracal, both varieties showed better yields compared to Criscior, with Amiata reaching up to 0.355 kg/m² at 150 kg/ha and Orakel achieving 0.38 kg/m² at the same rate.

Table 16. The average grain yield correlation

AMIATA				
Region	Repetiton	Grain yield	Grain yeald - Correlation	N applied
Criscior	R1	0.235	0.990536065	75
	R2	0.265		125
	R3	0.29		150
Caracal	R1	0.265	0.992064533	75
	R2	0.315		125
	R3	0.355		150
ORAKEL				
Region	Repetiton	Grain yield	Grain yeald - Correlation	N applied
Criscior	R1	0.255	0.997788423	75
	R2	0.295		125
	R3	0.31		150
Caracal	R1	0.285	0.987267385	75
	R2	0.335		125
	R3	0.38		150

Correlations between nitrogen application and bean yield were strong for both varieties and regions, indicating a consistent positive response to nitrogen fertilization. For Amiata, correlation coefficients were r = 0.9905 in Criscior and r = 0.9921 in Caracal. For Orakel, they were r = 0.9978 in Criscior and r = 0.9873 in Caracal (Table 16).

These strong correlations confirm that nitrogen fertilization is crucial for maximizing yield potential, with Orakel showing superior productivity in both regions.

In Crişcior, the average number of branches per plant varied for both the Amiata and Orakel varieties as it cand be seen in Figure 11. For Amiata, the number of branches increased from 2.3 for Amiata R1 (75 kg/ha) to 2.7 for Amiata R2 (125 kg/ha), reaching a peak of 3 branches per plant for Amiata R3 (150 kg/ha) as shown in Figure 11.

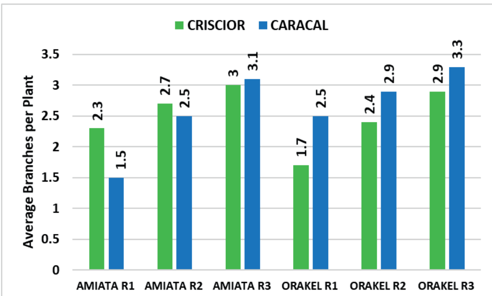

Figure11. The average number of branches per plant across all two regions

Similarly, in Orakel, the number of branches improved as the fertilizer rate increased, with Orakel R1 (75 kg/ha) producing 1.7 branches, Orakel R2 (125 kg/ha) producing 2.4 branches, and Orakel R3 (150 kg/ha) reaching 2.9 branches per plant (figure 11). Despite these increases, Orakel consistently had fewer branches compared to Amiata under the same conditions in Criscior.

In Caracal, both varieties exhibited distinct branching patterns. Amiata, for example, had fewer branches in Caracal compared to Crişcior, with Amiata R1 (75 kg/ha) producing just 1.5 branches per plant. However, as fertilizer application increased, branching improved, with Amiata R3 (150 kg/ha) reaching 3.1 branches per plant, slightly surpassing the maximum observed in Criscior. In contrast, Orakel performed better in Caracal than in Crişcior, with Orakel R1 (75 kg/ha) producing 2.5 branches, and Orakel R3 (150 kg/ha) achieving 3.3 branches per plant. This improvement highlights the beneficial environmental conditions in Caracal for Orakel.

Table 17. The average number of branches per plant correlation

AMIATA				
Region	**Repetiton**	**Branches**	**Branches Correlation**	**N applied**
Criscior	R1	2.3	0.994191626	75
	R2	2.7		125
	R3	3		150
Caracal	R1	1.5	0.998906107	75
	R2	2.5		125
	R3	3.1		150
ORAKEL				
Region	**Repetiton**	**Branches**	**Branches Correlation**	**N applied**
Criscior	R1	1.7	0.995566845	75
	R2	2.4		125
	R3	2.9		150
Caracal	R1	2.5	0.981980506	75
	R2	2.9		125
	R3	3.3		150

Table 18. The average number of branches per plant ANOVA analysis

Variation source	Sum of squares	Degrees of freedom	F - statistic	p-value
Intercept	19.6	1	10.341	0.0017
Genotype	4.05	1	2.1368	0.1467
Region	7.2	1	3.7987	0.0539
Nitrogen	14.8667	2	3.9218	0.0227
Genotype x Region x Nitrogen	2.2167	2	0.5848	0.559
Residual	204.7	108		

The ANOVA analysis presented in Table 18 confirmed that nitrogen application had a significant effect on the number of branches (F = 3.92, p = 0.0227), further supporting the positive response of branching to increased fertilizer levels. The region factor also approached significance (F = 3.798, p = 0.0539), indicating that environmental conditions could influence branching development. However, genotype (F = 2.136, p = 0.1467) and the triple interaction (F = 0.58, p = 0.559) did not significantly affect branching.

High correlations were observed in Table 17 between the number of branches and nitrogen application, with Amiata showing r = 0.9941 in Criscior and r = 0.9989 in Caracal. For Orakel, correlations were similarly high, with r = 0.9955 in Crişcior and r = 0.981 in Caracal, confirming that nitrogen fertilization significantly stimulates branching. These results underline the biological and statistical significance of nitrogen fertilization in enhancing plant development, especially in terms of branching.

DISCUSSIONS

This study builds upon previous research conducted in 2024 titled "Analyzing the Impact of Climate Variations and Fertilizer Application on Soybean Cultivation across Western, Southern, and Central Romania". (Jurcut et al., 2024) While the earlier work provided valuable insights into regional differences in soybean performance and highlighted the influence of environmental and fertilization factors, it lacked statistical analysis, limiting the interpretation of result significance.

The current study represents a continuation and deepening of that research, focusing specifically on the Southern and Central regions (Caracal and Crişcior) and incorporating a robust statistical framework. By introducing ANOVA and correlation analyses, the study not only confirms trends observed previously - such as improved productivity with nitrogen application and the influence of regional agro-environmental conditions - but also quantifies the strength of these relationships. Despite most results not reaching statistical significance, strong positive correlations were consistently found between nitrogen levels and key agronomic traits such as grain yield, plant height, number of pods, and branching.

These findings reinforce the importance of context-specific nitrogen management in soybean cultivation and provide a more rigorous foundation for optimizing fertilization strategies under the pressures of climate variability in Romania.

CONCLUSIONS

Nitrogen fertilization proved essential for enhancing soybean productivity, with all measured traits - plant height, number of pods, grain yield, and branching - showing strong positive correlations with increasing nitrogen levels, despite the lack of statistically significant differences in most ANOVA tests.

Statistical analysis using ANOVA did not indicate significant effects ($p > 0.05$) of nitrogen rate, genotype, or location on most traits, suggesting that high variability and environmental interactions may obscure measurable differences at conventional significance thresholds.

Correlation analysis revealed consistently strong and positive relationships between nitrogen application and key agronomic indicators, particularly in plant height ($r > 0.96$), pod number ($r > 0.98$), and grain yield ($r > 0.98$), supporting the physiological impact of nitrogen on soybean development.

The Amiata variety exhibited better nitrogen use efficiency, with greater increases in Thousand Kernel Weight (TKW) and branching in response to higher nitrogen doses, indicating its adaptability to nitrogen-enhanced cultivation systems.

Although not statistically significant, the combined statistical and observational data support the conclusion that nitrogen fertilization plays a critical role in optimizing soybean performance under variable climatic conditions, highlighting the value of integrated fertilization strategies in climate-sensitive regions.

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MORPHOLOGICAL VARIATION IN *Hemileia vastatrix* CAUSING COFFEE LEAF RUST FROM COFFEE GROWING REGIONS IN SOUTHERN KARNATAKA, INDIA

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Abstract

Throughout the world, coffee is an important agricultural commodity and most consuming beverages. Over the last two decades, coffee production has been declined due to the outbreak of the most devastating disease called coffee leaf rust caused by Hemileia vastatrix. To achieve sustainability, soil biodiversity plays a key role in the agriculture system as the indicator of soil health. A total of 29 different localities were surveyed for coffee leaf rust disease in major coffee-growing regions such as Chikkamagaluru, Kodagu, and Hassan in Karnataka, India. The present study reveals the morphological variation of Hemileia vastatrix causing coffee leaf rust disease from different geographical regions, observed under a light microscope and scanning electron microscope

Key words*: coffee leaf rust, urediniospores, Scanning Electron Microscope, disease severity, morphological variation.*

INTRODUCTION

Coffee is a vital crop and beverage worldwide, creating incomes for over 125 million people. In terms of commerce, it is the second most exported commodity after oil. Coffee production is a crucial aspect of the global economy, with main producers including Brazil, Vietnam, Colombia, and Indonesia. The coffee plant is intuitive to the subtropical region of Africa and some islands of Southern Asia. Brazil is the largest coffee producer globally, responsible for 40% of the world's total production, with 69.9 million bags in 2021. Other major coffee-producing countries include Vietnam, Colombia, and Indonesia. In South India, Karnataka accounts for 71% of the total coffee production, followed by Kerala (21%) and Tamil Nadu (5%). (Rossi Moda et al., 2022; Vijayan et al., 2022; Silva et al., 2023; Soares et al., 2023). The coffee production regions in Karnataka include Chikkamagaluru, Kodagu, and Hassan. Chikkamagaluru, known as the Coffee Land of Karnataka, is a primary location for *arabica* coffee cultivation (Nayak et al., 2023). Additionally, coffee is mainly grown in Kodagu and Hassan districts, part of the Western Ghats in Karnataka (Reddy et al., 2022). Traditionally, coffee being grown in tropical and subtropical regions, belongs to the Rubiaceae family and the genus Coffea which consists of 90 to 124 species (Silva et al., 2022).

Coffee being a perennial crop is continuously affected by many pathogens under unfavourable conditions (Wellman, 1953; Waller, 1982). Over the last two decades, coffee production has been declined as compared to consumption which has been on a steady expansion over the same period. Because of climate change, the production of coffee has been negatively affected which directly affects yield and quality (Pham et al., 2019). Coffee production has been wiped out due to coffee leaf rust disease in the past 150 years and still causing serious issues across coffee-growing regions (Talhinhas et al., 2017). The decline in the production of coffee due to coffee leaf rust which is one of the devastating diseases caused by *Hemileia vastatrix* Berk & Broome affecting coffee crops worldwide. In *arabica* variety coffee leaf rust is a major disease, causing substantial economic loss in more than 50 countries. The fungal disease is hosting specific causes severe losses of berries and foliage up to 70-75% (Rutherford & Phiri, 2006; McCook, 2006; Suresh et al., 2012).

Hemileia vastatrix is an obligate parasite, that deposits on the lower side of the coffee leaf and colonizes intercellular to complete their reproductive cycle until their host is alive. The symptoms of CLR (coffee leaf rust) form the chlorotic spots in leaves, reduce photosynthetic rate, defoliation, and decrease quality and quantity of coffee production. Spore germination of *Hemileia vastatrix* is favoured by a dark humid environment between 21°C-27°C and available water on the leaves up to 8 h. The process of infection begins at the penetration phase of *H. vastatrix* spores after the adhesion to the underside leaf surface, a crucial phase for the process of pathogenesis since it prevents the displacement of pathogens from their host and enhances thigmotropism. Soon after the first contact, the germination tube is formed in the dikaryotic phase, the end of the germination tube has a hypha hook like tip that senses and transduces the leaf surface via signal chain, and the appressorium is formed after the stomata identification (Voegele et al., 2009; Castillo et al., 2022; RoyChowdhury et al., 2022).

Before the sporulation, small chlorotic spots may appear as young lesions. On the underside of leaves yellow to orange powdery blotches appear, that forms chlorotic patches on the upper side of leaves. These steadily expand up to 3-4 mm in diameter. Older leaves show several lesions and together produce irregular disease areas. The *Hemileia* is a genus belonging to the phylum Basidiomycota, class Pucciniomycetes, order Pucciniales (rust fungi) described by Berkeley & Broome (1869).

Hemileia vastatrix is a hemicyclic, obligate biotroph fungi which means that it depends on another living host for its growth and reproduction, and also produces urediniospores, teliospores, and basidiospores, but only the dikaryotic urediniospores, which form the asexual part of the cycle, reinfect coffee leaves frequently and are responsible for the disease proliferation. The reproductive cycle of rust fungi includes two parasitic stages, dikaryotic and monokaryotic. A significant impact on coffee production has been observed, during severe rust infection leads to the premature fall of the leaf, which reduces plant photosynthesis area debilitates the plant, and can cause branch dieback (Sera et al., 2022). During favourable environmental conditions, the urediniospores reinfect the leaves which are dikaryotic and represent the asexual cycle. However, at different times, urediniospores and teliospores are produced in the same sorus. Teliospores produce a promycelium which forms four basidiospores. Basidiospores of *Hemileia vastatrix* do not infect coffee plants (Kushalappa & Eskes, 1989; Talhinhas et al., 2017). Thus, the current study aims to analyse morphological variation in *Hemileia vastatrix* urediniospore which causes coffee leaf rust, the most devastating disease seen in coffee-growing regions, examining the disease severity of *Hemileia vastatrix* and macroscopic and microscopic identification of isolated fungi from soil. Subsequently, study investigates the potential spread of coffee leaf rust disease.

MATERIALS AND METHODS

Study location

Karnataka state lies between longitudes 74°12'00" to 78°41'00" E and latitude 11°31'00" to 18°45'00" N and seventh largest state in India. The field survey was done to know the severity of coffee leaf rust disease in major coffee growing regions of Karnataka: Chikkamagaluru, Hassan, and Kodagu. These areas lie between 75°28'00" to 75°45'00" E longitudes and 12°30'00" to 13°22'00" N latitudes and elevation between 920 to 1000 AMSL. A zone of transition between the Deccan Plateau and the Western Ghats is formed by the physiography (Figure 1; Table 1).

Collection of diseased plant samples

Collection of coffee leaf rust disease samples was done in 29 different locality of three districts. Disease sample was randomly monitored. Total ten leaves per tree were collected randomly from adjacent branches exhibiting a fungal pustules, were collected aseptically in ziplock polythene bags and brought to the laboratory and stored in cryopreservation at (-196°C) for further analysis of disease samples. Morphological variation of *Hemileia vastatrix* uredospore and teliospores was studied using CX23 Olympus light microscope (Alhudaib & Ismail, 2024) (Figure 2).

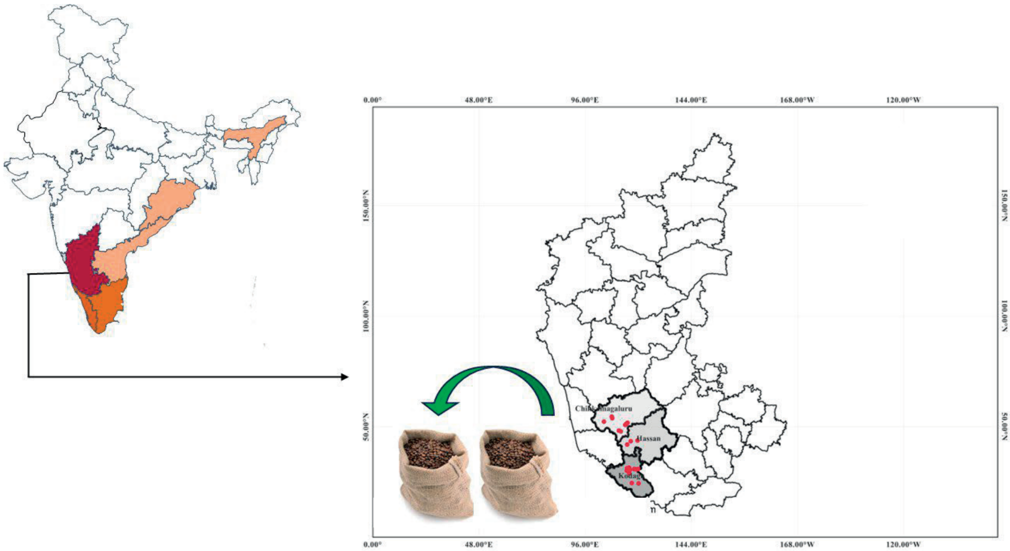

Figure 1. Field survey for coffee leaf rust disease was conducted in twenty-nine place of three districts of coffee growing regions of Karnataka

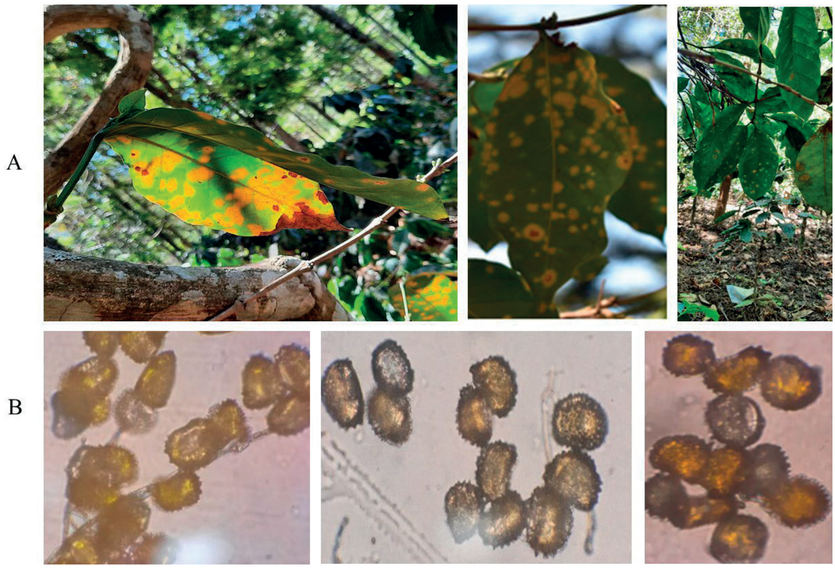

Figure 2. (A) Severity of Coffee leaf rust disease in coffee field, (B) Light microscopic image of *Hemileia vastatrix* spores under 40X magnification

Statistical analysis

The analyses CLR incidence in various elevation and disease severity index were performed in Microsoft excel using formula (Rhiney et al., 2021).

$$\text{AI \% in lot} = \frac{\text{Number of disease leaves in 60 trees}}{\text{Total number of leaves in 60 trees}} \text{X100}$$

Table 1. Survey for coffee leaf rust disease in agro-climatic zone of in coffee growing regions of Southern Karnataka, India

District	Taluk	Place of collection	Coffee varieties grown in Karnataka
Chikmagalur	Sringeri	Sringeri	Arabica
	Narasimharajapura	B. Kanubur	Arabica
		Balehonnur	Robusta
	Chikmagalur	Aladagudde	Arabica
		Chikmagalur	Chandragiri
		Mahaji	Robusta
		Chithavalli	Arabica
	Mudigeri	Mudigeri	Robusta
		Baggasagodu	Arabica
Hassan	Hassan	Hassan	Robusta
	Arkalgud	Bychanahalli	Arabica
	Sakleshpura	Heggadde	Arabica
	Alur	Rangenahalli	Arabica
		Sakleshpura	Cavery
Kodagu	Madikeri	Madikeri	Robusta
		Katakeri	Arabica
		Hebbettageri	Arabica
		Hakathur	Arabica
		Ibnivalvadi Rural	Arabica
	Virajpet	Virajpet	Chandragiri
	Somwarpet	Basavanahalli	Robusta
		Kodagarahalli	Arabica
		Guddehosur	Arabica
		Andagove	Robusta
	Ponnampet	Ponnampet	Arabica
		Gonikoppal	Arabica
	Kushalnagar	Kushalnagar	Arabica
		7th Hosakote	Arabica
		Suntikoppa	Arabica

Analysis of *Hemileia vastatrix* spore by scanning electron microscope

Diseased leaf samples were mainly collected from 29 places in coffee-growing regions from Chikkamagaluru, Kodagu, and Hassan. All selected leaves of coffee varieties show the colonies of *Hemileia vastatrix* with the development of teliospores. SEM examination was done by fragmenting the fresh coffee leaves bearing the symptoms of coffee leaf rust, round discs about 0.5 x 0.5 cm^2 containing spores and mycelia. The disc was mounted on aluminum stubs, using double-stick carbon tape. Samples were then dried in a critical point dryer (BAL-TEC model CPD 030) using liquid CO_2 as transition fluid and examined using a Zeiss EVO18 model (Fernandes et al., 2009; Gómez-de la Cruz et al., 2022).

Spore size of *Hemileia vastatrix* uredinospores

Total 29 selected diseased leaf samples were collected and individual spores were removed from lower surface using sharp needle and observed under light microscope for urediniospore measurement (µm). Urediniospores per leaf was measured (CX23 Olympus). Wall thickness width, and length of urediniospore was recorded.

RESULTS AND DISCUSSIONS

Morphological characterization of *Hemileia vastatrix*

The urediniospore wall of *Hemileia vastatrix* is hyaline and reniform (28-36 × 1-28 µm), strongly warted on the convex face, smooth on the concave face, and 1 µm thick with spherical teliospore. The morphological character of *Hemileia vastatrix* reveals the half-smooth and half-rough surface. Due to a unique combination of three morphological features the genus *Hemileia vastatrix* is distinguished from other genera of rust pathogen which include suprastomatal bouquet sori; urediniospores are

ovoid to reniform with ventral side smooth and echinulated convex dorsal side, and with angular-globose to very irregular teliospores.
The helium is distinguished toward the middle of the ventral side in oval urediniospores. Whereas in reniform urediniospores helium is found at the edge of the ventral side. Less perturbance was found in reniform urediniospores compared to oval urediniospores. The gradual increase in the diameter of pale-yellow spots is the first symptom of CLR, before the deposition of orange-colored uredinial on the lower surface of the leaf. On average, mature uredinospores can develop up to 200-300 spines at the dorsal surface, these groups of spines function to grip and hold onto foliar tissues of leaves (Table 2).
Beyene et al. (2021) studied the coffee leaf rust and its hyperparasite in dry and wet seasons at 60 sites across southwestern Ethiopia and found more severe leaf rust during the dry season and the wet season in two out of three years seems more hyperparasite. Generally, coffee leaf rust is more severe at lower altitudes in the dry season and more severe at high altitudes in the wet season.
Worldwide more than 50 physiological races of *Hemileia vastatrix* have been identified (Rodrigues-Junior et al., 1975; Zambolim & Caixeta, 2021). Carvalho et al. (2011) investigate the hidden sexual life cycle disguised within the asexual spore and elucidate new physiological races using computer-assisted DNA image cytometry followed by a modified nuclear stoichiometric staining technique with Feulgen (Figure 3).

CLR occurrence in various elevation

Coffee leaf rust severity was evaluated in three districts, different months at various elevations, from Jan-Dec 2022. Highest disease prevalence was observed in the month of Sep-Oct and Nov-Dec, in Hassan, Kodagu, and Chikmagalur, the lowest was observed during the month of May-Jun (Figure 4A, B).

Table 2. Morphological variation in uredospore of *Hemileia vastatrix* isolates from coffee growing regions in Karnataka, India

Isolates	Spore color	Shape of spores	Echinulation	Elevation	Measurement		
					Length (μm)	Width (μm)	Wall thickness (μm)
CHKM-1	Orange	Reniform	Present	Hunchback	8	4	1
CHKM-2	Orange	Reniform	Present	Convex	6	6	1.2
CHKM-3	Orange	Reniform	Present	Convex	10	2	2
CHKM-4	Yellow	Oval	Present	Convex	6	4	1.5
CHKM-5	Yellow	Reniform	Present	Convex	4	6	2.5
CHKM-6	Yellow	Oval	Present	Convex	4	8	1.2
CHKM-7	Orange	Reniform	Present	Convex	10	4	1.5
CHKM-8	Yellow	Reniform	Present	Convex	8	2	1.6
CHKM-9	Yellow	Reniform	Present	Convex	7	3	1.6
HSN-1	Yellow	Oval	Present	Convex	8	3	1.5
HSN-2	Yellow	Reniform	Present	Hunchback	6	5	2.5
HSN-3	Orange	Oval	Present	Hunchback	7	2	1.1
HSN-4	Yellow	Reniform	Present	Hunchback	5	2	1.8
HSN-5	Yellow	Reniform	present	Hunchback	3	3	1.5
KDG-1	Orange	Reniform	Present	Hunchback	6	2	1.6
KDG-2	Yellow	Oval	Present	Hunchback	8	3	1.3
KDG-3	Orange	Reniform	Present	Hunchback	4	2	1.4
KDG-4	Orange	Reniform	Present	Hunchback	6	3	1.3
KDG-5	Orange	Reniform	Present	Hunchback	3	1	1.0
KDG-6	Orange	Reniform	Present	Hunchback	5	3	1.4
KDG-7	Orange	Reniform	Present	Hunchback	5	3	1.8
KDG-8	Orange	Reniform	Present	Hunchback	7	4	1.3
KDG-9	Yellow	Oval	Present	Hunchback	9	4	1.6
KDG-10	Yellow	Reniform	Present	Hunchback	6	4	1.2
KDG-11	Orange	Reniform	Present	Hunchback	5	1	1.0
KDG-12	Yellow	Reniform	Present	Hunchback	7	3	1.5
KDG-13	Yellow	Oval	Present	Hunchback	8	4	1.3
KDG-14	Orange	Reniform	Present	Convex	4	2	1.2
KDG-15	Yellow	Reniform	Present	Convex	8	3	1.0

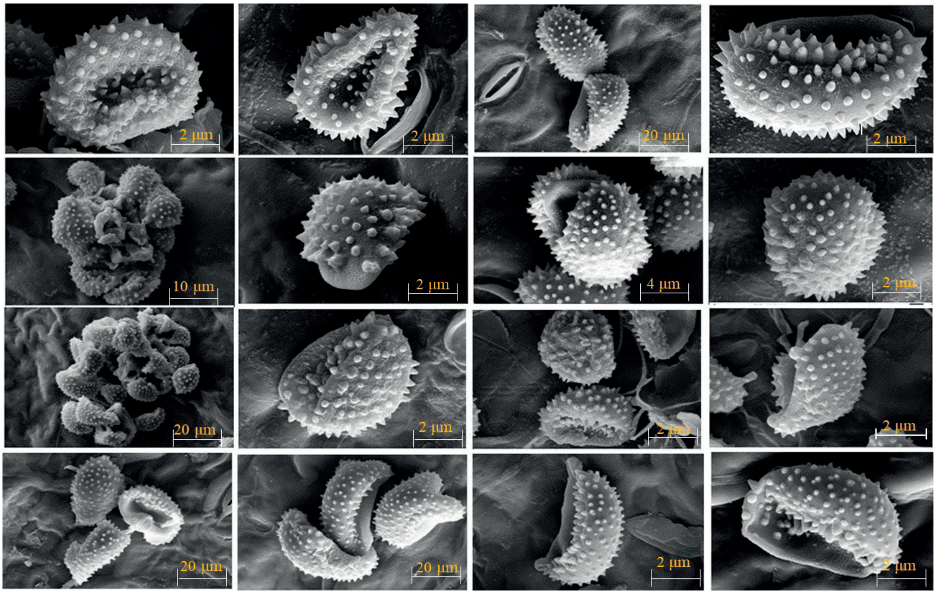

Figure 3. Morphological variation of *Hemileia vastatrix* under scanning electron microscope

A

B

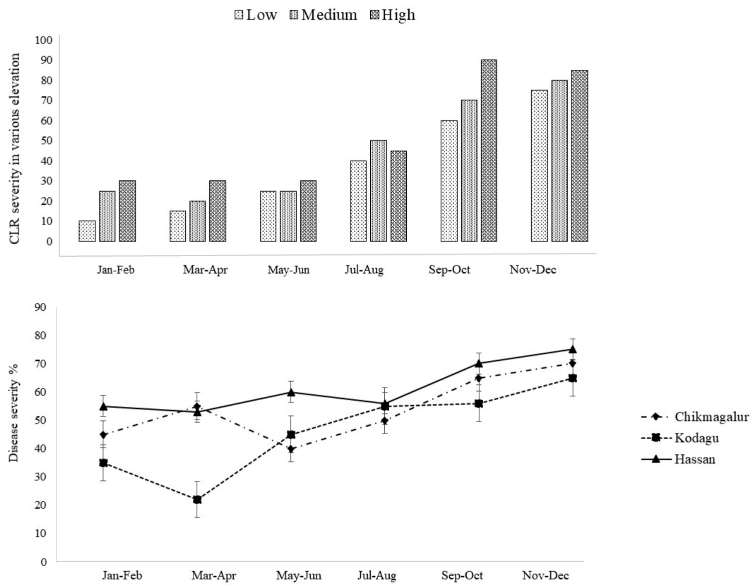

Figure 4. CLR incidence in various elevation (A) and disease severity index in Chikmagalur, Kodagu, Hassan during different seasons during Jan-Dec 2022 in Karnataka, India (B)

Variation in the size of urediniospores

Hemileia vastatrix spore size varies in length, width and wall thickness. Color of the spore varies from yellow to orange, shape of spores varies from reniform to oval. The presence of echinulation was observed on all spores. Elevation varies from hunchback to convex thirty urediniospores (30 × 60 leaves = 1,800 spores) were measured per leaf under a light microscope (×100) (CX23 Olympus). Through the epidermal cells, basidiospores germinate directly and it is related to the monokaryotic phase. The dikaryotic phase initiates with the formation of uredinospores germ tube and elongation for surface recognition. After the germination of uredinospores germ tube on subsidiary cells, results in the formation of appressorium over the stomata. The dikaryotic phase is a crucial stage for rust fungi for the development of CLR when in contact with the lower side of the leaf. The hydrophobic nature of uredinospores and the cuticle wax layer in the host leaves enhances the adhesion of uredinospores.

The presence of the spines on the uredinospores provides the mechanical grip for adhesion (Lorrain et al., 2019; Voegele et al., 2009; Mapuranga et al., 2022; Panigatti et al. 2025).

The diagrammatic scale is a tool to determine the close to the real value of CLR on *arabica* coffee leaves. The instigation of *Hemileia vastatrix* infection on coffee leaves, like other rust fungi, comprises specific events, including adhesion to the host surface, germination of urediniospore, formation of appressorium over stomata, penetration and inter- and intracellular colonization (Braun & Howard, 1994).

Aristizábal (2024) studied the CLR incidence in three coffee farms from the South Kona district of Hawaii Island. The study focuses on the early detection of CLR incidence, management practices, agronomic information, and evaluation of the cost of controlling CLR. The effective measure to control CLR sprays of fungicides with good coverage.

CONCLUSIONS

The major threat to sustainable and profitable coffee production is coffee leaf rust disease all over the world. CLR continues to be a major task to control for coffee growers in Karnataka. In the present study, the severity of the CLR epidemic was studied in 29 different localities in different months. The incidence of CLR is highly variable at various altitudes. The result showed the various morphological variation of *Hemileia vastatrix* was observed under a microscope and also through a scanning electron microscope. Even though there are several approaches to control, it is necessary to address integrated approaches to manage the disease using improved biocontrol agents through the implementation of new tools and techniques.

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INFLUENCE OF TEMPERATURE ON COMMON WHEAT – *Fusarium culmorum* (W.G. Smith) SACCARDO INTERACTIONS

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Abstract

The paper presents data on the reaction of common wheat genotypes to Fusarium culmorum isolates. By treating the grains with culture filtrates, under controlled conditions, it was found that low temperature contributes to increasing the virulence of F. culmorum isolates and to changing the factorial weight of phytopathosystem components in the source of variation in growth organs. The lowest values of the heritability coefficient (h^2) were recorded in the case of the embryonic radicle length for both thermal variants under study (I – control: 18-19°C, II – 18-19 / 8-9 / 18-19°C). In contrast to the radicle, the stem recorded much higher h^2 values at both temperatures, which is evidence of its more pronounced genetic determinism and the weaker influence of fungal culture filtrates and temperature. The vigor index recorded relatively high values of heritability coefficient (0.65-0.70) and genetic progress (21.8-27.6%) for both thermal conditions, which denotes additive control of growth and development characters and, at the same time, opportunities to create wheat genotypes resistant to F. culmorum in short terms.

***Key words**: wheat, Fusarium culmorum, temperature, character.*

INTRODUCTION

Root rot remains a major global warning to agricultural crop productivity. The disease is caused by several pathogens, often named as root rot complex. Fungal and oomycete species are predominant in these complexes, although bacteria and viruses are also known as root rot pathogens. Incorporating genetic resistance in crop plants is considered the most effective and sustainable solution to combat root rot, however resistance is often of a quantitative nature (Williamson-Benavides & Amit, 2021). Some of the most important genera of fungi capable of developing root rot in cereal crops are *Fusarium* spp. and *Bipolaris* spp. which not only cause direct economic losses but also pollute grains with mycotoxins harmful to human consumers and animals (Dinolfo et al., 2017). In recent years, the incidence of the species *F. culmorum* as the causative agent of wheat root rot has increased in the conditions of the Republic of Moldova. Starting from the mentioned, the particularities of the *common wheat – F. culmorum* interaction, the variability and heritability of plant growth and development characters are of interest for study. *F. culmorum* is an important pathogen of wheat, which causes seedling wilting, root rot, stem base rot, spike disease (shattering and bleaching of grains), mycotoxin accumulation (Bentley et al., 2006). The pathogen is dominant in colder and semiarid areas such as North Dakota (USA) (Shrestha et al., 2021), northern, central and western Europe (Wagacha & Muthomi, 2007). The fungus reproduces asexually by means of conidia, which form the main mode of dispersion. *F. culmorum* produces short, stout, thick-walled macroconidia that have curved ventral and dorsal surfaces. On Potatoe Dextrose Agar (PDA) medium, growth is rapid with dense aerial mycelium. The mycelium is generally white, but often yellow to tan. Orange to red-brown sporodochia appear as the crop ages. The underside is carmine red (Sempere & Santamarin, 2009). The main mycotoxins produced by *F. culmorum* are deoxynivalenol, nivalenol and zearalenone, which pose a potential health hazard to both humans and animals. The available management options for Fusarium head blight include the use of fungicides, cultural practices, resistant cultivars and biological agents. However, no wheat variety is completely resistant to this disease, while fungicides are at most 70% effective against natural infections. It was found that the susceptibility of wheat genotypes to this pathogen varies (Friscop, 2017). Lately, special attention is granted to *plant - fungi* interactions that can have strong effects on plant density, both through direct effects on plant performance and through indirect effects on fungi/plant competition and selective promotion of some species. Most of the evidence demonstrating certain linkages between plant abundance derives from direct fungal effects on initial growth, but with little evidence linking fungal effects on *plant - plant* interactions in intact communities. By researching a wide variety of plant species, fungi have been found to have net indirect effects - by influencing *plant - plant* interactions within intact plant communities (Bennett & Cahill, 2016). Interactions between plants and fungi can have either beneficial or detrimental effects on host plants and can give rise to various changes in both the plant and the fungus. Fungal plant pathogens are economically important due to the threat they pose to agricultural crop production and yield. Reducing or preventing fungal plant diseases depends on resistant crop varieties or fungicide treatment. *Plant - fungus* interactions need to be known to understand the mechanisms of plant diseases caused by fungi, their prevention and to improve plant productivity (Geetha & Dathar, 2022). The purpose of the present research consisted in establishing the particularities of the reaction of common wheat genotypes to the *F. culmorum* fungus under controlled conditions, with optimal temperature and thermal alternation, and the influence of *plant - pathogen* interactions on the variability and heritability of growth organs.

MATERIALS AND METHODS

F. culmorum strains were isolated in aseptic conditions on PDA (Potatoes Dextrose Agar) medium, being later identified based on macro - and microscopic characteristics according to the mycological determinant (Barnett & Hunter, 1998) (Figure 1).


A

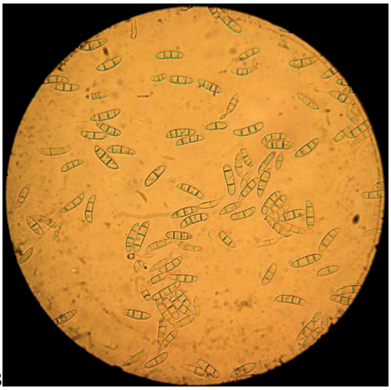
B

Figure 1. Macro- and microscopic aspects of the *F. culmorum* fungus
Colony (A), conidia (B), 300x

Four common winter wheat genotypes – Moldova 66, L Cub.101/Bas., L Bas./M30, L Sel./Accent and 4 culture filtrates (CF1, CF2, CF3, CF4) of the *F. culmorum* fungus as objects of study they served. Culture filtrates were prepared based on Cszapek liquid medium (g/l distilled water): $NaNO_3$ – 2; KH_2PO4 – 1; $MgSO_4$ 7 H_2O – 0.5; KCl – 0.5; $FeSO_4$ 7 H_2O – 0.01; sucrose – 20.0 (Methods…, 1982).

Wheat grains were treated for 18 hours with CF, after which they were rinsed with distilled water and placed in Petri dishes on filter paper moistened with water, the seedlings being grown for 6 days under different thermal conditions. In variant I, the temperature was constant: 18-19°C for 6 days, in variant II the seedlings were maintained on day 2 at a temperature of 8-9°C for 6 hours. The experiment was performed in 3 repetitions.

Genetic variance (σ^2_G), phenotypic (σ^2_{Ph}), heritability coefficient (h^2), genetic coefficient of variation (GCV, %), phenotypic coefficient of variation (PCV, %), genetic progress (GAM, %) were calculated on based on the formulas proposed by Balkan, 2018. The data were statistically processed through variance, factorial analyzes in the STATISTICA 7 software package.

RESULTS AND DISCUSSIONS

The action of F. culmorum culture filtrates on the growth of wheat seedlings. In relation to the fact that temperature significantly influences *plant x pathogen* interactions, research was carried out in view of the particularities of response of wheat genotypes to the action of culture filtrates (FC) of 4 *F. culmorum* strains. Biometric measurements demonstrated different reactions of wheat genotypes to grains treatment with CF – from no reaction to mild, medium or strong inhibition, the effect depending on the genotype, the analyzed character, the fungal isolate and the temperature.

At the optimal temperature (18-19°C), the germination capacity did not suffer any significant influences from the action of the 3 CFs in the case of genotypes M66 and L Bas./M30, but inhibition was recorded in L Cub.101/Bas. under the influence of FC1 (-14.5%) and L Sel./Accent – in the FC2 variant (-18.9%) (Figure 2).

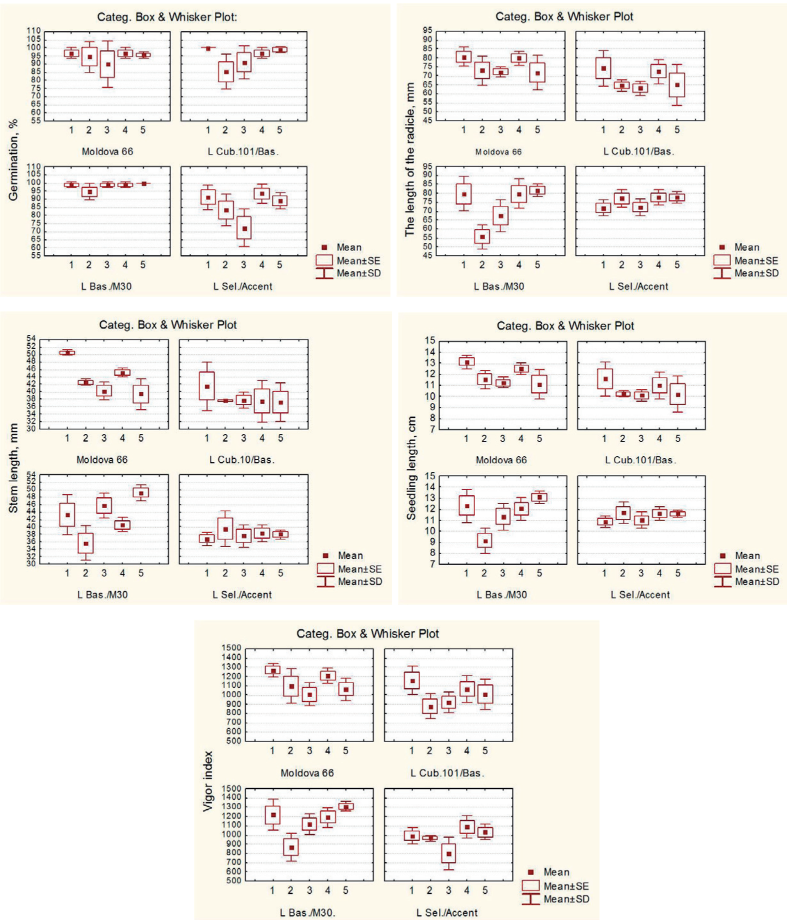

Figure 2. The influence of *F. culmorum* culture filtrates on the growth and development characteristics of common wheat, at a temperature of 18-19°C
1 - Control, 2 - CF1, 3 - CF2, 4 - CF3, 5 - CF4

In our previous research (Lupascu, 2020), it was found that compared to other growth and development organs of wheat, the embryonic radicle shows the highest sensitivity to CFs of Fusarium fungi. According to the obtained data, at the optimal temperature (18-19°C), statistically veridical inhibitions ($p<0.05$) were recorded for: i) *embryonic radicle length* at M66 (FC2: -10.5%) and L Bas./M30 (FC1: -30.4%); ii) *stem length* – at M 66 (FC1-FC4: -10.6 … -22.1%); iii) *seedling length* – M66 (FC1, FC2: -12.0, -14.3%, respectively), L Bas./M30 (FC1: -25.7%), *vigor index* – M66 (FC1, FC2: -12.0, -14.3% , respectively), L Bas./M30 (FC1: -28.8%).

At alternating temperatures 18-19 / 8-9 / 18-19°C, statistically significant inhibitions ($p<0.05$) were found in the action of *F. culmorum* CFs in the following variants: i) *germination capacity* – L Sel./ Accent, FC1, FC4 (-21.2%, -20%, respectively); ii) *the length of the embryonic radicle* – M 66 (FC2: -12.0%), L Cub.101/Bas. (FC1: - 19.9%), L Bass / M30 (FC1, FC2, FC4: -10.2%, -15.7%, -11.7%, respectively), L Sel./Accent (FC1, FC2, FC3, FC4: -31.4 %, -27.4%, -16.0%, -26.3%, respectively); iii) *stem length* – M66 FC1 (-17%), L Cub.101/Bas. (FC1: - 23.3%), L Sel./Accent (FC1, FC2, FC3, FC4: -28.2%, -23.0%, -18.9%, -24.9%, respectively); iv) *seedling length* – M 66 (FC2: - 12.0%), L Cub.101/Bas. (FC1: - 21.1%), L Bass / M30 (FC1: -6.9%), L Sel./Accent (FC1, FC2, FC3, FC4: -30.3%, -25.8%, -16.9%, -25.8%, respectively); iv) *vigor index* – L Cub.101/Bas. (FC1: - 25.2%), L Sel./Accent (FC1, FC2, FC3, FC4: -45.1%, -44.6%, -25%, -40.6%, respectively) (Figure 3).

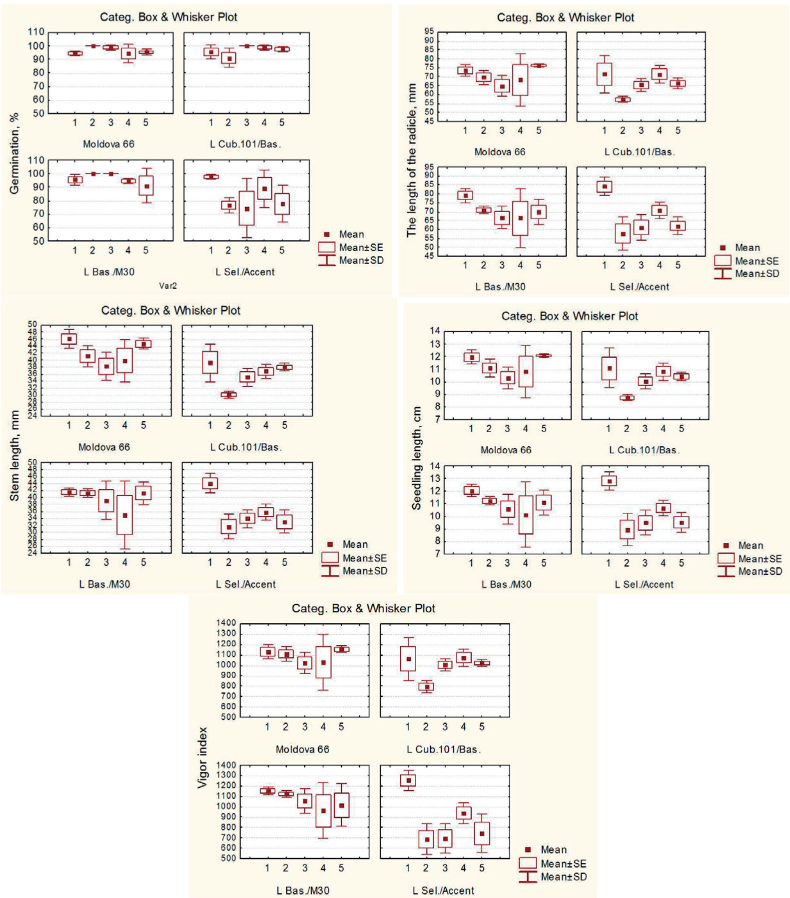

Figure 3. The influence of *F. culmorum* culture filtrates on the growth and development characteristics of common wheat, at temperature alternation 18-19/8-9/18-19°C
1 - Control, 2 - CF1, 3 - CF2, 4 - CF3, 5 - CF4

Starting from recent approaches to the virulence phenotypes of pathogen isolates (Kosman et al., 2019), we can make the assumption that the virulence potential of *F. culmorum* isolates has a multidimensional expression that manifests itself through the differentiated phenotypic plasticity of organs of growth and development as a function of genotype, isolate, temperature, determining a wide functional variation of *common wheat - F. culmorum* interactions.

Factorial analysis of wheat genotype x F. culmorum relationships. It was found that at the temperature of 18-19°C the factorial weight of the genotype, the isolate, their interaction was different in the source of variation of wheat growth characters. For example, the role of the wheat genotype constituted 57.69%; 32.83%; 61.25%; 38.54%; 40.37%, and of the isolate: 28.82%; 42.52%; 13.09%; 33.4%; 44.28%, respectively, of germination capacity, embryonic radicle length, stem length, seedling length, vigor index (Table 1).

Table 1. Factor share of wheat genotype and *F. culmorum* isolate in the growth and development of plants at a temperature of 18-19°C

Source of variation	**Degree of freedom**	**Mean sum of squares**	**F**	**p**	**Share in the source of variation, %**
Germination					
Wheat genotype	3	420.4*	8.79	0.000	57.69
Fungus isolate	4	210.0*	4.39	0.005	28.82
Genotype x isolate	12	50.5	1.06	0.420	6.93
Random effects	40	47.8			6.56
The length of the embryonic radicle					
Wheat genotype	3	189.1*	4.182	0.012	32.83
Fungus isolate	4	244.9*	5.417	0.001	42.52
Genotype x isolate	12	96.8*	2.140	0.036	16.81
Random effects	40	45.2			7.84
The length of the stem					
Wheat genotype	3	128.43*	10.545	0.000	61.25
Fungus isolate	4	27.44	2.253	0.080	13.09
Genotype x isolate	12	41.62*	3.417	0.002	19.85
Random effects	40	12.18			5.81
The length of the seedling					
Wheat genotype	3	4.456*	4.680	0.007	38.54
Fungus isolate	4	3.862*	4.056	0.008	33.40
Genotype x isolate	12	2.293*	2.408	0.019	19.83
Random effects	40	0.952			8.23
Vigor index					
Wheat genotype	3	107564*	6.674	0.001	40.37
Fungus isolate	4	117993*	7.321	0.000	44.28
Genotype x isolate	12	24799	1.539	0.151	9.31
Random effects	40	16117			6.04

*- $p<0.05$.

So at the optimal temperature, the wheat genotype factor had the highest weight for stem growth (61.25%), but the lowest – for embryonic radicle length (32.83%). The isolate factor had a greater influence for the vigor index (44.28%), but the least – for the stem length (13.09%). In the conditions in which the seedlings grew on the background of temperature alternation, a change in the factorial weight of the components of the phytopathosystem in the source of variation of the characters under study was observed. Thus, the weight of the genotype constituted 75.0%; 13.36%; 52.39%; 27.89%; 48.32%, and the isolate – 5.47%; 61.67%; 33.29%, 51.49%; 29.75%, respectively, of germination, radicle length, stem length, seedling length, vigor index (Table 2).

Thus, it can be observed that the low temperature significantly contributed to the reduction of the role of the genotype and the increase of the isolate factor in the growth of the embryonic radicle and stem, which was reflected on the whole seedling, a fact that denotes: i) *F. culmorum* isolates have a higher pathogenic potential against the background of the unfavorable temperature; ii) the virulence polymorphism of *F. culmorum* isolates, specific to plant growth organs, has a more pronounced phenotypic manifestation under these conditions.

Table 2. Factor share of wheat genotype and *F. culmorum* isolate in the growth and development of plants at temperature alternation 18-19° / 8-9 / 18-19°C

Source of variation	Degree of freedom	Mean sum of squares	F	p	Share in the source of variation, %
Germination					
Wheat genotype	3	676.3*	11.256	0.000	75.00
Fungus isolate	4	49.3*	0.820	0.520	5.47
Genotype x isolate	12	116.0	1.930	0.060	12.86
Random effects	40	60.1			6.67
The length of the embryonic radicle					
Wheat genotype	3	72.1*	1.389	0.260	13.36
Fungus isolate	4	332.9*	6.413	0.000	61.67
Genotype x isolate	12	82.9*	1.596	0.132	15.36
Random effects	40	51.9			9.61
The length of the stem					
Wheat genotype	3	144.49*	9.829	0.000	52.39
Fungus isolate	4	91.81*	6.245	0.000	33.29
Genotype x isolate	12	24.79*	1.686	0.107	8.99
Random effects	40	14.70			5.33
The length of the seedling					
Wheat genotype	3	4.067*	3.514	0.024	27.89
Fungus isolate	4	7.509*	6.488	0.000	51.49
Genotype x isolate	12	1.850	1.598	0.131	12.69
Random effects	40	1.157			7.93
Vigor index					
Wheat genotype	3	150451*	8.002	0.000	48.32
Fungus isolate	4	92623*	4.926	0.003	29.75
Genotype x isolate	12	49502*	2.633	0.011	15.90
Random effects	40	18802			6.03

*- $p<0.05$.

Variability and heritability of quantitative growth characters of wheat in the interaction with F. culmorum under the influence of temperature. The calculation of genetic parameters of variability and heritability demonstrated that the magnitude of genotypic (σ^2_G) and phenotypic (σ^2_{Ph}) variances of the analyzed characters was different and strongly influenced by temperature. This was reflected on the h^2 index, i.e. on the capacity for hereditary transmission (Table 3). For example, in the case of the fungus *F. culmorum*, h^2 varied between 0.52-0.76 and 0.12-0.77 at temperatures 18-19°C and 18-19 / 8-9/ 18-19°C, respectively.

The lowest values of the coefficient h^2 were recorded in the case of the length of the embryonic radicle for both thermal variants, which denotes the pronounced dependence of the character on the biotic environment. In contrast to the radicle, the stem recorded much higher h^2 values at both temperatures, which is evidence of its more pronounced genetic determinism and the weaker influence of fungal CFs and temperature.

Table 3. Variability and heritability of common wheat growth characters in interaction with *F. culmorum*

Parameter	Germination	The length of the radicle	The length of the stem	The length of the seedling	Vigor index
18-19°C					
σ^2_G	124.2	47.97	38.75	1.17	30482.67
σ^2_{Ph}	172.0	93.17	50.93	2.12	46599.33
h^2, %	0.72	0.52	0.76	0.55	0.65
GCV, %	11.95	9.50	15.3	9.52	16.44
PCV, %	14.06	13.24	17.54	12.82	20.32
GAM, %	14.11	12.73	19.3	12.71	21.76
18-19 / 8-9 /18-19°C					
σ^2_G	205.4	6.73	43.26	1.74	43883
σ^2_{Ph}	265.5	58.63	57.96	2.90	62685
h^2, %	0.77	0.12	0.75	0.40	0.70
GCV, %	15.38	3.78	17.18	12.33	20.91
PCV, %	17.49	11.15	19.88	15.91	24.99
GAM, %	17.28	3.05	21.47	10.66	27.59

Considering that the reaction of plants to fungal attack is more relevant at the stage of active growth, probably radicle and stem growth is more informative than germination, the grain presenting more of a nutrient substrate for fungal growth.

The h^2 coefficient for radicle length and stem length is directly correlated with the genotypic coefficient of variation (GCV, %), which is in all cases much higher for the stem than for the radicle.

The vigor index (*germination, % x seedling length, cm*) recorded high values of the coefficient h^2 and genetic progress (GAM, %) which denotes the significant control of additive genes in the formation of characters and the high opportunities of creating in narrow terms the genotypes of wheat, resistant to these pathogens that have recently been observed with relatively high frequency in common autumn wheat under the conditions of the Republic of Moldova.

CONCLUSIONS

Treatment of grains with the culture filtrates of *F. culmorum* isolates demonstrated a varied response of common wheat genotypes, function of isolate, growth and development character, isolate, temperature contributing to increase the virulence of the pathogen.

The differentiated phenotypic plasticity of the growth and development organs (embryonic radicle, stem) to the action of *F. culmorum* isolates denotes both the peculiarities of adaptation of wheat seedlings to the pressure of the biotic factor, and the phenotypic virulence of *F. culmorum* isolates.

The factorial analysis of the relationships between wheat genotype and *F. culmorum* isolate demonstrated that the temperature factor modifies the share of the components of the phytopathosystem in the source of variation in the growth and development characteristics of the wheat seedling (radicle, stem), the low temperature diminishing the role of the genotype and increasing that of the isolate.

The relatively high values of the heritability coefficient (0.65-0.70) and genetic progress (21.8-27.6%) for the vigor index (*germination, % x seedling length, cm*) against the background of different conditions thermals denote the additive control of growth and development characters and, at the same time, the opportunities to create wheat genotypes, resistant to *F. culmorum* in restricted terms.

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EFFECT OF BIODEGRADABLE COMPOSITION FOR SEED COATING ON EARLY STAGE OF CORN GROWTH

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Abstract

The objective of this research was to evaluate the influence of biodegradable carboxymethylcellulose-based composition on the growth characteristics of coated corn seeds during storage. The seeds of four corn hybrids (P280, P300, P398, P402) were coated with a carboxymethylcellulose-based composition containing an extract of phenolic compounds from Juniperus sabina and natural dye obtained from Phytolacca americana berries. Early growth characteristics (germination, root/shoot lengths, root/shoot biomass, root/seedling vigour, metabolic efficiency) of coated seeds were studied before storage and during four months of storage. During storage, the overall germination of seeds remained at the level of 94-98%, while the length of the roots and seedlings of the germinated seeds increased by 1.2-1.9 and 1.4-1.8 times, respectively. After storage, the coated seeds also showed an increase in the vigour of roots and seedlings (by 1.5-2.1 times). Thus, the developed composition contributes to the improvement of the growth characteristics of corn seeds and, due to the original dye, allows for easy identification of coated seeds.

***Key words**: corn, seed coating, biodegradable composition, growth characteristics.*

INTRODUCTION

Corn grain production, which in 2023 accounted for 41.9% of the total grain production, is an important component of the Republic of Moldova economy (Statistica RM, 2023). Increasing gross harvests of corn remains a priority task for agricultural production in republic.

The use of high-quality seeds for sowing is the basis for increasing the productivity of corn, which is carried out using various types of highly productive varieties and pre-sowing treatments of corn seeds. However, by protecting seeds through coating with synthetic components (fungicides, pesticides, colorants), in most cases, could be provide an inhibitory effect on the seed germination and the plant growth as a whole. However, adverse environmental impacts caused by using non-renewable and non-biodegradable polymers are driving the search for alternatives to overcome these effects, such as natural-based polymers.

At present, much attention is paid to the search for new environmentally friendly methods of stimulating germination, growth and protection of plants from diseases, pests, abiotic stresses, based on increasing resistance by inducing internal reserves of the seed itself (Ivanova & Makarov, 2020; Dziwulska-Hunek et al., 2023). In addition, the natural substances and biological products are used to obtain environmentally friendly and healthy products as well as to maintain of soil fertility.

It is relevant to use biologically active compounds, secondary metabolites of higher plants, in the composition of a complex cover mixture for seed protection, which in small quantities have the ability to influence many physiological processes of seed germination and plant growth.

When using natural growth regulators, the degree and some nature of the metabolic processes of seed germination change. Activation of metabolic processes and mobilization of reserves substances are key steps to maintain the growth of seedlings and roots before the mechanisms of photosynthesis are initiated (Zhang et al., 2018; Silva & Carvalho, 2023).

Significant variability in emergence and plant vigour existed in field condition by influence of abiotic factors, namely cold temperatures and high soil moisture (Ali et al., 2018; Khaeim et al., 2022; Beegum et al., 2023).

To protect corn seeds from unfavourable environmental factors and ensure uniform emergence of seedlings, which leads to increased yields, pre-sowing seed treatment with natural plant growth regulators and coating with biodegradable polymers are studied.

Biodegradable polymers, such as, starch, pectin, alginate, chitosan, gelatine gum arabic, polyethylene glycol, cellulose and its derivatives (hydroxyethylcellulose, carboxymetylcellulose) use as substitutes to synthetic materials owing to their environment friendly and nontoxic characteristics, as well as good adherence to the seeds (Pirzada et al., 2020; Rahman et al., 2021; Vinzant et al., 2023; Zacharias et al., 2024).

Carboxymethylcellulose is one of the most promising biopolymers due to its characteristic of surface properties, mechanical strength, viscous properties, availability and low-cost (Rahman et al., 2021). Carboxymethylcellulose-based seed coat has developed and tested for soybean (de Camargo et al., 2017), wheat (Ren et al., 2019) and sweet corn (Pallaoro et al., 2016; Mahisanon et al., 2021) and parental form of corn (Borovskaia et al., 2022) seeds using various fungicides, plant growth regulators and additives; and showed the effect of seed coating on germination, root/shoot growth and vigour.

The natural plant growth regulators with suitable concentrations incorporated in coating compositions improved seed early growth characteristics of sweet corn (Suo et al., 2017) and corn for grain and silage (Borovskaia et al., 2023). The positive effect of presowing seed coating of corn parental form with composition on carboxymetylcellulose containing natural bioregulator (genistifolioside) was confirmed by experimental data obtained in the field, which was expressed in improving the development of the root system and increasing the mass of both the green aerial part of plants and 1000 grains (Borovskaia et al., 2022).

This study is a continuation of the ongoing research concerning the influence of seed coating with composition containing bioregulator JS extracted from aboveground parts of *Juniperus sabina* L. plants and natural dye from berries of *Phytolacca americana* L. on the bio-morphological and physiological characters of coated corn seeds during storage.

It is well known that extracts from *J. sabina* contains terpenoids, flavonoids, coumarins, lignans, reducing sugars, etc. and possess increased antioxidant activity, have the ability to increase plant immunity and resistance to pathogens and unfavorable environmental factors (Orhana et al., 2017; Elisovetcaia et al., 2019; Zazharskyi et al., 2020; Pan et al., 2024). Early it was showed that extract from *J. sabina* exhibit biological activity on beech seed by increasing the mean daily germination (1.33) in comparison with control by 1.4 times (Elisovetcaia et al., 2024).

Natural dye from the berries of the American pokeweed (*Phytolacca americana* L.) contains red betacyanins, which are betalain compounds and also have biological activity, such as antimicrobial, antioxidant, and immuno-modulatory effects (Mchedlishvili et al., 2014; Rahimi et al., 2019; Sadowska-Bartosz & Bartosz, 2021; Martínez-Rodríguez et al., 2022). Betacyanins were extracted and analyzed from different plant, namely *Opuntia* spp. (Castellar et al., 2003; Sanchez-Gonzalez et al, 2013); cactus pear (Ruiz-Gutierrez et al., 2015), red pitaya (Low Pinn Yee et al., 2017), *Amaranthus* spp. (Chong et al., 2014; Das et al., 2019), red dragon fruits (Woo et al., 2011), red beetroot (Lazar et al., 2021). The results of research concerning to application of betacyanins as dye in food and textile industries wide reported (Baaka et al., 2019; Singh et al., 2020; Khan, 2022; Rocha et al., 2022; Roriz et al, 2023). However, no information was found on the use of betacyanins in seed coatings and their biological effect on seed germination and plant growth.

The objective of this study was to evaluate the effect of coating composition based on biodegradable carboxymethylcellulose and containning a natural plant growth regulator from *J. sabina* and natural dye betacyanins on the early growth characteristics of coated corn seeds.

MATERIALS AND METHODS

The studies were carried out in 2024 year in the Institute of Genetics, Physiology and Plant Protection (IGPPP), Moldova State University (MSU), Republic of Moldova.

Corn seeds. The National Center of Research and Seed Production, Institute of Crop Science „Porumbeni” generously contributed the seeds of food corn hybrids, which have different ripening periods and differ according to the structure of the endosperm:

Porumbeni 280su (P280) is mid-season sweet corn hybrid, FAO 280. In the technical ripening phase, the grains contain 14.0% total sugar and 31.5% starch. Light yellow grain with fine pericarp.
Porumbeni 300 sh-2 (P300) is a mid-season synthetic population, super-sweet corn, FAO 300. At the technical maturity stage, the grains contain 14.0-16.0% total sugar and 31.4% starch, dextrin does not contain. A distinctive feature is the large, angular, wrinkled pericarp grain, consisting of floury endosperm.
Porumbeni 398 (P398) is a mid-early maturing corn hybrid that belongs to the popcorn group, FAO 400. The grains have a glassy consistency and are characterized by a high specific gravity of the endosperm. The floury part of the endosperm is present only near the embryo. The grains have a high protein content (16%).
Porumbeni 402 (P402) is mid-late-ripening corn hybrid with toothed-glassy, orange grain, semi-flint corn, FAO 400. The grains contain 70–75% starch, up to 15% protein and 3–6% fat.

Coating composition. The water solution of sodium salt of carboxymethylcellulose (CMC) in concentration 1.0-1.2% was used as a film former support. Natural plant growth regulator as concentrated extract from aboveground parts of *Juniperus sabina* (bioregulator JS), was obtained in laboratory Natural Bioregulators, IGPPP. Extract contents the total polyphenolic substances 38.89±1.67 mgGAE*/g; flavonoids 8.55±0.09 mgQE**/g; and phenolic acids 13.25±0.25 mgCAE***/g. (Note: *GAE - gallic acid equivalent; **QE - quercetin equivalent; ***CAE- caffeic acid equivalent). Antioxidant activity of extract is equal IC_{50}=59.19±9.77 µg/ml was evaluated by potentiometric procedure (Ivanova, 2016; Elisovetcaia et al., 2018). Bioregulator JS diluted to a concentration of 0.001% was used to coat the corn seeds.
Natural dye of red color containing betacyanins was extracted from berries of *Phytolacca americana*. Procedure for obtaining of this natural dye in powder form was patented (Patent MD-1817). Antioxidant activity of obtained natural dye evaluated by potentiometric procedure and expressed in gallic acid equivalent is 129.72±2.59µMGAE/g. In coating composition, the natural dye was used in concentration of 0.3%.

Germination testing. Germination of control seeds and coated seeds was carried out in accordance with the provisions of international rules (ISTA, 2017). After germination, the overall seed germination, root and seedling length, root and seedling biomass, root and seedling vigor were assessed (Kerecki et al., 2021). The biomass of reserve substances that was spent for energetic support of physiological processes and respiration during seed germination as well as metabolic efficiency were calculated (Sikder et al., 2009; Dascaliuc et al., 2020; Borovskaia et al., 2023).

Statistical analysis. The obtained experimental data were processed by the statistical methods using the software package Statgraphics Plus 5.0. The ANOVA test was applied for variance analysis of characters, Student test in assessment of statistically significant differences between plots (Raudonius, 2017).

RESULTS AND DISCUSSIONS

The overall germination of corn seeds of the studied hybrids before coating and storage was 92-97%, which is within the error limits of the method. However, the lengths of roots and seedlings differed significantly (Figure 1). Hybrids were divided into two homogeneous groups by root length: 1) P280 and P398 with an average root length of 5.78-5.95 cm; and 2) P300 and P402 (4.24-4.48 cm). The root lengths of these groups of hybrids had statistically significant differences at $p \leq 0.001$. The length of the embryonic roots in corn affects the development and growth of the aboveground organs of the plant, as well as its overall productivity. During germination of the corn seeds, the embryonic root quickly goes deep into the soil by 30-40 cm and, together with the lateral hypocotyl roots, forms a system of primary roots that provide the plant with nutrients and water for about 2-3 weeks.
The length of the seedlings is also important, since due to the elongation of the epicotyl, corn seeds can germinate from a great depth, bringing the first stem node to the soil surface. The distribution of hybrids by seedling length was as follows: one group P280, P300, P398 and the other P402. The differences in seedling length between these two groups were at $p \leq 0.01$. Nevertheless, the P402 hybrid had statistically significantly lower seedling lengths ($p \leq 0.001$) than the P280 and P398 hybrids. Thus, the studied hybrids had the various early growth characteristics.

The early growth characteristics of coated seeds of studied corn hybrids before storage were not affected by the coating procedure.

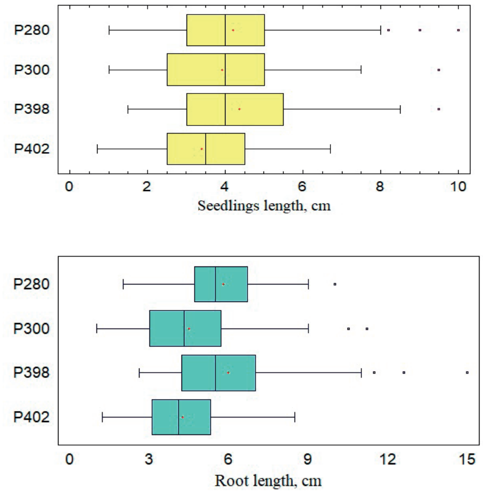

Figure 1. Roots and seedlings length of control corn seeds

The mean values of root/seedling length of coated seeds during germination changed, but not statistically significantly, for hybrids P280, P300 and P398 (Figures 2-4).
However, for hybrid P402 the root length of coated seeds before storage increased significantly ($p \leq 0.001$) to 5.92±2.28 cm from 4.24±1.54 cm in control seeds (Figure 5). The seedlings length of coated seeds also changed significantly ($p \leq 0.001$), but their growth was suppressed by an average of 0.88 cm after coating procedure.

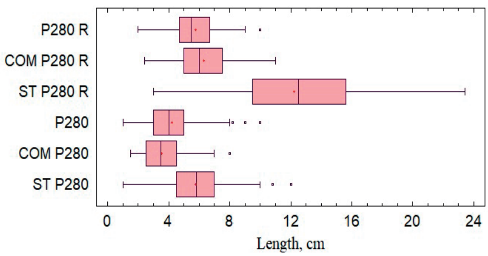

Figure 2. Roots and seedlings length of coated corn seeds of P280 hybrid: P280 R - root control; COM P280 R - root of coated seeds before storage; ST P280 R - root of coated seeds after 4 months of storage coated seeds; P280 - seedlings control; COM P280 - seedlings of coated seeds before storage; ST P280 - seedlings of coated seeds after 4 months of storage

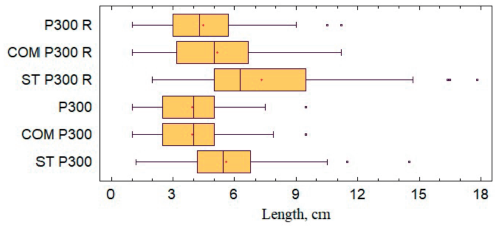

Figure 3. Roots and seedlings length of coated corn seeds of P300 hybrid: P300 R - root control; COM P300 R - root of coated seeds before storage; ST P300 R - root of coated seeds after 4 months of storage coated seeds; P300 - seedlings control; COM P300 - seedlings of coated seeds before storage; ST P300 - seedlings of coated seeds after 4 months of storage

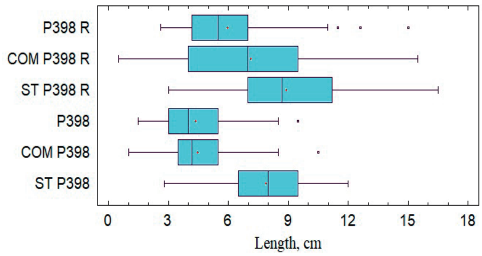

Figure 4. Roots and seedlings length of coated corn seeds of P398 hybrid: P398 R - root control; COM P398 R - root of coated seeds before storage; ST P398 R - root of coated seeds after 4 months of storage coated seeds; P398 - seedlings control; COM P398 - seedlings of coated seeds before storage; ST P398 - seedlings of coated seeds after 4 months of storage

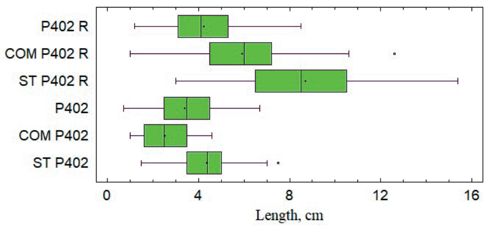

Figure 5. Roots and seedlings length of coated corn seeds of P402 hybrid: P402 R - root control; COM P402 R - root of coated seeds before storage; ST P402 R - root of coated seeds after 4 months of storage coated seeds; P402 - seedlings control; COM P402 - seedlings of coated seeds before storage; ST P402 - seedlings of coated seeds after 4 months of storage

After 4 months of storage, the coated seeds were germinated under optimal conditions. During storage, the overall germination rate of seeds did not change significantly, remaining at the level of 94-98%. The effect of the bioregulator from *J. sabina* and natural dye with antioxidant activity during storage period was positive and significant ($p \leq 0.001$) on the growth of roots and seedlings for all the studied hybrids (Figures 2-5). Storage of coated seeds contributed to an increase in the root length an average of 1.2-1.9 times, and seedlings - 1.4-1.8 times compared to these indexes before storage. On average, the root length increased by 1.79cm for seeds of the P398 hybrid, by 2.14 cm for P300, by 2.77 cm for P402, and by 5.94 cm for the P280 hybrid. The length of seedlings elongated by 1.64cm for P300, by 1.85 cm for P402, by 2.22 cm for P280, and by 3.42 cm for P398. Considering the fact that the root/seedling length may indicate corn tolerance to abiotic factors, the investigated coating composition can contribute to seed resistance to adverse environmental conditions.

The procedure of coating corn seeds and storing them for four months had a positive effect not only on the length of roots and seedlings, but also on their vigour (Figures 6-9).

It should be noted that before storage, the coated seeds changed the root/seedling vigour compared to control seeds according to the above-described pattern relative to the length of the roots/seedlings. Namely, the corn of the P280, P300, and P398 hybrids showed root/seedling vigour that was not significantly different from control seeds (Figures 6-8). However, the root vigour of coated seeds of the P402 hybrid before storage fortified significantly ($p \leq 0.001$), while the seedling vigour decreased significantly ($p \leq 0.001$) compared with control seeds (Figure 9).

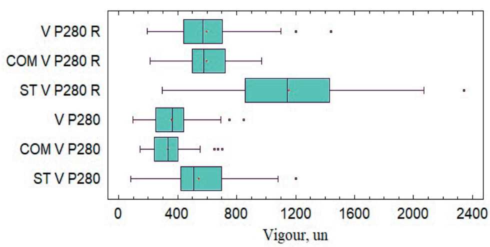

Figure 6. Vigour of roots and seedlings of P280 hybrid: V P280 R - root control; COM V P280 R - root of coated seeds before storage; ST V P280 R - root of coated seeds after 4 months of storage; V P280 - seedlings control; COM V P280 - seedlings of coated seeds before storage; ST V P280 - seedlings of coated seeds after 4 months of storage

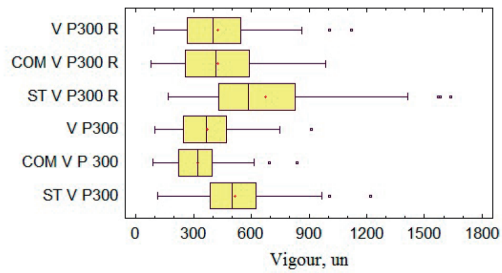

Figure 7. Vigour of roots and seedlings of P300 hybrid: V P300 R - root control; COM V P300 R - root of coated seeds before storage; ST V P300 R - root of coated seeds after 4 months of storage; V P300 - seedlings control; COM V P300 - seedlings of coated seeds before storage; ST V P300 - seedlings of coated seeds after 4 months of storage

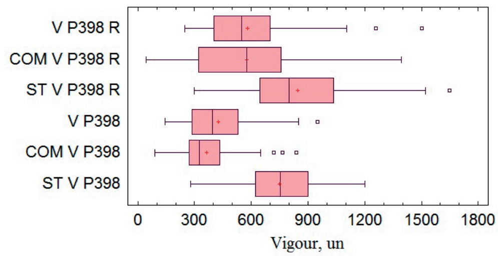

Figure 8. Vigour of roots and seedlings of P398 hybrid: V P398 R - root control; COM V P398 R - root of coated seeds before storage; ST V P398 R - root of coated seeds after 4 months of storage; V P398 - seedlings control; COM V P398 - seedlings of coated seeds before storage; ST V P398 - seedlings of coated seeds after 4 months of storage

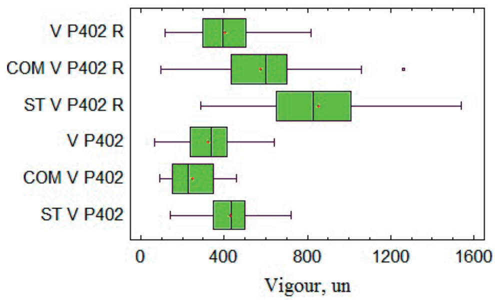

Figure 9. Vigour of roots and seedlings of P402 hybrid: V P402 R - root control; COM V P402 R - root of coated seeds before storage; ST V P402 R - root of coated seeds after 4 months of storage; V P402 - seedlings control; COM V P402 - seedlings of coated seeds before storage; ST V P402 - seedlings of coated seeds after 4 months of storage

The seedling vigour of coated seeds after storage was higher than vigour of coated seed before storage by 183.34 (P402); 208.67 (P280); 353.50 (P300) and 387.11 (P398). Compared with control seeds, the seedlings vigour of coated and stored seeds increased 1.33 (P402) - 1.71 (P398) times.

Application of coating procedure on corn seeds and storing them during 4 months led to a significant increasing of early growth characteristic such as root/seedling length and vigour. In addition, the biomass of roots and seedlings of coated and stored corn seeds was more 1.5-2.0 times than root/seedling biomass of control seeds. However, the proportion of reserve substances used for the growth of roots and seedlings from the total amount of reserve substances spent on germination was different (Figures10-11).

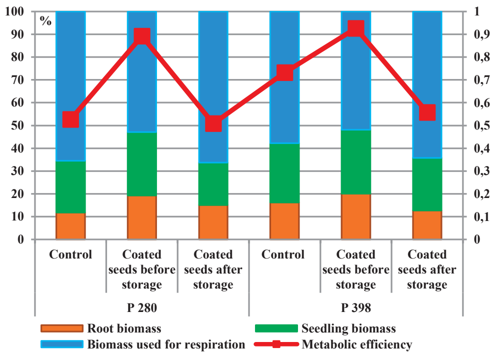

Figure 10. Proportion of reserve substances eliminated for growth of root and seedlings, and spent for respiration, metabolic efficiency of hybrids P280 and P398

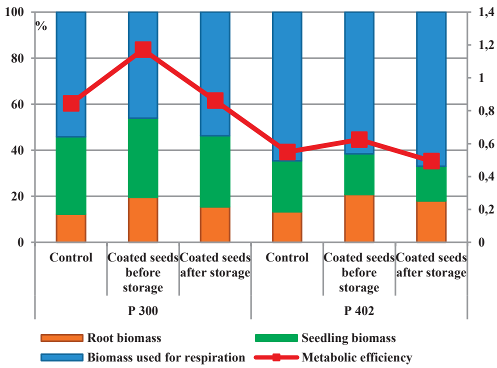

Figure 11. Proportion of reserve substances eliminated for growth of root and seedlings, and spent for respiration, metabolic efficiency of hybrids P300 and P402

This is explained by the fact that the proportion of reserve substances spent on energy support of the physiological processes of germination and respiration varied also, which naturally affected the values of metabolic efficiency in the studied variants (Figures 10-11). Early it was showed the high negative correlation between the biomass of reserve substances eliminated for respiration and the metabolic efficiency of the P427 and P458 hybrids, Pearson correlation coefficients were equal to -0.9557 and -0.9457, respectively (Borovskaia et al, 2023).

The studied hybrids showed a characteristic feature of changes in the metabolic efficiency of germination of coated seeds (Figures 10-11): before storage, it increased compared to the control, and after 4 months of storage, it practically returned to the values of the control seeds.

It can be assumed that coated corn seeds during storage occur balancing of the initial early growth characteristics concerning distribution of eliminated reserve substances, but at the same time the coating composition contributes to increase the root/seedling length, biomass and vigour.

Similar results were obtained on coated seeds of hybrids P427 and P458, which were coated with a composition containing biologically active substances of natural origin, genistifoliosides, and a biodegradable polymer, sodium salt of carboxymethylcellulose. However, long-term storage of coated corn seeds from four to 12 months did not lead to a further increase in root/seedling vigour (Borovskaia et al, 2023).

Thus, the coating composition based on carboxymethylcellulose containing an extract of phenolic compounds from *J. sabina* and a natural dye obtained from *P. americana* berries with antioxidant activity contributed to improve the early growth characteristics of coated corn seeds of different hybrids after four months of storage. Storage during four months is the most practicable period for pre-sowing treatment of corn seeds by coating procedure.

CONCLUSIONS

The obtained results of experimental studies provide grounds to assert that pre-sowing coating of corn seeds with a biodegradable composition containing phenolic compounds from *J. sabina* and a natural dye obtained from *P. americana* berries contributes to preserve the sowing qualities of seeds and improve the growth characteristics of corn seeds over a storage period. Due to the original dye, this composition allows for easy identification of coated seeds. Studied coating composition showed promising results in terms of physical aspects of corn seeds and their physiological potential.

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AGRONOMIC EVALUATION OF COMMON WHEAT VARIETIES FOR PRODUCTIVITY AND QUALITY CHARACTERISTICS

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Abstract

A field trial of five common winter wheat cultivars was conducted from 2021 to 2024 in SouthEastern Bulgaria. The experiment was carried out employing a block-plot method of design with four replications and a plot area of 15 m², following a coriander predecessor. The winter wheat varieties Asterion, Sofru, Lazuly, Avenu, and Pibrak were examined. The objective of the study was to determine and compare elements of productivity, grain yield, and some qualitative measurements of five common wheat types in southeastern Bulgaria. The results demonstrate that the assessed varieties displayed the highest values of productive structural elements in Avenu cultivar. The thousand kernel grain- and Test Weight of the Asterion variety were the highest. The grain of the Sofru cultivar had the greatest vitreousness. The Lazuly variety indicate better technological features of grain (wet gluten, dry gluten, and gluten extension), compared to the other examined common wheat varieties.

Key words*: common wheat, grain yield, thousand kernel weight, test weight, gluten content.*

INTRODUCTION

One of the main tasks of agriculture on a global scale is solving the food problem for feeding the world's population. Wheat occupies first place in the group of cereals. More than half of the global population utilizes it for sustenance. Its wide and diverse use is determined by the high nutritional value and the excellent taste qualities of the products obtained from it. The variety with its specific genetic predispositions has a decisive influence on the realization of the productive possibilities (Aktas et al., 2017; Mut et al., 2017; Studnicki et al., 2018). New varieties of common wheat are continually developed and introduced both domestically and internationally, assessing their productivity and adaptation to environmental circumstances (Georgieva, & Kirchev, 2020; Chamurliiski, 2019; Matev & Kirchev, 2010; Mitkov et al., 2018; Tityanov et al., 2020). Research by numerous scientists indicates that common wheat varieties exhibit varying productive capabilities throughout distinct agroecological regions of the country (Dallev & Ivanov 2015; Dimitrov et al., 2023; Manilov, 2022; Uhr et al., 2023; Uhr et al., 2021; Chipilski et al., 2022; Stoyanova et al., 2022). The appropriate selection of a variety is crucial for both yield quantity and production quality. The quality of wheat grain encompasses a range of indices that reflect its physical state, chemical composition, and biochemical-technological properties. The values of these indicators for each variety are genetically predetermined yet affected by the agricultural practices employed, climatic conditions throughout the growing season, and the particular agro-ecological circumstances of the region (Atanasov et al., 2020; Stamatov et al., 2017; Tsenov et al., 2020; Yanev et al., 2021). Therefore, research related to the cultivation of varieties of common wheat in different regions of the country has a certain scientific and practical importance (Dimitrov et al., 2016; Kaya & Akcura, 2014; Kirchev & Delibaltova, 2016; Tsenov et al., 2022). This requires the ongoing deployment of higher quality and stronger varieties that are most suitable and effective for individual microdistricts of the country (Ilieva, 2011; Ivanova et al., 2010; Yanchev & Ivanov, 2016). The study aimed to identify and compare the productivity components, grain yield, and certain qualitative markers of five varieties common wheat in South-Eastern Bulgaria.

MATERIALS AND METHODS

The experiment was performed on a carbonate resinous soil type from 2021 to 2024 in the region of town Elhovo, Southeastern Bulgaria. The experiment was conducted using a block design with four replications, with a crop plot size of 15 m² following the predecessor of coriander. The cultivars 'Asterion', 'Lazuly', 'Sofru', 'Avenu', and 'Pibrak' were examined. The pre-sowing soil treatment involved 2-3 passes of harrow disking. Sowing occurred at an optimal period at sowing densities of 550 g.s./m^2 and a seeding rate of 240-260 kg ha^{-1}. Phosphorus and Potassium fertilizers (P_{12}, K_8) were spread during the initial treatment, along with one-third of the Nitrogen fertilizer (N_5), while the remaining Nitrogen fertilizer (N_{10}) was applied as top dressing in early spring. Weed management was executed using the herbicides Derby Super (30 g ha^{-1}) and Puma Super (1 L ha^{-1}). To achieve the study's objecttive, the subsequent indicators were considered: grain yield (kg ha^{-1}), plant height (cm), spike length (cm), number of spikelets per spike, number of grains per spike, weight of grains per spike (g), Thousand Grain Weight (TGW) - g, Test Weight - kg, vitreousness - %, dry and wet gluten - % and relaxation of gluten - mm.

The acquired data were quantitatively analyzed employing the analysis of variance (ANOVA) method, and the differences among the variants were assessed using Duncan's multiple range test. The correlation among grain yield, productivity factors, and the physical and technological attributes of grain was established following the methodologies of Gomez & Gomez (1984) and Sokoto et al. (2012).

The main meteorological factors influencing the growth and development of wheat are the average diurnal and nocturnal temperatures, together with the amount and distribution of precipitation throughout the growing season. Throughout the three years of the study, the monthly recorded temperature values are close or slightly higher than those of the multi-year average, with no observed deviations from crop requirements (Figure 1).

The differences among the three years of the study are manifested in the rainfall distribution during the growth season. The smallest amount of precipitation was observed in the year 2023-2024 - 324 mm, with a sum for multi- year period of 432 mm, indicating that this last experimental year is less favorable to plant growth and development compared to previous years. The 2022-2023 year of study is marked by the highest volume of precipitation for vegetation, totaling 537 mm, which is irregularly distributed and exceeds the 1961-1991 average by 104 mm, rendering this economic year very advantageous for wheat cultivation. The period most conducive to plant development is 2021-2022. This year's precipitation was evenly distributed to satisfy the crop's needs during the growing season (Figure 2).

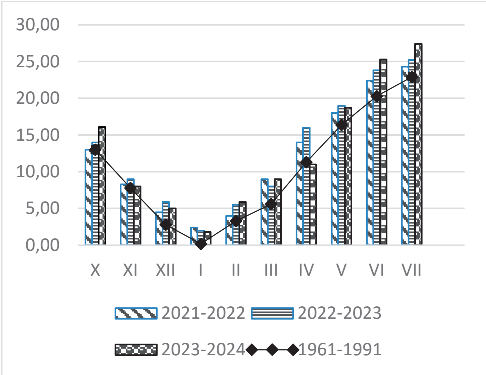

Figure 1. Average monthly air temperature, ^{0}C

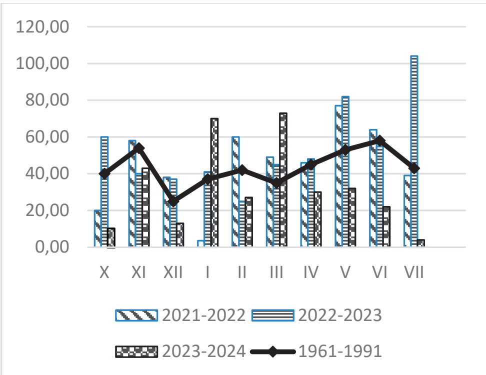

Figure 2. Rainfall, mm

RESULTS AND DISCUSSIONS

Table 1 indicates that, both annually and on average during the study period, the Avenu variety exceeds the other varieties in grain yield. The increased productivity of this variety is attributable to the superior values of the structural elements of yield. Due to improved moisture availability to plants during the growing season and the grain formation and filling period in 2021-2022, higher results were achieved compared to 2022-2023 and 2023-2024. This year, yields range from 6040 kg ha^{-1} for the Sofru variety to 7100 kg ha^{-1} for the Avenu variety. In the Lazuly, Asterion, and Pibrak varieties, the yields were 400, 600, and 840 kg ha^{-1} lower than the Avenu variety, and 660, 460, and 220 kg ha^{-1} higher than the Sofru variety, respectively. Statistical evidence substantiates the differences among all cultivars.

Table 1. Grain yield - kg ha^{-1}

Variety	Years of study			Average for the period
	2021-2022	2022-2023	2023-2024	(kg ha^{-1})
Asterion	6500 ^{c}	5720 ^{c}	5600 ^{b}	5940
Lazuly	6700 ^{b}	5990 ^{b}	5670 ^{b}	6120
Sofru	6040 ^{e}	5390 ^{e}	5100 ^{d}	5510
Avenu	7100 ^{a}	6830 ^{a}	6320 ^{a}	6750
Pibrak	6260 ^{d}	5510 ^{d}	5420 ^{c}	5730

*Means within columns followed by different lowercase letters are significantly different ($P<0.05$) according to the LSD test.

During the 2022-2023 growing season, which recorded the highest precipitation levels, although less from April to June, the evaluated cultivars yielded on average of 10.7% lower than in 2021-2022. In the second experimental year, grain yields varied from 5390 kg ha^{-1} for the Sofru variety to 6830 kg ha^{-1} for the Avenu variety. The differences between the tested varieties have been mathematically substantiated. The lowest grain yields observed throughout the analyzed period occurred in the third experimental year (2023-2024), and were caused by insufficient moisture during the crucial stages of wheat plant growth and development. In this year, the lowest yields were obtained from the Sofru variety - 5100 kg ha^{-1}, giving way to the varieties - Pibrak with 6.2% - Asterion with 9.8%, Lazuli with 11.2%, and for the Avenue variety with 23.9%. Over the three-year experimental period, the Avenue variety had the highest grain yield at 6750 kg ha^{-1}, followed by the Lazuli variety at 6120 kg ha^{-1}, and the Sofru variety with a yield of 5510 kg ha^{-1}. The Asterion and Pibrak cultivars yield 5940 kg ha^{-1} and 5730 kg ha^{-1}, respectively.

The results of the variance analysis of the grain yield data of five varieties of common wheat show a significant reliable influence of the studied factors: year and variety, while the interaction between them is insignificant (Table 2).

Table 2. Analysis of variance ANOVA

Source of Variation	Sum of Square	df	Mean Square	F	P-value	F crit
Variety*	10968000	4	2742000	79.44756	0.00	2.578739
Year**	8317960	2	4158980	120.5036	0.00	3.204317
Interactions ^{ns}	527040	8	65880	1.908828	0.08	2.152133
Within	1553100	45	34513.33			

*F-test significant at $P<0.05$; **F-test significant at $P<0.01$; ns - non-significant

Owing to the unidirectional format of the data over the three-year study period, Table 3 displays the average values obtained for plant height and yield structural elements. The height of the plants partially influences the variety's resistance to lodging. Of all the varieties examined, the Pibrak variety formed the tallest plants - 103 cm, succeeded by the Asterion variety - 96.0 cm, while the Avenu variety was the shortest at 81.0 cm. The plant height of the cultivars Lazuly and Sofru are 85.0 cm and 90.0 cm, respectively. The results obtained are statistically validated. Lodging was not observed in the investigated variaties during the investigation period. The primary structural components influencing grain yield are spike length, the number of spikes per plant, and the number and weight of grains per spike. The spike length in the examined cultivars varies from 8.3 cm in the Pibrak variety to 11.5 cm in the Avenu variant. Statistically proven, this variety is superior in this indicator from 10.6 to 38.5% to the remaining varieties involved in the investigation. The Avenu variety exhibits the highest spikelet number - 23.0, followed by the Asterion and Lazuly cultivars with 21 and 20 spikelets, respectively, while the Sofru and Pibrak varieties have the lowest numbers at 18 and 17 spikelets. The quantity of grains per spike is of significant relevance. The Avenu variety is distinguished by the highest number of grains - 51 pieces. This cultivar surpassed Sofru, Pibrak, Asterion, and Lazuly by 34.0%, 27.0%, 18.6%, and 11.0%, respectively. The results were statistically significant, as were the differences between cultivars. In terms of the structural components of yield and the grain weight indicator values per spike, the Avenu variety outperforms the other varieties in the experiment. For this variety, an average of 1.76 g was documented during the period 2021-2024. The values for the remaining cultivars range from 1.29 g for the Sofru variety to 1.61 g for Lazuly. The disparities among the examined varieties have been mathematically proven.

Table 3. Height of plants and structural elements of the yield, average during the period 2021-2024

Variety	Height of plants (cm)	Length of spike (cm)	Number of spikelets per spike	Number of the grains per spike	Weight of the grains per spike (g)
Asterion	96.0 [b]	9.2 [c]	21.0 [b]	43.0 [c]	1.53 [c]
Lazuly	85.0 [d]	10.4 [b]	20.0 [b]	46.0 [b]	1.61 [b]
Sofru	90.0 [c]	8.5 [d]	18.0 [c]	38.0 [e]	1.29 [e]
Avenu	81.0 [e]	11.5 [a]	23.0 [a]	51.0 [a]	1.76 [a]
Pibrak	103.0 [a]	8.3 [d]	17.0 [c]	40.0 [d]	1.48 [d]
LSD5%	*3.84*	*1.11*	*1.86*	*2.46*	*0.13*

*Means within columns followed by different lowercase letters are significantly different ($P<0.05$) according to the LSD test.

The findings on the physical and technical features of the grain are displayed in Table 4. The TGW acts as an indicator of the grain's size, technological worth, and quality as seed material. The results indicate that the advantageous meteorological circumstances during the wheat growing season in the economic year 2022 resulted in higher values for this indicator compared to the other variables included in the experiment. The Asterion variety produces the largest grain at 51.6 g, followed by Lazuly and Pibrak at 48.0 g and 47.2 g, respectively, while the Sofru variety yields the smallest grain at 37.2 g. The differences among the examined cultivars were statistically confirmed. In the economic year 2022-2023, the TGW for the Sofru variety is 34.7 g, while for the Asterion variety, it is up to 46.0 g. Mathematical evidence demonstrates that the Asterion variety exhibits the greatest values of this indicator, while the Sofru variety displays the lowest. The distinctions among the other variations remain unproven. The drought and higher temperatures during grain formation and maturation in the previous research year (2023-2024) adversely affected grain size. The Sofru variety exhibited the lowest weight per TGW at 32.0 g, succeeded by Avenu at 39.5 g, and the Pibrak and Lazuly at 41.0 g and 41.6 g, respectively. Statistically, the Asterion variety surpassed these by 3.6% and 34.7%. During the period from 2021 to 2024, the Asterion variety is distinguished by having the largest grain, followed by the Lazuly, Pibrak, Avenu, and Sofru varieties.

Test Weight is the commercial indicator of grain and plays an important role in determining the selling price. The values of this indicator for the tested varieties were the lowest in the 2024 business year compared to 2023 and 2022. The test weight in the last year of study ranged from 71.6 for the Avenu variety up to 75.0 in Asterion variety. During the first and second experimental years, the Test Weight values varied from 73.0 to 78.2 in 2023 and from 75.0 to 81.9 in 2022. During the experimental period, the Asterion variety exhibited the highest test weight of grain at 78.4, while the Avenu and Sofru variety recorded the lowest at 74.2 kg.

The overall vitreousness of the grain is an important indicator of the quality of the wheat grain. The highest percentage of vitreousness from 69.3 to 84.6% in the studied varieties was reported in the economic year 2024, when the amount of precipitation was insignificant during the growing season. Lower values of this indicator for the tested varieties were reported in the 2023 harvest year, which can be accounted for by most of the precipitation during the wheat growing season. The percentage of vitreousness in the varieties is from 63.0% in the Avenue variety to 77.5% in the Sofru variety. The vitreousness of the grain in the initial experimental year across the evaluated cultivars ranged from 67.1% to 83.0%. During the research period, the Sofru variety surpassed the Avenue, Pibrak, Lazuli, and Asterion varieties in total grain vitreousness by 22.8%, 11.2%, 10.5%, and 4.4%, respectively.

Table 4. Physical and technological properties of the grain

Variable		TGW (g)	Test Weight (kg)	Vitreousness (%)	Wet gluten content (%)	Dry gluten content (%)	Gluten Extension (mm)
Years (A)	2021-2022	45.8 a	77.7 a	75.7 b	23.2 b	7.9 b	8.5 b
	2022-2023	41.5 b	75.2 b	71.1 c	21.7 c	6.9 c	10.2 a
	2023-2024	39.4 c	73.1 c	77.5 a	25.7 a	8.7 a	7.7 c
Variety (B)	Asterion	47.0	78.4	78.2	22.9	7.4	7.9
	Lazuly	44.0	75.8	73.9	27.2	9.7	6.6
	Sofru	34.6	74.1	81.7	22.0	7.1	8.9
	Avenu	42.0	74.2	66.5	20.2	6.7	11.0
	Pibrak	43.7	75.1	73.5	25.2	8.5	9.6
2021-2022	Asterion	51.6 a	81.9 a	78.8 b	23.0 b c	7.6 c	7.3 d
	Lazuly	48.0 b	78.5 b	75.0 c	26.0 a	9.6 a	6.5 e
	Sofru	37.2 d	76.2 c	83.0 a	22.1 c	7.5 c	8.8 c
	Avenu	45.0 c	75.0 d	67.1 d	20.7 d	6.9 d	10.4 a
	Pibrak	47.2 b	76.8 c	74.5 c	24.0 b	8.2 b	9.7 b
2022-2023	Asterion	46.0 a	78.2 a	74.6 b	21.3 c	6.5 c	9.5 c
	Lazuly	42.5 b	76.0 b	70.2 c	25.4 a	8.4 a	7.4 d
	Sofru	34.7 c	73.6 c	77.5 a	20.8 c	6.2 c	10.0 c
	Avenu	41.4 b	73.0 c	63.0 d	18.0 d	5.6 d	13.2 a
	Pibrak	43.0 b	75.0 b	70.0 c	22.8 b	7.8 b	11.0 b
2023-2024	Asterion	43.1 a	75.0 a	81.2 b	24.5 c	8.0 c	7.1 d
	Lazuly	41.6 b	73.0 b	76.5 c	30.2 a	11.0 a	6.0 e
	Sofru	32.0 d	72.5 b c	84.6 a	23.0 d	7.7 c	7.9 c
	Avenu	39.5 c	71.6 c	69.3 d	22.0 d	7.5 c	9.5 a
	Pibrak	41.0 b	73.4 b	76.0 c	28.9 b	9.5 b	8.1 b
Anova	A	*	*	**	*	*	*
	B	**	*	*	**	**	**
	AB	*	*	ns	*	*	*

*Means within columns followed by different lowercase letters are significantly different (P<0.05) according to the LSD test.
*F-test significant at P<0.05; ** F-test significant at P<0.01; ns - non-significant.

The two-factor variance analysis of the grain's physical properties indicates that both the genetic predisposition of the varieties and the specific climatic conditions of the year show a statistically significant influence on the TGW, Test Weight, and total vitreousness. The interaction between the two factors (Variety x Year) has been statistically proven for the indicators of weight per 1000 grains (η 46) and test weight (η 52), whereas it is not significant for total vitreousness. The results obtained regarding the technological properties of the grain in the tested varieties over three years of research indicate that the wet gluten content is lowest in the Avenue variety, with values of 22.0%, 18.0%, and 20.7%, and highest in the Lazuli variety, with values of 30.2%, 25.4%, and 26.0% for the economic years 2022, 2023, and 2024, respectively. The results were statistically significant over all three years of the study. During the period from 2021 to 2024, the experimental varieties produced grains with wet gluten percentages of 7.9%, 18.8%, and 23.6% for the Pibrak, Asterion, and Sofru varieties, respectively, while the Avenu variety exhibited a percentage of 34.6%, all of which were inferior to that of the Lazuly variety. In the dry and warm year 2024, the content of both dry and wet gluten exhibits the highest proportion compared to other years. In the evaluated variations, this parameter ranges from 7.5% (for the Avenu variety) to 11.0% (for the Lazuly variety). During the second trial year, the dry gluten content exhibited its lowest values, ranging from 5.6% to 8.4%, whereas in the economic year 2022, it ranged from 6.9% to 9.6%. During the study period, the Lazuly variety exhibited the highest average percentage of dry gluten at 9.7%, while the Avenu variety had the lowest at 6.7%, with statistically significant differences between them. The gluten relaxation indicator shows that the data for the year that has more precipitation (2022-2023) for the investigated varieties exhibit slightly higher values compared to 2023-2024 and 2021-2022, ranging from 7.4 to 13.2 mm. This year, the Lazuly variety had the lowest gluten relaxation, succeeded by the Asterion and Sofru variety, while the Avenu variety demonstrated the highest gluten relaxation. In the years 2023-2024 and 2021-2022, the values of this indicator ranged from 6.0 to 9.5 mm and from 6.5 to 10.4 mm, respectively, with the lowest confirmed values recorded for the Lazuly variety. The average data for the three-year period indicates that the variety Lazuly demonstrates the lowest level of gluten relaxation at 6.6 mm, followed by Asterion at 7.9 mm, Sofru at 8.9 mm, Pibrak at 9.6 mm, and Avenu at 11.0 mm. The analysis of variance results indicated that the variety had the most significant impact on the levels of wet gluten, dry gluten, and gluten extension (Table 4). The year with the specific meteorological conditions demonstrably affects the values of these indicators. The interactions between the examined parameters are significant for all the analyzed technological properties of the grain.

In the correlation analysis between grain yield, structural elements and grain quality indicators, a very high correlation dependence ($r > 0.9$) was found between the following indicators: grain yield and spike length; number of grains per spike and grain yield; grain weight per spike and grain yield; the length of spike and number of spikletes per spike; number of grains per spike and spike length; number of spikelets per spike and number of grains per spike; wet and dry gluten content (Table 5). High positive values of r ($r > 0.8$) are reported between grain yield and number of spikelets per spike; spike length and spike weight; number of spikelets per spike and grain weight per spike. Average correlation dependencies exist between indicators: Test Weight and TGW ($r = 0.745$); TGW and grain weight per spike ($r = 0.514$). Weak correlation dependences ($r > 0.3$) were reported between grain yield and TGW; grain yield and gluten extension; plant height and vitreousness; TGW and number of grains per spike; vitreousness and wet gluten content.

Table 5. Values of the coefficient of correlation

	1. Grain yield	2. Height of plant	3. Length of spike	4. Nr of spikelets per spike	5. Nr of grains per spike	6. Weight of grains per spike	7. TGW	8. Test Weigh	9. Vitreousness	10. Wet gluten content	11. Dry gluten content	12. Gluten extension
1.	1											
2.	-0.684	1										
3.	**0.954**	-0.853	1									
4.	**0.897**	-0.739	**0.901**	1								
5.	**0.989**	-0,723	**0.977**	**0.902**	1							
6.	**0.959**	-0.524	**0.891**	**0.811**	**0.965**	1						
7.	0.322	0.193	0.214	0.269	0.354	0.514	1					
8.	-0.165	0.281	-0.172	-0.011	-0.118	-0,046	0.745	1				
9.	-0.807	0.364	-0.697	-0.552	-0.775	-0.829	-0.345	0.161	1			
10.	-0.277	0.272	-0.209	-0.399	-0.191	-0.079	0.181	0.042	0.308	1		
11.	-0.171	0.153	-0.087	-0.319	-0.081	0.016	0.228	0.055	0.242	**0.969**	1	
12.	0.317	-0.041	0.149	0.191	0.203	0.201	-0.138	-0.291	-0.599	-0.793	-0.781	1

*significance level alpha = 0.05.

CONCLUSIONS

The studied common wheat variaties - Asterion, Lazuly, Sofru, Avenu, and Pibrak - exhibit no tendency for lodging. In the research period (2021-2024), the Avenu variety achieved the highest grain yields at 6750 kg ha^{-1}, surpassing other varieties by 10.3 to 22.5% in term of yield, due to the higher values of its structural elements. Throughout the study period, common wheat varieties in South-Eastern Bulgaria formed a grain, the values of the indicators - TGW and Test Weight were the highest in the Asterion variety (47.0 g and 78.4 kg). The Sofru variety showed the highest vitreousness percentage at 81.7%, while the Avenu variety displayed the lowest at 66.5%. The analyzed technological properties of the grain (wet gluten, dry gluten, and gluten extension) in the evaluated common wheat varieties exhibit the best results in the Lazuly variety (27.2%, 9.7%, and 6.6 mm).

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PHENOTYPIC VARIABILITY OF COMPONENTS OF PRODUCTION IN WHEAT (*Triticum aestivum* L.), UNDER THE CONDITIONS OF THE SOUTH OF THE COUNTRY

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Abstract

The aim of this paper is to conduct an ecological study of the variability of productivity elements in native wheat varieties. The research was carried out under field conditions at the Teleorman Agricultural Development Research Station in the agricultural years 2019-2020, 2020-2021, 2021-2022 and 2022-2023 on a pseudoglazed cambic chernoziom soil. 10 wheat varieties created at INCDA Fundulea were analyzed both from the phenological point of view and the variation of productivity elements under the influence of climatic factors. The productivity elements, although they were influenced by the climatic conditions of the years of study, had a small and medium variation, proving/confirming the fact that the native breeding material has a high adaptability to the variations of the climatic factors. The varieties Abund (7755 kg/ha), Ursita (7295 kg/ha) and Otilia (7117 kg/ha) stood out, drought-resistant varieties with productivity and quality corresponding to market requirements and high adaptability to biotic and abiotic factors, in conditions of climate change that can be cultivated successfully in the southern part of the country.

***Key words**: wheat varieties, climate change, productivity elements, variability, production.*

INTRODUCTION

The ideal wheat variety for high productivity or any other desired trait must express genetic potential with a low value of variation in different crop environmental factors. Adaptation and productivity are, however, both complexly inherited traits and highly affected by the environment (Alard, 1997).

Understanding the causes of genotype-environment interaction can be used to set breeding goals, identify ideal test conditions, and formulate optimal cultivar recommendations for specific crop areas. (WeikaI and Hunt, 2001). The presence of genotype-environment interaction complicates the selection of superior genotypes, and understanding the environmental and genotypic causes of significant genotype-environment interaction is important at all stages of plant breeding (Dhungana et al., 2007).

Stability and adaptability represent the response of the genotype to environmental variation. Adaptability is a natural reaction of the genotype to survive and reproduce. Stability means a very small genotypic reaction to environmental changes, and in a broad sense, it could not be considered evolutionarily favorable under natural conditions. However, in agriculture, stability represents a desirable response of cultivated genotypes, forced and supported by humans, ensuring a similar level of production under different environmental conditions (Federer and Sculy, 1993; Lin and Binns, 1991; 1994).

Wheat production is a quantitative character with high variability, which is given by numerous production components, their formation under the influence of environmental conditions (Kraljevic-Balalic et al., 2001).

Global climate change, as well as regional climate change, influences agricultural behavior (Finlay and Wilkinson, 1963; Eberhart and Russel, 1966; Brady and Gabriel, 1978).

Production capacity is a complex quantitative character, determined by intrinsic factors (production components) and influencing factors (resistance to the unfavorable action of external factors). Each element of production is in turn a complex quantitative character, conditioned by hereditary factors and external factors.

Wheat production, according to Kamaluddin et al. (2007) depends on the number of grains/surface unit and the weight of the grain, the latter being the resultant between the grain filling rate and the time period in which it was achieved (Gebeyehou et al., 1982; Van Sanford and Mackown, 1985; Bruckner and Frohberg, 1987).

The purpose of this work was to conduct an ecological study on the variability of productivity elements in the native wheat varieties developed at NARDI Fundulea.

MATERIALS AND METHODS

In the years 2019-2023 at the Teleorman Agricultural Development Research Station, a monofactorial experiment was established with 10 genotypes of common winter wheat. The research was carried out on a vertic subtype cambic chernoziom soil, having swelling clays as parent rock, a loamy-clay texture at the depth of the plowed layer (0-25 cm). From the point of view of physical and chemical properties, the soil is characterized by a clay content of 45%, humus 3.1%, slightly acidic soil reaction (pH = 6.1-6.5), total nitrogen content 0.166%, phosphorus mobile 40-60 ppm and mobile potassium 250 ppm.

The main hydro-physical indices of the soil on the 0-80 cm horizon have the following average values: apparent density 1.43 t/m^3, field capacity 27.3% (310.4 mm), wilting coefficient 15.0% (171.0 mm), minimum ceiling 21.1% (240.7 mm).

Applied technology: After harvesting the preceding plant, plant remains were destroyed, after which work was carried out with a disc, perpendicular passes. The preparation of the germinal bed was carried out with the combiner, 2 perpendicular works before sowing, when chemical fertilizers were incorporated into the soil (45 N s.a. kg/ha; 45 P s.a. kg/ha, 45 K s.a.kg/ha). After sowing, as the sowing conditions were very bad (drought), a ring roller work was carried out. In the winter windows, 54 N s.a. was applied. kg/ha, and in the spring in the last decade of March - the first decade of April (depending on the evolution of the climatic conditions in the study year) a phytosanitary treatment was applied with the following pesticide products: Mustang (florasulam 6.25 g/l + acid 2.4 D EHE 300 g/l) in a dose of 0.6 l/ha; Nuance (750 g/kg tribenuron methyl) in a dose of 14 g/ha; Inazuma (Acetamiprid 100 g/kg + Lambda-cyhalothrin 30 g/kg) in a dose of 0.3 kg/ha; Basfoliar Extra (N 27%; Mg 3%; + Mn, Fe, B, Zn, Mo, Cu) in a dose of 3l/ha; Trend (adjuvant) 0.150 l/ha in 180 l of water/ha. In order to test the genetic resistance of the variety to the attack of pathogens, no treatments with fungicidal products were carried out.

The years of experimentation were different from the point of view of the evolution of average monthly temperatures and precipitation during the vegetation period of autumn grass cereals.

The agricultural year 2022-2023, from the point of view of the average temperature recorded per agricultural year (13.6^{0}C), was the warmest year of the four years of study, compared to the multiannual average (10.70^C), the deviation was +2.8^{0}C, in 2019-2020 the deviation was positive 2.7^{0}C, in the agricultural year 2020-2021 positive deviation of 2.3^{0}C, and in the agricultural year 2021-2022 +1.9^{0}C. During the entire vegetation period, the deviations from the multi-annual average were positive, except for May, when the average deviation for the research years was -0.9^{0}C (Table 1).

Table 1. The evolution of average monthly temperatures during the years of experimentation at ARDS Teleorman

The year agricultural	Month												Average
	IX	X	XI	XII	I	II	III	IV	V	VI	VII	VIII	
2019-2020	20.2	14.0	10.4	3.3	0.5	4.9	8.0	11.9	16.6	21.1	24.7	25.4	13.4
Deviation	**+2.2**	**+2.4**	**+6.0**	**+3.6**	**+3.8**	**+5.4**	**+3.4**	**+0.0**	**-0.2**	**+0.5**	**+2.1**	**+3.0**	**+2.7**
2020-2021	21.3	14.5	5.1	3.4	0.8	3.1	4.7	9.5	17.3	24.6	26.3	25.5	13.0
Deviation	**+3.3**	**+2.9**	**+0.7**	**+3.7**	**+4.1**	**+3.6**	**+0.1**	**-2.4**	**+0.5**	**+4.0**	**+3.7**	**+3.1**	**+2.3**
2021-2022	18.5	10.4	7.3	2.1	1.4	4.4	4.0	11.4	18.0	23.0	25.7	25.9	12.7
Deviation	**+0.5**	**-1.2**	**+2.9**	**+2.4**	**+4.7**	**+4.9**	**-0.6**	**-0.5**	**+1.2**	**+2.4**	**+3.1**	**+3.5**	**+1.9**
2022-2023	18.9	14.1	8.8	2.9	4.3	3.6	7.9	11.1	16.2	21.9	26.6	26.5	13.6
Deviation	**+0.9**	**+2.5**	**+4.4**	**+3.2**	**+7.6**	**+4.1**	**+3.3**	**-0.8**	**-0.6**	**+1.3**	**+4.0**	**+4.1**	**+2.8**
Average 126 year	*18.0*	*1.6*	*4.4*	*-0.3*	*-3.3*	*-0.5*	*4.6*	*11.9*	*16.8*	*20.6*	*22.6*	*22.4*	10.7
Average deviation	**+1.7**	**+1.7**	**+3.5**	**+3.2**	**+5.1**	**+4.5**	**+1.6**	**-0.9**	**+0.2**	**+2.1**	**+3.2**	**+3.4**	**+2.4**

From the point of view of rainfall, the study years are characterized as follows: the 2020-2021 agricultural year the rainiest with the amount of precipitation of 697.5 mm with a deviation of +147.0 mm from the multi-year average, and the years 2019-2020, 2021-2022 and 2022-2023 dry years, the deviations from the multiannual average being -105.1 mm, -124.5 mm and -120.5 mm respectively (Table 2).

Table 2. Precipitation evolution during the years of experimentation at ARDS Teleorman

The year agricultural	Month												Total
	IX	X	XI	XII	I	II	III	IV	V	VI	VII	VIII	
2019-2020	2.5	28.4	51.8	16.0	5.8	68.5	74.0	20.0	79.0	84.0	2.8	12.6	**445.4**
Deviation	**-41.7**	**-10.1**	**+10.1**	**-25.4**	**-31.8**	**+37.8**	**+39.2**	**-22.1**	**+18.0**	**+12.0**	**-57.4**	**-33.7**	**-105.1**
2020-2021	65.0	64.0	8.0	79.0	118.0	10.5	98.0	36.0	83.0	99.0	1.0	36.0	**697.5**
Deviation	**+20.8**	**+25.5**	**-33.7**	**+37.6**	**+80.4**	**-20.2**	**+63.2**	**-6.1**	**+22.0**	**+27.0**	**-59.2**	**-10.3**	**+147.0**
2021-2022	4.5	111.4	31.5	59.0	7.0	19.0	19.0	73.0	22.0	36.5	22.1	21.0	**426.0**
Deviation	**-39,7**	**+72.9**	**-10.2**	**+17.6**	**-30.6**	**-11.7**	**-15.8**	**+30.9**	**-39.0**	**-35.5**	**-38.1**	**-25.3**	**-124.5**
2022-2023	26.0	9.0	61.0	39.5	87.0	12.5	24.0	55.0	54.0	31.0	16.0	15.0	**430.0**
Deviation	**-18.2**	**-29.5**	**+19.3**	**-1.9**	**+49.4**	**-18.2**	**-10.8**	**+12.9**	**-7.0**	**-41.0**	**-44.2**	**-31.3**	**-120.5**
Average 126 year	*44.2*	*38.5*	*41.7*	*41.4*	*37.6*	*30.7*	*34.8*	*42.1*	*61.0*	*72.0*	*60.2*	*46.3*	**550.5**
Average deviation	**-19.7**	**+14.7**	**-3.6**	**+7.0**	**+16.9**	**-3.1**	**+19.0**	**+3.9**	**-1.5**	**-9.4**	**-49.7**	**-25.2**	**-50.8**

Although the amounts of precipitation that fell during the wheat vegetation period as total amounts are not insignificant quantitatively, their distribution, according to the necessary vegetation phases, was unevenly distributed, which had repercussions on the quantity and quality of the productions obtained.

The data obtained as a result of biometric measurements, determinations and laboratory analyzes were statistically processed by determining the average values, the standard deviation of the average and the coefficient of variability, according to ARDELEAN (2008):

- arithmetic mean;
- the standard deviation of the mean;
- coefficient of variability (s%)
- analysis of variance (ANOVA)

The simple correlation coefficients (r) were calculated for the characteristics analyzed in each of the four experimental years, in order to establish the degree of association between the characteristics and the correlated ones.

RESULTS AND DISCUSSIONS

The preceding plant in the years 2019-2020 and 2021-2022 was the chickpea, and in the years 2020-2021 and 2022-2023 the pea (Table 3).

In the agricultural years 2019-2020 and 2020-2021 the experiments were sown on October 17 and emerged 23 days after sowing on November 9. In the year 2021-2022, sowing took place on October 27, and the emergence was recorded 14 days after sowing on November 10, and in the year 2022-2023 the experience was sown on 24.10.2022 and emerged after 57 of days on 20.12.2022 (Table 3).

Table 3. Sowing and emergence data from common winter wheat experiments

The year agricultural	Date of sowing	Date of emergence	Number of days sowing - emergence	The preceding plant
2019-2020	17.10.2019	9.11.2019	23	chickpeas
2020-2021	17.10.2020	9.11.2020	23	peas
2021-2022	27.10.2021	10.11.2021	14	chickpeas
2022-2023	24.10.2022	20.12.2022	57	peas

The density used for sowing was 550 b.g./m^2, and the number of plants sprouted in autumn, on average per experience, was 455 pl/m^2 in the year 2019-2020, 489 pl/m^2 in the year 2020-2021, 472 pl /m^2 in the year 2021-2022 and 485 pl/m^2 in the year 2022-2023 (tab. 4). The reduced density at sunrise in the year 2019-2020 (455 pl/m^2) is due to the very low water regime in that year.

All tested genotypes had weak twinning, thus the highest density, on average over the years of experimentation, was the Glosa variety (577 spikes/m^2), at the opposite pole was the Pitar variety (548 spikes/m^2) with the lowest number of ears per m^2.

Table 4. Density at sunrise of common winter wheat genotypes, ARDS Teleorman - 2019-2023

No.	Variant	No. emerged plants				Average genotype	Coefficient of variation (%)
		2019-2020	2020-2021	2021-2022	2022-2023		
1	Glosa	467	487	480	501	484	2.93
2	FDL Miranda	450	450	503	489	473	5.74
3	Otilia	467	497	485	489	485	2.62
4	Pitar	440	502	495	486	481	5.81
5	Izvor	465	494	491	477	482	2.78
6	Ursita	455	498	477	493	481	4.03
7	Voinic	448	500	413	459	455	7.88
8	FDL Abund	468	496	465	504	483	4.07
9	FDL Amurg	433	488	469	492	471	5.73
10	FDL Bogdana	455	478	445	455	458	3.05
Average year		455	489	472	485		

On average, on the varieties tested, the highest density of ears/m^2 was recorded in 2023 (598.5 ears/m^2), and the lowest density of ears/m^2 was recorded in the agricultural year 2019-2020 (500.4 ears/ m^2) (Table 5).

The variation of spike density/m^2 was small in 8 of the varieties studied (CV=5.11% Otilia) and medium in 2 varieties (CV= 12.77% FDL Miranda) (Table 5).

Table 5. Ear density per m^2 of common winter wheat genotypes, SCDA Teleorman - 2019-2023

No.	Variant	No. ears/m^2				Average genotype	Coefficient of variation (%)
		2019-2020	2020-2021	2021-2022	2022-2023		
1	Glosa	537	610	556	605	**577**	6.26
2	FDL Miranda	478	631	549	628	572	**12.77**
3	Otilia	521	578	554	584	559	**5.11**
4	Pitar	454	594	553	589	**548**	11.86
5	Izvor	521	574	563	610	567	6.46
6	Ursita	485	594	558	610	562	9.90
7	Voinic	479	579	557	601	554	9.59
8	FDL Abund	512	589	556	600	564	7.01
9	FDL Amurg	510	578	547	608	561	7.49
10	FDL Bogdana	507	588	555	550	550	6.05
Average year		500.4	591.5	554.8	598.5		

Analyzing the number of sprouted plants and the density of ears/m^2, it can be seen that in the formation of the crop, the contribution of the brothers is reduced. Due to the sowing in limiting climatic conditions (drought) which delayed the emergence, the twinning took place in the windows of winter (periods with positive temperatures) and in the late spring, a situation in which the formed brothers did not end up forming ears, only the main brother formed an ear which had little contribution to final wheat production. Considering that among the elements of productivity, the number of plants per surface unit and the number of ears per plant, respectively the number of ears per surface unit influence to the greatest extent the formation of the crop, for the southern part of the country the sowing density must be established so as to ensure a high density of ears without relying on the contribution of siblings.

On average over the years of experimentation, the number of grains in the ear had values between 39 grains/ear in the FDL Abund variety and the Otilia variety, and the lowest number of grains in the ear in the FDL Amurg (34.25 grains/ear).

The highest number of grains in the ear was obtained in the agricultural year 2021-2022, and the lowest number in the year 2020-2021 (Table 6). The coefficient of variation of the tested varieties was small and medium as follows: 5 varieties had small variation, the FDL Amurg variety standing out with CV=5.53%, and 5 medium variation varieties with the highest variation coefficient in the FDL Abund variety (CV+16.08%) (Table 6).

Table 6. The number of grains in the ear of wheat genotypes, ARDS Teleorman-2019-2023

No.	Variant	No. grain in ear				Average genotype	Coefficient of variation (%)
		2019-2020	2020-2021	2021-2022	2022-2023		
1	Glosa	34	33	40	36	35.75	8.66
2	FDL Miranda	35	34	41	34	36.00	9.35
3	Otilia	35	35	42	44	**39.00**	12.03
4	Pitar	37	33	37	37	36.00	5.56
5	Izvor	33	32	40	33	34.50	10.72
6	Ursita	34	34	40	31	34.75	10.86
7	Voinic	34	34	39	43	37.50	11.62
8	FDL Abund	34	34	41	47	**39.00**	**16.08**
9	FDL Amurg	34	33	37	33	34.25	**5.53**
10	FDL Bogdana	35	36	39	40	37.50	6.36
Average year		34.5	33.8	**39.6**	37.8		

The average weight of the grains in the ear was 1.38 g, on average over the years of experimentation, with the maximum value of 1.61 g in the FDL Abund variety and the minimum of 1.28 g in the Izvor variety. The highest weight of grains in the ear was obtained in the agricultural year 2019-2020, and the lowest weight in the agricultural year 2021-2022 (Table 7).

Table 7. Grain weight in the ear of wheat genotypes, ARDS Teleorman-2019-2023

No.	Variant	Grain weight per ear (g)					Coefficient of variation (%)
		2019-2020	2020-2021	2021-2022	2022-2023	Average genotype	
1	Glosa	1.55	1.38	1.27	1.33	1.38	8.71
2	FDL Miranda	1.55	1.38	1.25	1.20	1.35	11.62
3	Otilia	1.39	1.44	1.26	1.43	1.38	**6.00**
4	Pitar	1.67	1.32	1.15	1.38	1.38	15.69
5	Izvor	1.39	1.19	1.33	1.22	**1.28**	7.30
6	Ursita	1.48	1.40	1.26	1.23	1.34	8.78
7	Voinic	1.42	1.41	1.21	1.45	1.37	7.99
8	FDL Abund	1.47	1.48	1.22	1.88	**1.51**	**18.05**
9	FDL Amurg	1.54	1.38	1.12	1.44	1.37	13.08
10	FDL Bogdana	1.38	1.49	1.16	1.34	1.43	10.22
Average year		**1.48**	1.39	1.22	1.39		

The variation of the varieties studied in terms of the weight of the grains in the ear was small in 4 of the varieties with the minimum value of the coefficient of variation of 6.0% in the variety Otilia and 6 varieties varieties with medium variation with the maximum value of the coefficient of variation of 18, 5% in the FDL Amurg variety (Table 7). On average over the years, the weight of 1000 grains had values between 39.59 g for the FDL Amurg variety and 35.11 g for the Otilia variety. The weight of 1000 grains was strongly influenced by the climatic conditions in the years of study but also by the attack of pathogens, very obvious differences between the agricultural years 2019-2020, when the value of the weight of 1000 grains was 43.26 g and the agricultural year 2021 -2022 when the TWG of the varieties studied was 30.99 g (Table 8). Although this character was strongly influenced by climatic conditions, but also by the attack of pathogens, the variation of the varieties studied was medium, the highest coefficient of variation obtained by the variety FDL Miranda (17.5%) (Table 8). As in the case of the TWG, the hectoliter mass was strongly influenced by the climatic conditions (relative air humidity and temperature) as well as by the attack of pathogens in the study year. On average over the years of experimentation, the Ursita variety had the highest hectoliter weight of 78.26 kg/hl. On average, during the 2019-2020 agricultural year, the tested varieties had the highest hectoliter mass at 79.65 kg/hl, while the lowest hectoliter mass value of 72.66 kg/hl was recorded in the year 2021-2022 (Table 9). Strongly influenced by the climatic conditions but also by the attack of pathogens in the study year, the hectoliter mass had a small variation (CV<10%) in the case of all the varieties studied, with the exception of the Bogdana variety which had a medium variation of the MH (10 .99%) (Table 9).

Table 8. Weight of 1000 grains for wheat genotypes, ARDS Teleorman-2019-2023

No.	Variant	TWG (g)					Coefficient of variation (%)
		2019-2020	2020-2021	2021-2022	2022-2023	Average genotype	
1	Glosa	45.72	41.80	32.01	36.44	38.99	15.42
2	FDL Miranda	45.03	39.40	30.84	32.79	37.02	**17.50**
3	Otilia	40.27	38.90	30.06	31.22	**35.11**	14.86
4	Pitar	45.38	39.90	30.82	35.67	37.94	16.32
5	Izvor	42.08	38.30	33.42	31.65	36.36	**13.03**
6	Ursita	43.44	39.90	31.46	35.25	37.51	13.99
7	Voinic	41.99	40.30	31.19	35.01	37.12	13.33
8	FDL Abund	43.32	42.31	30.11	38.82	38.64	15.54
9	FDL Amurg	45.63	41.83	30.25	40.64	**39.59**	16.62
10	FDL Bogdana	39.71	40.18	29.69	36.52	36.53	13.25
Average year		**43.26**	40.28	**30.99**	35.40		

Table 9. Hectoliter mass obtained from common autumn wheat, ARDS Teleorman-2019-2023

No.	Variant	Hectoliter mass (kg/hl)					Coefficient of variation (%)
		2019-2020	2020-2021	2021-2022	2022-2023	Average genotype	
1	Glosa	80.20	80.35	75.75	74.1	77.60	4.07
2	FDL Miranda	78.20	79.00	70.55	69.6	74.34	6.66
3	Otilia	81.10	80.70	72.60	73.3	76.93	5.98
4	Pitar	80.00	79.45	73.25	73.3	76.50	4.88
5	Izvor	78.55	81.30	79.20	72.2	77.81	5.04
6	Ursita	80.30	80.90	76.85	75.0	**78.26**	3.59
7	Voinic	79.80	81.30	75.35	75.1	77.89	4.03
8	FDL Abund	79.00	80.00	70.85	74.6	76.11	5.54
9	FDL Amurg	79.30	76.80	70.60	72.6	74.83	5.27
10	FDL Bogdana	80.00	76.55	61.60	**74.73**	73.22	**10.99**
Average year		**79.65**	79.64	**72.66**	73.45		

The average production obtained by the wheat varieties studied was 6926 kg/ha, on average over the years of experimentation, the FDL Abund variety being noted, which achieved a production of 7755 kg/ha, and the least productive was the FDL Amurg variety which achieved a production of 6275 kg/ha. The highest production level was recorded in the 2022-2023 agricultural year (7301 kg/ha), and the lowest in the 2021-2022 agricultural year (6249 kg/ha). The variation in the production of the varieties studied during the 4 years of experimentation was small (CV>10%) in the varieties Izvor, Voinic, Otilia, Glosa, FDL Miranda and medium (CV>20%) in the varieties FDL Amurg, Pitar, Ursita, FDL Abund and FDL Bogdana (Table 10).

Table 10. Production results obtained in common autumn wheat varieties, ARDS Teleorman-2019-2023

No.	Variant	Average production la U=14% (kg/ha)					Coefficient of variation (%)
		2019-2020	2020-2021	2021-2022	2022-2023	Average genotype	
1	Glosa	7726	7279	6413	6737	7039	8.25
2	FDL Miranda	6619	7690	6189	6906	6851	9.23
3	**Otilia**	**7561**	**7183**	**6344**	**7378**	**7117**	7.55
4	Pitar	6249	7075	5772	7329	6606	10.94
5	Izvor	6135	6221	6877	6951	6546	6.53
6	**Ursita**	**7020**	**7444**	**6356**	**8358**	**7295**	11.50
7	Voinic	7064	7131	6154	6938	6822	6.63
8	**FDL Abund**	**7448**	**7566**	**6980**	**9027**	**7755**	11.41
9	FDL Amurg	5864	6904	5561	6769	6275	10.57
10	FDL Bogdana	7482	7858	5845	6620	6951	12.97
Average year		6917	7235	**6249**	**7301**	6926	

In order to highlight the importance of the experimental factors (genotype and year of experimentation) in the formation of the crop, the statistical analysis of the 10 genotypes of common autumn wheat was carried out.
The calculation and interpretation of production results was based on the analysis of variance of bifactorial experiments arranged in randomized blocks (Săulescu and Săulescu, 1967).
The experimental factors were: Factor A – genotype with 10 gradations (a1=Glosa; a2=FDL Miranda; a3=Otilia; a4=Pitar; a5= Izvor; a6= Ursita; a7= Voinic; a8= FDL Abund; a9= FDL Amurg; a10=FDL Bogdana); Factor B – the year of experimentation with 4 graduations (b1=2019-2020; b2 =2020-2021; b3=2021-2022, b4=2022-2023).
Analyzing the variance table of the bifactorial experience (10 genotypes × 4 years) we observe the very significant influence of the genotype and the year of experimentation (climatic conditions), also the interaction genotype x year of experimentation is very significant for the production of common autumn wheat obtained (Table 11).

Table 11. Analysis of variance (ANOVA) for production at bifactorial experience genotype x year of experimentation

The cause of variability	SP	GL	s²	Test F
The total	62033489	119		
Repetitions	277283.0065	2		
Years	26222839.36	3	8740946.5	260.11***
Genotype	9071453.3	9	1007939.3	29.99***
Interaction year X genotype	23840773.6	27	882991.6	26.28***
Error	2621140	78	33604.4	

The variety influences the production of autumn wheat obtained, on average over the years of experimentation, very significant increases in production of 830 kg/ha were obtained with the FDL Abund variety and the Ursita variety (369 kg/ha), and the Otilia variety obtained a distinct increase in production significantly (191 kg/ha), compared to the experience average (Table 12).
Compared to the favorable agricultural year for the wheat crop, the 2022-2023 agricultural year is noteworthy when very significant production increases of 376 kg/ha were obtained, compared to the average of experience, and the 2020-2021 agricultural year when very significant production increases were obtained significant amounts of 310 kg/ha (Table 13).

Table 12. The influence of the genotype on the production of common autumn wheat, ARDS Teleorman-2019-2023

No.	Variant	Yields		Difference	Signifi-cance
		kg/ha	%	± CO	
1	Glosa	7039	101.63	+113	
2	FDL Miranda	6851	98.92	-75	
3	**Otilia**	**7117**	**102.76**	**+191**	**
4	Pitar	6606	95.39	-319	
5	Izvor	6546	94.52	-380	
6	**Ursita**	**7295**	**105.33**	**+369**	***
7	Voinic	6822	98.50	-104	
8	**FDL Abund**	**7755**	**111.98**	**+830**	***
9	FDL Amurg	6275	90.60	-651	
10	FDL Bogdana	6951	100.37	+26	
Average		6926	100	MT	

LSD 5% = 148.93 kg/ha; LSD 1% = 197.57 kg/ha; LSD 0,1% = 255.20 kg/ha

Table 13. The influence of the year of experimentation on the production of common autumn wheat, ARDS Teleorman-2019-2023

The year of experimentation	Yields		Difference ± CO	Significance
	kg/ha	%		
2019-2020	6917	99.88	-9	
2020-2021	**7235**	**104.47**	**+310**	***
2021-2022	6249	90.23	-677	
2022-2023	**7301**	**105.42**	**+376**	***
Media	6926	100	MT	

LSD 5% = 94.19 kg/ha; LSD 1% = 124.96 kg/ha; LSD 0,1% = 161.40 kg/ha.

The production correlates positively with all the morph-productive elements (no. of emerged plants, no. of ears/m^2, no. of grains in the ear, the weight of the grains/ear and the MH), with the exception of the TWG where the correlation is negative (-0 ,0701). The number of emerged plants correlates negatively with the number of ears/m^2 (-0.0830) and positively with the weight of grains/ear (0.2029), with the TWG (0.0777) and with the MH (0.4134). Grain weight/ear correlates positively with number of emerged plants (0.2029), very weakly positively with number of ears/m^2 (-0.00005) and positively with number of grains per ear (0.6304). The TWG correlates positively with the number of emerged plants (0.0777) and the weight of grains per ear (0.4231) and negatively with the number of ears/m^2 (-0.0497) and the number of grains per ear (- 0.0497). The MH correlates positively with the number of emerged plants (0.4134), with the number of ears/m^2 (0.3274) and negatively with the number of grains in the ear (-0.1334), with the weight of grains in the ear (- 0.0573) and with the TWG (-0.0524) (Table 14).

Table 14. Correlation of yield with different morpho-productive elements in common winter wheat cultivars

	Yields (kg/ha)	No. emerged plants	No. ears/m^2	No. grain in ear	Grain weight/ear (g)	TWG (g)	MH (kg/hl)
Yields (kg/ha)	1						
No. emerged plants	0.2988	1					
No. ears/m^2	0.4175	0.4803	1				
No. grain in ear	0.6339	-0.0830	-0.0181	1			
Grain weight/ear (g)	0.6528	0.2029	0.00005	0.6304	1		
TWG (g)	-0.0701	0.0777	-0.0497	-0.0497	0.4231	1	
MH (kg/hl)	0.1657	0.4134	0.3274	-0.1334	-0.0573	-0.0524	1

In Romania, climate changes have determined in recent years, the intensification of water deficits (often associated with heat) during the vegetation of agricultural crops, in almost all areas of the country. Cultivating winter wheat varieties that can withstand drought and heat well is a decisive factor in obtaining stable and economically efficient productions.

The productivity elements, although they were influenced by the climatic conditions of the years of study, had a small and medium variation, proving/confirming the fact that the native breeding material has a high adaptability to the variations of the climatic factors.

Despite all the unfavorable abiotic factors, the genotypes tested at ARDS Teleorman managed to obtain productions, on average over the years of experience, of 6926 kg/ha.

The Abund, Ursita, Otilia and Glosa varieties stood out, drought-resistant varieties with productivity and quality corresponding to market requirements and high adaptability to biotic and abiotic factors, under the conditions of climate change, which can be successfully cultivated in the southern part of the country.

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COMPETITIVE RELATIONSHIPS BETWEEN WEEDS AND *Sorghum bicolor* L. GROWN BY IGROWTH® TECHNOLOGY

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Abstract

In 2023-2024 in the experimental field of the Agricultural University-Plovdiv, weed associations in different BBCH stages of Sorghum bicolor L. were established. The crop was grown by igrowth® technology under non-irrigated conditions. The highest weed density from the late-spring group were Amaranthus retroflexus L., Chenopodium album L., Solanum nigrum L., Setaria viridis L., Portulaca oleracea L., and from perennial group - Sorghum halepense L. and Convolvulus arvensis L. In the control plot, during BBCH 14-15 stage of S. bicolor, the heights of the species Chenopodium album L. and Amaranthus retroflexus L. exceed the crop by 7.3 cm and 1.55 cm in 2023, but in 2024 the trend was the opposite. In BBCH 50, in both years, the two species were taller by 13.2 cm to 14.7 cm compare to S. bicolor. Sorghum halepense L. dominated with its height in both growing stages for the study period. The herbicide Pulsar 40 (imazamox), applied at rates of 1.20, 2.40 and 4.80 L ha-1, controlled the weeds up to BBCH 50 of the crop by 15% to 100%, depending on the species density

Key words: *Sorghum bicolor L., imazamox, igrowth® technology, weeds.*

INTRODUCTION

Sorghum is the fifth most important cereal crop grown in the world and a staple food in several regions of the Third World, mainly in Sub-Saharan Africa and India, where more than 300 million people rely on sorghum grain as a staple food (Rooney, 2004; Laidlaw and Godwin, 2008), and in the world, it has more than 60 million hectares of cultivated area, with the largest producers being the USA, India, China, Mexico, etc. Weeding is the main limiting factor for the intensive cultivation of sorghum for grain (Adu Tutu and Drennan, 1991; Rapparini, 1994; Rapparini et al., 1996; Limon-Ortega et al., 1998; Saayman, 2002). Weed control is a prerequisite for obtaining high and quality grain and green mass yields of sorghum. Due to this fact, in recent years it has become an increasingly widely studied crop in Bulgaria (Kikindonov et al., 2009; Enchev S. and G. Kikindonov, 2015; Enchev S., 2013).

In the early phases of its development, this crop is highly sensitive to weeding and yields significantly decrease if weeds are not destroyed in a timely manner (Limon-Ortega et al., 1998; Saayman, 2002).

In this regard, the aim of the present scientific work is to see the impact of the herbicide Pulsar 40 on the development and health status of the sorghum crop under the conditions of the igrowth® technology in the region of Central-South Bulgaria.

MATERIALS AND METHODS

The study was conducted at the Educational, Experimental and Implementation Base of the Agricultural University-Plovdiv at the Department of Agriculture and Herbology in 2023 and 2024. The experimental field of the Agricultural University - Plovdiv is located on alluvial-meadow soils, formed from sandy-clay and sandy-gravelly deposits of Quaternary origin. They are part of the second, higher terrace of the Maritsa River and are mainly made up of sandy and gravelly alluvial deposits, which in certain places also contain clay impurities. The thickness of the alluvial layer exceeds 54 meters. The width of this terrace varies between 300 meters and 4-5 kilometers, and it is of important importance for agriculture in the region (R. Popova et al., 2012.). The study was conducted with sorghum, a hybrid Sentinel, which was selected as suitable for cultivation using igrowth® technology (resistant to all imazamox-based herbicides). Pulsar 40 was used, which is a vegetative systemic herbicide from the imidazolinone group. It has a broad-spectrum effect against annual and perennial cereal and broadleaf weeds. It contains 40 g/l of the active ingredient imazamox. The recommended application rate is 1.2 L/ha. The active ingredient is taken up by the green parts of sensitive weeds, transported along the xylem and phloem to all growth points and inhibits the enzyme acetolactate synthetase (ALS). As a result, the weeds stop their growth, necrotize and die.

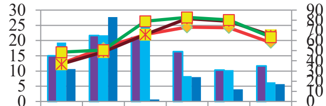
A two-factor field experiment was set up using the fractional plot method with a plot size of 20 m^2, in four replications. The experiment variants are as follows: A_1-Control (untreated), A_2-Pulsar 40 at a dose of 1.20 L/ha (imazamox 40 g/l), A_3-Pulsar 40 at a dose of 2.40 L/ha (imazamox 40 g/l), A_4-Pulsar 40 at a dose of 4.80 (imazamox 40 g/l).

The density of weeds and the height of the crop plant and individual weed species were recorded in stages BBCH 14-15 and BBCH 50.

Spraying with the herbicide Pulsar was carried out on 16.06.2023 and 13.06.2024 in BBCH 14-15 of the crop. The herbicide was applied with a backpack sprayer with a working solution of 20 l/da.

RESULTS AND DISCUSSIONS

The climatic conditions in the experimental years 2023 and 2024 pose a significant challenge for the crop, which is grown under non-irrigated conditions (Figure 1). In both years, the crop was sown at the appropriate time for it under favorable soil conditions. In 2023, at the beginning of the growing season, sorghum developed normally. From May to the end of the growing season, however, average monthly temperatures significantly exceeded normal values. Particularly high temperatures were recorded in the months of July, August and September, with temperatures reaching 40.9°C in July. These critical climatic conditions during the year have a significant impact on the growth and development of the crop, which have a negative impact on the overall development and productivity of the crop. In May 2024, the region is characterized by normal rainfall amounts of 83 l/m^2. Precipitation for the period June-August is only 38 mm, while in the first experimental year (2023) 135 mm fell for these 3 months. This extremely severe drought has had a negative impact on crop growth and development. However, weeds, which are not as dependent on rainfall, are developing in high density and competing with sorghum in phenological development and elements of growth and yield. Average monthly maximum temperatures for the period May-August 2024 are also at record highs, which further stress the crop plants.



	IV	V	VI	VII	VIII	IX
precip (mm/m2) aver. 1981-2010	45	65	63	49	31	35
precip. (mm/m2) 2023	58	65,1	79	25	31	19
precip. (mm/m2) 2024	32	83	2	24	12	17
t° C aver. 1981-2010	12,5	17,6	22	24,4	24,2	19,3
t° C 2023	12,3	16,3	22	27,2	26,3	21,7
t° C 2024	16,2	16,9	26,3	27,6	26,8	21,1

Figure 1. Agrometeorological conditions in the vegetation year 2023 and 2024

The selectivity of the herbicide Pulsar 40 (imazamox 40 g/L) applied at different doses to the sorghum crop, hybrid Sentinel IG, was monitored on the 7th, 14th and 21st day after the treatment of the crop, and the impact of the herbicide on the development and health status of the crop was recorded. The visual scale of the European Weed Research Society (EWRS) was applied, where at a score of 1 there was no damage to the crop, and at a score of 9 the crop was completely destroyed. (Table 1).

Table 1. Selectivity of studied herbicide towards *Sorghum bicolor* L., hybrid Sentinel IG, 2023

Variants	Selectivity, % by EWRS		
	7^{-th} day	14^{-th} day	21^{-st} day
A_1. Control (untreated)	1	1	1
A_2. Pulsar 40 1.2 L/ha (imazamox 40 g/L)	1	1	1
A_3. Pulsar 40 2.4 L/ha (imazamox 40 g/L)	2	1	1
A_4. Pulsar 40 4.8 L/ha (imazamox 40 g/L)	3	2	1

In the first experimental year, plants from all variants treated with herbicides reached the mass sweep phase (BBCH 50) on about the

40th day after spraying, with the exception of variants A_4, where the plants had a delayed growth and reached the 10th leaf phase. The growth lag and the non-simultaneous phenological development of some of the plants in the plots can be explained by a complex interaction between herbicide phytotoxicity and climatic conditions during the growing season (lack of precipitation and high temperatures). The applied herbicide Pulsar 40 at a dose of 2.4 L/ha (A_3) causes a milder and temporary phytotoxic effect, with a score of 2 on the 7th day after treatment. The crop recovers after the 14th day of herbicide application (score 1). No visible phytotoxic effects are detected in the A_2 variants after application of the product.

In 2024, on the 7th day after treating the crop with the herbicide Pulsar 40 at doses of 1.2 L/ha, 2. and 4.8 L/ha, no visible phytotoxic effect on the plants was observed and a score of 1 was recorded. At the next observation on the 14th day, slower growth was observed in variants A_4 compared to the other variants. By the end of the growing season, the plants treated with the herbicide Pulsar 40 remained the lowest in variants A_4. Weed height and density are shown in Table 2, at BBCH 14-15 of the crop. Extremely heavy weed infestation was reported by various annual and perennial species that were established in the trial area before spraying in 2023 and 2024

Table 2. Weed density and height before spraying with herbicide in the trial plots, hybrid Sentinel IG, BBCH 14-15 of the crop, year 2023 and 2024

Weeds	Weed density, pcs/m^2		Weed height, cm		Crop height, cm	
	2023	2024	2023	2024	2023	2024
Amaranthus retroflexus L.	157	6	26.2	15.5	24.7	25.6
Chenopodium album L.	152	72	32	13.2		
Xanthium strumarium L.	1	5	14.7	17.5		
Solanum nigrum L.	28	10	12.7	9.5		
Portulaca oleracea L.	117	17	5	10.5		
Setaria viridis L.	82	42	12	11.2		
Sinapis arvensis L.	2	6	16.5	13.5		
Anthemis arvensis L.	8	4	13.5	4.7		
Tribulus terrestris L.	3	4	7.2	9		
Datura stramonium L.	1	4	13.2	10.5		
Lolium perenne L.	2	7	24.2	14.2		
Echinochloa crus-galli L.	2	9	4	5.7		
Amaranthus blitoides L.	9	21	11.2	12.2		
Abutilon theophrasti L.	1	4	13.2	15.7		
Sorgum halepense L.	1	5	29.5	33.7		
Convolvulus arvensis L.	3	5	14.7	11.7		

The representatives present in the experiment for the two experimental years are described, with the most numerous species being the late spring weeds group – 6 species. These are: *Amaranthus retroflexus* L., *Amaranthus blitoides* L., *Chenopodium album* L., *Solanum nigrum* L., *Portulaca oleracea* L. and *Setaria viridis* L. With one representative each are the groups of early spring weeds - *Sinapis arvensis* L.; winter-spring weeds – *Anthemis arvensis* L., and from perennial group weeds - *Convolvulus arvensis* L. and *Sorghum halepense* L.

It is noteworthy that in 2023, before spraying sorghum (BBCH 14-15), the density of individual species was very different from the second experimental year. The highest density of species was *Amaranthus retroflexus* L., which was 157 pcs./m^2; for *Chenopodium album* L. the density was 152 pcs./m^2, *Setaria viridis* L. - 82 pcs./m^2, and the density of*Portulaca oleracea* L. e 117 pcs./m^2. The remaining weeds were at a significantly lower density, ranging from 9 pcs./m^2 for *Sorghum halepense* L. and up to 28 pcs./m^2 for *Solanum nigrum* L.

High weed density has the most negative impact on crop development. As a result of competition between weeds and sorghum, plants in the untreated control have delayed growth and set panicles significantly later than the treated variants.

In the second experimental year before spraying the sorghum, the density of individual species was significantly lower than the previous year, which was due to the rotation of the fields and the different stocking of the soil with weed seeds and organs for vegetative reproduction. The highest density is again maintained in the species *Chenopodium album* L., *Setaria viridis* L., *Portulaca oleracea* L. and *Amaranthus blitoides* L. In *Chenopodium album* L. the density is 72 pcs /m^2, in *Setaria viridis* L. - 42 pcs./m^2, and in *Portulaca oleracea* L. - 17 pcs./m^2. The density of *Solanum nigrum* L. is 10 pcs./m^2, and in *Amaranthus blitoides* L. is 21 pcs./m^2. The rest of the weeds are significantly lower, 5 pcs./m^2 за *Sorghum halepense* L. And in this experimental year, the tendency for the negative impact of high weed density on the phenological development of the crop in the control remained.

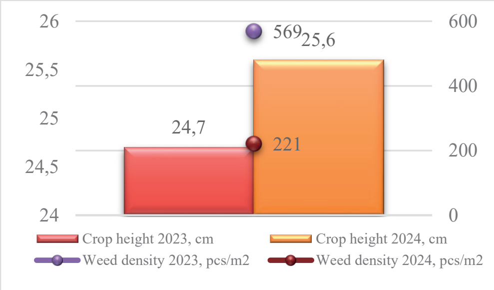


Figure 2. Crop height and total weed density before spraying with herbicide in the trial plots, BBCH 14-15 of the crop, year 2023 and 2024

The total weed density before treatment in 2023 was 569 pcs./m^2, while the height of the crop plant was 24.7 cm. In 2024, the total weed density was 221 pcs./m^2 and the height of the crop was 25.6 cm (Figure 2).

The results of the study show visible differences in the efficacy of the applied herbicide on different types of weeds, which provides important information for their control in agricultural crops. Table 3 presents data on the efficacy of the herbicide applied at different doses against individual weed species. Weeds were scored using the EWRS visual efficacy scale, and the results were compared with the untreated control. In 2023, the applied Pulsar 40 exerts practically complete control (100%) regardless of the doses applied to *Solanum nigrum* L., *Xanthium strumarium* L., *Sinapis arvensis* L., *Anthemis arvensis* L., *Tribulus terrestris* L., *Datura stramonium* L., *Lolium perenne* L., *Echinochloa crus-galli* L. и *Abutilon theophrasti* L. It has high efficacy against *Amaranthus retroflexus* L., which reaches 90% at herbicide doses of 1.2 L/ha and 2.4 L/ha, respectively 10 and 9 pcs./m^2, and at the highest dose the control reaches 100%. The efficacy against *Setaria viridis* L. at a dose of 1.2 L/ha educes the number of weeds om m^2 by half compared to the control, and with an increase to 2.4 and 4.8 L/ha pcs./m^2 reaches 17 pcs./m^2. Against *Chenopodium album* L., the herbicide at a dose of 4.8 L/ha (4 times higher than the registered one) achieves efficacy that reduces the number on m^2 by half, while at lower doses (1.2 L/ha and 2.4 L/ha) the control over the weed species is weaker. Against *Portulaca oleracea* L. of 140 pcs./m^2 in the control variant, the effect of the herbicide varies from 24 pcs./m^2 at the lowest dose of 1.2 L/ha to 16 pcs./m^2 at the highest dose 4.8 L/ha. The application of Pulsar 40 at a dose of 1.2 L/ha at *Amaranthus blitoides* L. has limited control-14 pcs./m^2, and with an increase in the herbicide dose to 4.8 L/ha it reaches 2 pcs./m^2. In *Convolvulus arvensis* L. the efficacy of the herbicide at a dose of 1.2 L/ha reduces the number to 10 pcs./m^2 compared to the control, where we have 14 pcs./m^2. Again with increasing the dose to 2.8 L/ha and 4.8 L/ha, the control is from 9 pcs./m^2 to 5 pcs./m^2. The perennial weed *Sorghum halepense* L., developed from rhizomes, is not controlled by Pulsar 40 at the registered dose of 1.2 L/ha. After its application, its control decreases in number reaching 1 pcs./m^2, but its height is significantly higher compared to the other species, reaching the height of the crop.

In 2024 г. the applied Pulsar 40 provided complete control (100%) against the species *Setaria viridis* L., *Sinapis arvensis* L., *Anthemis arvensis* L., *Tribulus terrestris* L., *Lolium perenne* L. and *Echinochloa crus-galli* L. (Table 4). At the highest dose (4.8 L/ha) it showed high efficacy against *Amaranthus retroflexus* L., reducing to 1 pcs./m^2 from 19 бр./m^2 in the control variant. In *Amaranthus blitoides* L. the efficacy was equally high at all doses of the herbicide, reducing from 33 pcs./m^2 in the untreated control to 2 pcs./m^2 in all treated variants. Against *Chenopodium album* L this year at a dose of 4.8 L/ha Pulsar 40 achieved greater efficacy, reaching 22 pcs./m^2, and at lower doses (1.2 L/ha и 2.4 L/ha) it also showed good control over the weed species – 30 and 26 pcs./m^2. Against *Portulaca oleracea* L. he effect varied from 19 pcs./m^2 at the lowest dose to 14 pcs./m^2 at the highest dose. The density of *Xanthium strumarium* L., is reduced from 7 pcs./m^2 in the control variant to 2 pcs./m^2 regardless of the herbicide dose. Pulsar 40 is also highly effective against *Solanum nigrum* L., which reaches up to 2 pcs./m^2 at all doses of the herbicide. In *Datura stramonium* L. at a herbicide dose of 1.2 L/ha and 2.4 L/ha the number is reduced to 3 pcs./m^2, and at 4.8 L/ha it reaches to 2 pcs./m^2.

Table 3. Weed height and density in BBCH 50 of the crop, 2023

	Control		Pulsar 40 1.2 L/ha		Pulsar 40 2.4 L/ha		Pulsar 40 4.8 L/ha	
Weeds	Weed height, cm	Weed density, pcs./m^2	Weed height, cm	Weed density, pcs./m^2	Weed height, cm	Weed density, pcs./m^2	Weed height, cm	Weed density, pcs./m^2
Amaranthus retroflexus L.	61.7	188	35	10	34.7	9	0	0
Chenopodium album L.	60.2	178	43.2	151	36.2	120	34.2	90
Xanthium strumarium L.	59.7	4	0	0	0	0	0	0
Solanum nigrum L.	17.2	34	0	0	0	0	0	0
Portulaca oleracea L.	25	140	24	43	22.5	18	22.2	16
Setaria viridis L.	36	98	31.2	40	30.5	18	24.2	17
Sinapis arvensis L.	30.5	2	0	0	0	0	0	0
Anthemis arvensis L.	26.2	10	0	0	0	0	0	0
Tribulus terrestris L.	16.7	4	0	0	0	0	0	0
Datura stramonium L.	35	3	0	0	0	0	0	0
Lolium perenne L.	26.7	2	0	0	0	0	0	0
Echinochloa crus-galli L.	21.7	5	0	0	0	0	0	0
Amaranthus blitoides L.	24.2	14	21.2	11	21.2	5	20.7	2
Abutilon theophrasti L.	33.7	3	0	0	0	0	0	0
Sorgum halepense L.	85.7	6	63.7	1	61.7	1	61	1
Convolvulus arvensis L.	36.2	14	32.2	10	31.7	9	24.5	5

Table 4. Weed height and density in BBCH 50 of the crop, 2024

	Control		Pulsar 40 1.2 L/ha		Pulsar 40 2.4 L/ha		Pulsar 40 4.8 L/ha	
Weeds	Weed height, cm	Weed density, pcs./m^2	Weed height, cm	Weed density, pcs./m^2	Weed height, cm	Weed density, pcs./m^2	Weed height, cm	Weed density, pcs./m^2
Amaranthus retroflexus L.	36.2	19	30.7	3	28.7	3	27.5	1
Chenopodium album L.	47.5	86	25	30	21.2	26	19.5	22
Xanthium strumarium L.	47.7	7	31	2	26.2	2	25	2
Solanum nigrum L.	13	14	13.2	1	12.7	1	10.7	1
Portulaca oleracea L.	18.7	32	16.5	19	15	15	12.2	14
Setaria viridis L.	27.2	50	0	0	0	0	0	0
Sinapis arvensis L.	26	11	0	0	0	0	0	0
Anthemis arvensis L.	16	6	0	0	0	0	0	0
Tribulus terrestris L.	14	9	0	0	0	0	0	0
Datura stramonium L.	65	12	57.5	3	43.7	3	42.5	2
Lolium perenne L.	24.2	9	0	0	0	0	0	0
Echinochloa crus-galli L.	19.2	15	0	0	0	0	0	0
Amaranthus blitoides L.	24.7	33	21.2	2	21	2	20.7	2
Abutilon theophrasti L.	49	8	48.2	4	42.2	2	37.2	1
Sorgum halepense L.	65	17	57.5	4	43.7	3	42.5	3
Convolvulus arvensis L.	31.2	11	29.7	4	29.2	4	29.5	4

With the highest dose of the herbicide (4.8 L/ha) in the species *Abutilon theophrasti* L. the efficacy reaches 1 бр./m^2, while at a dose of 1.2 L/ha and 2.4 L/ha is 4 pcs./m^2 and 2 pcs./m^2. In *Convolvulus arvensis* L. the efficacy of the herbicide is the same regardless of the dose, reaching 4 бр./m^2. The perennial weed *Sorghum halepense* L., developed from rhizomes, is not controlled by Pulsar 40, and after its application, the effect is 3 pcs./m^2 at a dose of 4.8 L/ha.

The height of the crop at BBCH 50 stage was recorded during the two experimental years. Data show that the plants are the lowest in the untreated control. In the variants with the application of the herbicide Pulsar 40, the plants are the lowest at the highest dose of the herbicide (4.8 L/ha) reaching 56.7 cm in 2023 and 53.5 cm in 2024. In both years, the plants remained tallest at the lowest herbicide dose (1.2 L/ha), 61 cm and 58.8 cm, respectively.

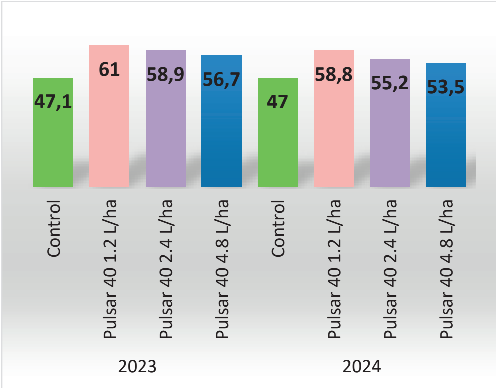


Figure 3. Crop height in BBCH 50 phase during the two experimental years, cm

CONCLUSIONS

From the research conducted at the experimental field of the Department of Agriculture and Herbology, the following important conclusions can be drawn:

With increasing dose of herbicide Pulsar 40, weed control increases, but the phytotoxic effect on sorghum increases. In the first year, a visible phytotoxic effect is observed, which is expressed in chlorosis and delayed plant growth. In the second experimental year, no visible phytotoxic effect is observed, but the plants remain shorter.

During both experimental years, the applied Pulsar 40 in all doses provided complete control (100%) against the species *Sinapis arvensis* L., *Anthemis arvensis* L., *Tribulus terrestris* L., *Lolium perenne* L. and *Echinochloa crus-galli* L.

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RESEARCH ON THE BIOLOGY AND PRODUCTIVITY OF THE *Carthamus tinctorius* L. SPECIES IN THE CLIMATE CONDITIONS OF CENTRAL MOLDOVA

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Abstract

Safflower (Carthamus tinctorius) is an annual herbaceous plant in the Asteraceae family. Safflower was previously cultivated for its seeds and flowers, which were used to add colour and flavour to food, in dyes, and medicine. Recently, the plant has been cultivated mainly for the vegetable oil extracted from the seeds. Saffron flowers are sometimes used as a substitute for safflower. Safflower is one of mankind's oldest crops but remains a minor crop compared to other oilseeds. Today, safflower is mostly cultivated for the production of vegetable oil. The main objective of the present research was to study the biology and productivity of the species Carthamus tinctorius L. with the aim of understanding its adaptability to the climatic conditions in Central Moldova. Safflowers matures 110-150 days after sowing; the optimal harvest time is when the seeds are matte white. Under the conditions of S.C.D.A. Secuieni, the highest seed production was obtained in the variant sown in the first decade of April at a distance of 70 cm between plants/row.

Key words: *safflower, productivity, biology.*

INTRODUCTION

Safflower has been cultivated since ancient times in China, Egypt and India. In the Middle Ages it was grown in Europe, Central America and South America. In the United States safflower started to be cultivated in the year 1925 (Walsh et al., 2008).
Safflower (*Carthamus tinctorius* L.) seed is an important source of oil also its flowers are an important source of spice. Safflower flowers can be in red, orange, yellow and rarely white colors (Erbas and Baydar, 2017; Hüseyin et al., 2018). With global demand, there is a need to develop varieties that have higher oil content, higher productivity, and are resistant to different challenging environments (Weselake, 2016; Zafar et al., 2019; Kotecka-Majchrzak et al., 2020; Hassani et al., 2020; Dobrin et al., 2021).
Safflower oil contains linoleic, palmitic and oleic acids with admixtures of stearic, arachidic, and meristic acids (Sazhin et al., 2017; Norov, 2019). The predominant acid in safflower oil is linoleic acid, the content of which is 78.5% (Mateev et al., 2017; Smetneva et al., 2020; Rathaur et al., 2023). Vegetable oils help reduce diseases of the cardiovascular system and metabolic diseases and help normalize painful human conditions (Smetneva et al., 2020). Growing safflower for use in feeding cattle and small ruminants, poultry farming also has a positive effect in the summer in eliminating the deficiency of protein and fat in animal diets (Belikina et al., 2021; Muscalu et al., 2022; Belyakov and Nazarova, 2023; Rathaur et al., 2023).
Safflower culture has importance to agriculture, the main reason of this cultivation being the following: high resistance to drought and soil salinity; tolerance to high temperatures and drought; mature seeds are not shaken and cannot be eaten by birds because of their specific inflorescence; it can be introduced into crop rotation in any agricultural system, including organic, having a deep root system; cultivation and harvesting can be fully mechanized; and it has lower production costs

(Gilbert, 2008; Cucu, 2014). Safflower seeds are used in food industry for the production of oil. Depending on the variety there are two kinds of oil: oil with a high content of linoleic acid, and oil with a high content of oleic acid. Safflower seeds are used both in the pharmaceutical industry, because of their therapeutic properties, and in varnish and paint industry (O'Brien, 2008; Dajue and Mündel, 1996).

Be aware of the different stages of growth period involves the emergence, shooting, button, flowering and maturation makes to evaluate the required conditions in each stage and it is close to the plant optimal conditions to increase the crop yield (Koocheki and Sarmadnia, 2000). Different investigation among safflower genotypes as terms of the phonological duration and time of phonological stages had difference because of genetic characteristics and environmental factors (Behdani et al., 2008). Increasing the plant density due to reducing the light absorption inside the plant canopy and creating competition among plants, caused to increase the plant height and early flowering (Dadashi, 2001). With the increasing of planting row interval, due to more light absorption into the plant canopy, the number of sub branches increased (Azari, 2001).

Different experiments results on safflower (Zareian, 2001: Azari, 2001), Soybean (Khadem Hamzeh, 1995), Chickpea (Gan et al., 2003) represents the reducing, sub branches is influencing of increasing plant density. Number of head in per plant is one of the main components of seed yield in safflower which high vegetative growth and plant ramifications are the reasons of the increasing yield (Pourdad, 1999; Omidi Tabrizi et al., 2002).

The plant growth analysis has become increasingly significant, particularly in controlled conditions, as it offers valuable insights into the physiological aspects of plant breeding (Fourcaud et al., 2008). It has been reported that through these analyses, determining the optimal planting time and plant density becomes feasible, leading to enhanced yield and quality when accompanied by timely irrigation and fertilization practices (Abbas et al., 2019). The determination of the optimal sowing time relies on the temperature and humidity conditions specific to the area. Moreover, timely sowing enhances grain retention in the field, leading to increased seed yield and high-quality oil production. Although several research have evaluated the adaptability of the safflower plant in Türkiye and in the world (Steberl et al., 2020; Culpan, 2023; Kamle et al., 2023; Yılmaz et al., 2023), comprehensive research focusing on its growth and development period remains scarce. Furthermore, there is a limited understanding of the physiological growth and development patterns exhibited by safflower plants.

This research was conducted to determine the effects of planting time on the growth parameters of the safflower plant, considering the effects of cultivation techniques on the growth parameters of the safflower plant.

The purpose of the research carried out during 2021-2023 period was to determine the influence of meteorological conditions on the biology, yield and quality of safflower seeds in the center of Moldova region.

MATERIALS AND METHODS

The Agricultural Research and Development Station (A.R.D.S.) Secuieni is located in the S-E part of Neamţ County, being located between the geographical coordinates of 26°51'00" east longitude and 46°51'15" north latitude. From an agro - ecosystem point of view, the territory belongs to the Central Moldavian Plateau, and from a morphostructural point of view, most of it is identified with the Moldavian platform. The area where the unit is located has a temperate continental climate (D.f.b.x. Köppen), characterized by short springs, cool summers and harsh winters, with an average annual temperature of 10.1°C and an annual amount of precipitation of 537 mm (Trotuş et al., 2020). In the conditions of A.R.D.S. Secuieni we experimented three sowing epochs: V1 (control) - sown in the first decade of April; V2 - sown in the second decade of April; V3 - sown in the third decade of April.

To establish the optimal nutrition space, a bifactorial experiment was set up according to the subdivided plot method with the following factors: A - the distance between rows with graduations: a1 - 25 cm, a2 - 50 cm and a3 - 70 cm and B - the distance between plants per row

with graduations: b1 - 15 cm, b2 - 25 cm and b3 - 35 cm.
During the safflower vegetation period, phenological observations were made, also establishing the required thermal degrees for its growth and development for the species under study.
From a thermal point of view during the safflower growing season, in 2021 there were monthly deviations from the multi-year average between -2.0°C (April) and 1.8°C (July). The spring was cool and the summer months were normal (June and August) and hot (July). In 2022, the safflower growing season was warm, with monthly deviations from the multi-year average ranging from 0°C (April) to 3.2°C (August) (Figure 1).

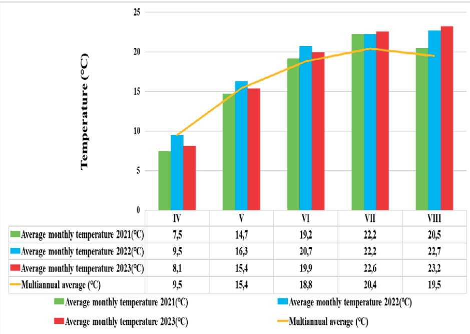

Figure 1. Temperatures recorded during the safflower growing season in the period 2021-2023

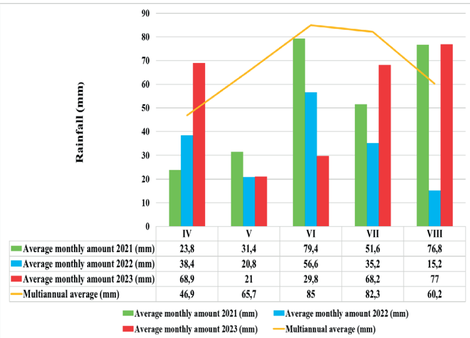

Figure 2. Precipitation recorded during the safflower growing season in the period 2021-2023

With regard to the rainfall recorded during the safflower growing season, in 2021 there were monthly deviations from the multi-annual monthly amount between -36.5 mm (September) and 16.6 mm (August). The safflower growing season in 2022 was characterized as dry. The recorded precipitation had deviations from the multiannual average between -47.1 mm (July) and -8.5 mm (April) (Figure 2). The precipitation that fell in 2023 characterized the safflower growing season as dry (Naie et al., 2024). Meteorological data comes from the unit's own weather station, located in the experimental field of ARDS Secuieni.

RESULTS AND DISCUSSIONS

In the case of the species *Carthamus tinctorius* L. sown in first decade of April, on average over three years, the plants emerged after 16 days from sowing, accumulating a sum of degrees of 128.5°C and the sum of precipitation totaling 14.6 mm. From emergence to the appearance of the flowering stem, 38 days were totaled, requiring a sum of degrees of 452.3°C and 29.6 mm of precipitation (Table 1).
From the appearance of the flowering stem to the beginning of flowering, 31 days were needed, the sum of thermal degrees recorded for this period was 534.5°C and 32.2 mm of precipitation (Table 1).
The seeds were harvested 32 days after the beginning of fruiting, accumulating 795.2°C and 26.2 mm of precipitation (Table 1).
From sowing to harvest, plants sown in the first epoch needed an average of 150 days, with a degree sum of 2498.9°C and 158 mm of precipitation.
Due to the higher temperatures during the vegetation period, the plants sown in the second and third epochs had a faster evolution. In the second epoch, from sowing to seed harvesting, 134 days were required, the sum of the accumulated temperatures was 2426.9°C and the precipitation amounted to 149 mm (Table 1).
In the third epoch, the plants had a vegetation period of 110 days to reach maturity. The sum of the temperatures recorded for this epoch was 2138.4°C and the precipitation amounted to 129.4 mm (Table 1).

Table 1. Growth phenophases of the species *Carthamus tinctorius* L.
under the conditions of A.R.D.S. Secuieni in the period 2021-2023

Phenological observations	Average growing season duration in the period 2021-2023 (days)	Σ average temperature (^{0}C)	Σ average precipitation (mm)
	Epoch I		
Sowing	-	-	-
Emergence	16	128.5	14.6
The appearance of flowering rods	38	452.3	29.6
The beginning of blooming	31	534.5	32.2
The beginning of fructification	33	588.4	55.9
Harvesting for seeds	32	795.2	26.2
TOTAL	150	2498.9	158.5
	Epoch II		
Sowing	-	-	-
Emergence	15	131.3	22.2
The appearance of flowering rods	35	526.6	30.8
The beginning of blooming	28	529.3	40.9
The beginning of fructification	30	621.9	26.4
Harvesting for seeds	26	617.8	28.7
TOTAL	134	2426.9	149.0
	Epoch III		
Sowing	-	-	-
Emergence	11	145.8	14.2
The appearance of flowering rods	30	522.5	31.4
The beginning of blooming	23	477.9	33.4
The beginning of fructification	26	616.1	26.4
Harvesting for seeds	21	376.1	24.0
TOTAL	110	2138.4	129.4

Table 2. Influence of the interaction between row spacing and plant spacing
on average seed production at *Carthamus tinctorius* L. (safflower) in the period 2021-2023

Distance between rows (A)	Distance between plants (B)	Production (kg/ha)	%	Diff.	Significance
a1 - 25 cm	b1-15 cm	623	53.75	-536	ooo
	b2-25 cm	718	61.95	-441	oo
	b3-35 cm	802	69.20	-357	oo
a2 - 50 cm	b1-15 cm	1124	96.98	-35	-
	b2-25 cm	1368	118.03	209	*
	b3-35 cm	1278	110.27	119	-
a3 - 70 cm	b1-15 cm	1498	129.25	339	**
	b2-25 cm	1589	137.10	430	**
	b3-35 cm	1436	123.90	277	**
Average		1159	100	Ct.	
LSD 1% 131.3 kg/ha LSD 0.1% 259.6 kg/ha LSD 5% 489.4 kg/ha					

Under the conditions at SCDA Secuieni, the interaction of the studied factors influenced the average seed production of the safflower species. Compared to the average of the experience (1159 kg/ha), production increases ranging from 209 to 430 kg/ha were obtained, being statistically assured and interpreted as distinctly significant positive. The interaction a3xb2 (70 cm row spacing and 25 cm between plants per row) with an average production of 1589 kg/ha was noted (Table 2).

The average seed production ranged between 1241 kg/ha for the variant sown in the III[rd] decade of April and 1593 kg/ha for the variant sown in the I[st] decade of April (control). Compared to the control, the production deficits were statistically confirmed and interpreted as negatively significant and distinctly significant and were obtained for the variants sown in the second period (210 kg/ha) and the third period (352 kg/ha) (Table 3).

Tabel 3. Average seed production at safflower obtained depending on the sowing season at A.R.D.S. Secuieni in the period 2021-2023

Variant	Production			
	kg/ha	%	Diff.	Sign.
V1 - I[st] decade of April	1593	100	Ct.	
V2 - II[nd] decade of April	1383	86.81	-210	o
V3 - III[rd] decade of April	1241	77.90	-352	oo
	LSD 5% 123.5 kg/ha LSD 1% 243.6 kg/ha LSD 0.1% 384.7 kg/ha			

CONCLUSIONS

Safflower has the ability to capitalize on poorly productive soils, unsuitable for sunflowers. The average production of safflower varies widely depending on climatic conditions, the sowing season and the varieties cultivated.

It is recommended to sow safflower in the first decade of April and at a distance of 70 cm between rows. Ensuring the nutrition space is very important for obtaining high productions for safflower. On safflower yields are strongly influenced by the conditions of experimental time of sowing.

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APPLICATION OF FOLIAR HERBICIDES FOR SOME DICOTYLEDONOUS WEEDS CONTROL IN *Triticum aestivum* L.

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Abstract

During the period 2023-2024, a field study with common wheat, Avenue variety was conducted. The experiment was set up in a production field in the village of Dobroplodno, Vetrino municipality, Bulgaria. The evaluated herbicidal products were Biathlon® 4 D (714 g/kg tritosulfuron + 54 g/kg florasulam), Ergon® WG (68 g/kg metsulfuron-methyl+ 682 g/kg tifensulfuron-methyl), Acurat Extra® WG (682 g/kg tifensulfuron-methyl + 70 g/kg metsulfuron-methyl), Aminopielic® 600 SL (600 g/l 2,4 amine salt), and Corida® 75 WDG (750 g/l tribenuron-methyl). The herbicidal products were applied as foliar treatments. The weed infestation of the experimental field was presented by Anthemis arvensis L., Lamium amplexicaule L., Consolida regalis S.F. Gray, Papaver rhoeas L., Sinapis arvensis L. and Convolvulus arvensis L. The infestation with these weeds resulted in a very low average grain yield for the untreated control (3.97 t ha-1). The highest biological yield of grain is obtained after using Biathlon 4 D (4.96 t/ha^{-1}) was found.

Key words: *Triticum aestivum, weedy plants, herbicidal products, efficient.*

INTRODUCTION

The role of food, and in particular the crops' production, is key to the survival of the population. This explains the great number of scientific studies aimed at the successful production of agricultural crops (Panayotov et al., 2024; Panchev & Shopova, 2024; Balabanova et al., 2023; Rankova et al., 2023; Shopova, 2023; Komitov et al., 2020; Dimtrova et al., 2019; Marinov-Serafimov et al., 2017; Mishra et al., 2016; Ditta et al., 2015; Fita et al., 2015; Shopova & Cholakov, 2015; Yanev, 2015; López-Bellido et al., 2014; Shopova & Cholakov, 2014; Panta et al., 2014; Yanev et al., 2014a). Winter wheat is one of the most important crops in the world. Weeds are a major competitor during the growing season of Triticum aestivum L. because they compete with crop plants for light, moisture, nutrients and space (Cheema and Farooq, 2007; Khan et al., 2001; Reddy, 2000). Weeds account for 10-80 percent yield reduction depending upon the weed species and infestation and caused depletion of soil water up to 6.5 cm (Ranjit et al., 1998; Afentouli and Eleftherohorinos, 1996; Khera et al., 1995; Mehra and Gill, 1988). Uncontrolled weeds are reported to cause up to 66% reduction in wheat grain yield (Kumar et al., 2011) or even more depending upon the weed densities, type of weed flora and duration of infestation. A formidable factor that limits its productivity is severe weed competition, which competes with crop plants for water, nutrients, space and solar radiation resulting in reduction of yield by 29% (Pandey et al., 2006). Wheat productivity depends on several factors such as irrigation, weed control, fertilizer management and other agronomic practices. Among these factors, the hidden war with the crop starts with weeds. Weeds are a major problem for sustainable crop production as weeds determine most agronomic practices for crop production and cause huge losses (Verma et al., 2015). Weeds also increase the cost of harvesting and degrade the quality of the produce. Therefore, they need to be controlled to obtain optimal wheat yield with good grain quality. Wheat is usually stressed by dicotyledonous weeds. The presence of weeds, especially in the early stages of crop development, proves detrimental to the yield obtained from it. Losses are highest when resources are limited and weeds germinate along with it (Hussain et al., 2015). There is a strong correlation between the duration of weed competition and wheat yield reduction (Fahad et al., 2014; Bekelle, 2004). Wheat crop is infested majorly with *Avena fatua*, *Chenopodium album*, *Cirsium arvense*, *Convolvulus arvensis*, *Coronopus didymus*, *Cynodon dactylon*, *Dichanthium annulatum*, *Melilotus indica*, *Phalaris minor*, *Polygonum plebejum*, *Polypogon fugax*, *Rumex dentatus* and *Spergula arvensis* weeds (Waheed et al., 2009). Wheat grain yield losses due to presence of these weeds were estimated to be 20 to 30% (Marwat et al., 2006). Apart from significantly reducing grain yield, weeds also reduce soil fertility. Timely weed control is essential for maximum yield (Vasudev et al., 2017). It is economically advantageous to use chemical weed control agents (Khalil et al., 1999). Herbicides are one of the most commonly used substances for weed management. Choosing the proper herbicide is a responsible moment, because it must meet a number of requirements such as: selectivity to the crop, efficacy against the weeds and to be safe for the produced food and soil health (Parven et al., 2025; Semenov et al., 2025; Morar et al., 2024; Rai et al., 2024; Yanev, 2024; Yanev, 2023; Atwood et al., 2022; Goranovska et al., 2022; Li et al., 2022; Yanev, 2022; Govindasamy et al., 2021; Yanev, 2021; Mandal et al., 2020; Tripathi et al., 2020; Yanev, 2020; Yanev & Kalinova, 2020; Martinez et al., 2018; Goranovska & Yanev, 2016; Kostadinova et al., 2016; Kumar et al., 2016; Rose et al., 2016; Hristeva et al., 2015; Kalinova & Yanev, 2015; Raj et al., 2015; Semerdjieva et al., 2015; Hristeva et al., 2014; Lee et al., 2014; Yanev et al., 2014b; Marin-Morales et al., 2013; Zabaloy et al., 2011). Herbicidal weed control is considered most effective and economical method in wheat (Ashiq et al., 2003). The integrated weed management approach is advantageous because one technique rarely achieve complete and effective control of all weeds during crop season and even a relatively few surviving weeds can produce sufficient number of seeds to perpetuate the species (Nayak, 2006; Walia et al., 1997) .An average decrease in grain yield by 15.42 % was observed due to season-long weed-crop competition. Lowest dicot weeds were observed with weed free treatment. The most popular herbicides on winter wheat are chemicals based on active ingredients: tribenuron-methyl, dicamba, florasulam, etc. (Zand et al., 2007). The best weed control efficiency in case of dicot (82.8%) was achieved with metsulfuron-methyl, respectively compared to other herbicide namely 2,4-D (Patel et al., 2017; Paighan et al., 2013; Maninder et al., 2007; Singh and Ali, 2004; Nayak et al., 2003; Kurchania et al., 2000). Ashiq et al. (2007) recorded the highest WCE of bromoxynil+ MCPA against broadleaf weeds *Chenopodium album, C. murale, Fumaria indica* and *Convolvulus arvensis* in wheat. It is also true that most of the dicot herbicides do not give a 100% control of all broadleaf weeds (Zimdahl, 1993). This is due to differential phytotoxic action of herbicides against a range of broadleaf weeds (Ashiq et al., 2007). According to Abbas et al. (2009) the best herbicides against broad leave weeds is Buctril Super 60 % EC - 825 ml ha^{-1}, as it out yielded all herbicides by producing 2300 kg ha^{-1} grain yield except T5 Starane-M - 875 ml ha^{-1}, which produced grain yield to the tune of 2245 kg ha^{-1}.

The present study was conducted with an objective to identify herbicides more effective in controlling broad leaf weeds and increasing wheat's yield. This trial was done to assess the efficacy of post-emergence herbicides for weed control in wheat and its effect on grain yield.

MATERIALS AND METHODS

In 2023 and 2024, a field experiment with the winter wheat variety "Avenue" was conducted in the village of Dobroplodno, Vetrino municipality, Bulgaria. The experiment was set up using the block method in 4 replications with a total size of the working plot of the four replications of 80 m². Before the treatment with herbicides, a weed count was carried out in the experimental field. Six widespread broadleaf weeds were identified in wheat. The average density of weeds in the two experimental years, per 1 m² is as follows: *Anthemis arvensis* L. - 6.5 exemplar; *Lamium amplexicaule* L. - 19.5 exemplar; *Consolida regalis* S.F. Gray - 5 exemplar; *Papaver rhoeas* L. - 5.5 exemplar; *Sinapis arvensis* L. - 7 exemplar; *Convolvulus arvensis* L. - 5 exemplar. The study included the following variants: 1. Untreated control; 2. Biathlon 4 D (714 g/kg tritosulfuron + 54 g/kg florasulam) - 0.055 kg ha^{-1}, Ergon® WG (68 g/kg metsulfuron-methyl + 682 g/kg tifensulfuron-methyl) - 0.09 kg ha^{-1}, Acurat Extra® WG (682 g/kg tifensulfuron-methyl +

70 g/kg metsulfuron-methyl) - 0.05 kg ha^{-1}, Aminopielic® 600 SL (600 g/l 2,4 amine salt) - 1.25 l ha^{-1}, and Corida® 75 WDG (750 g/l tribenuron-methyl) - 0.015 kg ha^{-1}. The herbicides were applied in the tillering phase of the wheat (BBCH 21-29). The herbicide spraying was carried out with a backpack sprayer with a working solution volume of 210 l ha^{-1}.

Before sowing the crop, fertilization was carried out with NPK 15:15:15 at a fertilizer rate of 200 kg ha^{-1}. Sowing was carried out at the optimal time for wheat with a small-sized Wintersteiger seeder for crops with a merged surface at a row spacing of 12 cm, with a seeding rate of 400 germinating seeds per m². In the spring, in the tillering phase, wheat was nourished with NH_4NO_3 at a fertilizer rate of 200 kg ha^{-1}. Weeds were assessed for efficacy on days 14, 28 and 56 after the application of the herbicide products. The 10-point EWRS (European Weed Research Society) scale was used for visual assessment of herbicide efficacy. The 9-point EWRS scale was used to assess herbicide selectivity.

The results for wheat yields were processed using the Duncan method.

RESULTS AND DISCUSSIONS

The efficacy of herbicides against Anthemis arvensis L. is shown in Table 1. On the 14th day after treatment, the highest herbicidal efficacy was recorded for variant 2 (Biathlon 4 D), and the lowest herbicidal effect was recorded for variant 5 (Aminopielic 600 SL). This trend was maintained at the last reading, carried out on the 56th day after the application of the herbicides. With the exception of variant 5, in all other treated variants with the products Biathlon 4 D, Ergon WG, Akurat Extra WG and Corida 75 WDG, we report almost complete weed destruction (95-100%).

Table 1. Avarage herbicidal control (%) against *A. arvensis*

Variants	Days after application		
	14	28	56
1. Untreated control	-	-	-
2. Biathlon 4 D - 0.055 kg ha^{-1}	70	90	100
3. Ergon WG - 0.09 kg ha^{-1}	60	80	95
4. Acurat Extra WG - 0.05 kg ha^{-1}	60	85	95
5. Aminopielic 600 SL - 1.25 l ha^{-1}	50	60	70
6. Corida 75 WDG - 0.015 kg ha^{-1}	55	75	95

Table 2 shows the dynamics of the herbicidal efficacy against *L. amplexicaule* L. High herbicidal efficacy was reported on all reporting dates. At the first reporting date, the efficacy of the individual products was almost the same, ranging from 80 to 85%. On the 28th day after herbicide treatment, this difference in the efficacy of the products persist, reaching 90-95%. At the last date, the weed completely vanished.

Table 2. Avarage herbicidal control (%) against *L. amplexicaule*

Variants	Days after application		
	14	28	56
1. Untreated control	-	-	-
2. Biathlon 4 D - 0.055 kg ha^{-1}	85	95	100
3. Ergon WG - 0.09 kg ha^{-1}	80	90	100
4. Acurat Extra WG - 0.05 kg ha^{-1}	80	95	100
5. Aminopielic 600 SL - 1.25 l ha^{-1}	85	95	100
6. Corida 75 WDG - 0.015 kg ha^{-1}	80	90	100

Against *C. regalis* (Table 3), none of the experiment's variants showed 100% efficacy. This indicates the greater resistance of the weed to the tested herbicides. On the 14th day after treatment with the herbicides, the efficacy was slightly higher in the variants with Biathlon 4 D and Aminopielic 600 SL. On the second date, the lowest herbicidal efficacy against weed was reported for the product Ergon WG. The highest herbicidal effect was reported from the products Biathlon 4 D and Aminopielic 600 SL (90%) 56th days after treatments. The other variants also have satisfactory herbicidal efficacy, around 85%.

Table 3. Avarage herbicidal control (%) against *C. regalis*

Variants	Days after application		
	14	28	56
1. Untreated control	-	-	-
2. Biathlon 4 D - 0.055 kg ha^{-1}	65	80	90
3. Ergon WG - 0.09 kg ha^{-1}	60	75	85
4. Acurat Extra WG - 0.05 kg ha^{-1}	60	80	85
5. Aminopielic 600 SL - 1.25 l ha^{-1}	65	85	90
6. Corida 75 WDG - 0.015 kg ha^{-1}	60	80	85

Herbicidal efficacy of the products against *P. rhoeas* is shown in Table 4. On the first reporting date for treatments 3 and 4 the weed efficacy was 65%. There was only 60% efficacy against the weed in the other treatments of the trial. The efficacy of the herbicide Aminopielic 600 SL on the same date was very low - 40%. On the 28th day after treatment, the percentages of efficacy in all variants increased. In variant 5, the efficacy is low again. With the exception of the herbicide Aminopielic 600 SL, where the efficacy was unsatisfactory (60%), the efficacy of the remaining treated variants at the last reporting was high (90%).

Table 4. Avarage herbicidal control (%) against *P. rhoeas*

Variants	Days after application		
	14	28	56
1. Untreated control	-	-	-
2. Biathlon 4 D - 0.055 kg ha^{-1}	60	80	90
3. Ergon WG - 0.09 kg ha^{-1}	60	85	90
4. Acurat Extra WG - 0.05 kg ha^{-1}	65	80	90
5. Aminopielic 600 SL - 1.25 l ha^{-1}	40	50	60
6. Corida 75 WDG - 0.015 kg ha^{-1}	60	80	90

Table 5. Avarage herbicidal control (%) against *S. arvensis*

Variants	Days after application		
	14	28	56
1. Untreated control	-	-	-
2. Biathlon 4 D - 0.055 kg ha^{-1}	50	85	100
3. Ergon WG - 0.09 kg ha^{-1}	55	80	100
4. Acurat Extra WG - 0.05 kg ha^{-1}	55	80	100
5. Aminopielic 600 SL - 1.25 l ha^{-1}	55	80	100
6. Corida 75 WDG - 0.015 kg ha^{-1}	50	85	100

S. arvensis (Table 5) is the easiest to control compared to all other weeds present in the experiment. Of all the herbicide products used in the trial, report 100% efficacy the weed, reported at the last reporting date.

Table 6. Avarage herbicidal control (%) against *C. arvensis*

Variants	Days after application		
	14	28	56
1. Untreated control	-	-	-
2. Biathlon 4 D - 0.055 kg ha^{-1}	80	50	40
3. Ergon WG - 0.09 kg ha^{-1}	60	40	30
4. Acurat Extra WG - 0.05 kg ha^{-1}	65	40	30
5. Aminopielic 600 SL - 1.25 l ha^{-1}	80	70	50
6. Corida 75 WDG - 0.015 kg ha^{-1}	70	50	30

On the 14^{th} day after the application of the herbicides, only the products Biathlon 4 D and Aminopielic 600 SL showed a satisfactory effect on *C. arvensis* (Table 6). The efficacy of Ergon WG, Acurat Extra WG and Corida 75 WDG was unsatisfactory varying from 60 to 70%. On the second date, we reported a decrease in efficacy for all products tested in the experiment. On the last date, field bindweed was controlled very poorly in all treated variants. The herbicide Aminopielic 600 SL reported 50% efficacy. With the other products, it was even and reached only 30 - 40%. These low efficacy percentages are due to the strong secondary growth of the weed.

Table 7 shows the average yields obtained for 2023-2024. The herbicidal efficacy of the products also determines the differences in yields in the individual variants of the experiment. Weeding with highly competitive species leads to a minimum yield of the untreated control (3.97 t/ha^{-1}).

Table 7. Productivity of wheat, t/ha^{-1}

Variants/ Yields	
1. Untreated control	3.97 a
2. Biathlon 4 D - 0.055 kg ha^{-1}	4.96 *c
3. Ergon WG - 0.09 kg ha^{-1}	4.92 *c
4. Acurat Extra WG - 0.05 kg ha^{-1}	4.91 *c
5. Aminopielic 600 SL - 1.25 l ha^{-1}	4.55 *b
6. Corida 75 WDG - 0.015 kg ha^{-1}	4.86 *c

Legend: Values marked with different letters differ significantly according to Duncan's test at P 0.05.

According to Duncan's test, three separate groups of herbicides are distinguished by the degree of statistical evidence (a, b, c). It was observed that all variants, except for Aminopielic 600 SL, were from group (c) furthest from the untreated control group (a), that was with the highest yields. The reason is mainly due to the fact that Aminopielic 600 SL has lower efficacy against *A. arvensis* and *P. rhoeas*, compared to the higher efficacy of the other evaluated products.

CONCLUSIONS

The herbicides Biathlon 4 D, Ergon WG, Acurat Extra WG, Aminopielic 600 SL and Corida 75 WDG were excellently effective against *L. amplexicaule* and *S. arvensis*.

The product Aminopielic 600 SL was not sufficient compared to the other studied herbicides against *A. arvensis* and *P. rhoeas*.

The weed *C. regalis* was controlled equally well by all tested products (from 85 to 90%).

The weed *C. arvensis* was not controlled successfully by any of the tested herbicides.

Visual signs of phytotoxicity were not detected in any of the trial treatments during the two experimental years.

The average yield of the variant treated with the herbicide Biathlon 4D is the highest compared to the other treated variants.

The lowest yield is from Aminopielik 600 SL (3.97 t/ha^{-1}).

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TESTING OF SOME HYBRIDS OF SWEET SORGHUM AND SORGHUM x SUDAN GRASS AT BRĂILA AGRICULTURAL RESEARCH AND DEVELOPMENT STATION

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Abstract

The paper aimed to present the study with sweet sorghum varieties carried out in Great Brăila Island, Romania in the 2022-2023 agricultural year. This year was considered by the World Meteorological Organization as the driest in the history of climate records at that time. This study was carried out by S.C. Eco-Sorghum Group S.R.L. in collaboration with the Agricultural Research and Development Station of Brăila within the research contract entitled "Testing of sweet sorghum hybrids (SAȘM 1 and SAȘM 2) and the sorghum x Sudan grass hybrid (SAȘM 4)" with the aim of highlighting the potential for total biomass production, technological strains, juice and bagasse and the cultivation technology appropriate to meet the eco-environmental requirements of sweet sorghum. This study of hybrids was also carried out at 6 other agricultural locations in Romania placed in the county of Arad, Argeș, Brăila, Dăbuleni, Iași and the Republic of Moldova. Following this experiment, it can be demonstrated that sweet sorghum has high potential in terms of biomass production, and also in terms of the production of juice with high sugar content. This is very important for the energy sector, by using sweet sorghum as a sustainable and affordable alternative in a context where the long-term use of fossil fuels presents quite a lot of uncertainties. Also the food industry can use sweet sorghum with very good results in the production of sugar.

***Key words**: sweet sorghum, biomass production, sugar content.*

INTRODUCTION

Sorghum is part of the cereal family, ranking fifth worldwide in terms of cultivated area, after wheat, corn, rice and barley, being cultivated on 40 million hectares (Axinte, 2006; Mocanu, 2023; Sorghum Id, 2022)

Sorghum is characterized by the fact that it can adapt better to climate change conditions, especially prolonged drought and heat and less fertile soil, thus giving it the nickname of "The camel of crops" (Bâlteanu, 2003; Mocanu, 2023; Antohe, 2007)

Sorghum bicolor is the main representative of the *Sorghum* genus and is increasingly used for a larger cultivation area, this being possible due to the multiple uses it has, namely, in human, animal food or for industrial purposes (Bâlteanu, 2003). According to the method of use, sorghum is divided into four groups (Roman, 2006; Sarca, 2004; Soare, 2012): grain sorghum (*Sorghum bicolor* variety *eusorghum*) for which the purpose of cultivation is represented by the production of grains used for human and animal food and for industrial processing.

Sorghum bicolor variety *sudanese* -fodder sorghum is characterized by the fact that it is very suitable for animal feed, the stem having the property of sprouting very well and producing green biomass in a large quantity.

Sorghum bicolor variety *technicum* - technical or broom sorghum is used in the production of brooms or for extracting cellulose from the stem.

High-growing sugar sorghum (*Sorghum bicolor* variety *sacchartum*) or sweet sorghum is part of the same *Sorghum bicolor* and is characterized by the fact that the panicle is not very important from a productive point of view, the stem being the most important constituent part. This is usually 1.7-4 m high, and can even reach 6.5 m (Roman et al., 2011).

The stem contains the sweet juice rich in sugar, which can be used mainly in the production of sugar but also for other food, in energy sector or other industrial purposes. Once the juice is extracted, the resulting auxiliary material, called bagasse, can be used successfully both in animal feed and for energy or industrial purposes (Antohe, 2006). Typically, sweet sorghum presents a yield of 70-100 t/ha with a very good yield in terms of fermentable sugar extraction. Thus, from one ton of sweet sorghum, 400-500 l of juice with 14-20% sugar is obtained (Antohe, 2006).

From this syrup, 7000 l of bioethanol per hectare can be obtained. From this point of view, sweet sorghum is of particular importance in that an increase in the consumption of biofuels is expected worldwide to the detriment of conventional fossil fuels (Pochișcanu, 2016).

From an agronomic point of view, studies have demonstrated the ability of sweet sorghum to combat soil erosion and prevent land desertification by incorporating the root mass and leaves resulting from harvesting into the soil, enriching the soil with humus. Also, to combat *Fusarium* leaf disease or *Agriotes spp*. worms, it is recommended to introduce sorghum into agricultural rotations. (Antohe, 2002; Pochișcanu, 2016). From an ecological point of view, sweet sorghum is very important in that it can capture 35-50 t of CO_2 from the atmosphere, a higher amount compared to deciduous forests or other cultivated plants. (Antohe, 2006)

MATERIALS AND METHODS

The experience with testing sweet sorghum hybrids for green mass, in order to obtain natural sweeteners, biochar and energy pellets, took place in 2023, within the Great Brăila Island (I.M.B.) Experimental Centre on an area of 3 hectares. This study was also carried out at 6 other agricultural locations in Romania placed in the county of Arad, Argeș, Brăila, Dăbuleni, Iași and the Republic of Moldova.

Testing area description

The I.M.B. Experimental centre is located on the territory of the Great Brăila Island, which is developed with complex land improvement works, and which in the past represented the largest natural unit in the Lower Danube Meadow with a total area of 76,700 ha. Located within the perimeter of Brăila County, the Great Brăila Island is surrounded by the waters of the Danube - the Măcin Branch (Old Danube) to the north, east and south, the Vilciu Branch and the New Danube between km 252 and km 170 to the west.

Climatic conditions of the area

The air temperature regime, through the monthly average values and especially through the absolute amplitude, most clearly reflects the characteristics of the temperate continental climate. The annual average air temperature in Brăila is 10.9ºC. The monthly average temperatures vary over a fairly large range, from the coldest month - January with a multiannual average of -2.1ºC, to the warmest month - July, whose multiannual value is 22.9ºC.

Precipitation regime in the Great Brăila Island liquid and solid precipitation in the area of North Bărăgan is below 500 mm, with average values ranging between 400-490 mm.

The annual variability of precipitation and the uneven distribution during the year reflect the continental character of the climate in the area (Table 1).

Wind is a climatic element with a great impact on agriculture practiced in the I.M.B., having a major influence on the hydrological regime of soils, the behaviour of crops or the quality of irrigation.

The biological material used

- SAŞM 1-simple, interlinear, late maturing hybrid with a vegetation period of 126-132 days. This hybrid is male-sterile, does not form pollen and grains with a height of 370-420 cm and tick juicy pith stem. The carbohydrate content in juice is 13.5-16%, and in favourable years up to 22%.Cultivation technology is similar to that of silage corn cultivation with a distance of 70 cm between rows, as well as in two-row strips (18-20 cm), alternating with an interval of 90-120 cm. The optimal plant density is 105-120 thousand plants/ ha. The yield when cultivated on non-irrigated is 90-120 t/ha and 20-30 t/ha of dry matter, when irrigated is 170-190 t/ha of biomass. This hybrid was approved in 2013 and is owned by the Institute of Genetics, Physiology and Plant Protection, Moldova.

Table 1. Main climatic elements of Great Brăila Island (IMB) Experimental Centre during the period October 1, 2022 - September 30, 2023

Climatic elements		2022			2023									TOTAL AVERAGE
		X	XI	XII	I	II	III	IV	V	VI	VII	VII	IX	
Precipitation (mm)	Monthly average	6	31	20	64	7	13	66	40	26	106	55	5	439
	Multiyear monthly average	30	33	36	28	27	26	35	48	62	46	39	32	442
	Deviation	-24	-2	-16	36	-20	-13	31	-8	-36	60	16	-27	-3
Temperature (^{0}C)	Monthly average	13	8.1	2.9	4.4	1.4	7.9	10.4	16.6	21.6	24.7	24.7	20.9	13.1
	Multiyear monthly average	11.5	5.6	0.6	-2.1	-0.2	4.7	11.2	16.7	20.9	22.9	22.1	17.3	10.9
	Deviation	1.5	2.5	2.3	6.5	1.6	3.2	-0.8	-0.1	0.7	1.8	2.6	3.6	2.1

- SAŞM 2-simple interlinear hybrid, medium late maturing variety with a vegetation period of 110-115 days. The hybrid is male sterile, does not form pollen and grains with a plant height of 360-400 cm. The stem is thick rich in juicy pith. The content of carbohydrates in the juice is 14-16.5% and in favourable years reaches up to 22%. 14-16 leaves with a length of 61-79 cm are formed on the main stem. The cultivation technology is similar to that of cultivating corn for silage with a row spacing of 70 cm or in two-row strips (18-20 cm), alternating with an interval of 90-110 cm. The optimal plant density is 110-120 thousand plants/ha.

Hybrid approved in 2013 and owned by the Institute of Genetics, Physiology and Plant Protection of Moldova.

- SAŞM 4-hybrid created by crossing the male-sterile line MSL-1 as a maternal form with the pollinating line SP-4.

The height of the plant during the period of full ripening is 250-280 cm, and the size of the plant at the time of the first mowing varies within the limits of 164-219 cm, and at the second mowing 113-193 cm.

The twinning of the plant at the first mowing constitutes 4-5 stems, and at the second mowing 9-11 stems. The vegetation period from the seedling phase to the first mowing is 65-72 days, and from the first mowing to the second

- length of the internode underlying the panicle

42-59 days. The hybrid is resistant to drought and lodging. The average green mass yield is 111.6 t/ha and 19.2 t/ha dry mass. The plant mass contains 11.4-15.8% protein, 3.7-4.4 carbohydrates, 24.4-29.7% cellulose and 48.9-52.7 non-nitrogenous substances.

Cultivation technology

- herbicide application with the total herbicide Barbarian 360 at a dose of 4l/ha;
- fertilization with D.A.P. 20.20.0 complexes at a quantity of 200 kg/ha;
- sowing of sorghum lines on 03.05.2023;
- application of Faster 10 CE insecticide at a dose of 0.5 l/ha;
- applied Urea 200 kg/ha;
- mechanical weeding.

Biometric observations made during the vegetation period

- average plant height;
- root length;
- base stem diameter;
- length of the second internode from the ground;
- length of the internode underlying the panicle;
- number of leaves;
- average leaf length and width;
- length of the second internode from the ground;

Laboratory analyses of plants:

- sugar content in the stem;
- dry matter content of the vegetative mass;
- dry matter content of the panicle;
- analysis of the concentrated sorghum juice;
- physic-chemical characteristics of the sorghum stems.

RESULTS AND DISCUSSIONS

Under the climatic conditions of 2023, from sowing (03.05.2023) until 28.08.2023 (at the interim report), 2513 growing degree units (G.D.U.) and 199.4 mm of precipitation were accumulated, and until 31.10.2023, when the stems could be harvested, 3698 GDU and 234 mm of precipitation were accumulated.

Field observations were carried out on 26.07.2023; 24.08.2023 and on 12.10.2023 when biometric measurements in the field and laboratory determinations were carried out, on the 3 variants of the sweet sorghum varieties, in three replications.

In the case of observations recorded on 26.07.2023, in most cases, panicles were not formed and the sugar content was below the 4% threshold.

Figure 1. Observations in the sorghum experience in the Great Braila Island (IMB) Centre

Table 2. Biometric determinations performed during the vegetation period

Observation	26.07.2023			24.08.2023			12.10.2023		
	SAȘM 1	SAȘM 2	SAȘM 4	SAȘM 1	SAȘM 2	SAȘM 4	SAȘM 1	SAȘM 2	SAȘM 4
1. Plant height	269.7	230.7	229	377.3	355.0	319.3	375.0	353.0	319.0
2. Root length	28.0	21.0	25.3	34.0	28.7	30.3	41.2	40.5	37.3
3. Number of leaves	9.7	8.0	7.3	15.3	14.3	10.3	15.0	13.3	9.5
4. Leaf length	87.0	84.7	80.3	95.0	93.7	86.0	92.5	91.2	88.3
5. Leaf width	7.5	7.8	4.6	8.2	7.7	4.2	8.0	7.5	4.0
6. Thickness of 2internode from the ground	2.6	2.2	1.3	2.2	1.9	1.3	2.5	2.2	2.0
7. Length of internode underlying panicle	-	-	58.5	38.7	44.2	57.7	39.2	44.5	58.0
8. Breaking resistance	8.2	8.2	9.3	7.5	7.3	9.3			
9. Falling resistance	8.0	7.7	9.2	6.3	6.3	9.2	6.0	6.0	8.5
10. Aphid resistance	9.5	9.2	9.5	9.5	9.2	9.0	9.0	9.0	9.0
11. Resistance to pathogen attack	9.7	9.3	9.0	9.7	9.3	9.0	9.0	9.1	9.0
12. Sugar content	3.9	3.9	2.8	9.1	9.6	5.1	16.3	16.4	6.9

Table 3. Results of the observations regarding green biomass and dry matter

Observation	24.08.2023		
	SAŞM 1	SAŞM 2	SAŞM 4
1. Plant height (cm)	377.3	355.0	319.3
2. Green plant mass (g) (stem + leaves)	1114.4	917.9	247.3
2. Plant dry matter (stem + leaves) (g)	219.2	182.6	44.1
3. Green panicle mass (g)	42.7	40.8	48.8
5. Panicle dry matter (g)	16.4	15.3	22.7

For the determinations made at the end of August - beginning of September, the statistical calculation performed with the Average test highlighted the following:

The average height of the plants was 377 cm at SAŞM 1, followed in descending order by SAŞM 2 (355 cm) and SAŞM 4 (319 cm).

The green mass per plant was between the values of 1.15 kg/plant for SAŞM 1, followed in descending order by SAŞM 2 with 0.91 kg/plant and by SAŞM 4 with 0.25 kg/plant, at density of 18 plants/m² for SAŞM 1 and SAŞM 2, and 26 plants/m² for the SAŞM 4 hybrid.

The average dry matter content was higher for the SAŞM 2 hybrid (60.8%), followed in descending order by the SAŞM 1 hybrid (47.1%) and the SAŞM 4 hybrid (32.2%).

Figure 2. Separation of plant parts for biomass determination

Figure 3. Determination of the sugar content in the stem by the refractometer method

The final field determinations were carried out before harvesting as green mass, respectively on October 12, 2023 and highlighted the fact that, under the pedoclimatic conditions of I.M.B., in 2023, an average of 98.6 t/ha of leafless stems from ŞAŞM 1, 86.5 t/ha of stems from ŞAŞM 2 and 24.9 t/ha of stems from ŞAŞM 4 were obtained, the sugar content determined by refractometer being 16.3% in ŞAŞM 1, 16.4% in ŞAŞM 2 and 6.9% in ŞAŞM 4 (Table 4).

Table 4. Results of the observations regarding green biomass and sugar content

Observation	12.10.2023		
	SAŞM 1	SAŞM 2	SAŞM 4
1. Number of plants/m²	16.3	16.0	26.6
2. Stem weight (kg)	9.86	8.65	2.5
3. Leaf weight (kg)	2.06	1.76	0.8
4. Panicle weight with underlying internode (kg)	2.19	2.58	0.9
5. Sugar content	16.30	16.4	6.9

Table 5. Average biomass production obtained from the three sweet sorghum lines, tested in 8 experimental centers, under non-irrigated conditions, in 2023

No.	Variety	Green biomass/ha		Harvest moisture %	Density thousand plants/ha	Plant height (cm)	Yield dry matter/ha		Stem weight (g/pl)
		Average	Margins				40% moist.	20% moist.	
1.	SAȘM 1	114.1	82/131.9	71	156	310	64	32	730
2.	SAȘM 2	132.1	80/163	78	151	315	67	33.4	860
3.	SAȘM 4	34.2	24/45.2	56	232	230	24.3	12.2	148

According to Table 5, and the production results obtained in the 8 testing centers, the average amount of green biomass at harvest for the SAŞM 1 hybrid was 114.1 t/ha. The lowest amount obtained was 82 t/ha, and the highest was 131.9 t/ha. The seeding density was 156 thousand plants per hectare. The average production for the SAŞM 2 hybrid was 132.1 t/ha, with a lower production limit of 80 t/ha and an upper production limit of 163 t/ha. For the SAŞM 4 hybrid, the average biomass production was much lower compared to the first two, this is due to the fact that this last hybrid is a hybrid created in combination with Sudan grass, a hybrid used in animal feed. The production limits for this hybrid were 24 t/ha and 45.2 t/ha, respectively.

Table 6. Average yields of stalks, juice and bagasse obtained from the three lines of sweet sorghum, tested in 8 experimental centers

No.	Variety	Stems (t/ha)	Juice (t/ha)	Bagasse (t/ha)
1.	SAȘM 1	79.8	35.9	43.9
2.	SAȘM 2	88.3	35.2	52.1
3.	SAȘM 4	22.7	9.1	13.6

After harvesting the sweet sorghum, the panicle leaves and the internode underlying the panicle were removed, as they are not important in terms of the juice obtained. For the SAŞM 1 hybrid, a quantity of 79.8 stems/ha resulted. After pressing, the resulting juice was 35.9 t/ha, and the remaining material, called bagasse, was in the amount of 43.9 t/ha. For the second hybrid, SAŞM 2, the resulting quantity of stems was 88.3 t/ha. After processing, the resulting quantity was 35.2 tons of sweet juice and 52.1 t of bagasse.

For the SAŞM 4 hybrid, the quantity of stems was 22.7 tons, from which 9.1 tons of juice and 13.6 tons of bagasse resulted (Table 6).

Table 7. Concentrated sugar sorghum juice sample analyses - I.B.A. Bucharest

No.	Determination	Unity of measure	Laboratory method	Determined value
1.	Total sugar	%	Schoorl method	61.18
2.	Reducing sugar	%	Schoorl method	31.50
3.	Dry substance	Refract. degree	SR EN 12143:2003	77.38
4.	Acidity, expressed as citric acid	%	SR EN 12147:1999 0	1.17
5.	Polyphenols	mg/100 ml	Florin-Ciocâlteu method	783.09
6.	Antioxidant capacity	mg/100 ml	DPPH method	411.12

After obtaining the concentrated sweet juice by pressing the sweet sorghum stems, it was subjected to a set of analyses. The analyses were carried out at the National Institute for Research and Development of Food Bioresources and were as follows: total sugar content, reducing sugar, amount of dry matter, acidity, expressed in citric acid, polyphenols and antioxidant capacity. The total sugar content obtained was 61.18%, and the reducible sugar was in the amount of 31.50%. Both determinations were carried out by the Schoorl method. The dry matter obtained was 77.38 and the acidity, expressed in citric acid, was 1.17%. Both were carried out by the SR EN method. The polyphenol content was 783.09 mg/100 ml. The antioxidant capacity was 411.12 mg/ 100 ml.

Table 8. Physico-chemical characteristics of the average sorghum sample (stems)

Parameter	%	% dry matter
Moisture	72.10	-
Ash	0.94	3.37
Total protein	1.15	4.2
Total fat	0.19	0.68
Total carbohy-drates	25.62	91.83
Invert sugar	9.90	35.48
Crude fiber	8.51	30.50

From the analysis of the sample of sweet sorghum stems with a moisture content of 71.10%, it was observed that the total carbohydrate content was 25.62%, invert sugar 9.90, crude fiber 8.51%. The protein content was 1.15%, ash 0.94%, and fat was 0.19%.

Table 9. Testing the calorific value of sweet sorghum biomass kcal/kg

No.	Date of sampling	Lower calorific value	Higher caloric value
1.	03/08/2023	3804	4097
2.	15/09/2023	3912	4023
3.	25/10/2023	4064	4328

During the vegetation period of the sweet sorghum crop, the calorific value of the biomass was also determined. This was carried out in August, September and October, close to the harvest of the crop. The analyses were carried out at the National Research and Development Institute for Industrial Ecology form Bucharest. According to the data found in Table 9, as the sorghum ripening period approached, its caloric value increased. Thus, at the third determination, the lower caloric value was 4064 kcal/kg, and the upper caloric value was 4219 kcal/kg. Values up to 3850 kcal/kg represent the capacity for use for feed purposes, and values higher than this threshold signify the capacity for use for energy purposes.

CONCLUSIONS

The testing of the three sweet sorghum hybrids (ŞAŞM 1, ŞAŞM 2 and ŞAŞM 4), in 2023, under the pedoclimatic conditions of Great Brăila Island (I.M.B.), highlighted an important production potential in terms of biomass and sugar content, this under non-irrigated conditions.
The highest biomass production (stems) belongs to the SAŞM 1 hybrid, which recorded 98.6 t/ha, followed by SAŞM 2, with 86.5 t/ha and the SAŞM 4 hybrid presented the production of 24.9 t of fresh biomass.
In terms of sugar content, the hybrids stand out with a content of 16.4% (SAŞM 2), 16.3%-SAŞM 1 and the SAŞM 4 hybrid with 6.9%, this being, however, a hybrid intended for animal feed. According to analyses of the concentrated sorghum juice sample that were performed at I.B.A. Bucharest the total sugar content was 61.18 % which represents an important value.
It can be stated that the juice obtained from sweet sorghum stems represents an important source in the production of food sugar, and the resulting bagasse can be successfully used for both energy and feed purposes.

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the cultivation technology appropriate to meet the eco-environmental requirements of sweet sorghum. The study was performed in other 7 locations as well.

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YIELD AND PROTEIN CONTENT OF WINTER PEA (*Pisum sativum*) VARIETIES IN AN ORGANIC FARMING SYSTEM

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Abstract

Pea (Pisum sativum) is one of the most important crops in the Fabaceae family, second only to soybean in significance. Its protein content, which ranges from 13% to 38%, is influenced by both environmental and genetic factors, making it a promising source of high-quality protein. A field experiment was conducted during the 2022–2024 years in an organic farming system in Satu Mare County, Romania, to evaluate the yield and protein content of winter pea. The study focused on three winter pea varieties: Andrada, Olguța, and Ghittia. The results indicated that the environmental conditions in the region were favourable for the growth, development, and yield formation of pea plants. The yield of the studied varieties exceeded 2500 kg ha⁻¹, with protein content surpassing 23%. These findings demonstrate that winter pea is a promising crop for Satu Mare County, offering a valuable source of protein. Additionally, the results provide practical insights for agricultural producers, enabling them to select pea varieties based on quality characteristics such as protein content.

Key words: *winter pea, protein content, yield, organic farming.*

INTRODUCTION

Pea, alongside common bean and soybean, is one of the most important cultivated species in the *Fabaceae* family (Jezierny et al., 2010; Shanthakumar et al., 2022). Its mature dry seeds have diverse applications (Wrigley et al., 2004), primarily as food and animal feed (Singh et al., 2013).

Pea seeds contain approximately 60-65% carbohydrates, with starch comprising 35-40%, 23–30% protein, 1–2% lipids, and smaller amounts of minerals and vitamins, depending on the cultivar, cultivation conditions, and the maturity stage of the seeds at harvest (Lam et al., 2018; Bogahawaththa et al., 2019; Lu et al., 2019; Saurel, 2020). The protein content, in particular, ranges from 23.3% to 31.7% across different genotypes and pedoclimatic conditions (Barac et al., 2010; Bogahawaththa et al., 2019).

Although pea protein has been studied since the 1980s (Johnson and Brekke, 1983; Koyoro and Powers, 1987; Sumner, Nielsen, and Youngs, 1981), interest in this crop has significantly increased in recent years. This increased attention is attributed both to its relevance in the food industry and to the increasing consumer awareness of the health benefits associated with products derived from pea seeds (Lam et al., 2018).

The rising global demand for plant-based proteins, linked to reduced risks of obesity, hypertension, and diabetes, has further reinforced its relevance. Pea protein is rich in lysine, an amino acid that supports a healthy immune system (Huntrakul et al., 2020). Overall, pea proteins exhibit antioxidant, antihypertensive, and anti-inflammatory properties (Liao et al., 2019). Furthermore, research has shown that regular consumption of pea protein-rich foods has been associated with reduced risk of cardiovascular disease and diabetes and may provide protective effects against several types of cancer, including breast, renal, and colon cancers (Ge et al., 2020). Additionally, the consumption of whole peas contributes to lowering blood glucose levels, improving gastrointestinal health, and increasing satiety (Tulbek et al., 2017). In food production, pea protein is used in a variety of products, including bread (Sahagun and Gomez, 2018), pasta (Tulbek et al., 2017), in meat analogues, dairy alternatives, dairy substitutes, and fortified beverages such as protein shakes and sports drinks (Philipp et al., 2017; 2018). Tulbek et al. (2017) provided solutions for replacing eggs in pasta, cakes, and bakery products with pea-based ingredients.

From an agronomic point of view, one of the most significant advantages of cultivating pea is its ability to enrich the soil with nitrogen through biological nitrogen fixation, which can subsequently benefit succeeding crops (Wysokinski et al., 2021; Ntatsi et al., 2019). Pea is a particularly valuable crop from an agronomic perspective (Roman et al., 2015). It vacates the field early and leaves behind significant quantities of organic matter and nitrogen in the soil. Furthermore, it leaves the land relatively free of weeds and without crop residues. Due to these characteristics, pea is an excellent precursor for many crops, especially winter wheat (Muntean et al., 2014).

According to available data (https://statistics.fibl.org/world/selected-crops-world.html), in 2023, the total area cultivated organically with peas in the European Union reached 70,528.77 hectares. The largest organically cultivated areas were recorded in Germany, with 14,000 hectares (representing 19.85% of the total EU area), Spain with 7,393.43 hectares (10.48% of the total), and France with 6,750 hectares (approximately 9.57%). Romania ranked fifth at the European level, with a total of 4,337 hectares of organically cultivated peas in 2023, accounting for 6.15% of the total EU area, highlighting the growing interest in organic practices and legume integration in sustainable cropping systems. Expanding the cultivation of pea in organic farming systems offers significant agronomic, economic, and environmental benefits, including reduced reliance on synthetic nitrogen fertilizers and improved nitrogen cycling through biological fixation (Faligowska et al., 2022). In this context, the aim of the present study is to evaluate the yield performance and protein content of three winter pea varieties cultivated under organic farming in the specific soil and climatic conditions of Satu Mare County, Romania. The specific objectives of the study were to: (1) assess the yield and yield components of three winter pea varieties; (2) determine the protein content of the pea genotypes studied.

MATERIALS AND METHODS

Study site

The research was carried out on a certified organic private farm located in Chereușa, Satu Mare County, Romania, over two consecutive agricultural years (2022-2023 and 2023-2024). In the research area, soil analyses indicate a slightly alkaline reaction, a very good supply of phosphorus (P), and a medium supply of nitrogen (N) and potassium (K). These analyses were delivered by the Laboratory for soil and plant analysis, Faculty of Agriculture, University for Agricultural Sciences and Veterinary Medicine Cluj-Napoca.

During the vegetation period (November to June), monthly average temperatures showed interannual variability.

The mean temperature recorded throughout the growth cycle was 7.475°C in the first season and 12.375°C in the second season, which significantly influenced the growth and development of the pea plants (Figure 1).

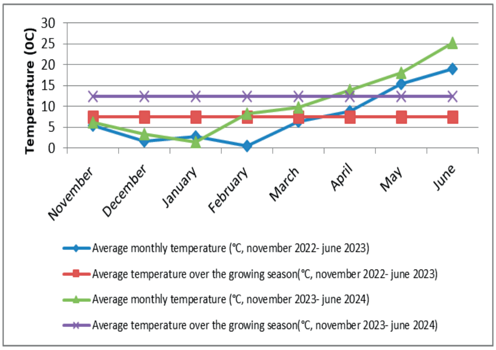

Figure 1. Average temperature over the growing season

The total precipitation recorded during the November 2022 - June 2023 period was 355.4 mm, while for the November 2023 - June 2024 period, it amounted to 471.2 mm (Figure 2). Climatic data were used to interpret growth dynamics and production levels of the studied genotypes.

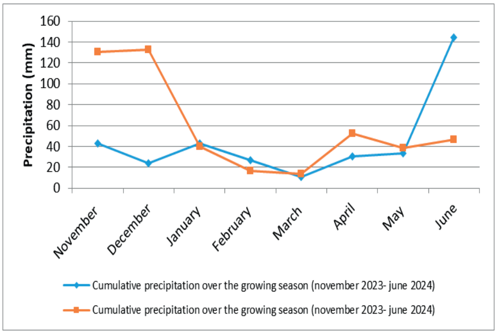

Figure 2. Cumulative precipitation over the growing season

Experimental design

The biological material consisted in three afila-type pea (*Pisum sativum* L.) cultivars - Olguța, Ghiția, and Andrada, under certified organic farming conditions. These cultivars, developed at the National Agricultural Research and Development Institute (NARDI) Fundulea, represent notable advancements in the genetic improvement of autumn-sown pea, offering high-performance alternatives to spring cultivars, particularly relevant in the context of climate change. The cultivars combine favourable agronomic traits such as adaptability, high productivity, superior seed quality, and resistance to biotic stress, thus representing promising options for growers across various agroecological zones of Romania.

The experiment was conducted within a crop rotation system, with winter wheat serving as the preceding crop. Sowing took place during the first ten days of November, in accordance with the technical requirements of organic production systems. No synthetic chemical inputs were applied throughout the growing season. Peas were harvested in the second decade of June, each experimental year. The moisture content of the seeds was 18%.

Phytosanitary observations

Throughout the growing seasons, the phytosanitary status of the crop was closely monitored. No pest or disease infestations above economic thresholds were recorded. Limited occurrences of powdery mildew (*Erysiphe polygoni*), anthracnose (*Ascochyta pisi*), and grey mold (*Botrytis cinerea*) were observed, but the severity of these attacks remained low and did not impact the overall plant health or yield.

Yield components and seed yield

Prior to harvest, the following yield components were determined: total number of pods per plant and seeds per pod and thousand seed weight (TSW). The TSW was determined in accordance with STAS standard methodology (SR 6123/99). Eight replicates of 100 seeds each were manually counted and individually weighed. The average of the eight values was multiplied by 10 to obtain the final TSW.

Seed yield (Q, kg/ha) was estimated based on the following formula (Muntean et al., 2018):

$$Q\ (kg/ha)\ \frac{NplxNppxNbpxTSW}{100}$$

where:

Npl = average number of plants per m²;

Npp = average number of pods per plant;

Nbp = average number of seed per pod;

TSW = thousand seed weight (g).

Protein content

The protein content of the seeds was determined using the Kjeldahl method (Kjeldahl, 1983). This method involves digesting the samples in concentrated sulphuric acid in the presence of catalysts to convert organic nitrogen into ammonium, which is further distillate and titrated. The resulting total nitrogen content was further multiplied by a nitrogen-to-protein conversion factor of 6.25 to estimate the crude protein content.

Data analysis

All collected data were compiled and statistically analyzed using analysis of variance (ANOVA), followed by Duncan's multiple range test, to evaluate the adaptability and performance of the tested cultivars under organic farming conditions within the specific pedoclimatic context of the study area.

RESULTS AND DISCUSSIONS

Yield components and seed yield

The climatic conditions during the vegetation period play an essential role in determining the agronomic success of winter pea crops, directly influencing germination, overwintering, vegetative regrowth, flowering, fecundation, and physiological seed maturation.

In both experimental years (2022-2023 and 2023-2024), the temperatures recorded in November, immediately after sowing, were above the minimum germination temperature for pea seeds, estimated at 1-2°C (Muntean et al., 2014; Samuil, 2007). This situation, correlated with satisfactory rainfall amounts during the same period, favoured a uniform and vigorous seedling emergence.

It is also noteworthy that in both experimental years, the winters were mild, with positive average monthly temperatures recorded in December, January, and February, which enabled proper overwintering without significant plant losses.

In March and April, thermal values were favourable for the resumption of vegetation and the branching process. Temperatures of 6.3°C in March and 8.8°C in April were recorded in the first year, while 9.73°C and 13.95°C were observed in the second year. The optimal temperature range for vegetative development of pea is 14-15°C, according to Muntean et al. (2014), or 10-18°C, according to Devi et al. (2023). In this context, we can conclude that in the first experimental year thermal conditions approached the optimal range, supporting a balanced development of pea plants. In the second year, the higher temperatures accelerated the phenological processes, which led to a shortened accumulation phase and, consequently, had a negative impact on yield levels.

During the flowering period, the optimal temperature is between 15-18°C (Muntean et al., 2014; Roman et al., 2015), while during the ripening phase it ranges between 18-20°C (Muntean et al., 2014). In the 2023-2024 season, these values were exceeded in May and June, which resulted in floral abortion and a reduced number of fertilized pods, with a negative effect on the crop's productive potential.

From a pluviometric perspective, pea water requirements are comparable to those of beans, with a total demand of 350-500 mm (https://www.fao.org/land-water/databases-and-software/crop-information/pea/fr/). The total precipitation recorded between November and June amounted to 355.4 mm in the first year and 471.2 mm in the second year, falling within the optimal range. However, rainfall distribution was not uniform. In both years, February and March were characterized by rainfall deficits (27.1 mm and 10.8 mm in 2022-2023; and 16.6 mm and 14 mm in 2023-2024, respectively). Nevertheless, the consistent precipitation recorded in the preceding months (November and December) contributed to the replenishment of soil moisture reserves, ensuring acceptable conditions for subsequent plant development.

The number of pods is one of the most important yield determining components in several grain legume species (French, 1990). The duration of pod formation depends on the onset and end of flowering (French, 1990). The initiation of flowering is mainly influenced by the cultivar, but also by environmental factors, particularly temperature (Berry and Aitken, 1979). In the year 2023, the average number of pods per plant was 9.32 (Table 1). In 2024, higher temperatures during the flowering period resulted in a reduced number of pods per plant (8.31 in average). Across the two experimental years, the differences among cultivars were small and statistically insignificant, as confirmed by both analysis of variance and Duncan's multiple range test.

Table 1. Influence of cultivar × year interaction on the number of pods per plant

Year	Variety	Pods number	% to control	Difference/ Signifficance	Duncan Test
2023	Average	9.32	100	Mt.	-
	Ghittia	9.33	100	0.01^{-}	a
	Andrada	9.13	98	-0.19^{-}	a
	Olguța	9.50	102	0.18^{-}	a
2024	Average	8.31	100	Mt.	-
	Ghittia	8.27	99	-0.04^{-}	b
	Andrada	8.13	98	-0.18^{-}	b
	Olguța	8.53	103	0.22^{-}	b
LSD (p 5%)				0.43	0.43-0.46
LSD (p 1%)				0.62	
LSD (p 0.1%)				0.93	

The number of seeds per pod is a key genetic trait that reflects the productive potential of pea cultivars. According to specialized literature, this parameter generally ranges between 2 and 5 seeds per pod (Muntean et al., 2014, Roman et. al., 2015). In the present study conducted during the 2022-2024 period, the average values obtained for this trait exceeded 3 seeds per pod in both experimental years, indicating a high productive capacity of the analyzed cultivars.

The results presented in Table 2 highlight a significant influence of the cultivar × year interaction on this parameter. In 2023, the cultivar Olguța recorded an average of 3.39 seeds per pod, with a highly significant difference (***). In contrast, the cultivar Ghittia registered a value of 2.90 seeds per pod, which was significantly lower (000). The same trend was observed in 2024, when Olguța achieved 3.43 seeds per pod, while Ghittia recorded only 2.83 seeds per pod. These differences confirm the stability of the cultivars with respect to the number of seeds per pod.

Table 2. Influence of cultivar × year interaction on number of seeds per pod

Year	Variety	Seed number/ pods	% to control	Difference/ Signifficance	Duncan Test
2023	Average	3.19	100	Mt.	-
	Ghittia	2.90	91	-0.28^{000}	a
	Andrada	3.26	102	0.07*	c
	Olguța	3.39	107	0.21***	d
2024	Average	3.13	100	Mt.	-
	Ghittia	2.83	90	-0.30^{000}	a
	Andrada	3.13	100	0.00^{-}	b
	Olguța	3.43	110	0.30***	d
LSD (p 5%)				0.07	0.07-0.08
LSD (p 1%)				0.11	
LSD (p 0.1%)				0.16	

The thousand seed weight (TSW) is an important parameter reflecting the production potential of a cultivar. According to Muntean et al. (2015), TSW in pea can range between 50 and 450 grams. In this study, the TSW values fell within this range, varying between 156.00 g and 195.00 g depending on cultivar and year.

As shown in Table 3, the first year was more favourable for accumulation in seeds, with an average of 184.56 g, compared to 165.00 g in 2024. The highest TSW was consistently recorded by the Ghittia cultivar, with 195.00 g in 2023 and 177.00 g in 2024. In both years, the differences compared to the control were highly significant from a statistically point of view, indicating a superior ability to accumulate dry matter in seeds, which is often associated with larger seed size.

Conversely, Andrada and Olguța cultivars recorded lower TSW values, with significantly negative differences. Particularly in 2023, Andrada had a TSW of 175.67 g, and Olguța reached 183.00 g. In 2024, a general decline in TSW was observed, with the lowest value noted in Andrada (156.00 g), suggesting a higher sensitivity to less favourable climatic conditions.

Table 3. Influence of cultivar × year interaction on thousand seed weight (TSW)

Year	Variety	TSW (g)	% to control	Difference/ Signifficance	Duncan Test
2023	Average	184.56	100	Mt.	-
	Ghittia	195.00	106	10.44***	……….e
	Andrada	175.67	95	-8.89^{000}	c
	Olguța	183.00	99	-15.6^{-}	d
2024	Average	165.00	100	Mt.	-
	Ghittia	177.00	107	12.00***	c
	Andrada	156.00	95	-9.00^{000}	a
	Olguța	162.00	98	-3.00^{-}	b
LSD (p 5%)				3.95	3.94-4.29
LSD (p 1%)				5.75	
LSD (p 0.1%)				8.62	

Under the specific environmental conditions of the Satu Mare region, the most productive autumn pea cultivar was Olguța, with a yield of 3834.10 kg/ha in 2023 and 3086.08 kg/ha in 2024.

In 2023, a year with more favourable climatic conditions (as detailed previously), significantly negative differences in yield were observed for Ghittia and Andrada compared to the average, while Olguța showed highly significant positive differences (Table 4). In 2024, Ghittia displayed small and statistically non-significant differences, Andrada exhibited significantly lower yields, and Olguța again achieved highly significant positive differences. The Duncan test clearly highlighted significant differences in yield between Olguța and the other studied cultivars in both experimental years.

Table 4. Influence of cultivar × year interaction on pea grain yield

Year	Variety	Yield (kg/ha)	% to control	Difference/ Signifficance	Duncan Test
2023	Average	3555.43	100	Mt.	-
	Ghittia	3433.38	97	-122.04^{0}	c
	Andrada	3398.80	96	-156.63^{0}	c
	Olguța	3834.10	108	278.67***	d
2024	Average	2788.29	100	Mt.	-
	Ghittia	2694.61	97	-93.68^{-}	a
	Andrada	2584.18	92.7	-204.11^{00}	a
	Olguța	3086.08	111	297.79***	b
LSD (p 5%)				120.84	120.59-131.32
LSD (p 1%)				175.77	
LSD (p 0.1%)				263.66	

Grain yield (kg/ha) is the most relevant parameter for evaluating economic efficiency, as it reflects the interaction between genetic traits and pedoclimatic conditions. The data presented in Table 4 show that the year 2023 was more favourable in terms of climate, with an average yield of 3555.43 kg/ha, compared to 2788.29 kg/ha in 2024.

The Olguța cultivar demonstrated outstanding productive capacity in both years, with 3834.10 kg/ha in 2023 (+278.67 kg/ha compared to the control) and 3086.08 kg/ha in 2024 (+297.79 kg/ha), both differences being highly significant. In contrast, Ghittia and Andrada produced yields below the average in most cases, with some significantly negative differences. Notably, in 2024, Andrada recorded a yield of 2584.18 kg/ha, with a significantly negative difference of -204.11 kg/ha compared to the control, indicating high sensitivity to the year's less favourable conditions.

Protein content

According to the data presented in Table 5, the average protein content of the studied varieties was 23.97% in 2023 and 24.24% in 2024, with relatively consistent values across the two years, despite significant differences in yield (3555.43 kg/ha in 2023 compared to 2788.29 kg/ha in 2024).

Table 5. The influence of the variety × year interaction on protein content

Year	Variety	Protein (%)	% to control	Difference/ Signifficance	Duncan Test
2023	Average	23.97	100	Mt.	-
	Ghittia	24.16	101	0.19^{-}	a
	Andrada	24.04	100	0.07^{-}	a
	Olguța	23.71	99	-0.26^{-}	a
2024	Average	24.24	100	Mt.	a
	Ghittia	24.34	100	0.11^{-}	a
	Andrada	24.27	100	0.03^{-}	a
	Olguța	24.10	99	-0.14^{-}	a
LSD (p 5%)				0.67	0.67-0.73
LSD (p 1%)				0.98	
LSD (p 0.1%)				1.47	

Regarding the varieties studied in this research, it was observed that although the Olguța variety recorded the highest yield values in both experimental years (3834.10 kg/ha in 2023 and 3086.08 kg/ha in 2024), it also exhibited the lowest protein content, namely 23.71% in 2023 and 24.10% in 2024.

In contrast, the Ghittia and Andrada varieties, which recorded yields below the annual average in both years, presented slightly higher protein content values. However, these differences were not statistically significant, neither in the analysis of variance nor according to Duncan's test. For example, in 2024, the Ghittia variety showed a protein content of 24.34%, and Andrada 24.27%, compared to the average of 24.24%. Although these values were higher than that of Olguța (24.10%), they were not statistically confirmed, indicating low variability and a relatively stable expression of this parameter. These results are consistent with those reported by Bărbieru (2022) for all the three varieties studied in this research.

CONCLUSIONS

The results obtained over the two experimental years (2022-2023 and 2023-2024) highlight the decisive influence of climatic conditions on the productive and qualitative performance of winter pea cultivars grown in the Satu Mare region. Positive average winter temperatures ensured proper overwintering without significant plant losses. Although the total precipitation amounts were within the optimal range for pea cultivation, their uneven distribution - especially in February and March - proved to be a limiting factor in both experimental years. Nevertheless, soil water reserves accumulated from precipitation in previous months contributed to maintaining an acceptable moisture regime.

Among the productivity traits analysed, the number of seeds per pod and the thousand seed weight (TSW) showed significant differences among cultivars, influenced by their interaction with the year of cultivation. The cultivar Olguța stood out with higher values for the number of seeds per pod, while Ghittia exhibited the highest TSW values.

Grain yield analysis confirmed the superiority of the Olguța cultivar in both experimental years, with very significant differences compared to the control. In contrast, the Ghittia and Andrada cultivars recorded yields below the control average, particularly in 2024, suggesting a higher sensitivity to unfavourable climatic conditions.

Concerning protein content, the values obtained were relatively stable across years and among varieties, with differences that were not statistically significant.

An inverse proportional trend was observed between yield and protein content, with the most productive variety (Olguța) exhibiting the lowest protein content values; however, these differences were not statistically confirmed.

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RESEARCH ON THE INFLUENCE OF SOWING TIME ON SUGAR BEET PRODUCTION IN THE CONTEXT OF CLIMATE CHANGE IN CENTRAL MOLDOVA

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Abstract

This paper presents the results obtained from research carried out at A.R.D.S. Secuieni, which aimed to identify solutions to increase the productivity of sugar beet, a crop strongly affected by drought in the specific conditions of the Center of Moldova. To this end, the influence of sowing time on production was analyzed in the experimental field. The growing period of sugar beet was characterized differently depending on the sowing epoch, being very dry for the first two epochs and normal for the third epoch, which benefited better from the large amounts of rainfall in September. The best plant density at harvest was recorded in the third sowing epoch (May 3th), which also achieved the highest root production (48.2 t/ha). Compared with the control variant (average of experience), the increase in production achieved by this variant was statistically assured and interpreted as highly significant. At the opposite pole was the variant sown in the first sowing period (April 01th), which achieved a difference from the control that was interpreted as a very significant negative difference.

***Key words**: root yields, climate change, beetroot, cultivation technologies.*

INTRODUCTION

Today, competition for increasingly scarce water resources is becoming very important. The scope for growing water availability is reduced because of the major challenge facing the agricultural sector today to produce more food using less water.

Climate change in recent years has had a significant impact on plant growing cycles (Pochișcanu et al., 2017), including sugar beet production.

Sugar beet (*Beta vulgaris* L.) is the second most important sugar crop after sugarcane, producing about 30% of the total global production and having a high adaptability to different environmental factors, including climate (Naghizadeh et al., 2013). In the early 19th century, the only global source of sugar was sugarcane. Later, sugar beet became one of the most important sources of sugar in the world. Due to its numerous industrial applications, sugar beet is considered a profitable crop (Mukherjee and Gantait, 2023). People need this crop because it provides them with high quality energy and can be used for animal feed. It is a species particularly adapted to temperate regions and its yield is influenced by factors such as soil type, climatic conditions and cultivation technology. Successful cultivation requires a moderate climatic regime with sufficient rainfall and temperate temperatures to develop quality roots and ensure optimal yield (Călin et al., 2019; Nistor et al., 2021). Sugar beets can reduce their yield by about 51% under abiotic stresses, especially drought and soil salinity (Vicente, 2022).

The growth of sugar beet in tropical and subtropical regions to replace or supplement sugar production from sugarcane is increasing

(Abou-Elwafa et al., 2020; Simova-Stoilova et al., 2016). Sugar beet cultivation has many advantages in these regions, including less need for irrigation and its high sugar productivity in a shorter growing season compared to sugarcane (Abo-Elwafa et al., 2013; Abou-Elwafa et al., 2020; Balakrishnan and Selvakumar, 2009). Sugar beet cultivation in developing countries can also be profitable for farmers and the sugar industry by diversifying farmers' income by allowing them to grow an additional crop for commercialization, and by supplying sugar factories with raw material in addition to sugarcane, which can extend the processing season of sugar factories by up to 10 months per year (Abou-Elwafa et al., 2020; Balakrishnan and Selvakumar, 2009; Mandere et al., 2010).

Sugar beet crop productivity is strongly influenced by a number of factors, both technological and environmental (Bocos et al., 2023). Optimization of agronomic practices, which is dependent on climatic conditions, is essential to achieve higher potential and sustainable sugar beet cultivation in the context of climate change. Results of numerous studies have highlighted that the yield potential of sugar beet is directly influenced by the adaptation of cropping technologies to environmental conditions, this fact contend more than the number of plants per unit area (Galal et al., 2022; Elmasry and Al-Maracy, 2023; Hussien et al., 2023). In the context of climate change, which negatively influences plant productivity and quality and severely affects the sustainability of agricultural production, determining the most appropriate sowing date for sugar beet is essential for sustainable cultivation and achieving high sugar beet yield (Curcic et al., 2018). To obtain higher yields there is a need to increase the value of some important parameters in sugar beet crop, such as the diameter of the beetlet, in addition to the number of plants at harvest (Sabaghnia et al., 2024), and this can be done by optimizing some technological sequences. Also, solutions should be found for proper maintenance of the crop, significant increase in weed infestation of sugar beet crop.

Evidence from the literature has emphasized that early sowing leads to earlier maturation of sugar beet and hence earlier harvest, whereas late sowing requires later harvest (Refay, 2010). The determination of an optimal sowing date is one of the most important requirements for successful sugar beet crop (Ezueh, 1982), with numerous recent studies highlighting the complex interaction between sowing time and climatic factors such as temperature, rainfall and drought.

The latest results obtained in the Moldovan area have shown that earlier sowing times favor a better crop start, reducing the risks of late frosts, but have the disadvantage that beet plants are exposed to large temperature fluctuations (Popovici and Grosu, 2023). In the context of climate change in Central Moldova, it has been shown that delayed sowing leads to poor crop growth and development, taking into account the water deficit during the growing season (Popa and Ilie, 2021).

In order to contribute to ensuring stable sugar beet yields in the pedoclimatic conditions of Central Moldova, the SCDA Secuieni studied the influence of sowing time on sugar beet root production.

MATERIALS AND METHODS

In order to achieve our proposed objectives, a single-factor experiment was set up in the experimental field at A.R.D.S. Secuieni, the factor being the sowing time.

The study was carried out in the agricultural year 2023-2024, on a typical Cambian chernozemic soil type, characterized as medium supplied with nitrogen, very well supplied with phosphorus and potassium, well supplied with magnesium, iron sulphur and copper, poorly supplied with boron and molybdenum, and neutral soil reaction (Leonte et al., 2024; Leonte et al., 2023).

The biological material used in the experiment was represented by the hybrid Terrapin Smart which is cultivated according to the Conviso technology, and the rest of the technological sequences applied were those specific for the conditions in the Center of Moldova, following the experimental protocol. Three sowing epochs were experimented, namely:

- Season I - Sown on April 01;
- Season II - Sown on April 16;
- Season III - Sown on May 03.

The crop was fertilized with 200 kg/ha of NPK 15:15:15 complex fertilizer, and the experiment was sown at a row spacing of 50 cm, ensuring a density of 110,000 pellets/ha.

The 2023/2024 crop year was marked by extreme climatic conditions, characterized by constant high temperatures and severe drought, which considerably influenced agricultural crops, including sugar beet. The growing season for sugar beet was characterized by monthly average temperatures higher than the multiannual values, with high positive deviations of up to 4.1°C-4.3°C in July and August. These extreme conditions amplified the heat stress on the crop, negatively affecting physiological processes, such as sugar accumulation, and significantly reducing yield potential. The growing period of sugar beet was 3.1°C warmer than the multiannual average (19.6°C), which emphasizes the need to adapt sugar beet growing technology to the new climatic conditions (Figure 1).

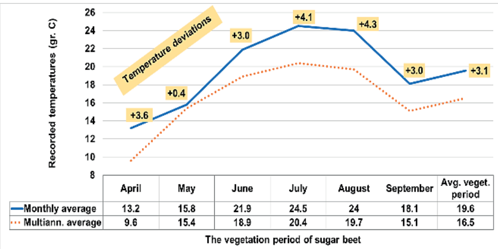

Figure 1. Temperatures recorded at the meteorological station of A.R.D.S. Secuieni, during sugar beet growing season

In terms of precipitation, 2023/2024 was significantly drier, with a total of only 451.8 mm, a deficit of 80.1 mm compared to the multi-year average. The 2023/2024 growing season for sugar beet in crop year 2023/2024 experienced significant rainfall deficits in most months, with the notable exception being September, when heavy rains significantly exceeded the multi-year average. Severe water deficits in May, July and August adversely affected initial plant development and sugar accumulation, amplifying water stress on the crop. Although June had rainfall close to the multiannual average and September brought excessive amounts of water, these conditions failed to offset the impact of the prolonged drought on production. Although 128.6 mm of precipitation accumulated in September, this heavy rainfall came too late to effectively support crop development, the only crop to benefit from it being beet sown in the third sowing season. Overall, total growing season rainfall amounted to 319.4 mm, 57.4 mm below the multiannual average of 376.8 mm (Figure 2).

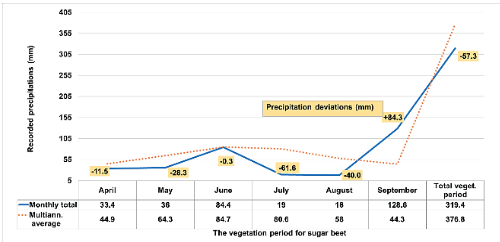

Figure 2. Rainfall recorded at the meteorological station of A.R.D.S. Secuieni, during sugar beet growing season

RESULTS AND DISCUSSIONS

The first sowing was harvested on October 11, the second sowing on October 14 and the third sowing on October 28. A series of determinations were made at maturity and the results showed very wide variations depending on the sowing season.

The number of leaves per plant is a key agronomic indicator for assessing the vegetative stage and yield potential of sugar beet. This parameter reflects the ability of the plant to photosynthesize and thus to accumulate reserve substances in the root. The results showed that the number of leaves varied considerably according to the sowing time, demonstrating the decisive influence of the time of sowing on the development of the leaf apparatus. Under the dry and warm conditions of 2024, late sowing of sugar beet proved beneficial for increasing the number of leaves per plant, with the maximum value of 75.2 leaves/plant being determined in the third epoch. This is mainly due to the fact that the first two sowing epochs were subjected to atmospheric heat for a longer period of time, losing some of their foliage (Figure 3).

The number of roots per square meter is a key agronomic indicator that reflects plant density and directly influences sugar beet yield. This parameter varied according to the sowing time, reaching the highest value in the late sowing, with an average of 11 roots/meter. Late sowing favored the formation of a higher number of roots per unit area, which can be attributed to the specific climatic conditions that stimulated germination and uniform rooting (Figure 3).

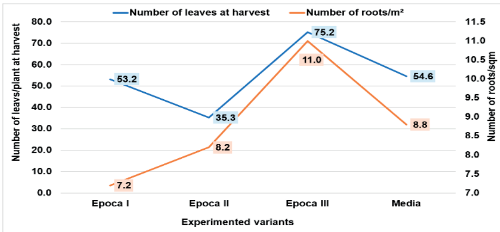

Figure 3. Sugar beet maturity determinations under soil and climatic conditions at A.R.D.S. Secuieni, 2024

After analyzing the data shown in Figure 4, we concluded that a higher number of leaves/plant, indicative of more intense photosynthetic activity, directly contributed to the formation of more roots. Our results emphasize the importance of managing crop factors in sugar beet, such as determining the optimal sowing time, which influenced plant development. This conclusion is supported by the value of Pearson correlation coefficient (r = 0.751155), which indicates a direct and strong relationship between the number of leaves/plant and the number of roots/meter. The coefficient was statistically secured and interpreted as significant.

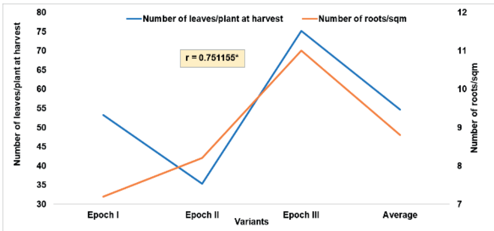

Figure 4. Correlation between the number of leaves/plant and the number of roots/meter in sugar beet, A.R.D.S Secuieni - 2024

The thickness of the beetlet is an essential indicator in assessing the quality and yield potential of sugar beet, having a direct impact on sugar accumulation and plant resistance to different stress conditions. Its values showed significant variations depending on the sowing epoch, with values ranging from 26.8 cm in epoch III (late sowing) to 29.6 cm in epoch II. These results suggest that sowing in seeding time II favored an optimal development of the seed coat, which may indicate a better adaptation of the plants to the specific growing conditions. Late sowing, on the other hand, resulted in a reduction in the thickness of the coleus, which could be explained by a shorter period of active growth (Figure 5).

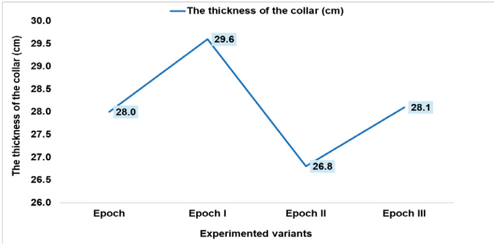

Figure 5. Sugar beet bunch thickness under pedoclimatic conditions at A.R.D.S. Secuieni, 2024

A higher number of roots per square meter at harvesting negatively influenced the diameter of the basket, which was also to be expected in view of the intensified competition between plants for resources. The correlation between the two variables was indirect, with a correlation coefficient r = -0.762156, interpreted as statistically significant negative (o). This relationship emphasizes the importance of ensuring an optimal density at harvest to improve sugar beet quality, with the diameter of the sugar beet boll being a relevant agronomic indicator in this respect (Figure 6).

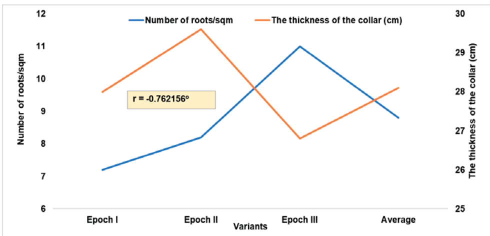

Figure 6. Correlation between the number of roots per square meter at harvest and the diameter of the sugar beet bunch, A.R.D.S. Secuieni - 2024

Sugar beet root yield varied significantly by sowing time, benefiting from different climatic and moisture conditions in plant growth and development.
The lowest yield was obtained in the variety sown in the first sowing season, at 32.2 t/ha. This can be explained by exposure to high temperatures and atmospheric scorch for a

longer vegetation period, including sensitive phenotypes. This variant realized compared to the experiment control (mean) a very significant negative yield difference (Table 1).
Sowing beets late, in the third sowing season, was beneficial, with the highest root yield of 48.2 t/ha. This is explained, firstly, by the higher plant density at harvest due to an extremely uniform emergence and, secondly, by the shorter exposure of the crop to atmospheric heat stress at the sensitive growth stages. Compared to the average of the experiment, this variant realized a very significant yield increase (Table 1).

Table 1. Sugar beet root production, A.R.D.S. Secuieni, 2024

	Epoch	Root Production		The difference from Mt. (t/ha)	Meaning
		t/ha	%		
1	Epoch I	32.2	80	-81	ooo
2	Epoch II	40.6	101	0.3	
3	Epoch III	48.2	120	7.9	***
4	Media	40.3	100	Mt.	
DL (t/ha)				5% = 1.9 1% = 2.8 0.1% = 4.0	

The number of roots per square meter is an indicator of crop density, and a well-managed density ensures better area coverage and efficient use of available resources. Each root contributes to the total yield, and a higher number per unit area leads to a proportional increase in yield/ha. In our case, the correlation between the number of roots/meter and the yield/ha was direct and very close, which shows that the beet benefited from sufficient resources for its development. The correlation coefficient (r) attests to this strong relationship between the two variables, with a positive value interpreted as highly significant (Figure 7).

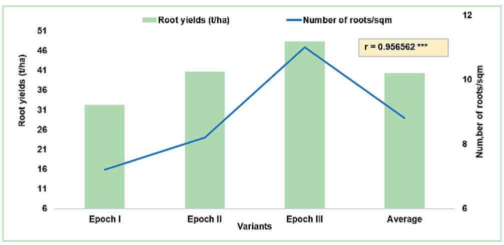

Figure 7. Correlation between number of roots/sq.m and root yield of sugar beet, A.R.D.S. Secuieni - 2024

CONCLUSIONS

The research conducted at A.R.D.S. Secuieni has highlighted the significant influence of sowing time on sugar beet production under climate change characterized by high temperatures and uneven rainfall.
The third sowing time (May 3th) proved to be the most favorable, with a root yield of 48.2 t/ha, a very significant increase compared to the average of the experiment, benefiting from the abundant rainfall in September.
All elements evaluated during the growing period of sugar beet, such as number of roots/meter, number of leaves/plant influenced the root production/ha, either directly or indirectly, thus contributing to the final productivity of the crop.
These results emphasize the need to adapt agricultural technologies to current climatic conditions, highlighting the importance of determining an optimal time for sowing. The implementation of this strategy can contribute to maximize yields and reduce the vulnerability of agricultural crops to water and heat stress.

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RESEARCH ON THE INFLUENCE OF FOLIAR FERTILIZER TREATMENT ON THE YIELD OF DIFFERENT WINTER WHEAT VARIETIES DEPENDING ON WATER SUPPLY LEVELS, ON CHERNOZEM

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Abstract

In 2023 and 2024, on the chernozem of Caracal, a three-factorial experiment with wheat was located for study the influence of the variety, foliar fertilizer treatment, water supply level and their interaction on yield, test weight and protein content. The yield and test weight were influenced by the variety and the level of water supply. Protein content was influenced only by the variety. The lowest yield was recorded by the Glosa variety fertilized with Foliq Nitrogen (3 kg/ha) sown under non-irrigation conditions – 5233 kg/ha and the highest was 7289 kg/ha for the Gabrio variety fertilized with Foliq Nitrogen (5 kg/ha) sown under irrigation conditions with a norm of 50% of the active moisture interval (AMI). The thousand weight grains had the highest value in the Glosa variety fertilized with Foliq Cereale (1 l/ha). The protein content values ranged between 10.52% (Avenue variety fertilized with Foliq 36 N 3 l/ha and irrigated at the level of 50% of the AMI) and 12.36% (Glosa variety fertilized with Foliq Cereale 2 l/ha and irrigated at the level of 50% of the AMI).

***Key words**: wheat, foliar fertilizer, water supply level, yield, protein.*

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important agricultural crops with a wide area of distribution, grown worldwide in more than one hundred countries, providing about 20% of the caloric and protein requirements for the human diet (Guarin et al., 2022).

World wheat production is growing steadily, according to statistics registering about 808 Mt in 2022, but to meet the estimated global cereal demand by 2050, wheat production needs to be continuously improved and this even in the context of climate change (del Pozo et al., 2016). A study (Van Dijk et al., 2021) suggested that cereal production would need to be increased by 35-56% to meet global food demand by 2050, respectively by 30-62% when accounting for climate change.

The soil and climatic conditions associated with the applied technology are decisive factors in the success of wheat cultivation, both in terms of productivity and quantity (Sobolewska et al., 2020; Rebouh et al., 2023).

Drought is a severe source of abiotic stress that threatens wheat (*Triticum aestivum* L.) growth and yield worldwide and is exacerbated by climate change (Wang et al., 2016; Zhang et al., 2017; Alam et al., 2020).

Wheat is one of the most adaptable crops under different environmental conditions, with very wide ecological plasticity, benefiting from efficient biological mechanisms in adapting to soil and climatic conditions (Stoian et al., 2015; Bărdaș et al., 2024).

The choice of varieties depends on the area, while soil and foliar applications of macro- and micronutrients contribute to adequate plant nutrition (Bielski et al., 2020; Wojtkowiak et al., 2015).

In order to produce more high-quality wheat, it is important to not only breed innovative high-quality varieties but also adopt optimized farming practices, such as improved water management (Buster et al., 2022; Si Z. et al., 2023), as well as new fertilization methods. Severe water scarcity and uneven rainfall distribution have become major challenges for meeting the growth needs of fall wheat, and supplemental irrigation is thus urgent for production (Jiatun et al., 2020; Motazedian et al., 2019).

Understanding the mechanism regulating the physiological process of wheat under different irrigation regimes will provide the theoretical basis for optimizing water-saving irrigation technology for sustainable wheat production. The adjustment of irrigation water is based on the regulation of the water demand of wheat during growth periods, a mild water deficit in vegetative stages causes the transpiration rate to decrease while the photosynthesis rate remains unchanged. Besides, winter wheat is highly resistant to moderate water stress in early vegetative growth periods and many negative effects can be eliminated after rehydration, such as photosynthesis and transpiration rates can quickly recover, or even exceed, which does not affect the accumulation of dry matter in the later periods of wheat (Si Z. et al., 2023).

In search for high yield, nitrogen fertilizers applied by farmers far exceed crop demand (Zhang et al., 2015). Excessive fertilization can lead to soil degradation and groundwater pollution (Yan et al., 2021). Previous studies have indicated that adequate soil moisture promoted the availability N capacity and simultaneous water use (Li et al., 2019; Di Paolo & Rinaldi, 2008). Therefore, there is a pressing demand for synergistic improvement of irrigation and fertilization synergy for wheat production in arid and semi-arid regions (Shicheng et al., 2021).

Fertilizer management is an important part of crop production, and proper application is considered a key component of achieving high yields and quality. Research in this regard demonstrates that, compared to soil application, foliar nitrogen fertilizer foliar application improves plant mineral nutrient content, increases productivity, quality and yield components of wheat and yield components of wheat (Arif et al., 2006; Tea et al., 2004; Baloch et al., 2019).

Among several advantages, foliar fertilization is able to alleviate nutrient deficiency faster than soil application (Fageria et al., 2009; Niu et al., 2021). In common wheat (*Triticum aestivum* L.), the application of nitrogen through foliar spraying is recognized as one of the most efficient agronomic tools to improve the grain protein content and the bread-making properties of flours (Bly et al., 2003; Arif et al., 2006; Ferrari et al., 2021). However, wheat response depends on the form of fertilizer, concentration and frequency of application, growth stage and leaf age, and other morphological and physiological traits (Fernández et al., 2013).

As N is allocated faster to grains through leaf application (Wuest & Cassman, 1992), the practice of applying N solutions to the canopy is commonly used only late in the growing season, particularly at anthesis (Wyatt et al., 2017; Ransom et al., 2016; Wuest & Cassman, 1992) or early milk (Turley et al., 2001), with the aim of improving flour quality and the bread-making properties (Rossmann et al., 2019; Ferrari et al., 2021).

Given the importance of fertilization in the success of any crop and the introduction on the market of numerous fertilizer products, many of which are foliar applied, the purpose of this paper is to present the yield and quality results obtained from experiments with two types of foliar fertilizers applied at different rates and at different times to three varieties of autumn wheat under different water supply conditions.

MATERIALS AND METHODS

The aim of the study is to determine the influence of foliar fertilizer application on the yield and quality of wheat crop depending on the variety and the optimal time of application through the level of soil water supply on the Caracal chernozem.

The study involves the influence of the studied factors (factor A - variety with 3 gradations - Glosa, Avenue, Gabrio; factor B - foliar fertilizer with 5 gradations; untreated, Foliq 36 Nitrogen 3 L/ha, Foliq 36 Nitrogen 5 L/ha; Foliq Cereal 1 L/ha, Foliq Cereal 2 L/ha; factor C - water supply level with 3 gradations: non-irrigated, irrigated at 50% of AMI and irrigated at 75% of IUA) and their interaction (variety x foliar fertilizer x level of water supply) on yield, 1000-grain mass and protein content (estimated by NIR- Near Infrared Spectroscopy).

A trifactorial experiment in 3 replications was located in Caracal in the autumn of 2022. Seed from 3 varieties of autumn wheat and 2 types of foliar fertilizer were used.

The *Glosa* variety, approved in 2005, was obtained at INCDA Fundulea from the complex hybrid combination Delabrad"S"/Dor"S"// Bucur, through individual selection. In the last decade, *Glosa* has been cultivated in 30 - 37% of the country's area, enjoying great success among farmers in all parts of the country, with very high adaptability and superior yield potential compared to previous varieties.

Avenue variety, an extra-early variety, the most sold wheat variety with foreign genetics in Romania, implicitly in the area of influence of SCDA Caracal. Very good yield potential. Recommended for cultivation in all growing areas, but especially in the south and south-east of Romania because it reaches flowering before the onset of very high temperatures.

The *Gabrio* variety is an early variety with a well-developed ear and a rustic appearance. It has high yield potential, medium intensive. High productivity and quality indices due to adaptability to different climatic and technological conditions. High protein content of superior quality.

Foliar fertilizers: 2 types in 2 different doses.

FOLIQ 36 NITROGEN**:** Content 36% N + 4% MgO + microelements (Table 1).

Table 1. Macro and micro elements content of FOLIQ 36 NITROGEN foliar fertilizer

Macro and micro elements	% of weight	% of volume
Total nitrogen (N)	27	36
Nitric nitrogen (NO_3^--N)	5	24
Ammoniacal nitrogen (NH_4^+-N)	4	5
Urea nitrogen (N)	18	7
Magnesium (MgO)	3	4
Boron (B)	0.010	0.012
Copper (Cu)	0.007	0.008
Iron (Fe)	0.020	0.027
Manganese (Mn)	0.013	0.015
Molybdenum (Mo)	0.001	0.001
Zinc (Zn)	0.005	0.006

It is recommended for crop fertilization obtaining high productivity. Application of the product speeds up the regeneration of crops damaged during winter or with phytotoxicity caused by pesticides. Applied when the plants are young, it stimulates growth during periods when nutrient uptake is limited because the root system is underdeveloped**.**

FOLIQ CEREALS: Content 12% N + 15%K_2O + 4%MgO +7 %SO_3 + microelements (Table 2). It is a foliar applied fertilizer with a formulation specially designed to prevent and treat microelements deficiency in the plant. Its composition largely meets the nutritional needs of intensive crops, ensuring their proper development throughout the growing season, favouring high and stable yields and optimal development before the onset of winter to better withstand frost.

These foliar were applied over a base fertilization with NPK 20-20-20-0 in autumn 250 kg/ha and 250 kg/ha ammonium nitrate, with 33.4% N.

Table 2. Macro and micro elements content of FOLIQ CEREAL foliar fertilizer

Macro and micro elements	% of weight	% of volume
Total nitrogen (N)	8	12
Urea nitrogen (N)	4	6
Ammonia nitrogen (NH_4^+-N)	4	6
Potassium (K_2O)	10	15
Magnesium (MgO)	3	4
Sulphur (SO_3)	5	7
Boron (B)	0.3000	0.435
Copper (Cu)	0.5000	0.725
Iron (Fe)	1.0	1.450
Manganese (Mn)	1.5	2.175
Molybdenum (Mo)	0.0100	0.015
Zinc (Zn)	1.0	1.450

Water supply level

Watering was applied with a perforated PVC pipe system, which ensures a good distribution of water on the plot. The water applied was measured with a flow meter. Soil moisture was determined by means of tensiometers, which were placed at different intervals along the watering depth.

The statistical processing of the experiment was carried out with the statistical analysis program specific for trifactorial experiments based on the methodology presented by N. Săulescu (PSUB 3) (Săulescu & Săulescu, 1967). The influence of the studied factors (variety, foliar fertilizer, water supply level) and the interaction of variety x foliar fertilizer x water supply level on yield, hectolitre mass, 1000-grain mass and protein content was interpreted.

Boxplot (Hawkins, 2009) was used to present the distribution of yield values.

Climatic conditions were different from year to year (Table 3 and 4). Thus, while 2023 was a favourable year for the crop, with rainy April and May months against a background of cooler than normal temperatures, 2024 was a rainfall deficit year but with a more differentiated month than all the others in the growing season, which also saved wheat production.

Table 3. The evolution of the main climatic factors during the growing season of straw cereals genotypes at S.C.D.A. Caracal, in the agricultural year 2022-2023

Specification		X	XI	XII	I	II	III	IV	V	VI	Total period
Temperature °C	Monthly minimum	-0.7	-2.7	-4.5	-3.1	-9.8	-7.5	-0.5	5.5	11.0	
	Monthly maximum	27.2	27.3	14.5	19.9	22.9	20.7	22.0	27.8	37.6	
	Monthly average	13.7	8,7	2,8	4,0	3.6	7.7	10.7	15.0	21.4	
	NORMAL	11,7	5,1	0.3	-1.3	0.8	6	12	17.7	21,6	
	Difference ±	+2.0	+3.6	+2.5	+5.3	+2.8	+1.7	-1.3	-2.7	-0.2	
Precipi-tations mm	Total monthly	15.0	78.8	33.8	103.4	13.2	20.8	68.8	78.6	44.4	456.8
	Multiannual average	46.0	37.0	39.1	30.8	26.3	34.2	47.8	58.6	69.7	389.5
	Difference±	-31.0	+31.8	-5,3	+72.6	-13.1	-13.4	+21.0	+20.0	-25.3	+67.3
Relative humidity of air %	Average	75.5	93.0	97.8	96.5	86.0	79.2	84.2	80.5	80.6	

Table 4. The evolution of the main climatic factors during the growing season of straw cereals genotypes at S.C.D.A. CARACAL, in the agricultural year 2023-2024

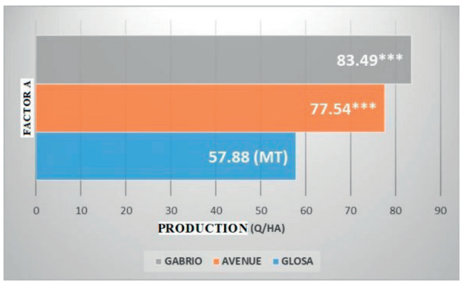
Specification		X	XI	XII	I	II	III	IV	V	VI	Total period
Temperature °C	Monthly minimum	0.6	-4.4	-3.2	-9.6	-6.7	-3.3	2,0	5.4	12.5	
	Monthly maximum	33.4	21.4	18.2	15.3	21.9	28.3	32,7	30.8	39.4	
	Monthly average	16.0	8.1	3.9	1.0	8.0	8.9	15,0	17.2	25.9	
	NORMAL	11.7	5.1	0.3	-1,3	0.8	6.0	12,0	17.7	21.6	
	Difference ±	+4.3	+3.0	+3.6	+2.3	+7.2	+3.9	+3,0	-0.5	+4.3	
Precipi-tations mm	Total monthly	21.4	124.2	21.4	26.8	11.6	22.4	26,0	71,8	31.6	357.2
	Multiannual average	46.0	37.0	39.1	30.8	26.3	34.2	47,8	58.6	69.7	389.5
	Difference±	-24.6	+87.2	-17.7	-4.0	-14.7	-11.8	-21,8	+13.2	-38.1	-32.3
Relative humidity of air %	Average	66.9	91.1	93.8	95.4	84.7	85.3	70,8	77.8	61.3	

RESULTS AND DISCUSSIONS

On average over the two years of testing, the influence of variety on yield was emphasized, mainly due to the strong attack of yellow rust on Glosa in 2023.

Avenue and Gabrio yields were significantly higher than the control - Glosa, with substantial differences (+19.66 q/ha and +25.61 q/ha, respectively) (Figure 1).

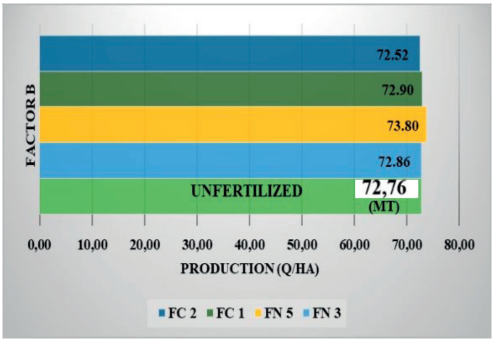
In addition, the rainfall in May, exactly at the grain filling phenotype, coupled with lower than normal temperatures, greatly helped the foreign varieties by creating a microclimate conducive to maximizing their productive capacity.



DL 5% = 1.89 q/ha; DL 1% = 3.13 q/ha; DL 0.1% = 5.84 q/ha

Figure 1. The influence of variety (factor A) on wheat yield - Caracal 2023-2024

On the other hand, fertilization with foliar fertilizer, irrespective of the product and the dose applied, did not show any significant differences in relation to the non-fertilized variant, and therefore did not show any influence on the yield obtained (Figure 2). As differences were not statistically assured, all variants were at the control level.



DL 5% = 1.94 q/ha; DL 1% = 2.63 q/ha; DL 0.1% = 3.52 q/ha

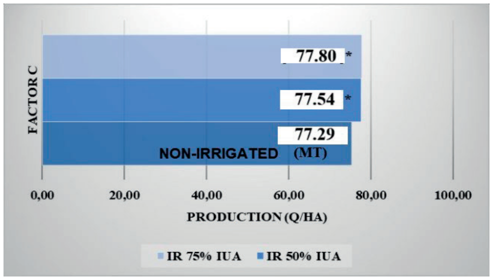
Figure 2. Influence of foliar fertilizer (factor B) on wheat yield - Caracal 2023-2024

Water supply at 50% of the IUA and 75% of the IUA boosted yields significantly, with an increase of 2.25 q/ha for the first level and 2.51 q/ha for the second level (Figure 3).

On average, the highest yield was recorded by Gabrio variety fertilized with maximum Foliq 36 N and irrigated at 50% of IUA - 87.87 q/ha.

The three-factor interaction of variety (factor A) x foliar fertilizer (factor B) x irrigation water supply (factor C) greatly influences yield. In all combinations of Avenue and Gabrio varieties, fertilization with foliar fertilizer of any type and dose and at all levels of water supply, yield increases were highly significant relative to the unfertilized and non-irrigated Glosa variety.

But in relation to the non-irrigated variety at each of the gradations of factor B - foliar fertilizer, for each of the varieties tested, either Glosa, Avenue or Gabrio, there were no statistically assured differences.



DL 5% = 1.92 q/ha; DL 1% = 2.56 q/ha; DL 0.1% = 3.32 q/ha

Figure 3. Influence of irrigation water supply (C-factor) on wheat yield - Caracal 2023-2024

The largest increases, were recorded for each of the varieties at most of the factor B gradations at the 75% supply level but without significance. However, the variety Glosa responded best, with yields over 6 q/ha, when fertilized with Foliq 36N 3L/ha and Foliq Cereal 1L/ha and irrigated at 75% IUA, but again without statistical assurance (Table 5).

Table 5. Influence of the interaction of variety (factor A) x foliar fertilizer (factor B) x irrigated water supply (factor C) on wheat yield - Caracal 2023-2024

FACTOR A	FACTOR B	FACTOR C	Yield (q/ha)	Dif. mt 1	Semnif.	Dif. mt 2	Semnif.
GLOSA	UNFERTILIZED	NON-IRRIGATED (mt1)	55.51	0.00			
GLOSA	UNFERTILIZED	NON-IRRIGATED (mt2)	55.51	0.00		0.00	
		IR 50% IUA	58.99	3.48		3.48	
		IR 75% IUA	58.20	2.69		2.69	
	FOLIQ 36 N 3 L/HA	NON-IRRIGATED (mt2)	53.26	-2.25		0.00	
		IR 50% IUA	58.64	3.13		-2.46	
		IR 75% IUA	60.74	5.23		6.02	
	FOLIQ 36 N 5 L/HA	NON-IRRIGATED (mt2)	56.46	0.95		0.00	
		IR 50% IUA	59.77	4.26		3.31	
		IR 75% IUA	59.84	4.33		3.38	
	FOLIQ CEREALS 1 L/HA	NON-IRRIGATED (mt2)	55.66	0.15		0.00	
		IR 50% IUA	58.32	2.81		2.66	
		IR 75% IUA	61.82	6.31		6.16	
	FOLIQ CEREALS 2 L/HA	NON-IRRIGATED (mt2)	56.70	1.19		0.00	
		IR 50% IUA	57.29	1.78		0.59	
		IR 75% IUA	57.01	1.50		0.31	
ANENUE	UNFERTILIZED	NON-IRRIGATED (mt2)	77.70	22.19	***	0.00	
		IR 50% IUA	76.40	20.89	***	-1.30	
		IR 75% IUA	75.97	20.46	***	-1.73	

	FOLIQ 36 N 3 L/HA	NON-IRRIGATED (mt2)	77.73	22.22	***	0.00	
		IR 50% IUA	76.07	20.56	***	-1.66	
		IR 75% IUA	82.28	26.77	***	4.55	
	FOLIQ 36 N 5 L/HA	NON-IRRIGATED (mt2)	77.58	22.07	***	0.00	
		IR 50% IUA	79.18	23.67	***	1.60	
		IR 75% IUA	77.98	22.47	***	0.40	
	FOLIQ CEREALS 1 L/HA	NON-IRRIGATED (mt2)	74.49	18.98	***	0.00	
		IR 50% IUA	76.84	21.33	***	2.35	
		IR 75% IUA	79.00	23.49	***	4.51	
	FOLIQ CEREALS 2 L/HA	NON-IRRIGATED (mt2)	75.05	19.54	***	0.00	
		IR 50% IUA	77.92	22.41	***	2.87	
		IR 75% IUA	78.97	23.46	***	3.92	
GABRIO	UNFERTILIZED	NON-IRRIGATED (mt2)	83.16	27.65	***	0.00	
		IR 50% IUA	85.32	29.81	***	2.16	
		IR 75% IUA	83.64	28.13	***	0.48	
	FOLIQ 36 N 3 L/HA	NON-IRRIGATED (mt2)	80.12	24.61	***	0.00	
		IR 50% IUA	83.37	27.86	***	3.25	
		IR 75% IUA	83.54	28.03	***	3.42	
	FOLIQ 36 N 5 L/HA	NON-IRRIGATED (mt2)	84.89	29.38	***	0.00	
		IR 50% IUA	87.87	32.36	***	2.98	
		IR 75% IUA	80.66	25.15	***	-4.23	
	FOLIQ CEREALS 1 L/HA	NON-IRRIGATED (mt2)	81.55	26.04	***	0.00	
		IR 50% IUA	83.08	27.57	***	1.53	
		IR 75% IUA	85.34	29.83	***	3.79	
	FOLIQ CEREALS 2 L/HA	NON-IRRIGATED (mt2)	80.84	25.33	***	0.00	
		IR 50% IUA	83.34	27.83	***	2.50	
		IR 75% IUA	85.60	30.09	***	4.76	
	DL 5%		7.44 q/ha				
	DL 1%		9.89 q/ha				
	DL 0.1%		12.86 q/ha				

The distribution of production values grouped by varieties evaluated by the box-plot method shows a narrower range of values for Glosa and Avenue compared to Gabrio (Figure 4). This distribution showed a marked stability of the yields of Glosa but in obtaining low yields, an undesirable characteristic in agricultural practice.

Figure 4. Box-plot synthesis of yield results by the interaction of variety (factor A) x foliar fertilizer (factor B) x irrigated water supply (factor C) grouped by variety - Caracal 2023-2024

The table below highlights the minimum, maximum, mean, 25% and 75% limits, maximum-minimum amplitude and inter-quartile range for the interaction variety x foliar fertilizer x irrigation water supply, grouped by variety (Table 6).

Table 6. Yield values for the box-plot calculation through the interaction of variety (factor A) x foliar fertilizer (factor B) x irrigation water supply (factor C) grouped by variety - Caracal 2023-2024

	Glosa	**Avenue**	**Gabrio**
Minimum value	53.25724	74.49391	80.12054
Q1 (limit of 25% of values)	56.58216	76.23268	82.31332
Median (limit of 50% of values	58.20402	77.69886	83.36556
Q3 (limit of 75% of values)	59.37881	78.47392	85.10697
Maximum value	61.82167	82.28474	87.87221
Mean value	57.88	77.54	83.49
Maximum-minimum amplitude	8.564433	7.790831	7.751666
IQR (interquartil = Q3 – Q1)	2.796652	2.241237	2.793653
IQR x 1,5	4.194979	3.361855	4.190479
IQR x 3	8.389957	6.72371	8.380959

The distribution of the yield values grouped by fertilization levels, evaluated by the box-plot method, showed a somewhat narrower range of values for Foliq Cereal fertilization at a dose of 1 l/ha, but also the fact that there is no outlier or extreme value (Figure 5).

From Table 7 it emerged that all box-plots have very close mean values and balanced towards the upper side.

The distribution of the yield values grouped by foliar fertilizer treatments, also evaluated by the box-plot method, showed a more pronounced instability for the Foliq Cereal 2 L/ha treatment.

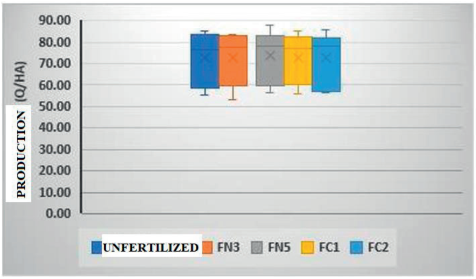


Figure 5. Box-plot synthesis of yield results by the interaction of variety (factor A) x foliar fertilizer (factor B) x irrigated water supply (factor C) grouped by type and dose of foliar fertilizer - Caracal 2023-2024

Table 7. Yield values for the box-plot calculation by the interaction of variety (factor A) x foliar fertilizer (factor B) x irrigated water supply (factor C) grouped by type and dose of foliar fertilizer - Caracal 2023-2024

Minimum value	55.50913	53.25724	56.46476	55.66203	56.6995543
Q1 (limit of 25% of values)	58.99082	60.74036	59.83685	61.82167	57.2931781
Median (limit of 50% of values	76.4	77.72917	77.98234	76.84268	72.8602965
Q3 (limit of 75% of values)	83.16063	82.28474	80.66106	81.55009	80.8418158
Maximum value	85.32068	83.53503	87.87221	85.34416	85.5980432
Mean value	72.77	72.86	73.80	72.90	72.53
Maximum-minimum amplitude	29.81155	30.27779	31.40745	29.68213	28.8984889
IQR (interquartil = Q3 – Q1)	24.16981	21.54439	20.82421	19.72842	23.5486378
IQR x 1,5	36.25472	32.31658	31.23632	29.59263	35.3229566
IQR x 3	72.50944	64.63316	62.47264	59.18525	70.6459133

As in the case of yield, on average over the two years there were statistically assured differences in hectolitre mass between varieties, but in the opposite direction.

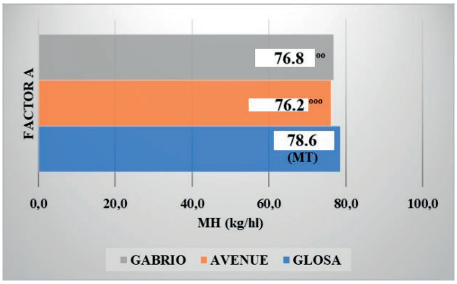
The foreign varieties showed low hectolitre mass, highly significant for Avenue and distinctly significant for Gabrio compared to Glosa (Figure 6).

Fertilization with foliar fertilizer, irrespective of the product and the dose applied, did not show significant differences in hectolitre mass compared to the non-fertilized variety (Figure 7).

The differences between the variants were extremely small and therefore not statistically assured.

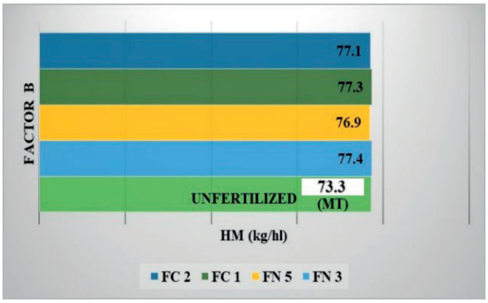
Water supply influenced hectolitre mass. At 75% of the IUA, the hectolitre mass (HM) was distinctly significantly higher than the non-irrigated variant, with an increase of 1.8 kg/hl (Figure 8).

However, the interaction of variety (factor A) x foliar fertilizer (factor B) x irrigated water supply (factor C) greatly influenced the hectolitre mass.



DL 5% = 0.7 kg/hl; DL 1% = 1.1 kg/hl; DL 0.1% = 2.0 kg/hl

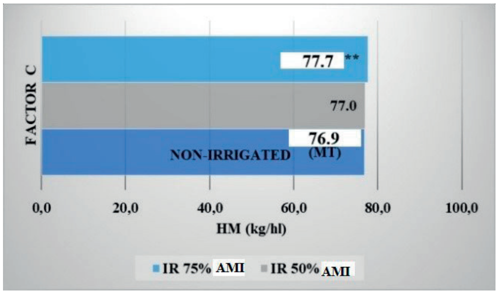
Figure 6. Influence of variety (factor A) on the hectolitre mass of wheat - Caracal 2023-2024



DL 5% = 0.7 kg/hl; DL 1% = 0.9 kg/hl; DL 0.1% = 1.3 kg/hl

Figure 7. Influence of foliar fertilizer (factor B) on the hectolitre mass of wheat – Caracal 2023+2024

Compared to the non-irrigated and non-fertilized variant of Glosa, most variants, but all of Avenue and Gabrio varieties, had much significantly, distinctly significantly or very significantly decreased hectolitre mass (Table 8). These decreases were in the range of 1.5-3.7 kg/hl, values that made the foreign varieties well below Glosa in this respect.



DL 5% = 0.6 kg/hl; DL 1% = 0.7 kg/hl; DL 0.1% = 0.9 kg/hl

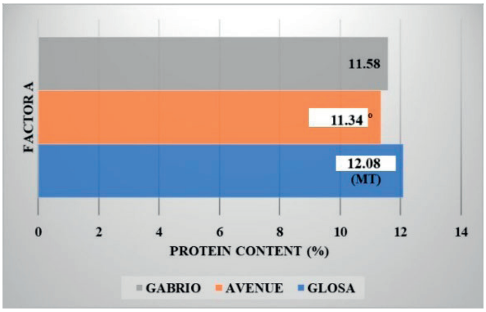
Figure 8. Influence of irrigation water supply (C-factor) on hectolitre mass of wheat - Caracal 2023-2024

Table 8. Influence of the interaction of variety (factor A) x foliar fertilizer (factor B) x irrigated water supply (factor C) on hectolitre mass in wheat - Caracal 2023-2024

FACTOR A	FACTOR B	FACTOR C	HM (kg/hl)	Dif. mt 1	Semnif.	Dif. mt 2	Semnif.
GLOSA	UNFERTILIZED	NON-IRRIGATED (mt1)	79.1	0.0			
GLOSA	UNFERTILIZED	NON-IRRIGATED (mt2)	79.1	0.0		0.0	
		IR 50% IUA	78.5	-0.6		-0.6	
		IR 75% IUA	78.2	-0.9		-0.9	
	FOLIQUE 36 N 3 L/HA	NON-IRRIGATED (mt2)	77.9	-1.2		0.0	
		IR 50% IUA	78.4	-0.7		0.5	
		IR 75% IUA	79.4	0.3		1.5	
	FOLIQUE 36 N 5 L/HA	NON-IRRIGATED (mt2)	78.5	-0.6		0.0	
		IR 50% IUA	78.0	-1.1		-0.5	
		IR 75% IUA	79.2	0.1		0.7	
	FOLIQUE CEREALS 1 L/HA	NON-IRRIGATED (mt2)	78.6	-0.5		0.0	
		IR 50% IUA	77.8	-1.3		-0.8	
		IR 75% IUA	80.0	0.9		1.4	
	FOLIQUE CEREALS 2 L/HA	NON-IRRIGATED (mt2)	78.3	-0.8		0.0	
		IR 50% IUA	77.8	-1.3		-0.5	
		IR 75% IUA	78.6	-0.5		0.3	
ANENUE	UNFERTILIZED	NON-IRRIGATED (mt2)	76.0	-3.1	oo	0.0	
		IR 50% IUA	76.2	-2.9	oo	0.2	
		IR 75% IUA	76.5	-2.6	o	0.5	
	FOLIQUE 36 N 3 L/HA	NON-IRRIGATED (mt2)	76.5	-2.6	o	0.0	
		IR 50% IUA	76.3	-2.8	oo	-0.2	
		IR 75% IUA	77.0	-2.1	o	0.5	
	FOLIQUE 36 N 5 L/HA	NON-IRRIGATED (mt2)	75.6	-3.5	oo	0.0	
		IR 50% IUA	75.4	-3.7	ooo	-0.2	
		IR 75% IUA	76.5	-2.6	o	0.9	
	FOLIQUE CEREALS 1 L/HA	NON-IRRIGATED (mt2)	75.5	-3.6	ooo	0.0	
		IR 50% IUA	76.3	-2.8	oo	0.8	
		IR 75% IUA	77.0	-2.1	o	1.5	
	FOLIQUE CEREALS 2 L/HA	NON-IRRIGATED (mt2)	76.0	-3.1	oo	0.0	
		IR 50% IUA	76.8	-2.3	o	0.8	
		IR 75% IUA	75.9	-3.2	oo	-0.1	
GABRIO	UNFERTILIZED	NON-IRRIGATED (mt2)	75.7	-3.4	oo	0.0	
		IR 50% IUA	77.6	-1.5		1.9	
		IR 75% IUA	77.7	-1.4		2.0	
	FOLIQUE 36 N 3 L/HA	NON-IRRIGATED (mt2)	76.3	-2.8	oo	0.0	
		IR 50% IUA	76.6	-2.5	o	0.3	
		IR 75% IUA	77.9	-1.2		1.6	

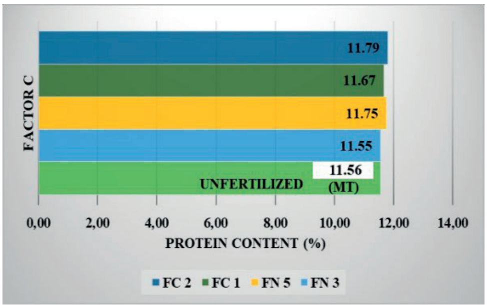
	FOLIQUE 36 N 5 L/HA	NON-IRRIGATED (mt2)	76.4	-2.8	oo	0.0	
		IR 50% IUA	76.4	-2.7	o	0.0	
		IR 75% IUA	76.5	-2.6	o	0.1	
	FOLIQUE CEREALS 1 L/HA	NON-IRRIGATED (mt2)	76.6	-2.5	o	0.0	
		IR 50% IUA	76.7	-2.4	o	0.1	
		IR 75% IUA	77.5	-1.6		0.9	
	FOLIQUE CEREALS 2 L/HA	NON-IRRIGATED (mt2)	76.0	-3.1	oo	0.0	
		IR 50% IUA	76.9	-2.2	o	0.9	
		IR 75% IUA	77.7	-1.4		1.7	
	DL 5%		2.1 kg/hl				
	DL 1%		2.8 kg/hl				
	DL 0.1%		3.6 kg/hl				

On average over the 2 years, protein content was influenced by variety. The protein content value of Avenue was significantly decreased compared to Glosa but not compared to Gabrio (Figure 9). Foliar fertilization and water supply did not influence the protein content (Figures 10 and 11).



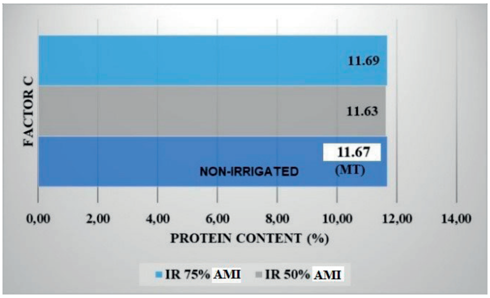
DL 5% = 0.53%; DL 1% = 0.88%; DL 0.1% = 0.65%

Figure 9. Influence of variety (factor A) on protein content in wheat - Caracal 2023-2024



DL 5% = 0.41%; DL 1% = 0.56%; DL 0.1% = 0.75%

Figure 10. Influence of foliar fertilizer (factor B) on protein content in wheat - Caracal 2023-2024



DL 5% = 0.28%; DL 1% = 0.38%; DL 0.1% = 0.49%

Figure 11. Influence of irrigation water supply (C-factor) on protein content in wheat - Caracal 2023-2024

The protein content values ranged from 10.52% for Avenue fertilized with Foliq 36 N 3 L/ha and irrigated at 50% of the IUA to 12.36% for Glosa fertilized with Foliq Cereal 2 L/ha and irrigated at 50% of the IUA. The foreign varieties had only one protein content value above 12% (12.16%) in Gabrio fertilized with Foliq Cereals 1 l/ha and irrigated at 50% of the IUA (Table 9).

The interaction of variety (factor A) x foliar fertilizer (factor B) x irrigation water supply (factor C) influenced protein content. Compared to the non-irrigated and non-fertilized variant of Glosa, seven variants of Avenue and Gabrio had significantly and distinctly significantly reduced hectolitre mass (Table 9). Based on these decreases, we do not recommend growing Avenue fertilized with Foliq36 N 3 kg/ha at 50% water level of IUA, a variant that has significantly reduced protein content also compared to itself unfertilized and non-irrigated.

Table 9. Influence of the interaction of variety (factor A) x foliar fertilizer (factor B) x irrigated water supply (factor C) on the protein content of wheat - Caracal 2023-2024

FACTOR A	FACTOR B	FACTOR C	Protein (%)	Dif. mt 1	Semnif.	Dif. mt 2	Semnif.
GLOSA	UNFERTILIZED	NON-IRRIGATED (mt1)	12.31	0.00			
GLOSA	UNFERTILIZED	NON-IRRIGATED (mt2)	12.31	0.00		0.00	
		IR 50% IUA	11.33	-0.98		-0.98	
		IR 75% IUA	11.96	-0.35		-0.35	
	FOLIQUE 36 N 3 L/HA	NON-IRRIGATED (mt2)	12.24	-0.07		0.00	
		IR 50% IUA	11.89	-0.42		-0.35	
		IR 75% IUA	11.94	-0.37		-0.30	
	FOLIQUE 36 N 5 L/HA	NON-IRRIGATED (mt2)	12.30	-0.01		0.00	
		IR 50% IUA	12.25	-0.06		-0.05	
		IR 75% IUA	12.26	-0.05		-0.04	
	FOLIQUE CEREALS 1 L/HA	NON-IRRIGATED (mt2)	12.31	0.00		0.00	
		IR 50% IUA	12.17	-0.14		-0.14	
		IR 75% IUA	11.91	-0.40		-0.40	
	FOLIQUE CEREALS 2 L/HA	NON-IRRIGATED (mt2)	12.07	-0.24		0.00	
		IR 50% IUA	12.36	0.05		0.29	
		IR 75% IUA	11.84	-0.47		-0.23	
ANENUE	UNFERTILIZED	NON-IRRIGATED (mt2)	11.39	-0.92		0.00	
		IR 50% IUA	11.08	-1.23	o	-0.31	
		IR 75% IUA	11.40	-0.91		0.01	
	FOLIQUE 36 N 3 L/HA	NON-IRRIGATED (mt2)	11.72	-0.59		0.00	
		IR 50% IUA	10.52	-1.79	oo	-1.20	o
		IR 75% IUA	11.67	-0.64		-0.05	
	FOLIQUE 36 N 5 L/HA	NON-IRRIGATED (mt2)	10.89	-1.42	o	0.00	
		IR 50% IUA	11.77	-0.54		0.88	
		IR 75% IUA	11.84	-0.47		0.95	
	FOLIQUE CEREALS 1 L/HA	NON-IRRIGATED (mt2)	11.23	-1.08		0.00	
		IR 50% IUA	11.00	-1.31	o	-0.23	
		IR 75% IUA	10.97	-1.34	o	-0.26	
	FOLIQUE CEREALS 2 L/HA	NON-IRRIGATED (mt2)	11.39	-0.92		0.00	
		IR 50% IUA	11.97	-0.34		0.58	
		IR 75% IUA	11.27	-1.04		-0.12	
GABRIO	UNFERTILIZED	NON-IRRIGATED (mt2)	11.48	-0.83		0.00	
		IR 50% IUA	11.51	-0.80		0.03	
		IR 75% IUA	11.60	-0.71		0.12	
	FOLIQUE 36 N 3 L/HA	NON-IRRIGATED (mt2)	10.87	-1.44	o	0.00	
		IR 50% IUA	11.17	-1.14	o	0.30	
		IR 75% IUA	11.90	-0.41		1.03	
	FOLIQUE 36 N 5 L/HA	NON-IRRIGATED (mt2)	11.46	-0.85		0.00	
		IR 50% IUA	11.60	-0.71		0.14	
		IR 75% IUA	11.38	-0.93		-0.08	
	FOLIQUE CEREALS 1 L/HA	NON-IRRIGATED (mt2)	11.58	-0.73		0.00	
		IR 50% IUA	12.16	-0.15		0.58	
		IR 75% IUA	11.68	-0.63		0.10	
	FOLIQUE CEREALS 2 L/HA	NON-IRRIGATED (mt2)	11.87	-0.44		0.00	
		IR 50% IUA	11.68	-0.63		-0.19	
		IR 75% IUA	11.69	-0.62		-0.18	
	DL 5%		**1.10%**				
	DL 1%		**1.46%**				
	DL 0.1%		**1.90%**				

CONCLUSIONS

The world market and, lately, the Romanian market, have been invaded by numerous products acting as fertilizers for wheat crops. The aim of the study carried out on Caracal chernozem was to determine the influence of foliar fertilizer application on yield and quality, depending on the variety and the optimal time of application in terms of soil water supply.

Analysis of the influence of single factors showed that yield and hectolitre mass were influenced by variety and water supply. Protein content was only influenced by variety.

The yields of Avenue and Gabrio were significantly higher than the control - Glosa, with substantial differences (+19.66 q/ha and +25.61 q/ha respectively). In addition, the rainfall in May, just at the grain filling phenotype, coupled with lower than normal temperatures, greatly helped the foreign varieties, creating a microclimate conducive to maximizing their productive capacity.

The distribution of production values grouped by variety evaluated by the box-plot method showed a marked stability of yields for the Glosa variety, but in obtaining low yields, an undesirable characteristic in agricultural practice.

As in the case of yields, statistically assured differences in hectolitre mass were recorded between varieties. The foreign varieties showed low hectolitre mass, highly significant for Avenue and distinctly significant for Gabrio compared to Glosa.

Fertilization with foliar fertilizer, irrespective of the product and the dose applied, did not reveal significant differences in hectolitre mass in relation to the non-fertilized variety. The differences between the variants were extremely small and therefore not statistically assured.

Water supply influenced hectolitre mass. The interaction of variety (factor A) x foliar fertilizer (factor B) x irrigated water supply (factor C) influenced the protein content.

Among the tested variants we recommend the variety Gabrio fertilized with Foliq 36 N 5 kg and irrigated at 50% of the A.I.U.

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INFLUENCE OF CLIMATIC CONDITIONS, VARIETY AND SOWING DENSITY ON WHEAT PRODUCTION AND QUALITY

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Abstract

The aim of the study was to highlight the reaction of the cultivated variety depending on the sowing density and climatic conditions on the level of production and quality indices. The wheat varieties tested were Biharia, Glosa and Anapurna, on three densities (530 b.g/m², 650 b.g/m² and 780 b.g/m²). Fertilization was carried out using N150 kg s.a., P2O5 78 kg s.a. at sowing, and in spring an additional N 46 kg s.a. was applied. The highest production was obtained for the Anapurna variety of 8403 kg/ha, followed by the Biharia variety with 7790 kg/ha and Glosa with 7587 kg/ha. Depending on the sowing density, the highest harvest, 8009 kg/ha, was obtained at a density of 780 b.g/m². The crude protein content ranged from 13.10% for the Glosa variety (780 b.g/m²) to 15.9% for the Anapurna variety (650 b.g/m²). Wet gluten recorded values between 25% for the Glosa variety (530 b.g/m²) and 36% for the Anapurna variety (780 b.g/m²).

Key words: *crude protein, density, gluten, variety, wheat.*

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important staple crops globally, providing essential nutrients to billions of people. Wheat production and quality are influenced by several key factors, including climatic conditions, variety selection, and crop density. Scientific research has extensively explored these variables to optimize wheat yield and improve grain quality.

Climatic factors, such as temperature, precipitation and solar radiation, play a key role in the growth and development of wheat. According to a study published in Nature Climate Change, an increase in global temperatures of 1°C could reduce wheat yield by 6%, highlighting the crop's sensitivity to heat stress (Asseng et al., 2015; Smuleac et al., 2020). High temperatures, especially during the cereal filling period, accelerate plant metabolism, leading to a reduction in grain size and weight (Pop et al., 2023). In contrast, adequate rainfall and optimal temperatures promote healthy wheat development (Fischer & Edmeades, 2010). Moreover, climate variability influences the prevalence of diseases. For example, warmer and wetter conditions increase the risk of fungal diseases such as rust and fusarium disease, which degrade grain quality and reduce market value (Beres et al., 2020).

Selecting the right wheat varieties is crucial for optimizing both grain yield and quality. Modern breeding programs focus on developing varieties that are drought-resistant, disease-tolerant, and able to thrive in various climatic conditions (Mosleth et al., 2015). Genetically improved wheat varieties can increase production potential by 20-30% while maintaining grain quality (Feng et al., 2018).

Grain quality parameters such as protein content, gluten strength and kernel hardness are influenced by both genetic factors and environmental interactions (Shewry & Hey, 2015; Yang et al., 2023). It is very important to select high-protein varieties for the quality of bread making, while softer wheat varieties are preferred for the pastry and confectionery industry. In addition, certain varieties are bred for biofortification, increasing the content of micronutrients such as zinc and iron to combat malnutrition (Gulyas et al., 2024).

Sowing density, or the number of seeds planted per unit area, significantly affects wheat growth, and ultimately yield (Sun et al., 2023). It is shown that the optimal seeding density varies depending on the wheat variety and environmental conditions (Hetea et al., 2024). High seeding densities can increase competition for resources, leading to thinner stems and an increased susceptibility to sheltering (fall), while low densities can lead to underutilization of available nutrients and sunlight.

However, adjusting the seeding density can optimize the yield under specific conditions. For example, in drought-prone areas, lower seeding densities reduce competition for water, improving plant hardiness (Constantin et al., 2024). In contrast, in fertile, irrigated environments, higher sowing densities maximize production potential by increasing the number of growers and grains per square meter.

The interaction between climatic conditions, variety and sowing density determines both quantitative (yield) and qualitative (grain quality) results in wheat production. For example, while higher temperatures can reduce overall yield, selecting heat-tolerant varieties and adjusting the seeding period can mitigate adverse effects. Combining hardy varieties with optimized seeding density and adequate mineral fertilization with nitrogen, phosphorus, and potassium, it can sustain wheat production in the face of climate change (Carvalho et al., 2016; Tadesse et al., 2019; Castro et al., 2022).

Grain quality, especially protein concentration, is also influenced by these factors (Blumenthal et al., 2014; Hetea et al., 2024). Recent studies note that moderate water stress during the grain filling stage can increase protein content, albeit at the expense of grain size. Similarly, seeding density affects nutrient uptake, with denser seeding reducing nitrogen availability per plant, potentially decreasing protein content (Dier et al., 2018; Ahmed et al., 2020; Chitu et al., 2024).

MATERIALS AND METHODS

The study was carried out on a vertisol soil, located in the area of Olari Commune in Câmpia Crişurilor, with a weak acid reaction and a humus content of 3.45.

The experience was three-factorial, where Factor A - the year of cultivation, Factor B - the cultivated variety (b1 - Biharia, b2 - Glosa and b3 - Anapurna), Factor C - sowing density (c1 - 780 g.s./m^2, c2 - 650 g.s./m^2 and c3 - 530 g.s./m^2).

For the climatic characterization, the data recorded at the Arad Meteorological Station were used. From the analysis of the data, it can be seen that in terms of the degree of water supply and the temperatures recorded, there were no very large deviations between the experimental years. Fertilization was carried out using N150 kg s.a., P_2O_5 78 kg s.a. at sowing, and in spring an additional N 46 kg s.a. was applied.

RESULTS AND DISCUSSIONS

Table 1 shows the wheat production (kg/ha) in two consecutive agricultural seasons (2022-2023 and 2023-2024), depending on climatic conditions. In the agricultural year 2022-2023, the production was 7877 kg/ha, and in the agricultural year 2023-2024, the production was 7841 kg/ha, the difference between the two years is -36 kg/ha, which indicates a slight decrease in production, but the variation in production is not large enough to be considered statistically significant. Since the observed difference (36 kg/ha) is well below the materiality threshold of 196 kg/ha (LSD 5%), we can conclude that the climatic variations between the two years did not have a significant impact on wheat production. It can be concluded that, under the analyzed experimental and climatic conditions, wheat production was stable between the agricultural years 2022-2023 and 2023-2024, without statistically significant differences.

Table 1. Wheat production according to climatic condition

Year	Yield kg/ha	%	Difference kg/ha	Significance
2022-2023	7877	100	-	-
2023-2024	7841	100	-36	Ns
LSD 5% = 196 kg, LSD1% = 274 kg, LSD 0.1% = 388 kg				

Wheat yields by cultivated varieties are shown in Table 2.

The wheat production achieved by the varieties was 7587 kg/ha of Biharia, 7597 kg/ha of Glosa and 8403 kg/ha (111% compared to Biharia) of Anapurna variety.

Production differences: Glosa vs. Biharia: the difference is 10 kg/ha, insignificant (ns) and Anapurna vs. Biharia: the difference is 816kg/ha, which represents an increase of 11% compared to Biharia. The difference of 816kg/ha in the case of the Anapurna variety significantly exceeds all thresholds, which confirms a very high significance (***) at a confidence level of 99.9%.

Table 2. Wheat production according to variety

Variety	Yield kg/ha	%	Difference kg/ha	Significance
Biharia	7587	100	-	-
Glosa	7597	100	10	ns
Anapurna	8403	111	816	***
LSD 5% = 221 kg, LSD 1% = 306 kg, LSD 0.1% = 421 kg				

On the other hand, the difference between Glosa and Biharia is only 10 kg/ha, well below the minimum significance threshold (LSD 5% = 221 kg/ha), so it is not statistically significant. In conclusion, the Biharia and Glosa varieties had a similar production, without statistically significant differences. The Anapurna variety recorded a significantly higher production compared to Biharia and Glosa, the difference being extremely statistically significant. This result suggests that Anapurna is a superior variety from the yield perspective under the experimental conditions analyzed.

Table 3 shows the influence of sowing density on wheat production, comparing three density levels: 530 g.s./m^2, 650 g.s./m^2 and 780 g.s./m^2. Wheat production according to sowing density was 7683 kg/ha at 530 g.s./m^2, 7885 kg/ha at 650 g.s./m^2 and 8009 kg/ha at 780 g.s./m^2.

The optimal density for maximum production is 780 g.s./m^2, having the highest production (8009 kg/ha).

Reducing the density to 650 g.s./m^2 does not significantly affect production, which means that this density could be an economically efficient option, having almost the same production with lower seed consumption.

Reducing the density to 530 g.s./m^2 significantly reduces production, so this density is not recommended for maximum yield.

The materiality threshold confirms that the differences between 780 g.s./m^2 and 650 g.s./m^2 are insignificant, while the differences between 780 g.s./m^2 and 530 g.s./m^2 are significant at a high confidence level (99%). If the main objective is maximum production, it is recommended to use the density of 780 g.s./m^2, and if you want to streamline seed costs, without significant production losses, you can use the density of 650 g.s./m^2.

Table 3. Wheat production according to sowing density

Density	Yield kg/ha	%	Difference kg/ha	Signifi –cance
780 g.s./m^2	8009	100	-	-
650 g.s./m^2	7885	98	-124	Ns
530 g.s./m^2	7683	96	-326	**
LSD 5% = 219 kg, LSD 1% = 316 kg, LSD 0.1% = 405 kg				

The Duncan test (Table 4), is a multiple comparison method used to identify significant differences between means. Values with different letters indicate statistically significant differences.

The Anapurna variety (8403 kg/ha) is in group A, which means that it has a significantly higher production compared to the other varieties Biharia (7587 kg/ha) and Glosa (7597 kg/ha) which are in the same group B, indicating that there are no significant differences between them.

Table 4. Duncan test results for Factors B and C

Duncan Test	LSD5%	Original Data	Value	Category	Sorted Data	Sorted Value	Sorted Category
Factor B	239.7 kg	Mean 1	7587	B	Mean 3	8403	A
		Mean 2	7597	B	Mean 2	7597	B
		Mean 3	8403	A	Mean 1	7587	B
Factor C	219.3 kg	Mean 1	8009	A	Mean 1	8009	A
		Mean 2	7885	AB	Mean 2	7885	AB
		Mean 3	7683	B	Mean 3	7683	B

*Note: The data represent the mean values of different levels tested under Duncan's multiple range test, with the sorted values reflecting the highest to lowest ranking

The difference between Anapurna and the other two varieties exceeds the significance threshold (LSD 5% = 239.7kg), which confirms the superiority of the Anapurna variety.

The sowing density of 780 g.s./m^2 (8009 kg/ha) is in group A, indicating a significantly higher production compared to the density of 530 g.s./m^2. The density of 650 g.s./m^2 (7885 kg/ha) is in group AB, which suggests that there is no significant difference from the density of 780 g.s./m^2 (the difference of 124 kg is below the materiality threshold).

There is a slightly significant difference from the density of 530 g.s./m^2, but not clear enough to be completely separated into group A. The

density of 530 g.s./m^2 (7683 kg/ha) is in group B, which indicates a significantly lower production compared to the density of 780 g.s./m^2.
These results suggest that in order to achieve optimal production, the Anapurna variety and a sowing density of 650-780 g.s./m^2 are recommended, depending on costs and other available resources.
Figure 1 shows the percentage contribution of the influencing factors on wheat production: crop year (Factor A), cultivated variety (Factor B) and sowing density (Factor C), together with the percentage attributed to the experimental error.
The cultivated variety has the greatest impact on wheat production (77.71%). This result suggests that the choice of variety is decisive for achieving a high yield. Considering the previous data, the Anapurna variety had a significantly higher production, which justifies the high weight of this factor.
Climatic conditions and annual variability have a moderate contribution in influencing production (12.70%). Although climatic conditions have a visible impact, the differences between the years analysed were not statistically significant, but they still contribute to a greater extent than density.

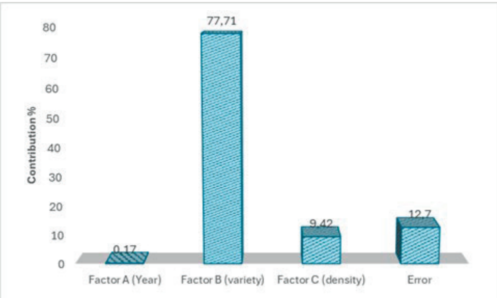

Figure 1. Contribution of factors A [crop year], B [cultivated variety], C [sowing density]

Sowing density has a relatively small impact on wheat production compared to the variety and crop year (9.42%). Although under certain conditions a higher density has led to an increase in production, this factor is not as influential as the choice of variety.
The very small percentage attributed to the error (0.17%) indicates a high precision of the experiment and a reduced variability not explained by factors. This suggests that the results are reliable and well controlled.
The cultivated variety is the most important factor, influencing production in a proportion of almost 78%. Choosing the right variety, such as Anapurna, can bring significant improvements in production.
The year of cultivation has a moderate contribution, which means that climatic variations and annual conditions play an important role, but not as decisive as the variety.
Sowing density has less impact on production, suggesting that after choosing the optimal variety, adjusting the sowing density can optimize production, but it will not have as great an effect.
In conclusion, it can be said that prioritizing the selection of the high-yielding wheat variety is essential for maximizing production. If the optimal variety is chosen, optimizing the sowing density and monitoring the climatic conditions can additionally contribute to increasing production. Given the relatively low influence of density, the additional costs associated with increasing seeding density must be weighed against the benefits.
The variation in protein content depending on the year of cultivation is shown in Figure 2.

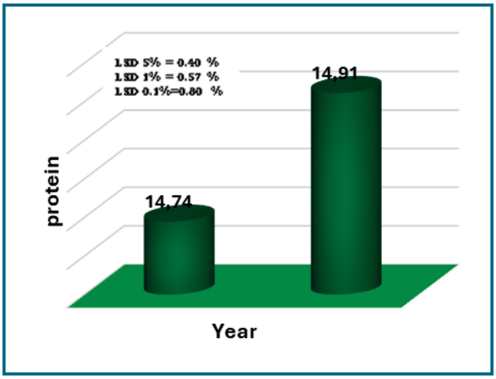

Figure 2. Variation of protein content depending on climatic conditions

The year of cultivation did not have a significant impact on the protein content in the wheat in the experiment carried out.
Although a slight increase in protein content was observed in the second year (14.91% vs. 14.74%), this difference is statistically

insignificant and can be attributed to natural variability or other factors not analyzed.
Climatic factors or other year-specific conditions did not significantly influence the protein composition, suggesting that other factors, such as the variety grown or agricultural technology, could have a greater impact on protein content.

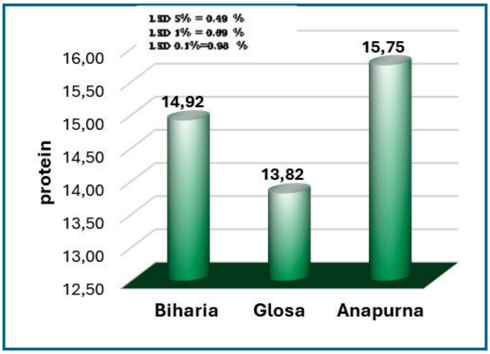

Figure 3. Variation of protein content depending on variety

The protein content (%) for the three wheat varieties, together with the values of the limits of statistical significance, is shown in Figure 3.
The Anapurna variety has the highest protein content (15.75%), being significantly higher than the other two varieties. This variety is an optimal choice if you want to obtain a higher quality of wheat in terms of protein content.
The Biharia variety has an intermediate protein content (14.92%), but still significantly higher than the Glosa variety.
The Glosa variety has the lowest protein content (13.82%), being significantly lower than the other two varieties.
Scientific interpretation of the results on the protein content (%) in wheat according to sowing density (Figure 4).
Density 3 (530 g.s./m^2) vs. Density 1 (780 g.s./m^2) is 0.69%, and this difference exceeds the significance threshold LSD 1% (0.69%), which indicates a significant difference at a 99% confidence level. A lower density (530 g.s./m^2) results in a significantly higher protein content compared to a high density (780 g.s./m^2).
Density 2 (650 g.s./m^2) vs. Density 1 (780 g.s./m^2) is 0.50%. This difference exceeds the 5% LSD materiality threshold (0.49%), making it significant at a 95% confidence level. Decreasing the density from 780 to 650 g.s./m^2 leads to a significant increase in protein content.
Density 3 (530 g.s./m^2) vs. Density 2 (650 g.s/m^2) was 0.19%, is insignificant and denotes that decreasing the density from 650 to 530 g.s/m^2 does not produce a significant increase in protein content.

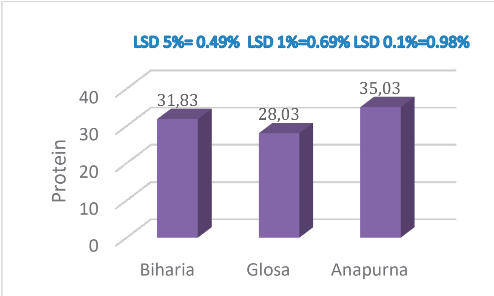

Figure 4. Variation of protein content depending on density

Lower seeding densities (530 and 650 g.s./m^2) result in a higher protein content compared to the high density of 780 g.s./m^2.
The significant difference between the high density (780 g.s. /m^2) and the other two densities suggests that too thick sowing can reduce the quality of protein in wheat.
Between 530 g.s./m^2 and 650 g.s./m^2 there are no significant differences, suggesting that a density of 650 g.s./m^2 could be optimal, providing a balance between protein content and other possible benefits such as yield.
Scientific interpretation of the results regarding the contribution of factors to the protein content in wheat (Figure 5).

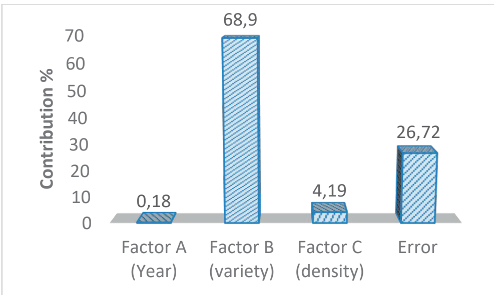

Figure 5. Contribution of factors A [crop year], B [cultivated variety], C [sowing density] to protein content

The cultivated variety (Factor B) is the most important factor influencing the protein content in wheat, contributing 76.47% to the total variation. Choosing the right variety is essential for improving the quality of wheat. Sowing density (Factor C) has a moderate impact (10.17%). Adjusting the density can bring improvements, but its effect is secondary to the choice of variety. The crop year (Factor A) has a minimal intake (0.85%), which suggests that the climatic conditions between the years analyzed had an insignificant impact on the protein content. The experimental error (12.51%) indicates that there are additional factors that may influence the protein content that were not included in this study. The choice of variety is crucial for obtaining a wheat with a high protein content Optimizing the sowing density can help to increase the quality, but it must also be analyzed according to the total yield and associated costs.

The variation of gluten content (%) in wheat according to climatic conditions in the crop year is shown in Figure 6.

The gluten content was slightly higher in the year 2022/2023 (31.78%) compared to 2023/2024 (31.49%), but this difference is not statistically significant. The climatic conditions in the two years analyzed did not significantly influence the quality of wheat in terms of gluten content.

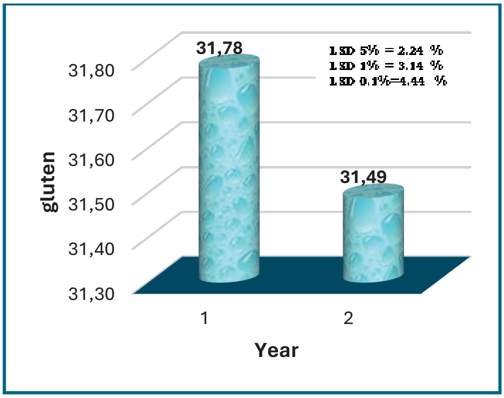

Figure 6. Variation of gluten content (%) in wheat according to climatic conditions in the crop year

The influence of the wheat variety on the gluten content (%), is shown in Figure 7.

The Anapurna variety (b3) has the highest gluten content (35.03%), being significantly higher than both varieties, Biharia and Glosa. The Biharia variety (b1) has an intermediate gluten content (31.83%), being significantly better than Glosa, but lower than Anapurna, and the Glosa variety (b2) has the lowest gluten content (28.03%), being significantly lower than the other two varieties.

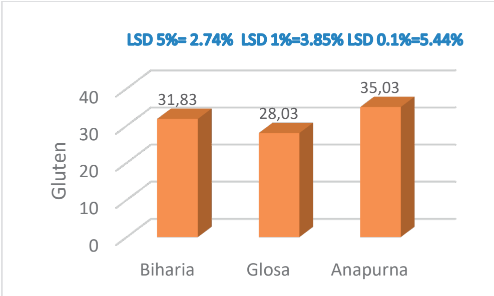

Figure 7. Variation of gluten content (%) in wheat according to variety

The variation of gluten content according to three density levels: 780 g.s./m^2 (c1), 650 g.s./m^2 (c2) and 530 g.s./m^2 (c3), is presented in Figure 8.

As the sowing density decreases, the gluten content increases. However, this increase is not statistically significant in this experiment.

The density of 530 g.s./m^2 had the highest gluten content (32.33%), but the difference from the higher densities is not significant.

The density of 780 g.s./m^2 had the lowest gluten content (30.67%), but the differences from the other densities are not large enough to be considered statistically significant.

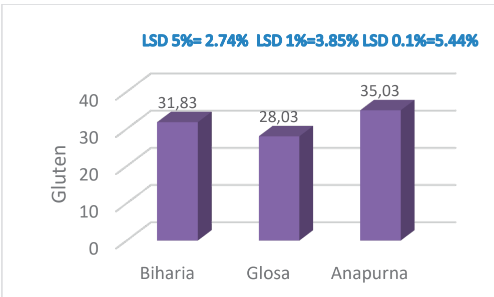

Figure 8. Variation of gluten content (%) in wheat according to density

The contribution of factors A [crop year], B [cultivated variety], C [sowing density] to the achievement of the gluten content of wheat is shown in Figure 9.

Analyzing the data obtained, it appears that the wheat variety (Factor B) has the greatest impact on gluten content, accounting for almost 70% of the total variation. This highlights the fact that the choice of variety is decisive for achieving a high gluten content. Previous results have shown that varieties such as Anapurna had a significantly higher gluten content compared to other varieties.

Sowing density (Factor C) has a limited effect, contributing only 4.19%. Adjusting the density can bring minor improvements, but its impact is reduced compared to choosing the variety.

The year of cultivation (Factor A) has a minimal influence (0.18%), which indicates that climatic variations between the analyzed years do not significantly affect the gluten content

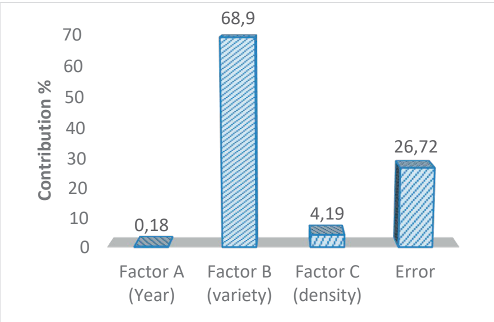

Figure 9. Contribution of factors A [crop year], B [cultivated variety], C [sowing density] to gluten content

CONCLUSIONS

The cultivated variety is the most important factor for both wheat production and its quality (protein and gluten content). The Anapurna variety stood out as the variety with the best results because it recorded a significantly higher production than the Biharia and Glosa varieties, had the highest protein and gluten content, with statistically significant differences compared to the other varieties, and the Biharia variety had intermediate results. The contribution of the variety to the variation of gluten content was 68.90%, and to the protein content of 76.47%, confirming the crucial importance of this factor in determining the quality of wheat.

Sowing density had a moderate impact on wheat production and quality because a lower density (530 g.s./m^2) led to a slight increase in protein and gluten content, but these differences were not statistically significant in most cases. The optimal density could be 650 g.s./m^2, as it provides a balance between production and quality, without significantly compromising yield. The contribution of density to gluten content was 4.19%, and to protein 10.17%, which shows that the impact of density is much lower compared to that of the variety.

The growing year, as there were no considerable climatic differences, had a negligible influence on both the production and the quality of wheat, the differences between years in terms of production, protein and gluten content were statistically insignificant. The year's contribution to gluten content was only 0.18%, and to protein 0.85%, confirming that moderate climatic variations did not significantly influence wheat.

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THE EFFECTS OF WATER DEFICIT AND AIR TEMPERATURE ON SEED PRODUCTION OF ALFAALFA (*Medicago sativa* L.) UNDER THE CONDITIONS OF ARDS SECUIENI, NEAMȚ

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Abstract

Good quality of alfalfa seed variety is very important to meet the farmers` requirements for feed production. Testing of new alfalfa varieties is important for assessing their seed production potential, which also influences their commercial value. The purpose of our research was to test the seeds production potential of new alfalfa varieties, under the pedoclimaic conditions of ARDS Secuieni, Neamț, Romania. The experiment was organized between 2020 and 2022 o a chernozem-type soil and aimed to evaluate the quality of some new varieties of alfalfa. An important aspect pursued in this study referred to the effects of climate change on new alfalfa varieties, in the absence of irrigation. Within the study, 17 Romanian alfalfa varieties were analyzed regarding seed productiion potential, based on multi-annual testing, to recommend the best-adapted ones in culture, to make these results available to farmers, and to expand in culture. Of the 17 alfalfa genotypes tested in our study, five (F 2907-20, F 2910-20, F 2905-20, F 2906-20, Catinca) showed a high adaptability to the pedo-climatic conditions of ARDS Secuieni, Neamț, Romania.

Key words: *alfalfa, new varieties, seed production, climatic conditions.*

INTRODUCTION

Alfalfa (*Medicago sativa* L.) is of particular importance as a good fodder plant, but also as a soil improver, as it enriches the soil in nitrogen due to its symbiosis with the *Rhizobium meliloti* Dangeard bacteria, thus saving important amounts of nitrogen fertilizer during the whole growing season. The highest amount of nitrogen fixing symbiotic nodules is formed in the 0-15 cm soil layer (Oliveira et al., 2004).
For seed production, alfalfa is usually sown in late fall or early spring. With the global warming, the water and thermal regime after sowing is highly fluctuating and thus cold and heat stress of the plants has also occurred (Gharoobi et al., 2012).
In general, weather fluctuations represented byan intense increase in monthly mean air temperature, a decrease in the amount of precipitation, uneven rainfall distribution and a prolonged period of drought in summer further limit legume seed productivity (Brouwer et al., 2000; Tyshchenko et al., 2020; Mîrzan et al., 2024; Lykhovyd, 2018). The air temperature is crucial in early sowing of alfalfa, and improving seed germination ability under low temperature conditions by modern breeding approaches could be beneficial for seed production (Zhao et al., 2024).
Alfalfa reacts negatively to drought and, in order to adapt and survive under stress conditions, undergoes morphological, physiological, biochemical or molecular changes, which need to be taken into account when breeding drought-tolerant varieties while increasing yields and quality of the products (Vozhehova et al., 2021).
During the dry season, alfalfa (*Medicago sativa* L.) plants reduce aboveground vegetative mass (Bellague et al., 2016; Durand, 2007), which limits the leaf area index and consequently reduces biomass productivity. Therefore, to stabilize and increase the productivity of

alfalfa, it is necessary to increase the drought resistance of alfalfa plants, and the study of this trait is an important step in plant breeding programs (Yu, 2017).

The potential for seed production of a new alfalfa variety induces its commercial potential (Varga et al., 1998; Ualiyeva et al., 2022). It has been established that species and varieties differ by seed germination under stress conditions (Budakli & Erdel, 2015; Molor et al., 2016). One of the most important steps in the plant breeding process is a preliminary evaluation of the drought tolerance of a large set of populations to choose the best primary material. The correct choice of primary material is a prerequisite for further success in plant breeding.

Breeding new alfalfa varieties with superior seed yield and high forage quality is one of the biggest challenges for alfalfa breeders and seed producers both abroad (Julier et al., 2000; Bolanos-Aguilar, 2002; Tucak et al., 2017; 2023) and in Romania (Schitea et al., 2007; 2020a; 2020b; 2022).

Alfalfa seed can be obtained from regular forage crops, or from crops specially established for this purpose, but it is recommended to obtain seed production from specially established crops (Moisa et al., 2018; Muscalu et al., 2022).

The purpose of the research was to test the seeds production potential of new alfalfa varieties, under the pedoclimaic conditions of ARDS Secuieni, Neamț.

MATERIALS AND METHODS

The research was carried out in the experimental field of the Fodder Plant Culture Laboratory of the Agricultural Research and Development Station (ARDS), Secuieni, Neamț, Romania, between 2020 and 2022.

The experiments were laid out according to the randomized block method, in four replications, with a harvestable plot area of 10 m^2, with a row spacing of 25 cm, and the seed density was 500/m^2. The cultivation technology specific to the forest steppe pedoclimatic conditions area was followed, and the obtained data were statistically processed according to the analysis of variance method and interpreted accordingly (Jităreanu, 1999). The experiment was stationary and was located on a medium-textured, acidic, chernozemic soil. The main characterstics of soil are $pH_{(H2O)}$ - 5.98; well supplied in phosphorus (77.6 ppm P_{AL}); Ca - 13.6 meq/100 g soil; Mg -1.8 meq/100 g soil Mg; poorly supplied in active humus (1.88 %) and nitrogen (16.2 ppm N-NO_3) and poorly supplied in potassium (124.6 ppm K_2O).

Alfalfa sowing was carried out in the third decade of March (20.03.2020) with the SCE-8 Haldrup experimental seed drill. During the growing season, bentazone 480 g/l + imazamox 22.4 g/l herbicide (1.25 l/ha) was administered to control annual dicotyledonous and some monocotyledonous weeds in the experimental field. Harvesting of the seedlings for seed was done with the Wintersteiger combine when 80% of the pods had browned (Photo 1.). Alfalfa seed is produced at the first or second harvest, depending on the climatic conditions of the year.

Photo 1. Pictures from the experimental field of ARDS Secuieni - mechanized harvesting

Average plant height (cm) was determined in the 100% flowering fenophase of alfalfa plants by measuring the height of the plants in the middle of each experimental plot.

The average number of racemes/shoot was determined by counting the racemes on 100 shoots from each experimental plot, then calculating the average value.

The average number of seeds/racema was determined by counting the seeds in the racemes of 10 shoots on each experimental plot, then calculating the average value.

Seed production ($kg \cdot ha^{-1}$) was determined by mechanized harvesting of each experimental plot, weighing seeds, and then reporting the amount of seeds to the surface unit.

The climatic conditions recorded in the 2019-2022 crop years were highly variable, and the years studied were characterized as hot and very dry.

In terms of the temperatures recorded at the unit's own weather station, the studied agricultural years were characterized as atypical, warm years, registering deviations ranging from 1°C to 1.5°C from the multi-year average of 8.9°C (Figure 1).

In terms of precipitation, the analyzed period was characterized as dry, compared to the multiannual sum of 544.3 mm, the deviations recorded were 168.3 mm in the 2019/2020 agricultural year, 144.5 mm in the 2020/2021 agricultural year and 283.5 mm in the 2021/2022 agricultural year (Figure 2).

Research was carried out on the behavior of 17 varieties and synthetic varieties of alfalfa for sowing, all of them of autochthonous origin, created at National Institute for Agricultural Research and Development Fundulea, thus aiming to introduce in the culture of genotypes that show high adaptability to the conditions of ARDS Secuieni Neamț.

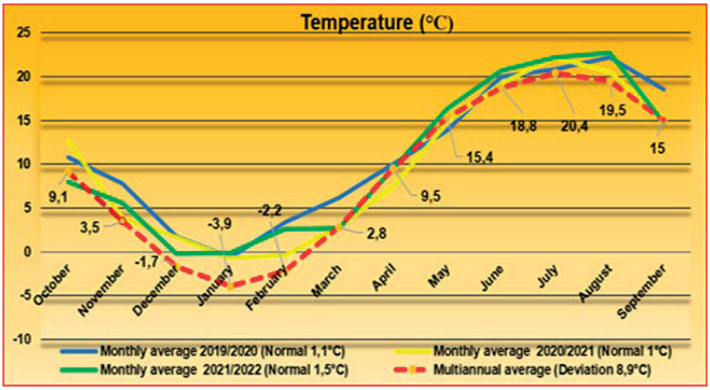

Figure 1 Monthly temperatures recorded at A.R.D.S. Secuieni, in the period 2019-2022

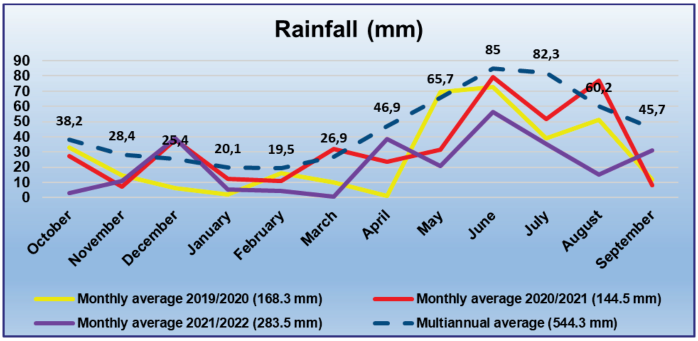

Figure 2. Monthly precipitation recorded at A.R.D.S. Secuieni, in the period 2019-2022

RESULTS AND DISCUSSIONS

In order to be able to characterize the studied varieties, a series of morpho-physiological determinations were made on the samples harvested in the experiment during the growing season.

As a result of the research carried out, on average over the three years studied (2020, 2021, 2022) it was observed that the average plant height ranged between 76.4 cm for the Catinca variety and 88.6 cm for the synthetic variety F 2909 - 1 - 20 (Table 1).

The average number of racemes per shoot ranged from 6.8 in the synthetic variety F 2814 - 19 to 16.5 in the synthetic variety F 2020 - 20, but the highest number of racemes per shoot was observed in the synthetic varieties F 2910 - 20 (15.4) and F 2020 - 20 (16.5).

The average number of seeds/raceme ranged from 14.5 for the synthetic variety F 2811 - 19 to 27.1 for Ileana.

Table 1. Biometric determinations of alfalfa (*Medicago sativa* L.), 2020-2022

No.	Varieties	Average plant height (cm)	Average number of racemes/shoot	Average number of seeds/raceme	Disease resistance*	Regeneration after mowing*
1	Catinca	76.4	9.6	18.6	1.5	3.51
2	Liliana	73.5	8.5	19.2	2.0	2.51
3	Pompilia	80.5	8.5	21.6	2.0	2.90
4	Ileana	81.0	10.6	27.1	1.5	1.53
5	F 2809 -19	81.8	13.7	26.8	1.0	2.31
6	F 2810 - 19	86.8	7.9	18.8	2.5	3.22
7	F 2811 - 19	85.5	10.7	14.5	1.0	254
8	F 2812 - 19	81.3	7.0	20.2	1.0	2.00
9	F 2814 - 19	78.8	6.8	21.9	2.5	2.59
10	F 2905 - 20	83.5	11.3	19.8	2.0	3.13
11	F 2906 - 20	79.3	11.8	16.7	1.0	2.58
12	F 2907 - 20	81.0	13.5	17.5	1.5	3.21
13	F 2908 - 20	79.3	7.8	19.2	1.0	2.54
14	F 2909 - 1 - 20	88.6	13.6	18.9	1.5	3.52
15	F 2909 - 2 - 20	78.3	10.7	19.0	1.5	2.57
16	F 2910 - 20	86.8	15.5	17.1	3.0	3.28
17	F 2020 - 20	88.3	16.5	18.6	2.0	3.51

*)Notes 1-9; 1 = very good, 9 = very poor.

Photo 2. Pictures from the experimental field of ARDS Secuieni - vegetation period

During the growing season, alfalfa was attacked by a complex of foliar diseases, thus the diseases alfalfa rust (*Uromyces striatus*) and alfalfa mealy bug (*Erysiphe pisi*) were identified. The disease resistance of the genotypes studied was scored on a 1-9 scale and values between 1 and 3 were recorded. From these data, the resistance of the analyzed genotypes under the conditions of Secuieni was determined.

From the data obtained, the regeneration after mowing was rated with scores ranging from 1.53 for the Ileana variety and 3.52 for the synthetic variety F 2909 - 1 - 20.

As for the correlation between the number of racemes per plant and the number of seeds in the raceme, in the three experimental years, it was observed that it was direct and the correlation coefficient (r) was statistically assured and interpreted as distinctly significant (Figure 3).

The thousand kernel mass values ranged widely by genotype from 1.61 g (Catinca) to 1.96 g (F 2907 - 20). The highest MMB values were 1.91, 1.92 g and 1.96 g, recorded in the synthetic varieties F 2909 - 2 - 20, F 2809 -19 and F 2907 - 20 (Figure 4).

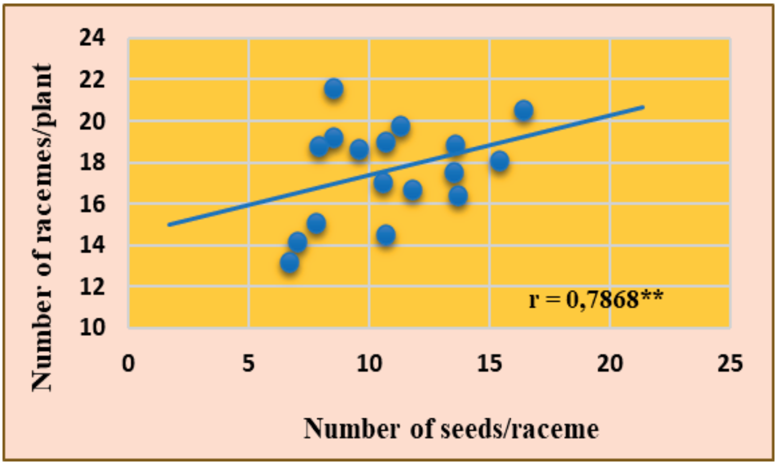

Figure 3. Correlation between number of racemes per plant and number of seeds per raceme

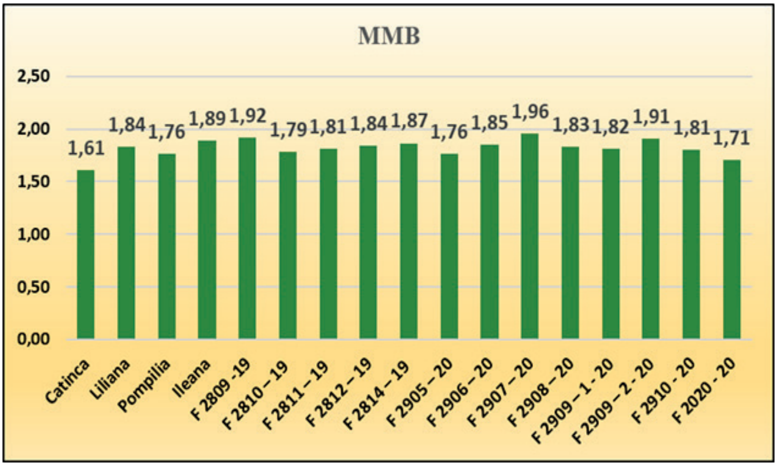

Figure 4. Weight of one thousand grains in the studied alfalfa genotypes

The experimental results obtained during the analyzed period indicate a significant difference in the production of the studied alfalfa varieties. Average alfalfa yields ranged from 416 kg/ha (synthetic variety F 2809-19) to 753 kg/ha (synthetic variety F 2907-20). Compared to the yield recorded in the control variant (mean of the experiment - 524 kg/ha), of the 17 alfalfa genotypes studied, very significant yield increases were recorded for the synthetic varieties F 2907-20, F 2910-20, and distinctly significant for Catinca and the synthetic varieties F 2905-20 and F 2906-20. The synthetic variety F 2809 - 19 with a very significant negative yield difference, the synthetic variety F 2810 - 19 with a distinctly significant negative yield difference, and the synthetic varieties Liliana and F 2811 - 19, F 2812 - 19, F 2909 - 1 - 20 and F 2020 - 20 with distinctly significant negative yields, were identified as having a lower adaptability to the conditions of the area (Table 2).

CONCLUSIONS

On average, during the three years of experimentation, the yields obtained from the 17 alfalfa genotypes tested at S.C.D.A. Secuieni, varied within very wide limits, from 416 kg/ha (F 2809 -19) to 753 kg/ha (F 2907 - 20).

Compared to the control (average of experience), very significant yield increases

Table 2. Production recorded for alfalfa varieties under soil and climatic conditions at ARDS Secuieni

Varieties	Average yield (kg·ha^{-1})	Relative output (%)	Diff. (kg/ha)	Significance
Catinca	584	111	60	**
Liliana	466	89	-58	o
Pompilia	546	104	22	-
Ileana	550	105	26	-
F 2809 -19	416	79	-108	ooo
F 2810 - 19	462	88	-62	oo
F 2811 - 19	465	89	-60	o
F 2812 - 19	482	92	-43	o
F 2814 - 19	555	106	31	-
F 2905 - 20	589	112	65	**
F 2906 - 20	587	112	62	**
F 2907 - 20	753	144	229	***
F 2908 - 20	553	105	29	-
F 2909 - 1 - 20	469	89	-55	o
F 2909 - 2 - 20	501	96	-23	-
F 2910 - 20	601	115	77	***
F 2020 - 20	478	91	-46	o
Average	524 Control	100	Control	-
LSD (kg/ha)	5% = 35; 1% = 63; 0.1% = 71			

were recorded for the synthetic varieties F 2910-20 and F 2907-20.

The highest plant height was recorded for the synthetic variety F 2909 - 1 - 20 (88.6 cm), the highest number of racemes per shoot was recorded for the synthetic varieties F 2910 - 20 (15.4) and F 2020 - 20 (16.5), and the average number of seeds/raceme ranged from 14.5 for the synthetic variety F 2811 - 19 to 27.1 for the synthetic variety Ileana.

Of the 17 alfalfa genotypes tested at ARDS Secuieni five showed a high adaptability to the pedo-climatic conditions without irrigation.

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EVALUATION OF THE DOWNY MILDEW ATTACK (*Peronospora camelinae*) IN CAMELINA (*Camelina sativa* (L.) CRTZ.) DURING 2023-2024, DRACEA, TELEORMAN COUNTY

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Abstract

In recent years, Camelina sativa has emerged as a crop that can be considered an alternative to traditional oilseed plants. The primary objective of our research was to evaluate the downy mildew (Peronospora camelinae) attack. Observations were conducted during 2023 and 2024 under the conditions of the Dracea location in Teleorman County. The biological material consisted of two Romanian genotypes, Mădălina and Camelia, monitored in spring cultivation. In the conditions of 2023, the disease frequency in the Mădălina variety was 25%, while the Camelia variety showed a frequency of 34%. In the conditions of 2024, the frequency of the attack was 20% for the Mădălina variety and 22% for the Camelia variety. Throughout the research period, the Mădălina variety exhibited a lower disease frequency compared to the Camelia variety.

Key words*: Camelina sativa, downy mildew, frequency, row spacing.*

INTRODUCTION

Originally from Eastern Europe and Western Asia, *Camelina sativa* (L.) Crantz is a well-known plant from the *Brassicaceae* family (Vollmann & Eynck, 2015).

In Europe and North America, camelina is gaining increasing attention from both industry and research (Zanetti et al., 2017) due to its adaptability to various environmental conditions, high yield potential, and its unique oil, which can be used in a wide range of bio-applications (jet fuel, biofuels, oleochemical compounds, feed, and food) (Larsson, 2013). Regarding cultivation technology, camelina does not have special requirements (Dobre et al., 2014a), but special attention must be given to the phytosanitary conditions of the crop (Podgoreanu et al., 2015).

Camelina is naturally resistant to many diseases and pests that affect other plants in the *Brassicaceae* family (Putnam et al., 1993). However, under field conditions, cases of downy mildew caused by *Peronospora camelinae* have been reported (Vollmann et al., 2008) as well as reports of the occurrence of the pathogen *Sclerotinia sclerotiorum* (Cristea & Jurcoane, 2015).

Downy mildew is an obligate parasite that can only survive by infecting and depending on living plants (Turk, 2002). The symptoms of this disease include the presence of a grayish-white mycelium on the underside of the leaves, as well as on the upper third of the plant, on the internodes, and on the developing siliques (Séguin-Swartz et al., 2009). Severely infected plants exhibit twisted or bent growth (Vollmann et al., 2008).

This research was conducted to evaluate the effects of *Peronospora camelinae* on two varieties of *Camelina sativa*, focusing on the disease's occurrence under dry and temperate conditions. The study aimed to assess how these factors influence disease incidence, particularly downy mildew, which is the main disease identified in the crop, and to examine the relationship between disease occurrence and the overall performance of the plant.

MATERIALS AND METHODS

The main aim of this study is to investigate the occurrence and effect of the *Peronospora camelinae* pathogen on camelina crops under the environmental conditions in the Dracea locality of Teleorman County. At the same time, the research aims to assess the influence of plant density and row spacing on the severity of downy mildew infection in camelina crops. *Peronospora camelinae* was first reported in camelina fields in 2014 (Cristea & Manole, 2014) in the southern part of Romania. Since then, the pathogen has been reported for the first time in various parts of the world, such as the Northwestern United States in 2022 (Benzhong & Qing, 2022) representing a significant increase in the importance of the pathogen in the crop. The data from this research are useful for farmers in the southern part of the country, providing information about camelina downy mildew and optimizing cultivation technology in correlation with the pathogen in the crop.

This research was conducted during the spring seasons of 2023 and 2024 in experimental fields located in Dracea, Teleorman (44.15° N latitude, 25.35° E longitude). The field was organized into a randomized block design, with each block measuring 48 meters in length. Two main factors were tested: row spacing and sowing rate. Factor 1, concerning row spacing, included 13 cm and 25 cm variants. Factor 2, concerning sowing rate, with 6 kg/ha (corresponding to 525 germinable seeds/m²) and 8 kg/ha (corresponding to 700 germinable seeds/m²). Camelina seeds were sown using a conventional seeder at a depth of 2.0 cm, ensuring uniform distribution for optimal germination.

The study analyzed two local camelina varieties, developed and adapted to the environmental conditions in Romania. The first variety, Mădălina (Matei et al., 2014), was developed at the University of Agronomic Sciences and Veterinary Medicine in Bucharest, while the second, Camelia (Toncea, 2014), was developed at ICDA Fundulea.

The experimental design was the randomized complete block design (RCBD) in a mirrored layout for the two varieties, as it helps control field variability, ensuring a more accurate comparison between factors. The inclusion of all factor combinations in each block and their randomization within the block, minimizes the effects of soil variations, microclimate, and other factors that could influence the results. Thus, the observed differences between the factor combinations are more likely to be attributed to the factors themselves, rather than environmental variations.

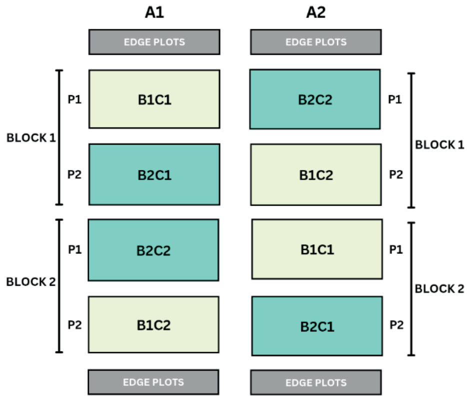

Figure 1. Layout of the plots in the experimental field. Factors in the experimental field are: A1 - Mădălina; A2 - Camelia; B1C1 - 6 kg/ha & 13 cm; B2C1 - 8 kg/ha & 13 cm; B2C2 - 8 kg/ha & 25 cm; B1C2 - 6 kg/ha & 25 cm

The disease evaluation was conducted twice during the plant growth period. The first evaluation took place before flowering (BBCH stage 6), at the maximum leaf development stage, and the second evaluation was performed during seed development and maturation (BBCH stage 8).

The evaluation was conducted according to an "N" pattern (Robertson, 2008) on the plot area to ensure representative sampling. In total, 100 plants were analyzed, with 25 plants sampled at each designated point (Figure 2). This approach allowed for a comprehensive assessment of the disease incidence and severity in the field.

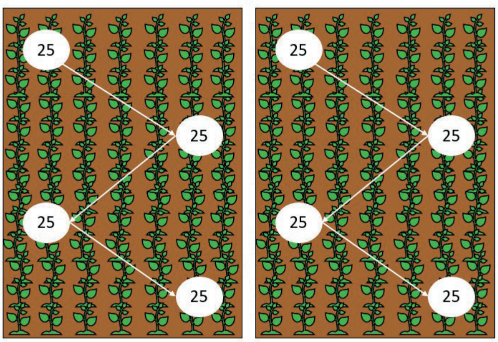

Figure 2. Evaluation in a “N” pattern

The disease frequency (F%) was calculated as the proportion of plants showing symptoms (n) relative to the total number of plants evaluated (N), expressed as a percentage.

$$F(\%) = \frac{n * 100}{N}$$

The intensity of the attack (I%) was calculated by assessing the extent of plant surface infection using a percentage scale from 0 to 100% of the plant's surface with symptoms. It was calculated using the following formula, where i = the percentage assigned, f = the number of plants with the assigned percentage, and n = the total number of infected plants analyzed.

$$I(\%) = \frac{\sum(i * f)}{n}$$

The degree of attack (DA%) was calculated to express the severity of the attack in the crop by correlating the frequency (F%) data with the intensity (I%) data using the following formula:

$$DA(\%) = \frac{F * I}{100}$$

The data were analyzed using ANOVA to determine the significance of the differences between the variants, with statistical calculations performed using R software and GraphPad Prism.

RESULTS AND DISCUSSIONS

During the experimental periods in the springs of 2023 and 2024, weather conditions varied at different stages of camelina development. Meteorological data were collected daily from March 1st to June 30th using the autonomous meteorological station Meteobot® Mini 2022, placed in the experimental field. In 2023, the total precipitation accumulated during the growing season was 222.6 l/m², and the average temperature was 14.8°C. In 2024, the total precipitation for the entire period was significantly lower, at 120.2 l/m², while the average temperature was higher, at 17.2°C.

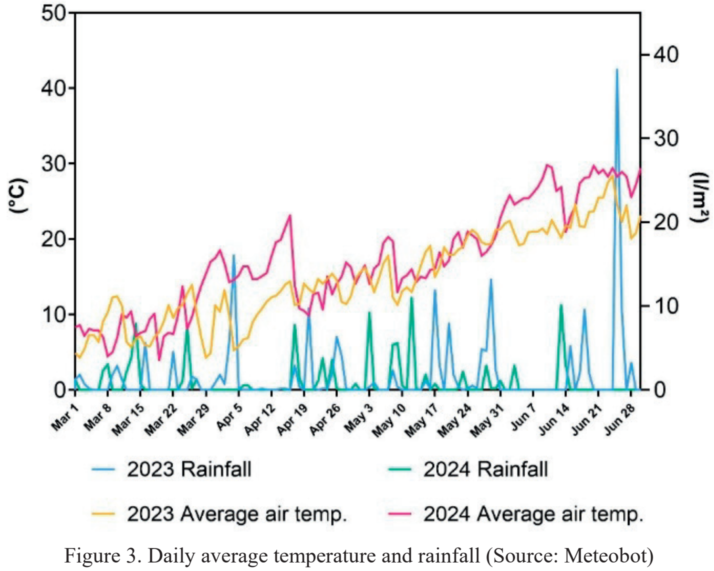

Figure 3. Daily average temperature and rainfall (Source: Meteobot)

The comparative analysis of the average air temperature between 2023 and 2024 reveals a significant increase in 2024, with higher and more variable temperatures throughout the observation period (March 1st - June 30th). Both periods show a gradual and normal increase in temperatures as the months progress; however, in 2024, temperatures exhibited a more abrupt rise. In 2024, temperatures were visibly higher (Figure 3). A t-test was conducted to assess the statistical significance of the difference between the average temperatures in 2023 and 2024. The results indicate a significant difference ($P = 0.0051$), confirming that the average temperature in 2024 (17.21°C) was 2.35°C higher than in 2023 (14.87°C), with a 95% confidence interval between 0.71 and 3.99°C.

The 2.35°C difference had an impact on the senescence of camelina plants in 2024, as camelina shortens its growing period when subjected to heat stress (Smith & Lu, 2024). Consequently, in 2024, camelina was harvested just 80 days after the seeding. In 2023, the precipitation levels were higher, characterized by irregular distribution and multiple pronounced peaks, including an extreme event at the end of June. This event did not affect the crop since, at that time, camelina was in the seed maturation phenophase. In contrast, 2024 had a considerably lower and more uniform precipitation regime, with no sudden increases in rainfall. These climatic discrepancies are crucial for camelina development, as precipitation affects soil water availability, disease incidence, and plant productivity. The difference between the two years was -102.4 l/m² for the entire period, indicating that 2024 was a year with drought conditions.

Table 1. Statistical analysis ANOVA of meteorological data

ANOVA summary	
F	310.6
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R squared	0.6582

The ANOVA analysis indicated extremely significant differences between the temperature and precipitation values analyzed in 2023 and 2024 ($F = 310.6$, $P < 0.0001$), suggesting considerable climatic variability between these two periods. The R^2 coefficient = 65.82% indicates that a large portion of the observed variation can be explained by the differences in temperature and precipitation, highlighting a significant climatic impact. The high statistical significance ($P < 0.0001$) confirms that these changes are not random but reflect a clear trend that influences biological and agricultural processes in the given system.

The pronounced fluctuations in 2023, with wet conditions and low temperatures, created favorable conditions for the development of pathogens such as *Peronospora camelinae*, while the more stable, hot and dry days in 2024 reduced the infection rate.

Sampling was done in the two phenophases BBCH 6 and BBCH 8. Plants showing symptoms were harvested and evaluated. The leaves and upper stem exhibited specific symptoms (Cristea & Manole, 2014), including discoloration spots on the upper side, which, over time, turned into necrotic areas. On the lower side, beneath these spots, a fine layer of whitish or grayish-white powder formed (Figure 4), a result of sporangiophores and sporangia of the pathogen. Plants with a higher intensity of downy mildew exhibited the specific phenomenon of downy mildew, namely, the twisting of the stem (Vollmann et al., 2008).

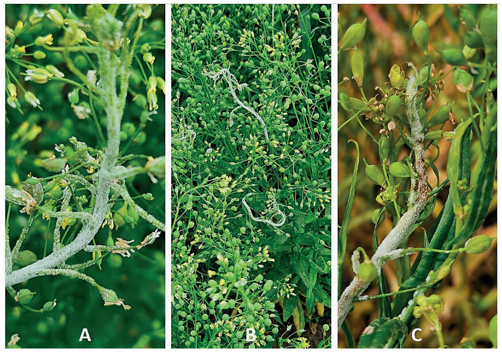

Figure 4. Specific symptoms of downy mildew in camelina plants (Source: Original)

Microscopic identification of the pathogen was done based on sporangiophores and sporangia morphology using the Zeiss Promo Star microscope (Figure 5).

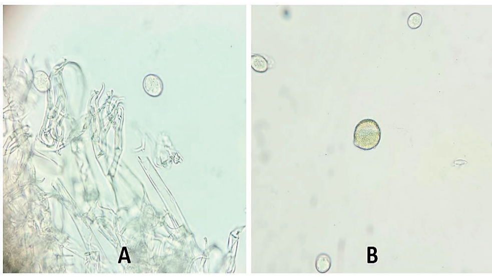

Figure 5. A- Sporangiophores; B- Sporangium (Source: Original)

Microscopic observations revealed characteristic structures, such as hyaline sporangia and dichotomously branched sporangiophores, belonging to the genus *Peronospora*. The observed sporangia had a lemon-shaped form and a yellow color (Figure 5 B), typical for the *Peronospora* genus. These observations were compared with descriptions in the scientific literature, confirming the pathogen's identity (Cristea & Manole, 2014, Salcedo et al., 2021).

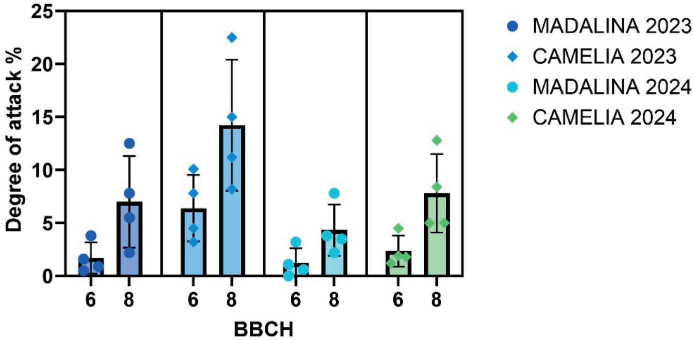

Figure 6. Evaluation of downy mildew attack (*Peronospora camelinae*)

The degree of attack of *Peronospora camelinae* for the two varieties, Mădălina and Camelia, in the BBCH 6 and BBCH 8 developmental stages, analyzed during the 2023 and 2024 seasons is presented in Figure 6. The ANOVA analysis indicates significant differences between treatments, with a *P* value of 0.0289, confirming notable variations between the varieties. In 2023, Camelia exhibited a higher degree of attack at the BBCH 8 stage compared to Mădălina, suggesting greater susceptibility at this developmental phase. In 2024, the degree of attack was lower than the previous year, but the trend between the varieties remained, with Camelia still being more affected. The error bars highlight significant variability, especially for Camelia at BBCH 8 in 2023, which could suggest external influences, such as the variable climatic conditions in 2023, with Camelia showing increased vulnerability to environmental fluctuations. In contrast, data from 2024 are more homogeneous, suggesting a stabilization of factors such as higher temperatures and lower precipitation, reducing the pathogen's virulence. Hot, dry weather keeps the disease from spreading in the crop (Petcu et all. 2022). To summaries, the attack was more severe in 2023, especially for Camelia at BBCH 8, and the differences between years and treatments remain significant.

Table 2. The influence of seeding rate per hectare and row spacing on downy mildew incidence in camelina (2023-2024) at Dracea, Teleorman location

Variety	Variant*	Phenological stage / year											
		BCCH6 / 2023			BCCH8 / 2023			BCCH6 / 2024			BCCH8 / 2024		
		F	I	DA	F	I	DA	F	I	DA	F	I	DA
		(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Mădălina	V1	5	18	0.9	15	36.7	5.5	0	0	0	8	22.5	2.2
	V2	12	31.7	3.8	25	50	12.5	10	32	3.2	20	39	7.8
	V3	7	22.9	1.6	18	43.3	7.8	2	55	1.1	12	29.2	3.5
	V4	2	25	0.5	10	22	2.2	5	11	0.6	10	22	3.8
Camelia	V1	15	30	4.5	22	56	11.2	8	22.5	1.8	12	41.7	5
	V2	25	40.4	10.1	34	66.2	22.5	11	40.9	4.5	22	58.2	12.8
	V3	22	35.5	7.8	28	53.6	15	5	38	1.9	19	44.2	8.4
	V4	10	32	3.2	15	54.7	8.2	5	24	1.2	16	31.3	5.0

*V1-6 kg/ha & 13 cm; V2-8 kg/ha & 13 cm; V3-8 kg/ha & 25 cm V4-6 kg/ha & 25 cm.

The Table 2 shows that variant V4 (6 kg/ha & 25 cm) provides the best results, with the lowest values for downy mildew frequency, intensity, and severity in both varieties during 2023 and 2024. On the other hand, variant V2 (8 kg/ha & 13 cm) is the most affected, recording the highest values for all indicators, indicating increased susceptibility to downy mildew. These results suggest that a lower seeding rate and wider row spacing help reduce downy mildew incidence, while a higher seeding rate and narrower rows promote disease development. Comparing the two varieties, Camelia appears to be more vulnerable to downy mildew than Mădălina, showing higher values for frequency, intensity, and degree of attack in almost all tested conditions. For example, in the seeding rate and narrow-row variant (V2: 8 kg/ha & 13 cm), the infection was more severe in Camelia, with a frequency of up to 66.2% compared to 50% in Mădălina, and nearly double the severity. Even in the most favorable variant (V4: 6 kg/ha & 25 cm), Camelia showed slightly higher values, suggesting it is more susceptible to the disease. Therefore, Mădălina appears to have low susceptibility to downy mildew.

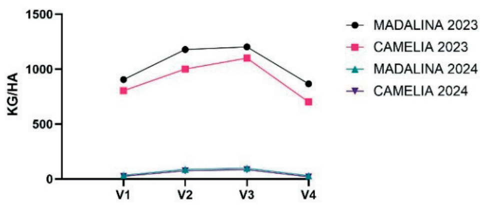

Figure 7. Yield of the two varieties in 2023 and 2024

Regarding the yields in 2024 were significantly lower than in 2023, indicating a strong negative impact of downy mildew and drought (Figure 7). It can be observed that Camelia was more affected than Mădălina, consistently recording lower yields across all tested variants. In 2023, the highest yields were obtained in variants V2 and V3, where the seeding rate and row spacing combination favored better crop performance. On the other hand, variants V1 and V4 showed lower yields, suggesting that both a lower seeding rate (V1) and a wider row spacing (V4) were less effective in maximizing yield. Similar studies, such as (Dobre et al., 2014b) reported a yield of 1,387 kg/ha for the Camelia variety under the 25 cm row spacing and 8 kg/ha seeding rate variant.

Table 3. Yield obtained in 2023-2024

Variety	Variant	Yield kg/ha	
Mădălina		2023	2024
	V1	905	34
	V2	1,179	88
	V3	1,203	98
	V4	867	31
Camelia			
	V1	804	25
	V2	1,002	75
	V3	1,101	86
	V4	702	21

In 2024, yields decreased dramatically across all variants, highlighting the combined impact of drought and downy mildew (Table 3). Unlike 2023, the differences between variants are smaller, suggesting that under extreme conditions, none of the variants were able to significantly counteract the effects of the climate and the disease. In short, the 2024 yield was heavily impacted by both drought and downy mildew. In a favorable year, variants V2 and V3 were the most productive (Table 4), but under challenging conditions, the impact of stress factors became much stronger, underscoring the importance of management strategies adapted to extreme climatic conditions.

Table 4. Average yields from 2023 and differences in 2024

Variety	Year	Average Yield (kg/ha)	% Change from 2023
Mădălina	2023	1,038.5	-
	2024	62.75	-93.97%
Camelia	2023	902.25	-
	2024	51.75	-94.25%

CONCLUSIONS

The results of this study highlight the significant impact of meteorological conditions on the development of *Peronospora camelinae* in *Camelina sativa* crop. Under conditions of abundant rainfall and moderate temperatures, as in 2023, the pathogen thrived, particularly affecting the Camelia variety. In contrast, the drought conditions of 2024 significantly limited the spread of the disease.

These findings align with previous research that has emphasized the sensitivity of *Peronospora camelinae* to humidity (Vollmann et al., 2001) (Séguin-Swartz et al., 2009). Furthermore, our study reveals varietal differences in occurrence of downy mildew, with Camelia being more susceptible compared to Mădălina.

Additionally, we observed that seeding density and row spacing influence disease incidence. A lower seeding density and wider row spacing reduced downy mildew attack, suggesting that these practices contribute to disease management.

Understanding the impact of meteorological conditions and agricultural practices on disease development can help camelina growers develop more effective management strategies. Specifically, in years with high rainfall, growers should take additional measures to protect crops, especially varieties like Camelia.

The variant that yielded better results in the study was 6 kg/ha & 25 cm, which provided the best outcomes with the lowest values for frequency, intensity, and degree of attack in both varieties, both in 2023 and 2024. On the other hand, variant 8 kg/ha & 13 cm was the most affected, showing the highest values for all indicators. These results suggest that a lower seeding density and wider row spacing help reduce downy mildew incidence, while a higher seeding density and closer rows favor disease development.

Comparing the two varieties, Camelia appears more susceptible to downy mildew than Mădălina, showing higher values for frequency, intensity, and severity in almost all tested conditions. For example, in the variant with higher seeding density and closer rows 8 kg/ha & 13 cm, the attack was more severe in Camelia, with a frequency of up to 66.2%, compared to 50% in Mădălina, and nearly double the severity, even in the most favorable variant 6 kg/ha & 25 cm.

In conclusion, this research provides valuable insights into the seeding rate and row spacing regarding the behavior of *Peronospora camelinae* and the importance of integrated disease management in *Camelina sativa* crop.

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INFLUENCE OF SOWING TIME ON THE PRODUCTION AND PRODUCTIVITY ELEMENTS OF WINTER WHEAT IN SOUTHERN ROMANIA

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Abstract

Wheat production is given by the possibilities of optimizing the interaction between ecological, technological and economic factors. The final production and quality of wheat is affected by pedoclimatic conditions and can be optimized and stabilized by respecting the sowing date associated with other important technological links. In general, the efficient strategy for obtaining maximum production is established by knowing the characteristics of the variety and the optimal time for applying technological highlights, and these must be adapted to the crop area. This article presents the results of research showing the effect of sowing time on the production and quality of winter wheat grains. Shifting the sowing time to an early date (September 25) or a late date (November 1) results in reduced yields. The results revealed that wheat planted on October-10 and October-25 produced higher spike length, 1000-grain weight, plant height and grain yield with a comparable number of tillers and number of grains per spike. It is recommended to plant wheat between October-10 to October-25 to attain higher grain yield.

Key words: *wheat, sowing times, variety, production.*

INTRODUCTION

Wheats genetic production potential of varieties is directly influenced by the relationship between precipitation and other technological links, and the final harvest is varied depending on the sowing period. The availability of water in the soil, the variety's resistance to drought, and the technological connections used at critical times are all closely related to the quick and consistent emergence of wheat seeds and the development of the crop under favourable conditions (Iagaru, 1998; Lupu, 2001; Popa, 2003). Prior studies on how climate and sowing time affect winter wheat production capacity have demonstrated the importance of technological links and their substantial impact on crop evolution (Epplin and Peeper, 1998; Tack et al., 2015). Several studies have underscored the importance and considerable effect of sowing time and plant density on the growth and progression of wheat crops, taking into account different climatic factors like precipitation and temperature (Raza et al., 2019).
Sowing time is a very important technological link to maximize yield and harvest quality, which is why research has primarily focused on the response of wheat crop to sowing at different times associated with technological factors (Paraschivu et al., 2017; Abendroth et al., 2017). The optimal sowing time for wheat crops in favorable conditions may vary across different agricultural regions, influenced by soil type and the fluctuations in soil moisture over time (Donatelli et al., 2012; Partal & Paraschivu, 2020). Wheat seeds need adequate soil moisture and favorable temperatures to germinate and establish properly in the early growth stages (Abendroth et al., 2017). Late sowing has become a common practice among farmers, as positive temperatures are often recorded during this period (Partal and Paraschivu, 2020). Depending on the crop area, early sowing leads to a higher number of spikes/square meter, heavier grains and a good yield, while late sowing negatively affected these characters (Wajid et al., 2006). Sowing at the optimal period leads to increased yields and avoids unjustified delay in seed germination and increased premises for inadequate quality. Under early sowing conditions, seeds are able to absorb water, which promotes rapid germination and quick plant growth, along with early branching (Donatelli et al., 2012;

Abendroth et al., 2017). The amount and distribution of precipitation, the soil's ability to retain water, along with plant variety and density, directly affect the crop's active growth period in both autumn and spring (Lobell and Burke, 2008; Raza et al., 2019).

Sowing date has a significant impact on growth stages, especially in the autumn period when temperatures are high and thus allow early sown wheat to grow and produce more wheels than later sown wheat (White & Edwards, 2008).Variations in final yield and grain quality among varieties are determined by their genetic characteristics, environmental conditions, soil nutrient levels and the technological methods used in crop management (Theago et al., 2014; Partal & Paraschivu, 2020). As the global population explosion progresses, more food, energy and goods are needed (Bonciu, 2023). The problem is that of limited natural resources, in addition to the spectrum of the problem of plant pathogens, which means is needed to produce more food with less resources, to ensure food security in environmental protection conditions (Paunescu et al., 2021). Plant bioengineering has led to significant improvements in crop yields, by including genes for resistance to herbicides, diseases, drought, etc. in plants genome (De Souza and Bonciu, 2022).

This paper outlines the results from the last three years, examining the effects of sowing time, plant density, and variety on the production and quality of winter wheat, in relation to the climatic conditions of each year.

MATERIALS AND METHODS

The field tests were carried out in 2022, 2023, and 2024, on a soil type specific to southern Romania (cambic chernozem). The soil's physical properties show a higher humus content in the top 15 cm, due to previous cultivation, which gradually decreases with depth.

The soil is composed of several horizons:

- Ap + Aph (0-30 cm): clay-loam with 36.5% clay, permeability of 492, pH 5.9.
- Am (30-45 cm): clay-clay, compacted with 37.3% clay, bulk density of 1.41 g/cm³, pH 5.9.
- A/B (45-62 cm), Bv1 (62-80 cm), Bv2 (82-112 cm), Cnk1 (149-170 cm), and Cnk2 (170-200 cm). Depending on the agricultural year, the soil's water supply is suitable for field crops, with groundwater levels at 10-12 meters.

The researchs was installed in a non-irrigated system on a land with uniform microrelief and with direct exposure to climatic conditions, especially severe drought. The experiment was trifactorial and placed in the field in randomized complete block design in three replications.

Factor A - sowing date with the following five graduations: SD I - September 20, SD II - October 01, SD III - October 10, SD IV – October 25 and SD V - November 10.

Factor B - plant density with the following two graduations: PD - 500 seeds/m^2 and PD - 600 seeds/m^2.

Factor C - varieties sown with the following two graduations: VS1- Ursita and VS2- Voinic, both developed at the NARDI Fundulea.

The main plots are 240 m^2 (30 m x 8 m) and the sub-plots 48 m^2 (6 m x 8 m). Wheat was cultivated after sunflower, as part of a four-year crop rotation.

To assess quality, samples were taken from each repetition and variant, and the following measurements were made:

- thousand grain weight - WTG was determined using the Kern precision electronic scale.
- hectoliter weight - HW was measured using a special cylinder, followed by weighing on the Kern scale.

Temperatures and precipitation for the 2022-2024 agricultural years were recorded at the NARDI Fundulea meteorological station

The data and analyses obtained were processed and statistically interpreted using the variance analysis method.

RESULTS AND DISCUSSIONS

Climatic aspects

The climatic conditions observed during the research period revealed notable differences between years, attributed to variations in temperature and precipitation distribution.

In 2021/2022, the months with the lowest rainfall were September, with 4.0 mm compared to the multi-year average of 48.5 mm; January, with 4.8 mm against the average of 34.1 mm; and August, with 14.4 mm, while the multi-year average was 49.7 mm. The highest precipitation was recorded in April, with 47.6 mm, which is 2.5 mm above the multi-year average. Between October 2021 and July 2022, the thermal regime showed that the monthly averages were above the multi-year average, with temperatures differing by 1.8°C from the multi-year average.

The agricultural year 2022/2023 was marked by dryness, with significant water shortages and higher temperatures compared to the multi-year average. The months with the lowest precipitation in 2022 were October, with 5.2 mm compared to the multi-year average of 42.3 mm; February, with 5.8 mm compared to the average of 32.0 mm; August, with 6.6 mm compared to the average of 49.7 mm; and March, with 10.0 mm compared to the average of 37.4 mm. In May, a significant amount of precipitation was recorded, totaling 77.2 mm, compared to the multi-year average of 45.1 mm, resulting in a difference of 32.1 mm.

The rainfall deficit affected the early stages of crop establishment and development, leading to reduced yields. The above-average temperatures worsened the drought conditions. The average temperature for the 2022/2023 agricultural year was 12.7°C, compared to the multi-year average of 10.8°C, showing an increase of 1.9°C.

The 2023/2024 year was notably dry, with a pronounced water deficit and elevated temperatures compared to the multi-year average. The months with the lowest rainfall were September, with 4.2 mm compared to the multi-year average of 49.5 mm; January, with 17.6 mm compared to 34.1 mm; and February, with 1.4 mm compared to the multi-year average of 32.0 mm.

November recorded the highest amount of precipitation, with 85.6 mm, which is 43.6 mm above the multi-year average. In terms of the thermal regime, from September to August, the average temperatures for the 2023-2024 agricultural year were 3.3°C higher than the multi-year average.

To determine the influence of climatic factors on the development of the wheat crop, the values obtained during various phenological phases were analyzed and compared with the final yield, considering both quantity and quality (Table 1). The annual climate data were also compared to the 50-year multi-year average, which recorded 584.3 mm of precipitation and a temperature of 10.8°C

Table 1. The meteorological parameters in the experimental period (NARDI Fundulea, 2022-2024)

Years/Months		Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	Ma	Jun	July	Aug	Total/ Average
Precipitations (mm)	’21/’22	4.0	56.4	33.8	37.6	4.8	5.4	12.3	47.6	30.1	59.6	29.2	14.4	335.2
	’22/’23	35.4	5.2	19.6	21.8	64.2	5.8	10.0	77.2	32.4	40.2	43.8	6.6	362.2
	‘23/’24	4.2	29.0	85.6	24.4	17.6	1.4	38.6	62.4	34.2	15.6	45.4	18.0	376.4
50 years average		**48.5**	**42.3**	**42.0**	**43.7**	**35.1**	**32.0**	**37.4**	**45.1**	**62.5**	**74.9**	**71.1**	**49.7**	**584.3**
Temperatures (oC)	’21/’22	17.3	10.2	7.7	2.6	2.1	4.7	4.4	12.1	17.9	22.6	25.0	25.6	12.6
	’22/’23	18.6	13.5	9.0	3.5	4.9	3.3	8.2	10.8	16.9	22.3	26.1	26.1	12.7
	’23/’24	21.7	16.1	8.5	4.3	1.0	7.6	8.5	15.0	16.4	26.1	27.7	26.3	14.1
50 years average		**17.3**	**11.3**	**5.4**	**0.1**	**-2.4**	**-0.4**	**4.9**	**11.3**	**17.0**	**20.8**	**22.7**	**22.3**	**10.8**

Production and Quality

The influence of sowing date. The experimental results from 2022 showed a statistically significant effect of sowing date on wheat yield. When compared to the control variant (SD - September 20), the highest yields were achieved with sowing dates between October 10 and October 25.

The maximum yield of 4600 kg/ha was obtained on October 10, representing an increase of +39.4% relative to the control, a difference that proved to be statistically significant (**). Furthermore, the quality parameters of the grains, namely hectoliter weight and thousand kernel weight, exhibited a moderate upward trend during this period, reaching peak values of 79.0 kg/hl and 45.7 g, respectively. These findings underscore the importance of optimizing sowing time to enhance both yield and grain quality.

The influence of sowing density. Increasing the sowing density from 500 to 600 germinable seeds/m² resulted in a modest yield increase of approximately 7.3%, although this difference was not statistically significant. However, slight improvements were observed in grain quality indicators, with the hectoliter weight reaching 79.4 kg/hl and the thousand kernel weight 45.5 g. These results suggest that a slightly higher sowing density may offer a technological advantage, particularly under favorable environmental and agronomic conditions.

The influence of varieties sown revealed the comparison between the two tested wheat varieties - Ursita and Voinic - and demonstrated a clear superiority of the Voinic variety in terms of grain quality. The Voinic variety recorded a hectoliter weight of 79.0 kg/hl and a thousand kernel weight of 45.9 g, differences that were statistically significant (* and **). Although the yield of the Voinic variety exceeded that of Ursita by +4.8%, this increase was not statistically significant. Nonetheless, the combination of slightly higher yield and significantly improved grain quality indicates the Voinic variety's enhanced agronomic potential, particularly in intensive cultivation systems (Table 2).

Table 2. Production wheat crop values in 2022

Specification variant	Producţion /Diference			HW		WTG	
	(kg/ha)	(%)	Semnific.	kg/hl	%	g	%
A. Sowing date (SD)							
A1 - Mt	3300	100.0	0	77.3	100.0	44.2	100.0
A2	4150	125.7	850*	78.2	101.2**	45.3	102.5**
A3	4600	139.4	1300**	79.0	102.2***	45.6	103.2***
A4	4500	136.4	1200*	79.0	102.2***	45.7	103.4***
A5	3850	116.7	550*	78.0	100.9*	45.0	101.8**
DL (kg/ha / kg/hl / g)	DL= (P 5%= 525 /P 1% = 1015 / P 0.1% = 1909)			DL = (0.72 /1.19 /2.20)		DL= (1.01 / 1.53/3.20)	
B. Plant density (PD)							
B1 - Mt	4100	100.0	0	78.0	100.0	45.2	100.0
B3	4400	107.3	300	79.4	101.8*	45.5	100.7
DL (kg/ha)	DL= (P 5%= 520 /P 1% = 1005 /P 0,1% = 1880)			DL= (0.70/1.11 /2.23)		DL= (1.06/ 2.09 /3.18)	
C. Varieties sown (VS)							
C1 - Mt	4200	100.0	0	78.0	100.0	45.3	100.0
C2	4400	104.8	300	79.0	101.2**	45.9	101.3*
DL (kg/ha)	P 5%= (622 /P 1% = 1105 /P 0.1% = 1900)			DL= (0.68 / 1.12 / 2.01)		DL= (1.00 / 2.11 / 3.09)	

The experimental data from 2023 indicate a significant increase in yield with a delay in the sowing date. The highest yield of 5200 kg/ha was recorded with sowing on October 10th (A3), which represents a 36.8% increase compared to the control, with a statistically significant difference (**). This suggests that the optimal sowing period is around October 10th, as it enhances both the yield and quality of wheat in comparison to earlier or later sowing dates.

Regarding the grain quality parameters (HW and WTG), the observations reveal a slight increase in values as sowing is delayed, with peak values of 78.1 kg/hl for HW and 44.7 g for WTG at sowing on October 10th and 25th. Additionally, the increase in WTG values with later sowing suggests an enhancement in grain quality.

However, sowing at the beginning of November (A5) resulted in a decrease in yield (4550 kg/ha), while still maintaining a high level of grain quality. This indicates that although the optimal sowing date is closer to October, sowing too late may negatively affect yields, even if it does not drastically impact grain quality.

Increasing the sowing density from 500 seeds/m² to 600 seeds/m² led to an 8.9% increase in yield (from 4180 kg/ha to 4550 kg/ha). This difference is statistically significant (***), indicating that a higher plant density can significantly increase yield, suggesting that 600 seeds/m² is a favorable density for maximizing yield.

In terms of grain quality, hectoliter weight and thousand grain weight were slightly higher at the 600 seeds/m² density, suggesting that a higher plant density contributes to a modest improvement in wheat quality. For example, hectoliter weight increased to 78.7 kg/hl, and thousand grain weight reached 44.4 g. This

suggests that higher plant density not only enhances yield but also contributes to a slight improvement in the quality of the grains.
The differences between the two tested varieties, Ursita and Voinic, are significant, with Voinic exhibiting higher yield and slightly better grain quality values. Voinic recorded a yield of 5300 kg/ha, representing a 10.4% increase compared to Ursita. Additionally, values for hectoliter weight and thousand grain weight are also slightly higher in Voinic compared to Ursita, suggesting that Voinic is more efficient in terms of both yield and quality (Table 3).

Table 3. Production wheat crop values in 2023

Specification variant	Production /Diference			HW		WTG	
	(kg/ha)	(%)	Semnific.	kg/hl	%	g	%
A. Sowing time							
A1 - Mt	3800	100.0	0	77.0	100.0	43.1	100.0
A2	4700	123.7	900*	78.0	101.3**	44.6	103.5***
A3	5200	136.8	1400**	78.1	101.4**	44.7	103.7***
A4	4700	123.7	900*	78.1	101.4**	44.7	103.5***
A5	4550	119.7	750*	78.0	101.3**	44.6	103.5***
DL (kg/ha / kg/hl / g)	DL= (P 5%= 620 /P 1% = 1120 / P 0.1% = 1955)			DL = (0.69 /1.10 /2.10)		DL= (1.03 / 1.67/3.10)	
B. Density							
B1 - Mt	4180	100.0	0	77.0	100.0	44.0	100.0
B2	4550	108.9	370	78.7	102.2***	44.4	100.9
DL (kg/ha)	DL= (P 5%= 604 /P 1% = 1001 /P 0.1% = 1985)			DL= (0.67 /1.12 /2.18)		DL= (1.01/ 2.01 /3.11)	
C. Variety							
C1 - Mt	4800	100.0	0	78.0	100.0	44.4	100.0
C2	5300	110.4	500	78.3	100.4	44.7	100.7
DL (kg/ha)	P 5%= (605 /P 1% = 1108 /P 0.1% = 1995)			DL= (0.68/ 1.05 / 2,05)		DL= (1.00 / 2.05 / 3.11)	

In 2024, sowing date influenced production, which varied significantly, with a trend of increasing yields as sowing was delayed. The highest yield was obtained for the sowing on October 10th (3990 kg/ha), followed by October 25th (3900 kg/ha), while the later sowing date (November 10th) resulted in a production of 3700 kg/ha, similar to the sowing on October 1st (3700 kg/ha).
Compared to the first variant (September 20th), the differences in production are statistically significant, falling within the 5% and 1% significance levels (*$p < 0.05$, **$p < 0.01$), suggesting that sowing at the optimal time provides production benefits despite potentially more challenging growing conditions.
The hectoliter weight is slightly higher for the sowing variants of October 1st and October 10th (76.1 and 76.8 kg/hl, respectively), compared to the other variants, which fluctuate around 75.2 kg/hl (September 20th). The thousand grain weight does not vary significantly between variants, but there is a slight increase as sowing is delayed. The sowing variants of October 10th and October 25th had a thousand grain weight of 43.2 g and 43.3 g, respectively, compared to 42 g for the sowing on September 20th.
Plant Density: the B1 variant (500 seeds/m²) recorded a yield of 2700 kg/ha, while for the higher plant density of 600 seeds/m² (B3), the yield increased to 3550 kg/ha. This difference is statistically significant ($p < 0.01$), meaning that the variation in production is largely due to plant density rather than other fluctuations.
Sown Varieties: compared to the Ursita variety, which recorded a yield of 3700 kg/ha, the Voinic variety produced a higher yield of 3950 kg/ha.
The increase in production for Voinic (6.7%) is significant ($p < 0.01$), indicating that the Voinic variety is more productive than Ursita under the tested conditions (Table 4).

Table 4. Production wheat crop values in 2024

Specification variant	Production/Diference			HW		WTG	
	(kg/ha)	(%)	Semnific.	kg/hl	%	g	%
A. Sowing time							
A1 - Mt	2900	100.0	0	75.2	100.0	42.0	100.0
A2	3700	119.4	800*	76.1	101.2**	43.1	102.6***
A3	3990	128.4	1090**	76.8	102.1***	43.2	102.9**
A4	3900	125.8	1000**	76.8	102.1***	43.3	103.1***
A5	3700	122.6	800*	76.8	102.1**	43.3	103.1***
DL (kg/ha / kg/hl / g)	DL= (P 5%= 500 /P 1% = 910 / P 0.1% = 1650)			DL = (0.55 /1.08 /2.10)		DL= (1.03 / 1.51/3.06)	
B. Density							
B1 - Mt	2700	100.0	0	75.0	100.0	43.0	100.0
B2	3550	131.5	850**	76.4	101.9**	43.4	100.9
DL (kg/ha)	DL= (P 5%= 452 /P 1% = 835 /P 0.1% = 1611)			DL= (0.60 /1.00 /2.04)		DL= (1.01/ 2.08 /3.01)	
C. Variety							
C1 - Mt	3700	100.0	0	75.7	100.0	43.1	100.0
C2	3950	106.7	250	76.0	100.4	43.5	100.9
DL (kg/ha)	P 5%= (495 /P 1% = 988 /P 0.1% = 1795)			DL= (0.68 / 1.10 / 2.20)		DL= (1.00 / 2.07 / 3.03)	

In 2023, the highest protein content was observed in the variant sown on October 25 at a density of 500 seeds/m² for the Ursita variety, with 13.9%. This was followed by the variant sown on October 1 at a density of 600 seeds/m² for the Voinic variety, with 13.5%. Of the functions available in the Windows program linear, logarithmic, polynomial, power, and exponential—the polynomial function exhibits the highest regression coefficient in modeling the relationship between agrotechnical factors (such as sowing date, seed density, and variety) and protein content in wheat (Figure 1).

Expanding the options for selecting the sowing date and determining the sowing density, particularly through the combination of these factors, leads to very positive correlations, with regression coefficients ranging from 0.01 to 0.93. Early sowing, when combined with a high sowing density, resulted in a lower protein content, with a regression coefficient of 0.37.

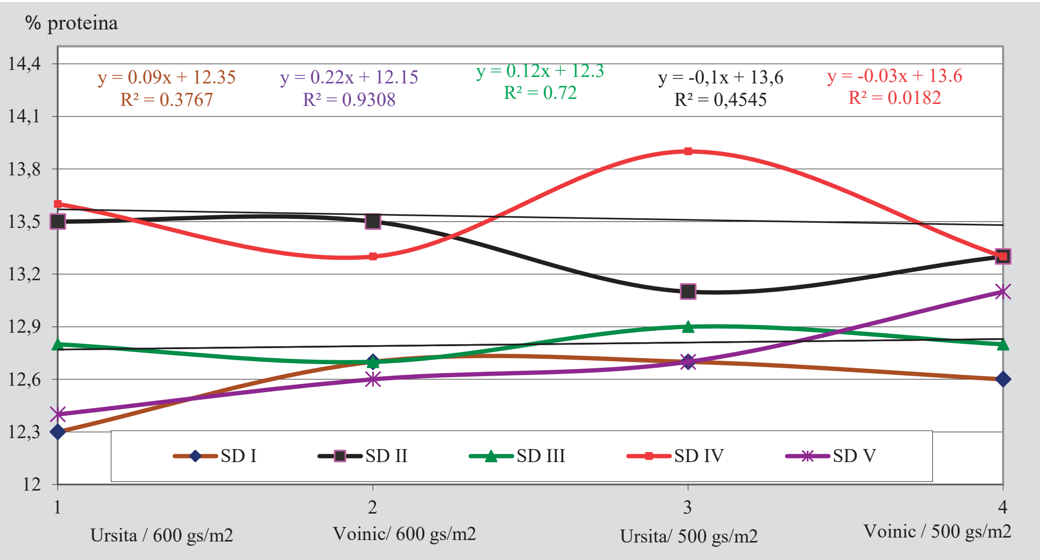

Figure 1. The correlation between wheat protein content and agronomic measures

CONCLUSIONS

The research findings indicate that sowing date, plant density, and variety significantly impact both the quantity and quality of wheat production, with the pedoclimatic conditions playing a key role. The applied agronomic practices have consistently contributed to the enhancement and stability of final yields and grain quality.

Regarding wheat cultivation technology, the following key observations were made: the optimal sowing window for maximizing both yield and quality is between October 10 and 25; slightly higher sowing densities offer some agronomic advantages, although yield increases are marginal; and the Voinic variety is recommended due to its superior grain quality, making it ideal for premium wheat production, with Ursita as a suitable alternative.

Protein content in wheat grouped the variants as follows: very good quality (>13%), good (12-13%), and satisfactory (8-12%). Sowing within the optimal period or later, along with plant densities of 600 seeds/m², led to an increase in protein content ranging from 12.1% to 13.9%, compared to the control variant.

In both 2022 and 2024, drought conditions impacted final yields regardless of the technological practices implemented. Temperature fluctuations and uneven precipitation distribution adversely affected wheat plants at all growth stages, reducing both yield and grain quality. Drought, depending on its intensity and duration, can severely damage the crop, and the variety's drought tolerance becomes a crucial factor in determining the plant's resilience.

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THE PERFORMANCE OF SOME BARLEY GENOTYPES UNDER ORGANIC AND DIFFERENT PEDOCLIMATIC CONDITIONS

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Abstract

Barley is an important food and industrial crop in Romania and the choice of varieties is one of the most important decisions in the barley crop management because it greatly improves the production obtained in the ecological farming system and not only that. The aim of this study was to compare how some barley varieties differ in physiological traits and yield under organic farming conditions. This study was conducted in three different area from Romania throughout 2023-2024 winter wheat season. In one of the three localities the soil is acidic so another goal was to highlight the genetic variability among barley genotypes, to identify barley genotypes tolerant to acid soils using stress indices and to assess the association among stress indices as well as grain yield. The results showed that in the three location, the chlorophyl content, the height of plants, leaf area index and dry matter were highly variable. The grain yields were positively correlated with chlorophyll content and biomass, at a significance level of 0.01. Assessment of acid soil stress indices was also found to be promising in identifying tolerant genotypes with good yield potential. Based on these results, high-yield barley varieties may be choise to be cultivated under organic agriculture under climate change conditions from Romania.

***Key words**: barley, organic agriculture, height of plant, chlorophyll content, leaf area index, index of aluminium tolerance, yield.*

INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the basic crops in European agriculture (Acharya et al., 2021). Currently, most barley production is for animal feed and malt (Vasilescu et al., 2022) and Romania achieved a total barley production of 2.001 million tons in 2023, an increase of 294,000 tons compared to 2022.

Organic farming relies on diverse crop rotation systems for maintaining soil health and breaking up disease, pest, and weed cycles, and winter barley can help of these challenges. Barley has a fibrous root system that contributes to increase the soil organic matter (Snapp et al., 2005), can escape by drought due to short period of vegetation (Vasilescu et al., 2019; Voica L., 2021) and rotating small grain crops can break up disease and weed cycles while increasing the biodiversity of the system (Voica and Lazar, 2021).

Growth analysis is the most simple and precise method to evaluate the contribution of different physiological processes in plant development. The physiological indices such as leaf area index (LAI), total dry matter (TDM) are influenced by genotypes, plant population, climate and soil fertility (Petcu et al., 2003). Chlorophyll content is an important physiological parameter that is frequently measured as an indicator of chloroplast development, photosynthetic capacity, leaf nitrogen content or general plant health (Ling et al., 2011; Guidi et al., 2019). Barley production is affected by biotic and abiotic constraints among which soil acidity is a serious threat in some area of Romania where barley production is very important.

The aim of this study was to evaluation of yield and the some of physiological indices of barley (*Hordeum vulgare* L.) genotypes under ecologycal farm conditions in three locations from Romania and to highlight which genotypes show tolerance to soil acidity.

MATERIALS AND METHODS

Twenty barley genotypes including 19 barley cultivar obtained by NARDI Fundulea and one standard check were studied.

The barley genotypes were grown for growing season 2023-2024 in ecological fields certified at three locations. These three locations includes the National Agricultural Research and Development Institute from Fundulea (44°26`N latitude and 26°31`E longitude) on the cambic chernozem soil, at Agricultural Research and Development Station Valu lui Traian (44°16`N - 28°48`E) on typical chernozem soil and at Agricultural Research and Development Station Albota (44°65`N - 24°50`E) on the albic luvisol soil with a 25-25.2% clay content (acid soil, soil pH is 5.02).

The plot for each cultivars was by 12 m^2 in three replications. Each barleyt variety was sown on 15 October 2023, the sowing depth was approximately 5 cm, the row spacing was 12 cm, and the sowing density was the same, at approximately 200 kg ha^{-1}.

The total area of leaves/plant was measured by a leaf area meter (Li-COR Inc., Lincoln, NE, USA), height of plants was measured by a ruler.

For each cultivars at maturity, three 1-m^2 areas were harvested randomly to measure the grain yield, 20 wheat plants were chosen to determine the grain number per spike, and 10 repeated samples were chosen to measure the weight of 1000 and weight of seeds per plant.

The analysis of variance (ANOVA) and coefficient of correlation (r) for traits under study were statistically analyzed using Microsoft Excel program. The aluminium adaptation index (AAI) was calculated using method of Howeler (1991), AAI = (Yns)*(Yst)/(AYns)*(AYst), where Yns and Yst are yields of a given genotype under non-stress (Fundulea and Valu lui Traian) and stressed soil conditions (Albota); the Yns and Yst are average yields of all genotypes under non-stress and stressed soil conditions.

RESULTS AND DISCUSSIONS

The years of experimentation were totally different from the viewpoint of quantity and monthly repartitions of rainfall in studied areas. In Fundulea, the cumulated rainfall from sowing to harvested stage was 308.8 mm, below multi annual average with 103,1 mm (Table 1). In location Albota the cumulated rainfall during winter wheat growing seazon were below the normal of the zone with 209.9 mm, with two drought periods, one of them in the autumn-winter and the other one in the spring starting with April. In Valu lui Traian the cumulated rainfall during winter wheat growing seazon exced the normal of the zone with 46.9 mm, but with hydric deficit in January, February and June. Thus, October, May and June registered a moisture deficits in all studied areas, while November was rainier with 44.4 mm in Fundulea, 17.2 mm in Albota and with 94.5 mm in Valu lui Traian vs. multi-annual average (Table 1).

Table 1. Monthly distribution of rainfall (mm) during the winter wheat vegetation period compared with multiannual average

Month	Fundulea		Valu lui Traian		Albota	
	Rainfall	MMA	Rainfall	MMA	Rainfall	MMA
Oct	29	*42.4*	13.6	*37.7*	6.8	47.9
Nov	85.6	*41.2*	136.2	*41.7*	66.5	49.3
Dec	24.4	*44.2*	42.2	*35.4*	17.6	45
Jan	17.6	*34.8*	17.2	*29.7*	70	41.5
Feb	1.4	*31*	1.4	*24.6*	13	37.7
Mar	38.6	*37.2*	28	*29.3*	41.5	38
Apr	62.4	*44.4*	85	*34.2*	45.1	55.9
May	34.2	*61.8*	35	*41.8*	15	88.5
June	15.6	*74.9*	25.8	*63.1*	12	93.6
Sum	308.8	*411.9*	384.4	337.5	287.5	497.4

According to the analysis of variance for height of plants, the magnitude of variance due to locations and genotypes was larger compared to their interaction (Table 2).

Table 2. The analysis of variance of height of plants for barley genotypes during 2023-2024 growing season in three locations (Fundulea, Valu lui Traian, Albota)

Source of variance	Df	Mean squares	Factor F and significance
Location	2	16354.2	2665.5***
Genotype	19	418.90	24.11***
Location x Genotype	38	287.76	16.56***

*** significant at 0.001 level of probability; Df, degrees of freedom

The heigh of plants at maturity stage varied over the locations and genotype (Figure 1). Thus, the genotypes that presented the highest height were Iulian and H 417-12. It is obvious that under the conditions from Albota, on acidic soil, the barley genotypes studied had a lower height (average of 69 cm) compared to the performances achieved by same barley genotypes on the cambic chernozem soil from Fundulea (101 cm) and the typical chernozem from Valul lui Traian (93 cm) (Figure 1).

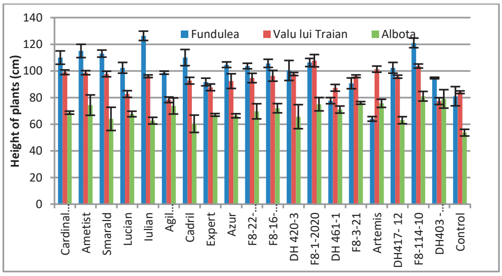

Figure 1. The height of studied barley genotypes in three locations

The analyses of variance showed than the variability for leaf area index (LAI) were the result of both the zone of cultivation, cultivars as well as of interactions of these (Table 3).

Table 3. The analysis of variance of leaf area index for barley genotypes during 2023-2024 growing season in three locations (Fundulea, Valu lui Traian, Albota) and two date of analyses

Source of variance	Df	Mean squares	Factor F and significance	Mean squares	Factor F and significance
Location	2	20.75	501.28***	139.28	191.06***
Genotype	19	1.91	36.3***	0.92	1276***
Location x Genotype	38	1.17	22.29***	0.90	12.50***

***significant at 0.001 level of probability; Df, degrees of freedom

The leaf area index (LAI) of barley in the BBCH 35 stage was significantly higher in Fundulea and Valu lui Traian locations than in Albota location. In Albota, the values of this index did not exceed 2.8. The highest values were achieved by the Cadril and Ileana genotypes under Fundulea conditions. On the other hand, the lowest LAI was found with genotype Iulian (0.9) in Albota location (Figure 2). Kizilgeci et al. (2017) reported that leaf area index at heading stage of barley varied within the range of 1.44-6.00

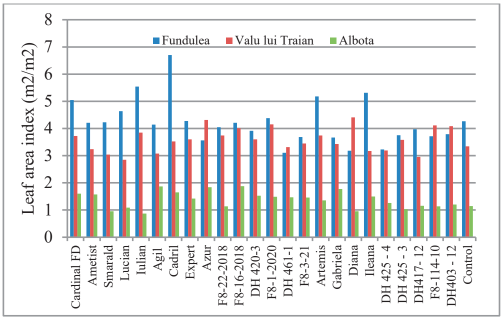

Figure 2. The leaf area index in the fully earing stage (BBCH 55) of studied barley genotypes in three locations

The results showed that both the genotypes and the environmental conditions significantly ($p < 0.01$) influenced the yield performance of barley genotypes (Table 4). The variation of 1000 grain weight (TGW) was significantly ($p < 0.01$) influenced by both main factors (G and E). The main effect of variation was due to genotype (88%) (Table 4).

Table 4. The analysis of variance of yield and 1000 grain weight (TGW) for barley genotypes during 2023-2024 growing season in three locations (Fundulea, Valu lui Traian, Albota)

Source of variance	Df	The yield		TGW	
		Mean squares	Factor F and significance	Mean squares	Factor F and significance
Location	2	6.13	31.86***	95.74	25.97***
Genotype	19	1.09	5.64***	209.10	856.7***
Location x Genotype	38		1.78**	21.59	88.4***

***significant at 0.001 level of probability; Df, degrees of freedom

The average grain yield varied between 1.53 g/pl in Fundulea, 1.93 and 1.10 g/pl in Valu lui Traian and Albota (Table 5). The varieties Cardinal, Smarald, Lucian, Iulian, Expert and FD 8-16-2018 genotypes performed very well in locations witout acidic stress. Lower yields, below the experience average, were achieved by the Artemis and Ametist genotypes in soil acidic comditions from Albota, (Table 5)

Highest values of AAI designate stress tolerant genotype. In this context Cardinal, Smarald, F 8 -22 -2018 and F8-16-2018 (with values of this index over 1.6) could be more tolerant to acidic soil as compared with Artemis (with lower values of this index) (Table 5).

Table 5. The grain yield and aluminium adaptation index for studied barley genotypes

Genotype	The grain yield (g/pl)			AAI
	Fundulea	Valu lui Traian	Albota	
Cardinal FD	2.91	2.77	1.48	2.20
Ametist	1.30	2.42	0.69	0.67
Smarald	2.23	2.20	1.40	1.63
Lucian	1.68	2.21	1.20	1.22
Iulian	1.83	2.25	0.85	0.91
Agil	1.60	1.78	1.20	1.07
Cadril	1.51	2.13	0.85	0.81
Expert	1.64	2.08	1.33	1.30
Azur	1.54	1.56	1.20	0.98
F8-22-2018	1.85	2.36	1.50	1.66
F8-16-2018	1.86	2.34	1.45	1.60
DH 420-3	1.40	2.06	1.15	1.04
F8-1-2020	1.03	2.16	1.29	1.08
DH 461-1	1.46	2.09	1.35	1.26
F8-3-21	1.32	1.69	0.96	0.76
Artemis	0.78	1.42	0.63	0.36
DH 417- 12	1.28	1.18	0.95	0.61
F8-114-10	1.18	1.24	1.04	0.66
DH 403 - 12	1.00	1.32	0.76	0.46
Control	1.13	1.42	0.75	0.50
Average	**1.53**	**1.93**	**1.10**	**1.04**

Leaf chlorophyll content is one of the important indicators of the health and potential physiological performance of a plant (Petcu et al., 2011; Collalti et al., 2020). The chlorophyll concentration in the leaf is essential for crop growth and development (Kummer et al., 2002) hence quantifying it makes available vital information about the effects of environment on plant growth (Schlemmer et al., 2005; Kalaji et al., 2017). Results show that there was strongly positive (r = 65***, 0.62***, 0.54***) correlation between the chlorophyl content and grain yield, (table 6). Significant and positive correlations was found between yield and biomass (r =0.53***, 0.59***, 0.47**) (Table 6).

Table 6. Relationship between yield and analized traits

Variable x	Variable Y	Coefficients of correlation (r)		
		Fundulea	Valu lui Traian	Albota
The yield	Biomass	0.53***	0.59***	0.47**
	Height of plant	0.44	0.19	0.1
	LAI	0.20	-0.08	0.2
	Chlorophyl content	0.65**	0.62***	0.54***

Acording to Farshadfar et al. (2001) most suitable indices for selecting stress-tolerant cultivars is an index that has are relatively strong correlation with the grain yield under stress and non-stress conditions (with coefficient of correlation over 80).

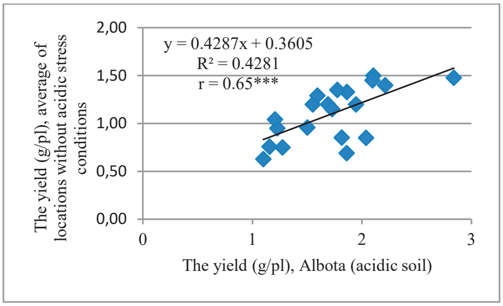

Figure 3. Relationships between the yield under acidic stress and non stress conditions

In our case, the association of grain yield under stress versus non-stress environments showed a significant positive (r = 0.65***) correlation indicating that genotypes that performed well under non-stress also performed well under stress conditions.

Generally, the strong positive correlation between grain yield under stress and non-stress environment implied the possibility of direct selection for stress conditions based on performance under non-stress conditions (Negarestani et al., 2019).

However, Drikvand et al. (2012) reported a lack of association between yield under stress and non-stressenvironment suggesting the feasibility of an independent breeding approach. The second approach would probably be more efficient in our case if we take into account the fact that in our case the correlation in question was not so strong (r = 0.65, Figure 3).

CONCLUSIONS

The present study aimed to evaluate the performances of different barley genotypes grown in three locations in ecological agricultural farming system. One of the three locations being with acid soil. The results showed that the barley genotypes presented variability for the studied physiological characters and for production.

The current study confirmed the severity of acid soils in barley development with negative repercussions on production. The percentage of yield loss under acid soil stress as compared to non-stress experiments being over 35%. Moreover, this study also revealed the existence of adequate levels of genetic variation in Romanian barley provided from NARDI Fundulea under both acid soil stress and non-stress conditions in ecological farming system indicating the potential for future barley genetic improvement. Accordingly, the currently identified high-yielding and tolerant barley genotypes need to be used for further adaptation studies and simultaneous breeding line extraction for subsequent crossing works and variety development.

At the same time, the study provides useful information regarding the production performance of some barley varieties necessary for farmers practicing organic agriculture

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DAMEANOR OF YIELD STRUCTURE OF DARMI VARIETY UNDER TWO SOIL TYPES AND THE SAME LEVELS OF MINERAL FERTILIZATION IN PERIOD 2012-2020

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Abstract

The considered demeanor of the yield structure development of Darmi cotton variety during long researches periods grown in two types of soil. The influence of nitrogen and phosphorus fertilization on cotton under conditions on pellic vertisoil and meadow-cinnamon experiment in cotton Darmi variety during the period 2012-2020 was studied. Treatments were: N_0; N_8; N_{16}; P_0; P_8. The numbers of 24 plot of land was design. With contents of mineral nitrogen in soil and phosphorus fertilization at rates P_8 – P_{16} was obtained total. During the researches period were studied development phases budding, flowering and ripening, as well as the content of nitrogen and phopshor elements in cotton Darmi variety.

Key words: *phosphours, nitrogen, cotton, soil, yield.*

INTRODUCTION

The crucial role of mineral fertilizers in enhancing soil fertility is well known. When the amounts of fertilizers applied to the soil are reduced, it is the mineralization of organic substances, as the main component of soil fertility, that ensures the plants nutritional needs are met. Fertilizer plays a major role in influencing the plant growth and development in cotton (Kumar et al., 2020). Compared to other major nutrients, nitrogen has a major impact on yield and quality of fiber cotton (Khan et al., 2017).

Cotton has an impact on our lives beyond recognition, and genetic engineering technology has the potential to improve cotton further (Chaudhry & Guitchounts, 2003). Use of chemical fertilizers has impacted the soil by increasing salinity, diminished soil fertility, loss of water holding potential and inconsistency in soil nutrients (Savci, 2012).

Apart from fertilizing cotton nevertheless reacts to an environmental change such as temperature and rainfall in instance (Kamburova et al., 2022). In 2020 Stefantova-Dobreva, Mihova (2023), established that the highest plants were recorded in the variants fertilized with P160 and the values were 34.9% higher compared to the unfertilized control.

Nitrogen fertilizer has been shown to significantly impact physiologically active substances in cotton growth. Nitrogen influences the formation of various critical components of the cotton plant such as chlorophyll, protein, enzymes, and phytohormones (Shen, 2001). As with most cultivated species, growth and yield of cotton cultivars depend on N availability during the crop cycle.

Controlled N applications can increase crop yield and soil quality (Palomo et al., 2002; Erhart, 2005). Another key component is the cultivar choice as several breeding programs have developed high-yielding cotton varieties well adapted to different environmental conditions (Bange & Milroy, 2004; Stiller, 2005). To achieve maximum productive potential, in addition to choosing a variety for a particular region, a high level of agricultural technology is also of particular importance, one of the main elements of which is nitrogen fertilization (Biberdzic et al., 2012; Mühleisen et al., 2014).

Mean average temperature and precipitation are prime determinants of large-scale variability in plant production or annual net primary production, followed by nitrogen (N) and P (Cleveland et al. 2011).

Phosphorus (P) is an essential, non-replaceable nutrient in biology, with finite global reserves. Whereas soils may contain pools of P that could be several thousand times higher than required for plant growth, only a small soluble fraction is available for plant uptake (Sohrt et al., 2017).
According to Bindraban et al. (2020) innovative P fertilizer products and fertilization strategies must, and can be developed to markedly reduce these unintended negative environmental and health effects of excessive P. The fact that the physiological P requirement is generally lower than the amount of P taken up by plants provides an opportunity to design and apply fertilization strategies to reduce total P supply and uptake, without penalty on the yield and nutritional value of the produce.
The production of cotton, its structural and qualitative aspects of the production are essentially influenced by the conditions of the elements of the year, the elements of fertilization and the variety (Panayotova, 2002; Sawan, 2008).

MATERIALS AND METHODS

The study of the cotton variety Darmi was conducted from 2012 to 2020 on two types of soils Haplic Vertisols and Luvisols. A field trial with cotton Darmi has been set up in 2008. The plants have a conical shape. The stem is green, with a weak anthocyanin coloration when maturing. The reproductive branches are of medium length and are located high on the plant. The leaves are palmate, green, with a raised perimeter. The seeds are medium-sized, covered with gray fuzz. The fiber is white, medium-fine, with very good uniformity and maturity, measuring 31-32 mm in length, and has good strength. The yield is 39.5%. The growing period of the variety is 116-122 days. On average, for a six-year period, the September yield is 1.45 t ha^{-1}, and the total yield is 2.00 t ha^{-1}. Based on the experimental data, regression equations were derived and theoretical curves were constructed expressing dependencies between the indicators - Statistical programs ANOVA and SPSS- 10 is used.
Haplic Vertisol in the experimental field of Chirpan is suitable for growing the main agricultural crops and has the potential to form high yields. The soil is characterized by a clay mechanical composition, high moisture retention, low permeability and a stable structure that ensures the exchange of air and water within the soil. It swells significantly when moistened and reduces in volume when dried.
A characteristic feature is the presence of a thick humus horizon (70-100 cm) and a strongly compacted zone in the profile (cemented horizon), *Haplic Vertisol* belong to the group of medium-humus and most fertile soils in Southern Bulgaria. The total nitrogen reserve in 0-30 is 0.5-0.6 t ha^{-1}. The total average nitrogen reserve in the form of NH_4-N in 0-30 cm is 3.20-3.60 t ha^{-1}, while in the form of NO_3-N it is 2.80-3.20 t ha^{-1}.
The amount of total phosphorus is 100 - 250 mg/100 g of soil, but a large part of the phosphorus is bound in the form of primary phosphorus minerals, which are difficult to dissolve and poorly accessible to plants. The amount of mobile phosphorus is low. The soil of the experimental field in the city of Stara Zagora is meadow-cinnamon soil with a humus horizon (0-45 cm). The mechanical composition of the soil is medium sandy-clay. The bulk density for the surface layer is 1.07 g/cm^3 and reaches 1.34 g/cm^3 in layers 60-80 cm.
In the topsoil layer of 0-30 cm, mineral nitrogen is 75.32 to 80.12 mg/1000 g of soil, which corresponds to a good supply of nitrogen to the soil. In terms of mobile phosphorus, the soil has a low to medium supply. In the layer of 0-30 cm, its content is 4.01-5.12 mg/100 g of soil and decreases insignificantly in the lower soil layer. The soil values characterize the soil as good for the growth and development of cotton variety Darmi.

RESULTS AND DISCUSSIONS

The results obtained from the present study for the period 2012-2014 show that mineral fertilization has a very good effect on the yield of unbleached cotton. The average realized yield of unginned cotton is 1.62 t ha^{-1}. Without fertilization, the average yield of unbleached cotton is 1.32 t ha^{-1} during the study period (Table 1). The lack of nutrients inhibits the development of cotton plants, reduces both the total yield of cotton and the yield of fiber.

Table 1. Impact of mineral fertilization on the yield of unbleached cotton, average for 2012-2014, t ha^{-1}

Fertilization	Year			Average %	
	2012	2013	2014	t ha^{-1}	%
N_0P_0	1.13	1.10	1.74	1.32	100.0
N_8	1.36	1.20	2.47	1.67	126.5
N_{16}	1.42	1.44	2.32	1.73	131.1
P_8	1.20	1.17	1.71	1.36	103.0

The yield of unbleached cotton in 2014 averaged 2.47 t ha^{-1} single fertilization with N_8, significantly higher than that obtained in 2012 and 2013 - 1.36-1.20 t/ha^{-1} .

The nitrogen content during the individual phases of cotton development is proven to be influenced by the level of nitrogen fertilization, while phosphorus fertilization has a negligible effect. In the budding phase, the nitrogen content in the unfertilized variant is on average 2.0%, ranging from 2.14% in 2012 to 1.84% in 2013 (Table 2). With increasing nitrogen fertilization levels, the concentration during this phase reaches 2.97% at N_{16}. With combined NP fertilization, the changes depend on the nitrogen level. Phosphorus fertilization alone at the tested rates leads to a nitrogen concentration close to the values of the unfertilized control. The highest value - 3.40% was recorded at N_{16} in 2012.

Table 2. Nitrogen and phosphorus content of the variety Darmi by development phases, %

Fertilization	Cotton budding phase				Flowering				Cotton ripening			
	2012	2013	2014	Avarage %	2012	2013	2014	Avarage %	2012	2013	2014	Avarage %
N_0P_0	2.14	1.84	2.04	2.00	2.52	2.11	2.45	2.36	1.65	1.52	1.44	1.54
N_8	2.26	2.44	2.21	2.30	2.73	2.48	2.64	2.62	2.33	1.82	2.17	2.11
N_{16}	3.15	2.72	3.05	2.97	3.40	2.58	3.09	3.02	2.65	2.19	2.52	2.45
P_8	1.89	1.60	2.02	1.83	2.77	1.89	2.41	2.36	1.68	1.62	1.56	1.62
N_8P_8	2.36	2.56	240	2.44	2.98	2.35	2.87	2.73	2.36	1.91	2.24	2.17

With separate fertilization with N_8 and N_{16}, the total average dry matter in the maturation phase is higher than in the unfertilized phase by 23.8% and 62.7%, respectively (Table 3). Separate phosphorus fertilization contributes to an increase in dry matter by 7.6% at P_8.

Combined nitrogen-phosphorus fertilization has a proven effect on dry matter yield. When fertilizing with N_8P_8, the total dry biomass increases, averaging 519.67 t /ha^{-1} for the period, 27.8% above unfertilized. With increasing rates of combined fertilization, the dry mass of cotton also increases.

The elevated yields observed in 2018 were primarily attributed to the favorable alignment of thermal and precipitation conditions throughout the vegetative period. Cumulative rainfall of 259.7 mm during May, June, and July created optimal conditions for timely germination, uniform crop establishment, and effectively promoted the formation, retention, and development of a high number of fruiting bolls (Table 4). In contrast, the reduced yields in 2020 were mainly a consequence of elevated temperatures during the vegetation phase, combined with a prolonged summer drought. These adverse conditions resulted in significant abscission of floral buds and blossoms, and impeded the adequate filling and maturation of bolls.

Under the influence of sole nitrogen fertilization, the average total cotton yield reached its maximum value at a nitrogen application rate of 2.16 t ha^{-1} for 2018 the cultivar Darmi. The applied nitrogen rate of N_{16} had a very positive effect on the crop productivity.

Table 3. Effect of nitrogen-phosphorus fertilization on the total dry biomass of cotton in the ripening phase, t/ha⁻¹, average for 2012-2014

Fertilization	2012	2013	2014	Avarage	
				t /ha⁻¹	%
N_0P_0	380	365	475	406.67	100.00
N_8	474	450	585	504.33*	124.0
N_{16}	576	620	790	662.00***	162.8
P_8	420	396	495	437.67	107.6
N_8P_8	506	458	595	519.67**	127.79
Avarage	**471.2**	**457.8**	**588**	**506.07**	**-**
*; **; *** - proven by probability P ≤ 0,05; P ≤ 0,01; P ≤ 0,001, accordingly					

Combined nitrogen-phosphorus fertilization has a proven effect on dry matter yield. When fertilizing with N_8P_8, the total dry biomass increases, averaging 519.67 t /ha⁻¹ for the period, 27.8% above unfertilized. With increasing rates of combined fertilization, the dry mass of cotton also increases.

The elevated yields observed in 2018 were primarily attributed to the favorable alignment of thermal and precipitation conditions throughout the vegetative period. Cumulative rainfall of 259.7 mm during May, June, and July created optimal conditions for timely germination, uniform crop establishment, and effectively promoted the formation, retention, and development of a high number of fruiting bolls (Table 4). In contrast, the reduced yields in 2020 were mainly a consequence of elevated temperatures during the vegetation phase, combined with a prolonged summer drought. These adverse conditions resulted in significant abscission of floral buds and blossoms, and impeded the adequate filling and maturation of bolls.

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Table 4. Impact of mineral fertilization on the yield of unbleached cotton, t/ha⁻¹, average for 2018-2020

Fertilization	Year			Average %	
	2018	2019	2020	t/ha⁻¹	%
N_0	1.76	1.52	7.93	1.36	100
N_8	2.06	1.64	8.10	1.53	112.5
N_{16}	2.11	1.68	1.05	1.61	118.4

CONCLUSIONS

The Darmi cotton variety is suitable for cultivation on both *Haplic Vertisol* and *Luvisols* soil under the specified fertilization conditions. The application of N_{16} and P_8 fertilizer rates results in higher and more stable yields, highlighting their effectiveness in supporting crop productivity. Statistical data and processing prove that nitrogen fertilization rates N_{16} significantly prove higher yield results. The study confirms that nitrogen and phosphorus fertilization levels play a crucial role in enhancing cotton yield, with balanced nutrient supply ensuring optimal plant development and fiber quality.

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THE INFLUENCE OF SOWING DEPTH AND OF PRECIPITATIONS ABOVE THE QUALITY AND THE YIELD OF AUTUMN WHEAT IN BOIANULUI'S MEADOW

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Abstract

Beside the yield gained of the autumn wheat's crop, the quality of it is the decisive factor of the seeding of a said kind or hybrid. The use of crop technology, but also of some recent innovations, lead to the improvement of the wheat crops. The cereal production is affected by the drought, one of the leading factors of abiotic stress, who is also affecting even tolerant crops. The constantly changing weather forces the farmers to adapt the crop technology specifically to the conditions of the local weather, the type of soil and the agricultural practices used to find the best strategy of seed distribution. This article presents the results of the effectuated studies with the purpose of determining the optimal depth of sowing in non-irrigable conditions and reduced precipitations in the southern part of the country. A year with water shortages is a different year when we can observe the influence of climate conditions over the growth of wheat crops at different depths.

***Key words**: autumn wheat, precipitations, protein, soil, technology.*

INTRODUCTION

Winter wheat (*Triticum aestivum*) is one of the fundamental crops for European agriculture, being particularly important both from an economic perspective and from its food use. With a very high food share, it enjoys special attention from farmers due to the grains rich in carbohydrates and proteins, the long shelf life of the grains and the fact that they can be transported without difficulty. The stalks left after harvesting are used as a raw material in the manufacture of cellulose, as well as in animal feed or as organic fertilizer (Iancu et al., 2024).
With an average production of 4364 kg/ha in Romania, wheat is cultivated on 2,3 million ha (www.madr.ro). Wheat provides 28% of the world's edible dry matter and up to 60% of daily calories in developing countries (Cakmak, 2008). Food consumption is expected to double by 2050, in addition to the increasing requirement for high-quality food for a healthy diet (Singh et al., 2016). A rapid increase in the necessity of wheat products is also predicted worldwide (Rosegrant, 2003). The composition and nutritional quality of the wheat grain have a significant impact on human health and well-being, especially in developing countries. Therefore, factors an acting not only wheat yield, but also wheat quality, require more attention (Wang et al., 2011; Peleg et al., 2008).
To obtain a rich and quality crop, wheat sowing is an essential stage. The quality of production and its yield are influenced by a multitude of pedoclimatic and technological factors, among which the sowing depth and the precipitation regime play a decisive role. Local relief conditions, groundwater, vegetation, lithology determine the variety of soils in each area, which proves that the distribution of soils is determined not only by bioclimatic zoning but also by local factors, which sometimes play a fundamental role in the distribution of soils in each area and implicitly the yield of cultivated plants (Popescu et al., 2024).
Worldwide under different climatic conditions, the variation in wheat and maize yield is mainly attributed to cropping systems, soil fertility, fertilizers, weeds, pests and diseases management (Partal et al., 2023a; Partal et al., 2023b; Partal & Paraschivu, 2020; Velea et al., 2021).
Currently, by grouping agricultural land, in farms and associations of different sizes in terms of surface, there are no more uncultivated lands and the need to obtain quantitative and

qualitative productions has increased (Popescu et al., 2024). Texture as a physical property of the soil determined by the granulometric fractions, sand, clay dust, which enter its composition in different proportions, is stable in any soil conditions and plays a very important role in ensuring a proper relationship of the soil with water and air, to grow and develop plants (Bălan et al., 2024).

Depending on the soil moisture, soil type and texture, the soil-water depth is between 3-7 cm. Increasing sowing depths can enhance wheat establishment because of the higher soil-water content in the seed zone, resulting in better germination and emergence of seed lings (Mahdi et al., 1998; Schillinger et al., 1998). Soil functions may be directly or indirectly impacted by climate change. Thus, the farmland management exacerbates losses in soil multifunctionnality throughout Europe due to dry weather and rising temperatures (Sünnemann et al., 2023). Changes in temperature, precipitation, and moisture regime are examples of direct effects. Adaptations including tillage techniques, crop rotation adjustments, crops mixtures, pest and diseases management and irrigation might have indirect effects (Partal et al., 2023; Sărățeanu et al., 2023; Sălceanu et al., 2022).

The Boian Plain, characterized by fertile soils and a specific rainfall regime, constitutes an optimal framework for evaluating the influence of these variables on the performance of winter wheat.

This paper aims to examine the impact of sowing depth and rainfall on grain quality and yield of winter wheat, in the context of the specific agroecological conditions of the Boian Plain. The research aims to identify the optimal parameters for maximizing production and improving its quality.

Drilling is an advisable sowing method due to its uniform population per unit area. As seeds are place data uniform depth and covered with soil, high germination and uniform stands are expected (Tanveer et al., 2003). In recent years, the new planting pattern of wide precision has been widely adopted. This new planting pattern of wide precision sowing changes the seed dispersal from planting all seeds in a line, as done in drilling and dibbling, to separating single grains from each other (Dandan et al., 2013; Bian et al., 2016).

MATERIALS AND METHODS

Soil and climatic description

The experiment was carried out in the Boian Plain located in southern Romania, part of the Romanian Plain, on a chernozem type soil with a humus content favorable to cereals of 3,9 in horizon A at a depth of 0-30 cm, in horizon B it decreases significantly to 1,3 at a depth of 30-60 cm. In horizon A the following values were determined: DA - 1.21 g/cm³, pH 6.4. In horizon B the values DA - 1.38 g/cm³, pH 5.8. The apparent density, total porosity of the soil, $CaCO_3$ content, soil reaction play an important role both in seed germination and in the development and fruiting of agricultural crops as well as in the amount and quality of the productions obtained (Bălan et al., 2024).

Due to the recent climate changes that led to excessive drought, this experiment was carried out over two years, with two different sowing dates and three sowing depths, respectively 4, 6 and 8 cm, to observe the quality of the wheat grains. The sowing was carried out with a precision seeder, thus respecting the planting depths.

The two wheat varieties studied are Glosa and Boema created at the Fundulea Agricultural Research and Development Institute, productive wheat varieties with high drought tolerance. The sowing of the experiment was done for all variants in the first and last decade of October, with a density of 500 germinable grains/m². In both years of experimentation, the plots had 30/8 m and 240 m^2 respectively.

Regarding quality, samples were taken and determined using NIRS (Near-Infrared Spectroscopy) analysis, a modern technique used for the rapid and non-destructive determination of the chemical composition of feed, cereals and other agri-food products. The parameters determined were: Protein, Starch (%), Sugar (%), NCGD (%), Fiber Fat (EE) (%), Fah_as. These parameters are determinants for the baking quality and industrial use of wheat.

RESULTS AND DISCUSSIONS

Climatic aspects

The area is characterized by a temperate-continental climate and variable precipitation. The lack of adequate autumn precipitation can delay the sowing of wheat crops in environments where irrigation is not available.

The year 2022 is in third place in the top of the warmest years in Romania. The monthly amount of precipitation (mm) in October 2022 was 15 mm short compared to the multiannual average of 46.0 mm with a deviation of -16.8 mm being characterized among the driest months since the determinations were made, and the average monthly temperature (ºC) was 13.6º C, a temperature considered high. This lack of precipitation caused the germination of wheat throughout the experience was affected, having an impact on the normal development of the plants and therefore on the quality of the grains. The month of November had an average monthly temperature of 8.6ºC being considered among the warmest November with a deviation of 3.6ºC. Very low precipitation amounts from a pluviometry point of view were recorded in February, namely 13.2 mm with a deviation of -14.7 mm. Also, the high temperatures of both the soil and the air in this month caused the biological rest of the wheat to be interrupted, and the growth processes were resumed. In all the spring months, the precipitation amounts were within satisfactory limits, which made the moisture reserve fall within a range close to optimal. Throughout the vegetation period, the average monthly temperatures were higher than the multiannual average (Table 1).

Table 1. The meteorological parameters in the experimental period 2022-2023

Specification	Month	Oct	Nov	Dec	Ian	Feb	Mar	Apr	May	June	July	Aug	Sept	Average Total
Temperature ºC	Monthly Average	**13.7**	**8.6**	**2.8**	**4.0**	**3.6**	**7.7**	**10.6**	***16.4***	**21.2**	**25.8**	**25.7**	***21.5***	**13.4**
	Multiannual Average Last 30 Years	11.7	5.1	0.3	-1.3	0.8	6.0	12.0	*17.7*	21.6	22.8	23.5	18.1	11.6
	Deviation	**+4.2**	**+3.1**	**+3.6**	**+0.4**	**+7.2**	**+2.8**	**+2.9**	***-0.5***	**+4.4**	**+4.4**	**+3.3**	**+2.1**	**+1.8**
Precipitation mm	Amount Monthly	**15.0**	**78.8**	**33.8**	**103.4**	**13.2**	**20.8**	**68.8**	***78.6***	**44.4**	**120.0**	**40.8**	***35.8***	**653.4**
	Multi annual Media Last 30 Years	46.0	37.0	39.1	30.8	26.3	34.2	47.8	*58.6*	69.7	62.1	46.6	43.5	541.7
	Deviation	**-16.8**	**+87.2**	**-17.7**	**-4.0**	**-14.7**	**-11.8**	**-21.8**	*+13.2*	**-38.1**	**+33.1**	**-40.6**	**-24.5**	**+111.7**

Regarding the years 2023-2024, we can say that this was a year with a deficit in precipitation, in October when the experiment was established the deviation was -16.8 mm. The precipitation in November of 124.2 mm made the soil moisture reserve satisfactory. Throughout the winter and spring, the amount of precipitation was deficient, which affected plant development. As in the previous year, February recorded the least precipitation, 11.7 mm, recording a deviation of -14.7 mm. The amount of precipitation 71.8 mm recorded in May helped during this period when water requirements are maximum because it is in the phenological phases of earring, flowering, formation and filling of the grain. Monthly temperatures recorded positive deviations throughout the vegetation period. However, May 2024 was highlighted by average temperatures below the multiannual averages of the last 30 years, with a negative deviation of -0.5^{0}C (Table 2).

Table 2. The meteorological parameters in the experimental period 2023-2024

Specification	Month	Oct	Nov	Dec	Ian	Feb	Mar	Apr	May	June	July	Aug	Sept	Average Total
Temperature ºC	Monthly Average	**15,9**	**8,2**	**3,9**	**0,9**	**8,0**	**8,8**	**14,9**	***17,2***	**26,0**	**27,2**	**26,8**	***20,2***	**14,8**
	Multiannual Average Last 30 Years	11,7	5,1	0,3	-1,3	0,8	6,0	12,0	*17,7*	21,6	22,8	23,5	18,1	11,6
	Deviation	**+4,2**	**+3,1**	**+3,6**	**+0,4**	**+7,2**	**+2,8**	**+2,9**	***-0,5***	**+4,4**	**+4,4**	**+3,3**	**+2,1**	**+3,2**
Precipitation mm	Amount Monthly	**21,4**	**124,2**	**21,4**	**26,8**	**11,6**	**22,4**	**26,0**	***71,8***	**31,6**	**95,2**	**6,0**	***19,0***	**477,4**
	Multi annual Media Last 30 Years	46,0	37,0	39,1	30,8	26,3	34,2	47,8	*58,6*	69,7	62,1	46,6	43,5	541,7
	Deviation	**-16,8**	**+87,2**	**-17,7**	**-4,0**	**-14,7**	**-11,8**	**-21,8**	*+13,2*	**-38,1**	**+33,1**	**-40,6**	**-24,5**	**-64,3**

Production and quality

In 2023, the highest production was recorded for the Glosa variety in the variants shown at a depth of 4 cm, respectively 5720 kg/ha and 5280 kg/ha in the variant shown at a depth of 6 cm. The variant shown at 8 cm recorded a production of 3080 kg/ha, significantly reduced compared to the other two depths tested. The differences between the two varieties were small, the Boema variety gave yields of 5720 kg/ha when shown at 4 cm and 5280 kg/ha at 6 cm. The variant is shown at a depth of 8 cm recorded 3980 kg/ha. Regarding the yields obtained at the two showing dates, the yields were similar (Table 3).

Table 3. Production results obtained for wheat crops in 2023 and 2024

Year	Variety	Depth/ Date sown 10 Oct	Production/ kg/ha	Depth/ Date sown 20 Oct	Production / kg/ha
2023	Glosa	4 cm	5720	4 cm	5650
		6 cm	5280	6 cm	5000
		8 cm	3980	8 cm	3860
	Boema	4 cm	5670	4 cm	5200
		6 cm	5050	6 cm	4850
		8 cm	3700	8 cm	3680
2024	Glosa	4 cm	4820	4 cm	4650
		6 cm	4480	6 cm	4360
		8 cm	3650	8 cm	3400
	Boema	4 cm	4770	4 cm	4600
		6 cm	4470	6 cm	4350
		8 cm	3420	8 cm	3280

The year 2024 recorded, due to the drought that is increasingly felt, lower productions compared to the previous year by approx. 1000 kg/ha for all variants. Thus, the lowest productions were recorded for the variants that were sown at a depth of 8 cm, which denotes the idea that the humidity that would be found with the increase in the sowing depth does not guarantee better production. The lowest production was recorded for the Boema variety, the variant sown at a depth of 8 cm, respectively 3680 kg/ha.

Interpreting the parameters analyzed for the two wheat varieties in 2023, it results that the protein varies between 12.207% and 12.657 %, values that fall into the category of good quality wheat for baking. At all depths, when sowing on October 20 compared to October 10, the Glosa variety presents slightly higher protein values, varying between 12.657% and 12.386%. In contrast, for the Boema variety, higher protein values were recorded at early sowing October 10 at a depth of 4 cm, the situation being reversed at greater depths. We can also observe that when sowing on October 20, the Glosa variety exceeds the Boema variety in protein content as follows: at a depth of 4 cm the difference is 0.056%; at a depth of 6 cm, it is 0.018 %, and at a depth of 8 cm, it has a value of 0.053%. The average differences at the three depths are 0.042%, which represents the average advantage of the Glosa variety over Boema in terms of protein content at the showing on October 20.

For both varieties, a clear trend of decreasing protein content is observed with increasing sowing depth. The highest protein values are recorded at a depth of 4 cm, and the lowest at a depth of 8 cm. The Glosa variety presents slightly higher protein values than Boema at all sowing depths, with the largest difference of 0.056% at a depth of 4 cm.

Starch, which serves as the main source of energy for animals and humans, is a crucial parameter for breadmaking quality and milling yield and varies between 57.537% and 61.198%. Especially when shown early, the Boema variety generally has higher starch values compared to Glosa. When shown on October 10, the maximum starch value of 61.198% is recorded for the Boema variety at 4 cm depth. In Glosa, early snowing causes an increase in starch with depth, from 57.537% to 60.944%. The starch values for the sowing on October 20 are more homogeneous for both varieties.

Digestibility shows values varying between 89.284% and 93.181%, the Boema variety shows the maximum value of 93.181% at a depth of 4 cm shown on October 20. For the sowing on October 10, digestibility increases with depth for the Glosa variety, but the Boema variety generally shows higher values. The nutritional quality and processing are given by fiber content varying between 1,3307 and 2,6308. The maximum values of 2,6308 and 2,3154 are recorded for the Boema variety shown at 4 cm and 6 cm on October 20. The ash content, an indicator of mineral content, varies between 1,1418 and 2,2438. Glosa has higher ash values compared to Boema at both sowing dates, thus, the maximum value of 2,2438 is recorded at Glosa 4 cm at the sowing date of October 10 (Table 4).

Table 4. Variation of wheat quality parameters depending on variety, depth and sowing time 2022-2023

Variety	Depth/ Date sown 10 Oct	Protein	Starch (%)	Sugar (%)	NCGD (%)	Fibre	Fat (EE) (%)	Fah_as
Glosa	4 cm	12,603	57.537	1.4681	89.284	2,1678	1.4952	2,2438
	6 cm	12,523	59.72	0.84841	90.273	1,7502	1.1921	1,5901
	8 cm	12,355	60.944	0.98477	90.376	1,4451	1.2191	2,1746
Boema	4 cm	12,594	61.198	1.3463	91.415	1,3307	1.2104	1,7957
	6 cm	12,416	59.763	0.21491	90.241	1,8549	1.3241	1,5766
	8 cm	12,207	60.183	0.82987	90.257	1,8509	1.2275	1,6534
Variety	Depth/ Date sown 20 oct	Protein	Starch (%)	Sugar (%)	NCGD (%)	Fibre	Fat (EE) (%)	Fah_as
Glosa	4 cm	12,657	60.49	0.75394	89.664	1,5176	1.2046	2,1954
	6 cm	12,531	59.75	1.777	90.119	1,5778	1.2301	1,8891
	8cm	12,386	59.695	1.1676	90.27	1,8174	1.0707	2,0516
Boema	4 cm	12,601	60.867	0.7195	93.181	2,6308	0.8113	1,1418
	6 cm	12,513	58.309	0.42983	89.449	2,3154	1.0993	1,4315
	8 cm	12,333	59.378	0.79453	90.046	1,6942	1.4423	1,9351

The comparison between varieties and showing dates shows that at a depth of 4 cm, the Glosa variety shown on October 20 has a maximum protein value of approximately 12.99%, slightly higher than the Glosa variety shown on October 10, approximately 12.98%. Regarding the performance of the Glosa varieties, it generally has a higher protein content than the Boema variety at all sowing depths (Figure 1). The 4 cm depth is optimal for maximizing protein content in both varieties, regardless of the sowing date. If for agrotechnological reasons a deeper sowing is necessary, the Glosa variety shown on October 10 seems to maintain a higher protein content at 8 cm depth compared to the other variants. The differences in protein content, although apparently small (around 0.3-0.5%), can have a significant impact on the commercial value of wheat and its classification into quality categories.

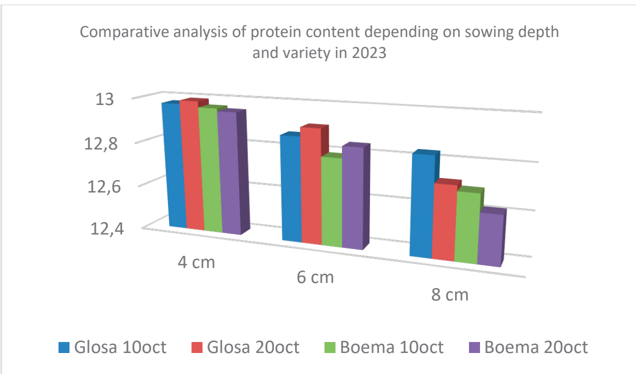

Figure 1. Comparative analysis of protein content depending on sowing depth and variety in 2023

The results of the parameters analyzed for the 2024 productions recorded a protein content varying between 12.617% for Boema shown on October 10 and 12.992% for Glosa shown on

October 20, both shown at a depth of 4 cm. In general, the protein content decreases with increasing sowing depth for both varieties and both sowing dates. The starch content shows relatively close values for both varieties, the maximum value of 59.988% was recorded for the Boema variety shown at a depth of 6 cm on October 10. In the Glosa variety, starch decreases with depth on both showing dates. In general, there is no clear correlation between showing depth and starch content. The highest sugar content value is 2.0164% and is recorded in the Boema variety shown at 4 cm on October 20. The sugar content values are generally lower at a depth of 6 cm for both varieties when sown on October 10. Digestibility varies between 89.032% and 94.161% respectively, the value recorded in the Boema variety sown at 4 cm depth on October 10, a value that indicates excellent digestibility. Also, the value of 92.455% recorded in the Glosa variety show at 6 cm depth on October 10 shows high digestibility. When shown on October 20, the fiber content increases with depth for both varieties. The minimum values are recorded at a depth of 4 cm for both varieties when shown on October 20. The fat content varies between 1.0265% for the Glosa variety shown at 4 cm depth on October 10 and respectively 1.4423% recorded for the Boema variety sown at 8 cm depth on October 20. These values are relatively homogeneous, with no clear trends related to depth or slowing date. The ash content varies between 1,1267 and 2,1625, the highest value being recorded for the Boema variety sown at 4 cm depth on October 10. When sowing on October 20, the Boema variety presents higher values at all sowing depths (Table 5).

Table 5. Variation of wheat quality parameters depending on variety, depth and sowing time 2023-2024

Variety	Depth/ Date sown 10 Oct	Protein	Starch (%)	Sugar (%)	NCGD (%)	Fibre	Fat (EE) (%)	Fah_as
	4 cm	12,976	59.802	1.0629	89.741	1,7551	1.0265	1,7594
Glosa	6 cm	12,869	59.497	0.59616	92.455	3,2863	1.27	1,7036
	8 cm	12,654	59.243	0.56947	89.772	2,0132	1.1003	1,6528
	4 cm	12,966	58.504	1.6537	94.161	4.4442	1.2539	2,1625
Boema	6 cm	12,790	59.988	0.40881	90.012	1,8479	1.1267	1,1267
	8 cm	12,695	58.916	0.48617	89.33	1,8338	1.1127	1,7731
Variety	Depth/ Date sown 20 oct	Protein	Starch (%)	Sugar (%)	NCGD (%)	Fibre	Fat (EE) (%)	Fah_as
	4 cm	12,992	59.945	1.064	89.672	1,3451	1.0391	1,7144
Glosa	6 cm	12,909	59.228	0.97033	89.653	1,7812	1.161	1,7563
	8cm	12,719	59.207	0.80891	89.032	2,1808	1.2378	1,5113
	4 cm	12,954	58.982	2.0164	90.613	1,4459	1.1514	2,0277
Boema	6 cm	12,842	58.477	1.0849	89.408	2,1567	1.3599	1,697
	8 cm	12,617	59.819	1.3439	90.346	1,7708	1.1164	1,7078

The variation of protein content in the two varieties at the three sowing depths and the two periods shows a clear trend of decreasing protein content with increasing sowing depth for all combinations of variety-sowing date. The optimal depth for maximizing protein content is 4 cm for both varieties, regardless of sowing date. The best option for obtaining a high protein content is Glosa shown on October 20 at a depth of 4 cm. The differences between protein values at 4 cm and 8 cm are significant, approximately 0.3-0.4% and although they may seem small, they can have a significant impact on the qualitative classification of wheat and, implicitly, on the market price (Figure 2).

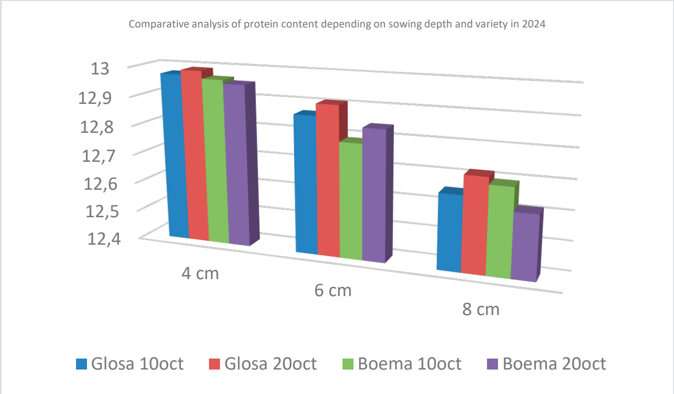

Figure 2. Comparative analysis of protein content depending on sowing depth and variety in 2024

CONCLUSIONS

Over the two years, the experimental results showed that the three sowing methods used produced statistically similar results in the main measured parameters. Variability in the rainfall regime affects crop yield, especially in years with below-average rainfall. The lack of adequate autumn rainfall can delay the sowing of wheat crops in environments where irrigation is not available. Wheat grown in the Boian Plain has a high potential to produce high-quality grains with a significant protein content, due to the combination of fertile soils, favorable climate and appropriate agricultural practices. In order to maintain this potential, it is essential to adopt measures that counteract climatic variability and support the sustainability of the region's soils. Regarding the optimal depth for breadmaking quality, it seems to be 4 cm for both varieties, with proteins decreasing with increasing depth. Influence of sowing date for Glosa, sowing on October 20 favors protein accumulation. For Boema, early sowing on October 10 at a depth of 4 cm provides the best protein-starch balance. Glosa has advantages for bakery due to its protein-starch balance, while Boema has superior starch and digestibility values.

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RESEARCH ON THE RELATIONSHIP BETWEEN THE VEGETATION PERIOD IN CEREAL SPECIES AND THE FLAG LEAF AREA THROUGH THE YIELD, ON THE CHERNOZEM OF CARACAL

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Abstract

In 2024, a bifactorial experiment was set up on the chernozem of Caracal, where A factor was the species: wheat, triticale, barley and B factor was the vegetation period: early, medium, late. The yield and flag leaf area were determined for each combination of factors in 3 repetitions. The highest yield was obtained for wheat – 61.92 q/ha. Compared to the control - yield of wheat species, only barley was significantly lower. There were no differences in yield between the vegetation periods, regardless of the species. The correlation coefficient between yield and flag leaf area recorded a value of 0.331, lower than the P5% limit for 27 studied cases, therefore insignificant. There were both varieties with small flag leaf area but with high yields (such as: medium-early triticale with flag leaf area of 1307.27 cm^2 and yield of 68.54 q/ha and late wheat with area of 1840.27 cm^2 and yield of 68.25 q/ha) but also varieties with large flag leaf area and high yields (such as: medium-early wheat with flag leaf area of 2951.07 cm^2 and yield of 66.08 q/ha).

***Key words**: wheat, triticale, barley, flag leaf area, vegetation period.*

INTRODUCTION

Cereals represent the phytotechnical group of agricultural plants with the largest distribution area in all cultivated areas of the globe, with an age of about ten thousand years. Grains of straw cereals (wheat, triticale, barley) are an important source of nutrition throughout the world, being rich in non-nitrogenous extractive substances but also proteins, fats, vitamins, etc. Also, cereal crops, specially wheat, contribute to economic stability and growth in rural communities.

Along with climate change, a series of regional and global political and economic factors have intensified food insecurity and long-term vulnerability in certain regions (Bonciu, 2023; Bonciu et al., 2021a; Mirzabaev et al., 2023; Păunescu and Păunescu, 2019). Drought affects plant growth, productivity, and their quality. Genetic engineering can increasing resilience and improving crop adaptation (Bonciu et al., 2021b; De Souza et al., 2022; Liu et al., 2024).

Food security has a fundamental importance for human existence, and increasing cereals production under climate change is, from this point of view, one of the permanent challenges for farmers (Bonciu et al., 2021; Dihoru et al., 2023; Rosculete et al., 2023). From this point of view, wheat is one of the safest crops, being genetically structured to withstand low winter temperatures. They exhibit a remarkable ecological plasticity, being cultivated even in unfavorable climatic and soil conditions.

Production in cereal crops is due to complex physiological and biochemical processes, but is essentially associated with the process of carbohydrate accumulation during the grain filling phase, which in turn is attributed to leaf functionalities (Biswal and Kohli, 2013). In this sense, the key components underlying cereal productivity are positively correlated with flag leaf size estimated by length, width and area (Khaliq et al., 2008; Wang et al., 2012), the flag leaf length-to-width ratio (Sohrabi et al., 2012) and the flag leaf basal angle (Isidro et al., 2012).

The flag leaf is one of the primary sources of carbohydrates in cereals (Liu et al., 2021). Flag leaf traits are considered to play an important role in grain filling of strawy cereals under drought conditions. Thus, physiological, morphological and biochemical traits of flag leaves are involved in determining grain yield and biomass (Biswal and Kohli, 2013; Liu et al., 2015; Racz et al., 2022).

The flag leaf plays an essential role in establishing production and therefore it must benefit from protection throughout the vegetation period. Thus, in order for the plants to remain healthy until harvest, it is necessary to apply additional treatments that protect the grass cereals in all phases of vegetation. Maintaining the green state of the flag leaf is a trait that allows grass cereals to maintain their photosynthetic capacity for a longer period of time after anthesis, especially under conditions of drought and heat stress.

The flag leaf provides the main source of assimilates necessary for plant growth and ear development in straw cereals. The contribution rate of flag leaves to daily photosynthetic products ranges from 50% to 60% (Towfiq et al., 2015), while its defoliation generated yield losses ranging from 18% to 30% (Banitaba et al., 2017). In wheat, removal of the flag leaf affected grain production under normal or water-limited conditions (Cruz-Aguado et al., 1999; Ma et al., 2021).

According to Niu et al. (2022), the flag leaf plays a vital role in seed development. Thus, the flag leaf thickness showed significantly positive correlations with grain size in barley.

Many results suggest that cereal species will continue to play an essential role in ensuring food security and an accessible intake of calories and protein (Grote et al., 2021; Pandey et al., 2020; Paunescu et al., 2021, 2023; Tadesse et al., 2018).

MATERIALS AND METHODS

The aim of this work was to determine the influence of the flag leaf area on the production of 3 species of straw cereals, on the chernozem of Caracal.

In 2024, a bifactorial experiment was set up on the chernozem of Caracal in which factor A was the species: wheat, triticale, barley and factor B was the vegetation period: early, medium, late. The production and flag leaf area were determined for each combination of factors in 3 repetitions.

Based on the results, was studied the factors influence (factor A – species with 3 graduations – wheat, triticale, barley; factor B – vegetation period with 3 graduations: early, medium, late) and their interactions (species *x* vegetation period, vegetation period *x* species) on the production and flag leaf area. Correlations were performed and the simulated flag leaf area was calculated based on the correlation coefficient equation. This was compared with the real flag leaf area simultaneously, depending on the production classes within the interval obtained within the experiment.

The used materials were:

- Wheat varieties with differentiated: DUSK (early); CAROM (medium/+3-4 days); BOGDANA (late/+7-8 days)
- Triticale varieties with differentiated earing: line 11588T2-23 (early); UTRIFUN (medium/ +4-5 days); INSPECTOR (late/+14-15 days)
- Barley earing with differentiated earing: line F 8-4-12 (early); AMETIST (medium/+3-4 days); ONIX (late/+7-8 days)

The statistical processing of the experiment was done with the statistical analysis program specific to bifactorial experiments based on the methodology presented by Săulescu (PSUB 2).

RESULTS AND DISCUSSIONS

The climatic conditions manifested in the 2023-2024 agricultural year in Caracal showed a lack of precipitation throughout the vegetation period (-32.3 mm) but with a surplus in May when grain filling took place (Table 1).

From the point of view of the species, there were differences between the yields, but only that of barley was significantly reduced in relation to the yield obtained in wheat (Figure 1). In contrast, yield did not differ according to the average vegetation period of the 3 species analyzed (Figure 2).

Table 1. Evolution of the main climatic factors during the vegetation period of straw cereal genotypes

Specification		X	XI	XII	I	II	III	IV	V	VI	Total growing season
Temperature °C	Monthly minimum	0.6	-4.4	-3.2	-9.6	-6.7	-3.3	2.0	5.4	12.5	
	Monthly maximum	33.4	21.4	18.2	15.3	21.9	28.3	32.7	30.8	39.4	
	Monthly average	16.0	8.1	3.9	1.0	8.0	8.9	15.0	17.2	25.9	
	NORMAL	11.7	5.1	0.3	-1.3	0.8	6.0	12.0	17.7	21.6	
	Difference ±	+4.3	+3.0	+3.6	+2.3	+7.2	+3.9	+3.0	-0.5	+4.3	
Rainfall mm	Monthly total	21.4	124.2	21.4	26.8	11.6	22.4	26.0	71.8	31.6	**357.2**
	Multiannual average	46.0	37.0	39.1	30.8	26.3	34.2	47.8	58.6	69.7	**389.5**
	Difference±	-24.6	+87.2	-17.7	-4.0	-14.7	-11.8	-21.8	+13.2	-38.1	**-32.3**
Solar radiation	**Average**	123	59	36	52	86	126	196	211	273	
Relative humidity of the air %	**Average**	66.9	91.1	93.8	95.4	84.7	85.3	70.8	77.8	61.3	

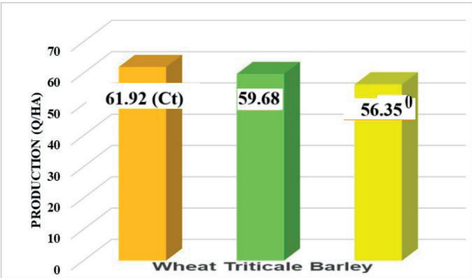

Figure 1. The influence of the species on production on the chernozem of Caracal

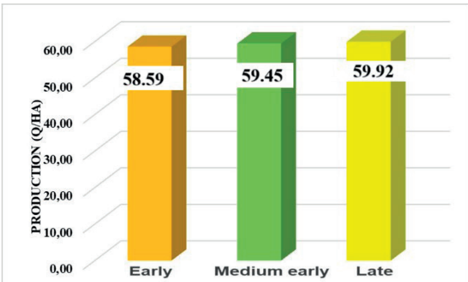

Figure 2. The influence of the vegetation period on production on the chernozem of Caracal

The species x vegetation period interaction has a limited influence on production. The only combination that significantly exceeded the control was late-growing barley. The increase was 16.8%, substantial, which made this species obtain a production almost equal to that of wheat (Table 2). The highest production was obtained with mid-early growing wheat (63.3 q/ha) and the lowest with early barley (52.44 q/ha).

The coefficient of variability of production values, regardless of the studied interactions, was 9.14%, therefore below 10% - the limit for stability. Thus, under Caracal conditions, all species can be cultivated regardless of their vegetation period, the results being within the limit of optimal stability.

If reporting is done on the control considered as the early wheat species, there are two combinations of factors where production is

significantly reduced: early barley and medium-early growing season barley.
In terms of flag leaf area, triticale and barley showed distinctly significantly, respectively very significantly reduced values compared to wheat - the control species (Figure 3). As in the case of production, the growing season did not influence the surface of the flag leaf under Caracal conditions (Figure 4).

Table 2. The influence of the species *x* vegetation period interaction on production on the chernozem of Caracal

Factor A (Species)	Factor B (Growing period)	Production (Q/Ha)	Difference	Significance
WHEAT	Early	63.00	Ct	
	Medium early	63.30	0.30	
	Late	59.47	-3.53	
TRITICALE	Early	60.33	Ct	
	Medium early	61.46	1.13	
	Late	57.27	-3.06	
BARLEY	Early	52.44	Ct	
	Medium early	53.59	1.15	
	Late	63.02	10.58	*
LD 5%			**9.03**	
LD 1%			**12.68**	
LD 0.1%			**17.9**	

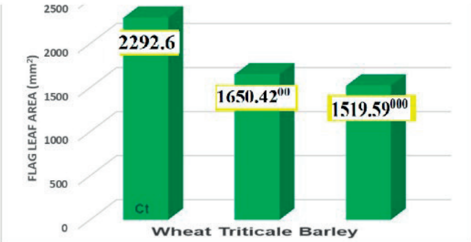

Figure 3. The influence of species on the flag leaf surface on the Caracal chernozem

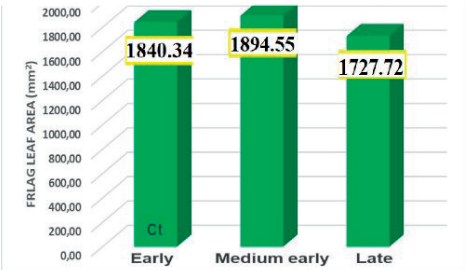

Figure 4. The influence of the vegetation period on the flag leaf area on the Caracal chernozem

The species x vegetation period interaction influenced the flag leaf area (Table 3).

In wheat and triticale, there were statistically significant interactions. While medium early wheat showed a significant increase in area compared to early wheat, late wheat showed a distinctly significant decrease compared to the same control. In triticale, at the average vegetation period as earliness, a significant decrease of 20% was recorded compared to the control - early triticale. The limits of the values determined for the flag leaf area were between 1352.83 mm^2 in early barley and 2671.94 mm^2 in medium-early wheat. These corresponded to the limits for production in the pedo-climatic conditions of Caracal.
The coefficient of variability of the flag leaf area values, regardless of the studied interactions, was 25.6%, therefore over 20% - the limit for character instability.
Unlike the production results, in the flag leaf area, in relation to early wheat, all interaction variants presented statistically significant differences - very significant decreases, except for medium-early wheat (Figure 5).
The correlation coefficient between production and flag leaf area, regardless of species and vegetation period, recorded a value of 0.331, lower than the P5% threshold for the 27 cases studied, therefore insignificant (Figure 6).

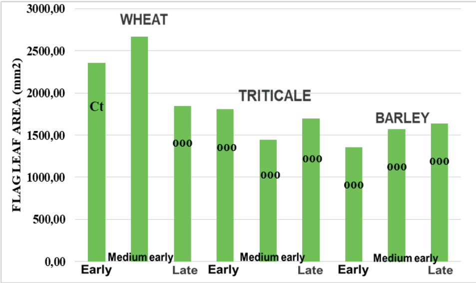

Figure 5. Flag leaf area x vegetation period interaction in relation to the control variant – wheat species with early vegetation period

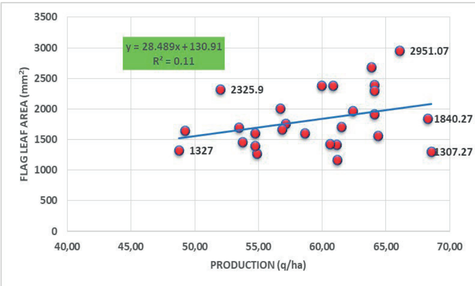

Figure 6. The relationship between production and flag leaf area in the variants studied at Caracal

There were both varieties with small flag leaf area but with high yields (example: triticale with medium precocity with flag leaf area of 1307.27 mm^2 and production of 68.54 q/ha and late wheat with area of 1840.27 mm^2 and production of 68.25 q/ha) but also varieties with large flag leaf area and high yields (example: wheat with medium precocity with flag leaf area of 2951.07 mm^2 and production of 66.08 q/ha).

The determination coefficient shows us that the first studied character explains the variability of the other in proportion of 11%. The linear equation showed that each 100 kg/ha increase in production is due to an increase in flag leaf area of about 28.5 mm^2, for the range studied.

By comparing the real and simulated results for the flag leaf area according to the production classes, it is noted that most of the variants where the difference between real and simulated is below DL 5% (307.72 mm^2) are below the average production of 59.32 q/ha.

Results over several years would allow obtaining simulations close to the real values, so that a simple determination would give predictions regarding the productions that can be obtained depending on the flag leaf area. Also, from these determinations it emerged that the greatest matches between real and simulated were recorded at lower productions, especially those of barley (Table 4).

Table 3. The influence of the species x vegetation period interaction on the flag leaf area on the Caracal chernozem

FACTOR A (Species)	FACTOR B (Growing period)	FLAG LEAF AREA (mm^2)	DIFFERENCE	SIGNIFICANCE
WHEAT	Early	2358.49	0.00	
	Medium early	2671.94	313.45	*
	Late	1847.39	-511.10	oo
TRITICALE	Early	1809.70	0.00	
	Medium early	1443.42	-366.28	o
	Late	1698.13	-111.57	
BARLEY	Early	1352.83	0.00	
	Medium early	1568.30	215.47	
	Late	1637.63	284.80	
LD 5%			**307.72**	
LD 1%			**431.93**	
LD 0.1%			**609.78**	

Table 4. Results of the simulation of the flag leaf area according to the linear equation of the production – flag leaf area relationship reported to the real flag leaf area by production classes

Production Classes	Real Production	Leaf Surface Simulation (mm^2)	Real Leaf Surface (mm^2)	Difference
48-48.99	48.73	1519.038	1327	192.04
49-49.99	49.21	1532.886	1648.4	-115.51
50-50.99				0.00
51-51.99				0.00
52-52.99	52.02	1612.794	2325.9	-713.11
53-53.99	53.46	1654.023	1696.83	-42.81
	53.73	1661.661	1462.1	199.56
54-54.99	54.71	1689.602	1392	297.60
	54.72	1689.791	1608.13	81.66
	54.86	1693.837	1269.4	424.44
55-55.99				0.00
56-56.99	56.70	1746.269	2005.07	-258.80
	56.86	1750.708	1664.5	86.21
57-57.99	57.10	1757.716	1756.2	1.52
58-58.99	58.62	1801.071	1600.2	200.87
59-59.99	59.96	1839.025	2385.47	-546.45
60-60.99	60.59	1857.094	1431.1	425.99
	60.83	1863.951	2380.93	-516.98
61-61.99	61.12	1872.068	1414.87	457.20
	61.16	1873.317	1168.3	705.02
	61.50	1883.019	1706.7	176.32
62-62.99	62.37	1907.864	1966.2	-58.34
63-63.99	63.85	1950.002	2679.27	-729.27
64-64.99	64.08	1956.443	2299	-342.56
	64.09	1956.718	1915.9	40.82
	64.09	1956.77	2395.53	-438.76
	64.38	1964.914	1565.9	399.01
65-65.99				0.00
66-66.99	66.08	2013.559	2951.07	-937.51
67-67.99				0.00
68-68.99	68.25	2075.323	1840.27	235.05
	68.54	2083.629	1307.27	776.36
	59.32			-0.42

CONCLUSIONS

With a growing world population and, at the same time, every scarce resource, food security is one of the key challenges for present and future generations.

The earliness-yield relationship can be highlighted with much greater accuracy by experimenting with cultivars differentiated in terms of earing date.

The species *x* growing season interaction has a reduced influence on production. The only combination that significantly exceeded the control was barley with a late growing season. The increase was 16.8%, almost equal to that of wheat.

The highest production was obtained with wheat with a medium-early growing season (63.3 q/ha) and the lowest with early barley (52.44 q/ha).

The coefficient of variability of production values, regardless of the studied interactions, was 9.14%, therefore below 10% - the limit for stability. Thus, under the conditions at Caracal, all species can be cultivated regardless of their vegetation period, the results being within the limit of optimal stability.

The correlation coefficient between production and flag leaf area, regardless of species and vegetation period, was insignificant. There were both varieties with small flag leaf area but high yields (example: medium-early triticale with flag leaf area of 1307.27 mm^2 and production of 68.54 q/ha and late wheat with area of 1840.27 mm^2 and production of 68.25 q/ha) and varieties with large flag leaf area and high yields (example: medium-early wheat with flag leaf area of 2951.07 mm^2 and production of 66.08 q/ha).

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DYNAMICS OF MORPHOPRODUCTIVE CHARACTERS IN *Phalaris arundinacea* UNDER THE CONDITIONS OF THE WESTERN PLAIN OF ROMANIA

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Abstract

Phalaris arundinacea, (reed canary grass), is suitable as an option for cultivating lands that are not ideal for other crops, numerous studies identify it in mild climates and humid environments but also drier areas, on various types of soil, with a pH between 4.0 and 7.5. The study aimed to identify the interdependence between morpho-productive characters in vegetation conditions in the Western plain of Romania, the biological material was represented by the Premier variety. Vegetative growth was investigated in 6 BBCH codes: 1.19; 2.21; 2.22; 2.29; 3.31; 3.37, and the measurements targeted the following characters: bush diameter, bush height, plant height, number of vegetative shoots, and the number of leaves of the main shoot. Statistical processing through descriptive statistics, Pearson correlation coefficient, and ANOVA analysis of variance illustrate a correct distribution of the experimental data and the existence of strong correlation relationships between the studied characters. This element is practically important because in the breeding process prioritizing positive and strong correlations can help to obtain faster results.

Key words: *reed canary grass, correlation, morpho-productive characters.*

INTRODUCTION

Reed canary grass (RCG) or *Phalaris arundinacea* L., Moench (Țîței, 2020), is a perennial, heterogamous, and stoloniferous species belonging to the family *Poaceae*, subfamily *Pooideae* (Strašil, 2012; Winterfeld et al., 2018). The species has a fast growth that follows the C3 photosynthetic pathway (Kinmonth-Schultz & Soo-Hyung, 2011), being native to regions of North America, Asia, and Europe (Kitczak et al., 2023).

The species reaches heights of up to 200 cm (Țîței, 2020), although it can sometimes grow up to 3 meters (Kitczak et al., 2023). The leaves are flat, smooth, and gradually narrow, measuring between 30-45 cm in length and 0.8-1.2 cm in width. The root system of RCG consists of broad rhizomes that are strong, long, and segmented, facilitating the spread of the plant underground to depths exceeding 3 meters (Zhang et al., 2013; Kitczak et al., 2023). Fresh rhizomes mainly arise below the soil surface from buds at the nodes of other rhizomes. The roots and rhizomes can create an almost impenetrable carpet. The lifespan of a RCG plant is commonly estimated to be about ten years (Von Cossel et al., 2019). In temperate regions of Europe, RCG usually flowers between May and July, lasting several weeks (Ustak & Muňoz, 2019). The inflorescence is in the form of branched panicles with lengths ranging from 7 to 40 cm (Linding-Cisneros & Zedler, 2001). The fruit consists of a caryopsis, measuring between 1.5- 4.0 mm in length and 0.7-1.5 mm in width, producing a relatively large number of seeds (Lavergne & Molofsky, 2004). The weight of 1000 seeds is on average 0.8 g (Ustak & Muňoz, 2019).

The germination process of RCG is dependent on adequate light exposure (Linding-Cisneros & Zedler, 2001) and well-drained soil. Optimal germination can occur even under water-saturated conditions (Lavergne & Molofsky, 2004). The ideal temperature for plant photosynthesis is typically between 20 and 25°C (Ge et al., 2012).

This versatile plant thrives in a variety of environments, making it a suitable option for cultivating lands that are not ideal for other crops (Ustak & Muňoz, 2019, Perdereau et al., 2017). Numerous studies show that RCG is most commonly found in mild climates and humid environments such as wet meadows, marshy areas, lake shores, river banks, and floodplains (Lavergne & Molofsky, 2004; Kieloch et al., 2015; Ustak & Muňoz, 2019; Kitczak et al., 2023) and humid forests (Perdereau et al., 2017). Although the species has a preference for humid conditions, the species also adapts to areas with reduced humidity even in mountainous areas (Lord, 2015).

RCG can grow in various soil types, such as poorly drained, heavy, compact, but also well-drained, dry soils (Lord, 2015), flooded (Ustak & Muňoz, 2019), clayey (Perdereau et al., 2017), artificial (Lord, 2015) or even soils contaminated by cattle urine (Maeda et al., 2006) with a pH between 4.0 and 7.5 (Ustak & Muňoz, 2019).

The ideal habitat for RCG consists of fertile, humus-rich soils (Kieloch et al., 2015) and with high concentrations of organic nitrogen, total phosphorus, and phosphate and low levels of dissolved oxygen (Perdereau et al., 2017).

Phalaris arundinacea has a high tolerance to flooding compared to other cool-season grasses and is known for its resistance to frost and drought. RCG thrives at temperatures between 5 and 30 degrees Celsius and can withstand extremely cold temperatures, even down to -20 degrees Celsius (Kitczak et al., 2023).

Its ability to survive prolonged periods of flooding is due to its rhizomes, which can withstand low oxygen conditions. In terms of productivity, environmental conditions play a crucial role in the growth and development of RCG.

Plants thrive in fertile soils with high humus content and neutral pH, which leads to increased height growth as well as larger leaves, which in turn leads to higher biomass production (Kieloch et al., 2015). Medvedev and Smetannikova, cited by Țîței (2020) reported that a green mass yield of 90 t/ha was obtained. The content of digestible nutrients was found as follows: 72% crude protein, 55% crude fat, 65% crude cellulose, and 72% nitrogen-free extract.

When grown in fertile soil and receiving adequate care, RCG can produce significant amounts ranging from 6 to 20 $Mg \cdot ha^{-1}$ DM (Kitczak et al., 2023). The multitude of specialized studies (Ustak & Muňoz, 2019), but also by other authors (Strašil, 2012; Piskier, 2017; Hesham et al., 2019; Kitczak et al., 2023) highlight the potential of RCG as an excellent energy source.

Apart from its energy applications, Ustak et al. (2019), as well as Wrobell et al. (2009), mention numerous uses of RCG, such as its use as a forage crop, as a long-term perennial cover for permanent grasslands, for the restoration of degraded soils and waters, for phytoextraction of soil contaminants, for revegetation and stabilization of banks, for the production of acidic suspension ponds, for the treatment of wastewater to remove ammonia and nitrates, for the mineralization of organic solutions, for bioenergy purposes and the production of pulp, paper, and fibers, as mentioned by Lavergne & Molofsky in 2004. Another potential bioenergy application for RCG is biogas production, which involves the decomposition of digestible organic material (Ustak et al., 2019).

Wrobel et al. (2009) mention that RCG can produce substantial quantities of high-quality hay under optimal crop management conditions.

MATERIALS AND METHODS

Biological material and the purpose of the work

The biological material was represented by the Premier variety produced by ICDPP Brasov, with this variety a collection field was established located at Lovrin Agricultural Research and Development Station. The soil on which the experience was carried out is a typical chernozem soil, weakly gleied, representative of the Low Banat Plain area. Regarding the properties of this type of soil, a pH value indicating a weakly alkaline reaction (7.3-8.4) in the range of 20-100 cm, to even strongly alkaline (9.1-9.4) at depths of up to 200 cm is evident (Bostan et al., 2024).

The purpose of the study

The study aimed to observe the externalization of productive characters in the conditions of the Low Banat Plain area so that reed canary grass, respectively the Premier variety, would constitute a viable alternative for the production of fodder in the plain area affected by limiting factors of production. Another objective of this study is to observe the behavior of the Premier variety in specific soil conditions, in terms of the presence of precipitation and temperatures as the most important growth factors.

Regarding the biological growth of the Premier variety, the vegetative growth was investigated in 6 BBCH codes (1.19; 2.21; 2.22; 2.29; 3.31; and 3.37). The measurements targeted the following characters: bush diameter, bush height, plant height, number of vegetative shoots, and the number of leaves of the main shoot. Regarding the expression of these characters, a statistical analysis was carried out, evaluating the correlation between these characters.

Climatic conditions

In the studied area, the expression of the climatic regime for the period 2023-2024 showed abiotic conditions for the growth of RCG atypical with precipitation amounts located well below the multiannual monthly limit. For the Low Banat Plain area, the total precipitation amounts in the growth period 2023-2024 was 417.4 mm. A negative deviation of 104 mm from the multiannual average is observed.

The most important is, however, the growth period (April - August) when the amount of precipitation was 121.6 mm, highlighting a negative deviation of 134.6 mm from the MMA.

Statistical processing

The graphical representations were made with JASP version 0.18.3 and presented aspects such: the distribution of variables, regression curves, confidence intervals, and prediction intervals for the pairs of variables analyzed. Also, in the JASP program, the test was used to identify the existence of significant differences between the means of the investigated morpho-productive characters.

A correlation matrix was created and graphically represented in the form of a heatmap, using the "corrplot" package in R. The p-value shows that significant correlations are marked with: *p < .05 if the correlation is significant at the alpha level =.05; **p < .01 if the correlation is significant at the alpha =.01 level; and ***p < .001 if the correlation is significant at the alpha =.001 level.

In the analysis of the strength of association between productive traits in reed canary grass, the Chi-squared Test was also used because it is desirable in the analysis of small samples with fidelity (Bandalos & Finney, 2018; Dinno, 2014; Golino et al., 2020).

The dendrogram-type cluster analysis of the characters was performed by cluster analysis by the program MiniTab 17.1.

RESULTS AND DISCUSSIONS

Descriptive statistics were used to analyze the distribution of the values of the analyzed characters: bush diameter, bush height, plant height, number of vegetative shoots, and the number of leaves of the main shoot (Table 1).

Table 1. Normality dynamics of the distribution of the analyzed characters at reed canary grass

	95% Confidence Interval Mean				
	Mean	Upper	Lower	Std. Deviation	MAD
Bush diameter (cm^2)	690.33	1058.38	322.28	740.11	205.50
Bush height (cm)	26.16	32.88	19.44	13.51	6.00
Plant height	22.95	29.68	16.23	13.51	6.35
Number of vegetative shoots	13.72	15.86	11.58	4.30	4.00
Number of leaves of the main shoot	2.72	3.09	2.34	0.75	1.00

Depending on the number of variables determining characters, the number of layers displayed is also displayed, for the five productive characters their average on the 95% confidence interval, the standard deviation and the coefficient of variation were determined. The distribution of the experimental data highlights normal values of enrollment compared to the average of the analyzed character on a high degree of confidence which led us to conclude that the experimental data were correctly sampled and statistically interpreted (Moore et al., 2012; Whitlock & Schluter, 2015).

During its biological development cycle, RCG along with the growth in height, develops compound leaves and stems, practically the plant preparing for the flowering process. It is considered that this stage of development is important for photosynthesis, which provides the energy necessary for the subsequent stages of development. The biological growth dynamics of reed canary grass were assessed by investigating the main characteristics of the species, namely: bush diameter, bush height, plant height, number of vegetative shoots, and the number of leaves of the main shoot Thus, the degree of linear association between normally distributed quantitative variables in our study is expressed by the Pearson's correlation matrix (Table 2). Also, to have the smallest possible statistical error, we used the common means of the exteriorization of the characters in expressing the correlations between them. In the present study, the Pearson correlation coefficient is obtained by calculating the covariance of the two variables divided by the product of their standard deviations (Sellke et al., 2001; Caruso & Cliff, 1997; Xu et al., 2013).

Table 2. Expressing the degree of linear association of productive characters in reed canary grass in Pearson's correlation matrix

Variable		Bush diameter (cm2)	Bush height (cm)	Plant height	Number of Vegetative shoot	Number of leaves Of the main shoots
Bush diameter (cm2)	Pearson's r	-				
	p-value	-				
Bush height (cm)	Pearson's r	0.986***	-			
	p-value	<.001	-			
Plant height	Pearson's r	0.988***	1.000***	-		
	p-value	<.001	<.001	-		
Number of vegetative shoots	Pearson's r	0.731***	0.729***	0.734***	-	
	p-value	<.001	<.001	<.001	-	
Number of leaves of the main shoots	Pearson's r	0.328	0.387	0.366	0.356	-
	p-value	0.184	0.113	0.135	0.147	-

*p < .05, ** p < .01, *** p < .001

From the analysis of the power of association between the five productive characters, we can state that there are positive associations, or even the lack of a linear correlation, between these characters. Considering bush diameter as a determined and positively correlated character in specialized studies with productive properties such as bush, plant height, and the number of vegetative shoots, we measured and described the degree of linear association between this character and the other quantitative variables. As a result of these considerations, the presence of statistically significant correlation relationships is observed between bush diameter and bush and plant height as well as the number of vegetative shoots. Thus, between the bush diameter character and the bush height and plant height characters, close Pearson correlation values of 0.986 and 0.988 are observed, which corresponds to p values considered as a very significant correlation. The same degree of significance is also observed between bush diameter and the number of vegetative shoots (Pearson correlation 0.731), and the p-value is considered a very significant correlation. The same degree of association is obtained between plant height and number of vegetative shoots, the value of the Pearson coefficient being 0.734, which ensures from a statistical point of view by the value of p as a very significant correlation. These results demonstrate the hypothesis that the increase in height in the case of the Premier reed canary grass variety is accompanied by a positive proportional increase in bush diameter, number of vegetative shoots, and number of leaves of the main shoot.

The graphical representation of correlation relationships provided with statistical significance by the Pearson correlation can be found in Figure 1. The graphical representation shows two elements, the first is given by the expression of the correlation between the characters, and the second shows the testing of the null hypothesis according to which the correlation between pairs of variables is equal to zero (there is no significant connection between the two characters).

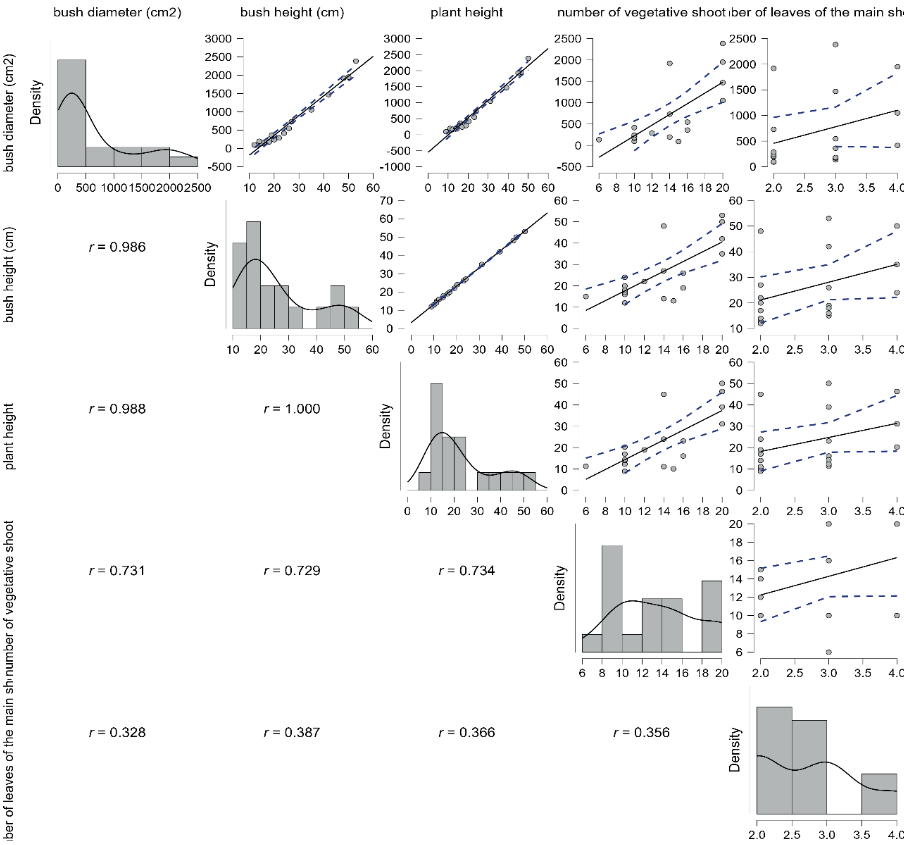

Figure 1. The graphical representation of the Pearson correlation at reed canary grass

From the experimental data illustrating the biological growth of the Premier variety under the specific experimental conditions of the Low Banat Plain area, it is evident in the previous graph that an increase in the scale of observations leads to a coarser table, while a decrease in the scale leads to a smoother table (Samfira et al., 2024).

Specifically, the distribution graphs presented show that for continuous variables, a histogram determined by the dynamics of bush diameter, plant height, and number of vegetative shoots is displayed, where the experimental data are correctly distributed in a Gaussian model.

Also, from the analysis of the nominal and ordinal variable values, a continuous frequency distribution is displayed as follows: in the case of correlation links between characters, the distribution of the experimental data is grouped, and if they are positively correlated, it is ascending.

The analysis of the power of association of productive characters in RCG by Chi-squared Test took into account the expression of df: Degrees of freedom cat and p: P-value when constructing the Principal Component Analysis (PCA) model (Hayton al., 2004; James et al., 2013). The analysis of the degree of uniqueness using Chi-squared Test confirms the close relationship between the studied productive characters (Table 3).

Table 3. The proportion of variance included in PCA

Component Loadings		
RC1		Uniqueness
bush height (cm)	0.980	0.040
plant height	0.979	0.042
bush diameter (cm^2)	0.970	0.060
number of vegetative shoots	0.831	0.309
number of leaves of the main shoots	0.485	0.764

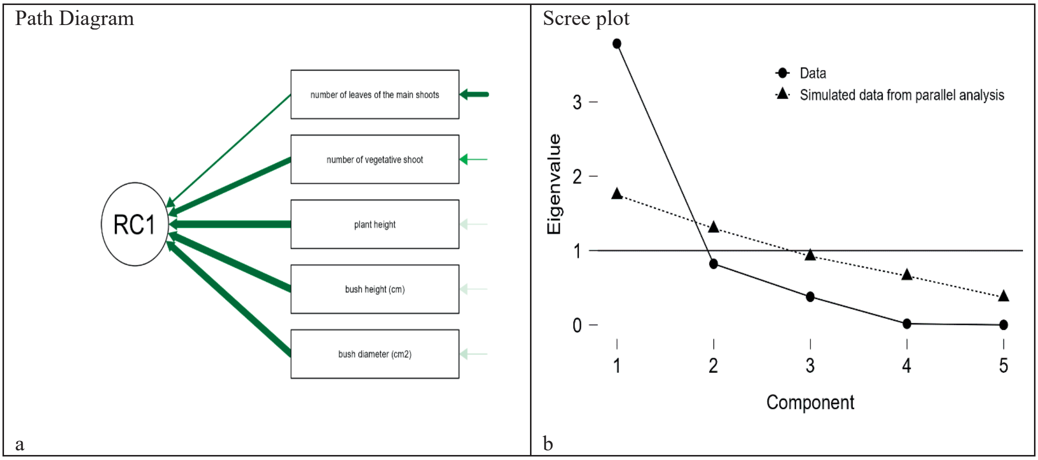

Figure 2. Analysis of the association strength of productive traits in reed canary grass by Principal Component Analysis (PCA): a) the degree of uniqueness of characters in biological development; b) the disposition of experimental data towards simulated analysis of the growth model

The graphical representation of Principal Component Analysis (PCA) gives us an image of the degree of uniqueness of the characters in biological development. In other words, the influence of each character in the biological growth of the reed canary grass species is analyzed according to df: and P-value (Osborne et al., 2008; Saris et al., 2009; Shlens, 2014).
The arrangement of productive characters in reed canary grass strengthens the value expression through the Pearson correlation of the association between characters. It can thus be seen from Figure 2 that the main role in biological growth is attributed to the character's bush diameter, bush height, and plant height.
The graphical representation of the PCA arranges the studied productive characters compared to a simulated growth model. In this case, the arrangement of the experimental data that make up the biological growth model of reed canary grass is under the influence of the specific growing conditions of the Low Banat Plain area.
Also, the dendrogram-type cluster analysis of the characters was performed by cluster analysis. The dendrogram cluster analysis was performed the distance between clusters is measured as a correlation. The OY axis shows the degree of similarity and the OX axis shows the biological growth periods and the BBCH code. In the case of the reed canary grass variety Premier, following the cluster analysis, three clusters were obtained in Figure 3.

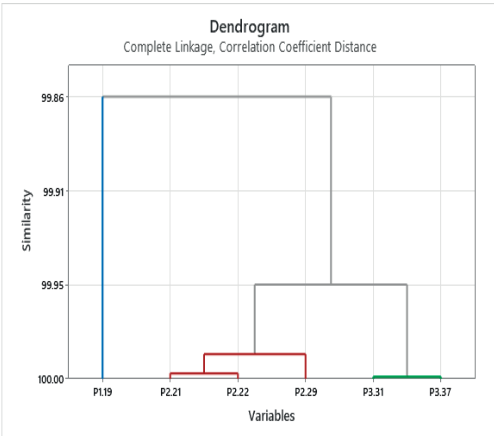

Figure 3. Graphic representation of the dendrogram regarding the externalization of productive characters in reed canary grass

The three clusters determine the grouping of the biological growth stages expressed in BBCH according to the five productive characters studied. The values of the productive characters were obtained on averages of the measurements of several plants within the same BBCH growth period. The intermediate biological growth periods (2.21, 2.22, and 2.29) are grouped in cluster number 2. Towards physiological maturity and the end of the vegetative growth period of the reed canary grass species, cluster 3 groups these stages 3.31 and 3.37.

CONCLUSIONS

Knowledge of the interrelationships between the productive characters is important because these relations ensure not only the productivity of the species but are essential for its regeneration and expansion within the different natural ecosystems where it is present or in the case of agroecosystems where it is cultivated.

In the experimental conditions of the Low Banat Plain area on a gleied chernozem soil, the biological growth of the reed canary grass species is influenced by humidity and temperature conditions. The continuous water regime below the multiannual average of precipitation is noted as an influencing factor.

The calculation of the power of association between the five productive characters by determining the Pearson Correlation highlighted the bush diameter character as a determining character in the biological growth of RCG. The strengthening of the statistical significance of the results obtained were also analyzed by the Chi-squared test. Thus, the power of association of the studied characters is also confirmed by Principal Component Analysis (PCA) where the degree of uniqueness of each character involved in the biological growth corresponds to the significance obtained by the Pearson correlation. The different arrangement of the experimental data compared to a predicted model validates the importance of abiotic growth factors in the biological development of RCG. The biological growth model obtained by following six BBCH stages and read by cluster analysis validates the normal distribution of the growth stages in the life cycle of RCG. In conclusion, RCG has demonstrated a high capacity to adapt to global changes in life factors and we can predict an increase in the cultivation area of the species.

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THE IMPACT OF DIGESTATE ON THE CHEMICAL PROPERTIES OF MAIZE (*Zea mays* L.) GRAINS

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Abstract

Maize grains accumulate nitrogen (N), especially in the form of storage proteins, correlated to zeins and starch content. Digestate, a new bio-based fertilizer rich in essential nutrients, is an effective alternative for mineral fertilizers. The aim of this study was to examine the effect of digestate application on chemical properties and maize grain yield. Field experiment was established during year 2018 on eight different fertilization treatments: control treatment without fertilization, mineral fertilizer (MF), liquid cattle manure, solid fraction of digestate (SFD), liquid fraction of digestate (LFD), digestate, a mixture of MF+SFD, a mixture of MF+LFD. Nitrogen content in grains was positively correlated with proteins (99.93%) and zein (73.91%) content. In addition, the results showed that only the LFD and digestate treatments had a positive correlation between the N content in the grains and the starch content. Statistically higher grain yield was observed on MF (13.1 t ha^{-1}), MF+SFD (11.2 t ha^{-1}) and MF+LFD (12.2 t ha^{-1}) treatments compared to others. Digestate showed positive effect on chemical properties of the maize grain.

Key words: *chemical properties, crop, digestate fractions, grain, maize, nitrogen.*

INTRODUCTION

In recent decades, sustainability has become an indispensable factor for survival, and the agricultural sector plays a crucial role in achieving multiple sustainable development goals by ensuring access to nutritious and healthy food, promoting sustainable agricultural practices and upholding human rights and fair working conditions (FAO, 2023). As the population grows, the demand for food also increases, leading to higher energy consumption and the use of non-renewable energy sources. The growing amount of agricultural waste (farmyard manure and various types of green waste) poses a major environmental challenge for the agricultural industry (European Directive, 2006; Brancoli et al., 2017; Tur-Cardona et al., 2018). In view of the fact that nowadays sustainability is being sought, loops are being closed and a circular economy is being pursued, an effective solution to this problem is processing it by anaerobic digestion in biogas plants. The production of biogas and the by-product digestate are an important source of renewable energy based on raw materials such as liquid manure, straw, hay, maize silage, food industry waste and energy crops (Holm-Nielsen et al., 2009; Wallace, 2011; Makadi et al., 2012; Nkoa, 2014).

Digestate is a high-quality fertilizer product and rich in plant-available nutrients such as nitrogen (N), phosphorus (P), potassium (K) and other macro- and micronutrients, making it a promising and sustainable alternative to mineral fertilizers (Vaneeckhaute et al., 2013; Risberg et al., 2017; Đurđević et al., 2018; Przygocka-Cyna & Grzebisz, 2020; Šatvar Vrbančić et al., 2024).

Maize (*Zea mays* L.) is the second most important crop in the world in terms of agricultural production and yield. In 2023, 1.2 billion tonnes of maize was produced - an increase of 46% since 2010. It is used in all areas, as food, biofuel, animal feed, etc. The largest producers are North and South America, led by the USA and Brazil (42% of global production), followed by China (23%) in Asia. In Croatia, it serves as an important raw material for various industries and is the most widely used of all cereals (Ranum et al., 2014; Mesterházy et al., 2020; FAO, 2023; Jukić et al., 2024).

Grains accumulate considerable amounts of N, especially in the form of storage proteins. These proteins play a crucial role in seed germination and serve as a vital food source for humans and animals (Duvnjak et al., 2020). The protein content ranges from 6% to 20% of the dry matter (DM). This range is influenced by several factors, such as the type of grain, the specific variety, agrotechnical processes, etc. In addition, the storage proteins of maize are classified according to their solubility in different solvents. About 70% of the proteins in maize grains are storage proteins, with zeins (prolamins) accounting for over 60% of this fraction. In addition to zeins, albumins, globulins and glutelins also belong to the storage proteins in maize (Wu et al., 2014). Grain endosperm is mainly composed of protein and starch (about 70% of the composition and consists of two types of carbohydrate chains: amylose and amylopectin) (Martinez et al., 2017).

The most important factors influencing zein and proteins are genotype and environment. Genotype is the most important determinant of zein properties and can contribute to 50-60% of the variability in hybrids, especially those resistant to abiotic and biotic stresses. Environmental factors such as N fertilization, irrigation and high temperatures have less influence, but still cause a small amount of variation within the same cultivar. The remaining 40-50% of the variability is determined by agricultural practices such as fertilization, disease control and pest management carried out by humans. This underscores the critical role of both genetic traits and advances in agricultural practices in optimizing maize quality (Duvnjak et al., 2020; Duvick, 2005).

The aim of this study is to determine the effect of digestate as fertilizer product on chemical properties of maize grain. In this case, the effect of different fertilization treatments on the content of protein, zein and starch in the maize grain.

MATERIALS AND METHODS

Field experiment were conducted on the experimental field Maksimir of the University of Zagreb Faculty of Agriculture, Croatia. Eight different fertilization treatments were carried out: 1-control without fertilization (C), 2-mineral fertilizers NPK 15-15-15+CAN (MF), 3-liquid cattle manure (LCM), 4-solid fraction of digestate (SFD), 5-liquid fraction of digestate (LFD), 6-mixture of solid and liquid fractions of digestate (SFD+LFD), 7-mixture of mineral fertilizer NPK 15-15-15 and solid fraction of digestate digestate (MF+SFD), and 8-mixture of mineral fertilizer NPK 15-15-15 and liquid fraction of digestate (MF+LFD).

The treatments were arranged in a completely randomized block design with four replicates (Figure 1). In addition, the average nitrogen (N) content in the soil was determined using the N_{min} method and amounted to 37 kg N_{min} ha^{-1}. In accordance with the Nitrates Directive (91/676/EEC), which sets a limit of 170 kg N ha^{-1}, 140 kg N ha^{-1} was applied in the field for each treatment.

As a test crop, maize hybrid Pioneer PR 0725 was sown on April 27, 2018 and harvested on September 28, 2018. Maize grain yield was adjusted to a moisture content of 14%.

Figure 1. Field experiment during fertilization

A soil sample was taken in the field from a depth of 0-30 cm, air-dried and sieved through a 2 mm sieve. It was then classified as a silt loam soil with an acid reaction and low humus content (Table 1).

Table 1. Soil characterization of field experiment

Depth cm	pH		Humus %	N_{min} kg ha^{-1} of soil	P_2O_5 mg 100 g^{-1} of soil	K_2O mg 100 g^{-1} of soil
	H_2O	KCl				
0-30	5.47	4.21	1.65	37.34	16.68	21.63

Table 2 presents the characteristics of the organic fertilizer products used at the beginning of the experiment. All materials were analyzed according to standard procedures: dry matter (DM) was measured by determining the residual mass after drying at 105 °C for 48 hours. The pH value was measured with a Mettler Toledo EL20/EL2 pH meter. Total N was determined using Kjeldahl destruction (HRN ISO 11261:2004), phosphorus (P_2O_5) was determined spectrophotometrically, and potassium (K_2O) was analyzed using a flame photometer (AOAC, 2015).
The digestate fractions were collected from the biogas plant Bojana in Čazma in northwest Croatia, where it was produced from a mixture of maize silage and LCM. The LCM was obtained from a near cattle farm.

Table 2. Fertilizer products characterization at the beginning of the experiment

Fertilizer products	pH H_2O	% N FW	% P_2O_5 DM	% K_2O DM
LCM	6.6	0.4	2.5	4.3
SFD	8.7	1.3	1.7	1.4
LFD	7.7	0.8	2.3	7.1

Note. LCM-liquid cattle manure; SFD-solid fraction of digestate; LFD-liquid fraction of digestate; DM: on dry matter; FW: on fresh weight.

The data of average air temperature and precipitation were taken from the Croatian Meteorological and Hydrological Service for the Maksimir station (DHMZ, 2018).
Table 3 shows the average values of temperature and precipitation for each month during maize vegetation growth from May to September 2018 as well as the optimal temperature and precipitation for normal maize growth according to Pucarić et al. (1997).
According to Walters climate diagram (Figure 2), based on the course of the precipitation and temperature curve, it can be concluded that the precipitation curve crosses the temperature curve in August, so there is a dry period on average during August 2018.

Table 3. Average air temperature and precipitation from May to September 2018 with optimal conditions according to Pucarić et al., 1997

	Optimal (Pucarić et al., 1997)		2018.	
Month	AT, °C	P, mm	AT, °C	P, mm
May	18.3	87.5	19.5	68.7
June	21.7	87.5	21.4	127.8
July	22.8	112.5	22.5	85.2
August	22.8	112.5	23.7	40.7
September	warmer and droughter		17.7	59.0

Note. AT: average air temperature; P: precipitation

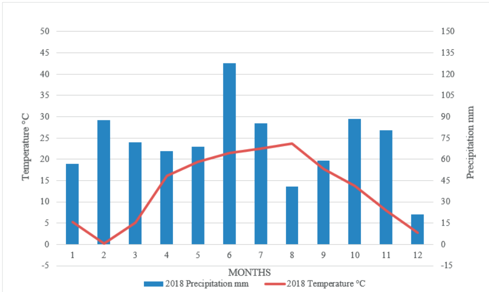


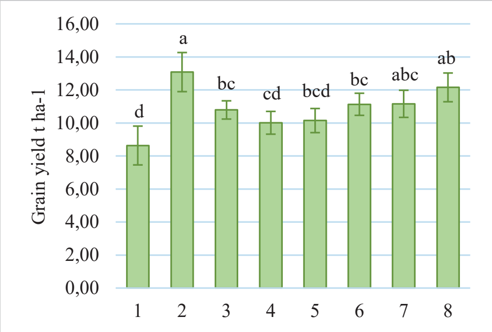
Figure 2. Walters climate diagram for Maksimir 2018

The proteins were calculated according to the following formula: % N x 6.25 (Vajić, 1964). Also, the zeins present in the vitreous and floury endosperm were extracted according to the protocol described by Wallace et al. (1990). In addition, starch and non-starch lipids were isolated from the floury and vitreous endosperm using the sequential extraction method described by Gayral et al. (2015). Both methods are explained in more detail in the work by Zurak et al. (2020).
Statistical analysis was performed using the Statistical Analysis System (SAS) 9.3. Analysis of variance (ANOVA) and mean comparisons (Tukey test, $p<0.05$) were performed to evaluate differences in corn yield between treatments. Additionally, correlation was performed between grain yield and chemical properties of the maize grain (protein, zein and starch content).

RESULTS AND DISCUSSIONS

The highest grain yield (Figure 3) was recorded on MF treatment (13.1 t ha^{-1}), a mixture of MF+SFD (11.2 t ha^{-1}) and a mixture of

MF+LFD (12.2 t ha^{-1}) treatments, while the lowest on C treatment (8.63 t ha^{-1}). It is also worth mentioning that the MF+SFD treatment gave statistically comparable results with MF+LFD treatment.



Note. Letters indicate significant differences between treatments ($p < 0.001$). 1-unfertilized control (C); 2-mineral fertilizers (MF); 3-liquid cattle manure (LCM); 4-solid fraction of digestate (SFD); 5-liquid fraction of digestate (LFD); 6-solid fraction of digestate+liquid fraction of digestate (SFD+LFD); 7-mixture of mineral fertilizer with solid fraction of digestate (MF+SFD); 8-mixture of mineral fertilizer with liquid fraction of digestate (MF+LFD).

Figure 3. Grain yield on different fertilization treatments

According to the Statistical Yearbook of the Republic of Croatia (DZS) (2023), the data for maize production and yield in the last five years (2018-2022) fluctuated between 6.1 and 9.1 t ha^{-1}. Yield variability could also be influenced by annual climatic conditions, as the total yields reported in the Statistical Yearbook of the DZS show. The weather conditions in 2018 contributed to higher yields, which is consistent with the results of this study. Although there was a dry period, precipitation that fell months earlier helped the maize overcome the drought.

Similar results were reported by Chantigny et al. (2008) in a three-year trial with treatments such as control, mineral fertilization, raw manure and digestate. They applied a total N dose of 130 kg N ha^{-1}every year. Over the three-year period, the average maize grain yield was 8.4 t ha^{-1} for the control, 9.6 t ha^{-1} for the mineral fertilizer, 9.7 t ha^{-1} for the raw manure and 9.5 t ha^{-1} for the digestate. On average, all treatments resulted in higher grain yields compared to the control. Przygocka-Cyna & Grzebisz (2020) also carried out a three-year trial with digestate at application rates of 0.2, 0.4 and 0.8 t ha^{-1}. These values correspond to a grain yield of 11.5, 10.8 and 9.2 t ha^{-1} maize grain in 2014, 2015 and 2016, respectively.

Yields can be influenced by many factors such as weather conditions, soil properties, suitable agrotechnical practices, human and many other factors.

Table 4 shows the chemical properties of maize grain (protein, zein and starch content) in different fertilizer treatments. The statistically highest protein content was found in the MF and MF+SFD treatments and the lowest concentration in the C treatment. In addition, the statistically highest zein content was found in MF, LCM and MF+SFD. The starch content was also statistically highest in the MF, LCM, LFD and MF+LFD treatments.

Table 4. Chemical properties of maize grain

Treatment	Protein %	Zein g kg^{-1} DM	Starch g kg^{-1} DM
1	6.8^{b}	28.8^{b}	718.4^{c}
2	8.3^{a}	48.2^{a}	732.1^{abc}
3	7.3^{b}	44.7^{a}	732.8^{abc}
4	7.1^{b}	40.1^{ab}	721.4^{bc}
5	7.3^{b}	38.6^{ab}	746.8^{a}
6	7.2^{b}	39.8^{ab}	719.7^{bc}
7	7.6^{ab}	42.4^{a}	725.5^{bc}
8	7.4^{b}	41.1^{ab}	736.0^{ab}

Note. Letters indicate significant differences between treatments ($p < 0.001$). 1-unfertilized control (C); 2-mineral fertilizers (MF); 3-liquid cattle manure (LCM); 4-solid fraction of digestate (SFD); 5-liquid fraction of digestate (LFD); 6-solid fraction of digestate+liquid fraction of digestate (SFD+LFD); 7-mixture of mineral fertilizer with solid fraction of digestate (MF+SFD); 8-mixture of mineral fertilizer with liquid fraction of digestate (MF+LFD); DM: on dry matter.

The maize grain yield was positively correlated with the zein concentration (67.9 %) in all treatments. Table 5 shows the correlation for each fertilization treatment separately. Additionally, it shows which of the treatments had the strongest correlation between maize grain yield and zein. The MF+LFD (99.6 %), MF (90.7 %) and LFD (88.0%) treatments showed the strongest and highest positive correlation between maize grain yield and zein, while the lowest correlation was found for C treatment (2.9 %).

Table 5. Correlation of maize grain yield and zein between treatments

Treatment	Grain yield and zein correlation %
1	2.9
2	90.7
3	42.8
4	34.6
5	88.0
6	60.6
7	32.8
8	99.6

Note. 1-unfertilized control (C); 2-mineral fertilizers (MF); 3-liquid cattle manure (LCM); 4-solid fraction of digestate (SFD); 5-liquid fraction of digestate (LFD); 6-solid fraction of digestate+liquid fraction of digestate (SFD+LFD); 7-mixture of mineral fertilizer with solid fraction of digestate (MF+SFD); 8-mixture of mineral fertilizer with liquid fraction of digestate (MF+LFD).

Since N directly influences yield, grain protein content (Tsai et al., 1978; Pearson & Jacobs, 1987; Uhart & Andrade, 1995) and grain hardness (Tamago et al., 2016), it also affects the zein concentration in the endosperm. In addition to this study, in which it can be clearly seen that N content is an important part for the zein concentration, this effect was also observed under field (Gerde et al., 2016; Tsai et al., 1978; Tsai et al., 1984; Ahmadi et al., 1995) and in vitro conditions (Singletary et al., 1990). As shown in Figure 4 N content was strongly and positively (<.0001) correlated with the concentration of proteins (99.93 %) and zeins (73.91 %) in the grains in all treatments, while starch gave negative correlation with N content.

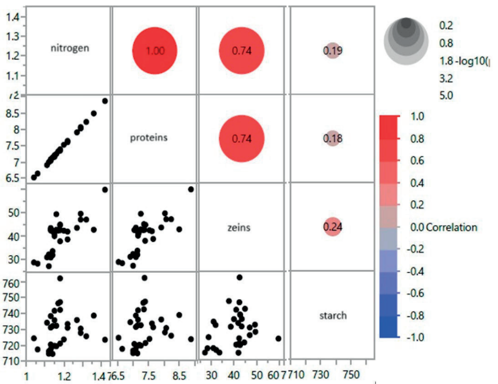


Figure 4. Correlation between N, proteins, zeins and starch as scatterplot matrix

From June to August 2018 there was a drought that could lead to a reduced of the starch content. Further, heat stress can also lead to a reduction in the starch content of the grains, while the protein content can increase (Tester & Karkalas, 2001; Wang & Frei, 2011; Thitisaksakul et al., 2012), what was also the case in this research.

CONCLUSIONS

In conclusion, this study emphasizes the important role of N accumulation in maize grains, especially in the form of storage proteins, which are strongly correlated with the zein and protein content. Digestate as a bio-based fertilizer product, rich in essential nutrients has proven its potential as an effective alternative to mineral fertilizers. The field experiment revealed that the N content in the maize grains was strongly associated with protein and zein content. The highest grain yields were obtained in treatment with mineral fertilizer (MF) and combined treatments (MF+SFD and MF+LFD), highlighting the synergistic effects of integrating digestate with mineral fertilizers.

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THE CONTROL OF WEEDS IN MAIZE USING NEW HERBICIDES FORMULATIONS

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Abstract

Worldwide grain yield in maize may be severely impacted by competition with weeds. To evaluate the efficacy of novel herbicide formulations for weed control in maize, a randomized complete block design trial comprising three replications and seven treatments was carried out at the experimental field of the Agricultural and Research Station Caracal in 2023 year. All of the treatments, which were made up of separate and related herbicides, were considered to be selective in maize after emergence (POST). Their effectiveness was evaluated at 7, 14, 21, and 28 days after each treatment targeting the most common weeds, such as HIBTR, DIGSA, SOLNI, ATRPL, AMARE, CONAR, POROL, XANTIST, CIRAR. The findings indicated that of the herbicidal treatments, the best efficacy was recorded by Click Trio (2 L/ha) with efficacy between 80-100% even at 7 days after treatment for most of the targeted weeds. Also, a good control of targeted weeds from maize crop was done by Pyxides WG 562.5 g/kg + Adigor ADJ and the combination SAE 0.53 H/01+ Baracuda controlled weeds 100% especially at 14, 21 and 28 days after treatment, excepting Convolvulus arvensis (CONAR), Hibiscus trionum (HIBTR).

Key words*: weeds, new herbicides, Zea mays L., efficacy, control.*

INTRODUCTION

Worldwide, climate changes directly affect the production of food and natural vegetation features, having costs consequences (Feng et al., 2020; Li et al., 2011; Păunescu et al., 2022; Răduţoiu and Băloniu, 2021; Răduţoiu, 2022; Răduţoiu and Stan, 2022; Răduţoiu and Ștefănescu, 2022; Răduţoiu, 2023; Velea et al., 2021; Wolf and Van Diepen, 1995).
As the global population explosion progresses, more food, energy and goods are needed (Bonciu, 2023). Today, maize is considered one of the most important crops for food, fodder, and industry, therefore it is cropped for grain, seeds, green feed, silage and biofuel (ethanol) (Millet et al., 2019; Smith et al., 2004; Veljković et al., 2018). Moreover, maize is so versatile cereal crops that can adapt widely to a variety of agroclimatic conditions (Borleanu et al., 2012; Fun et al., 2007; Kumar et al., 2018; Partal et al., 2012a, 2012b). Despite its versatility, the biotic and abiotic stress factors suppress the growth and performance of maize reducing yield and crop productivity. Thus, the main goal of agricultural research is to produce more food with less resources using breeding and bioengineering by including genes for resistance to herbicides, diseases, drought, etc. in plants genome, aside to alternative cropping methods that could increase the yield and quality of agricultural products (Alkan et al., 2022; Bonciu et al., 2021; De Souza and Bonciu, 2022; Lipianu et al., 2023; Paraschivu et al., 2022; Partal and Paraschivu, 2020; Păunescu et al., 2021; Sălceanu et al., 2022).
Globally, weeds are considered one of the most important biotic constrainers due to their competition for light, water and nutrients in maize fields (Gharde et al., 2018). Various studies have previously emphasized the negative effects of annual and perennial weed species on maize yield in the field, with global losses ranging from 28 to 100% of total maize production worldwide, depending on the composition of geographic locations, the weed

composition and each specie density and intensity and the stage of maize crop development (Brankov et al., 2021; Idziak et al., 2022; Imoloame and Omolaiye, 2016; Jagadish et al., 2016; Mhlanga et al., 2016; Samant et al., 2015; Sharma and Rayamajhi, 2022; Soare et al., 2010a; Tesfay et al., 2014; Zhang et al., 2013).

Worldwide, the main obstacle to increasing maize yield has to do with managing and controlling weed development. Because of their quick outcomes, easy application, low cost, and reduced labor requirements, herbicides are absolutely essentially as a necessary component of maize technology (Idziak et al., 2022; Qu et al., 2021; Sharma & Rayamajhi, 2022).

In practice, farmers heavily apply herbicides both before and after the emergence of maize fields. Pre-emergence herbicides have a 40-50 day half-life in the soil, however post-emergence foliar spray is necessary to control secondary weed infestation (Delchev, 2021). To acquire the targeted results from herbicides application, the proper herbicide must be used at the right time and dosage. However, weeds will be more affected when pre-emergence and post-emergence herbicides are used in tandem to target both annual and perennial weeds (Idziak et al., 2022).

As part of an integrated weed management approach, herbicides will continue to be considered a valuable tool in agriculture for weed control in the future.

The current study's goal was to assess the efficacy and selectivity of new herbicide formulations for managing weeds in maize using various bioactive components under natural settings from ARDS Caracal, Romania.

MATERIALS AND METHODS

In 2023 year, an experimental trail was conducted at the University of Craiova, Romania's Agricultural Research Station Caracal (ARDS) (44°11'N and 24°37'E) to assess the relative effectiveness on weed control in maize of new herbicide formulations combined with various bioactive ingredients. Split plots with three replicates were used for the trial, which was set up in a randomized full block (RCBD-Fisher model). Every trial plot was 25 m² in size. Standard recommended cultural methods were used, including two disc harrowings and two cultivations procedures prior to sowing, as well as fertilization with 250 kg ha-1 NPK 15:15:15 and spring top-coat fertilization with 200 kilogram/ha NH4NO3.

All of the treatments, which consisted of separate and related herbicides, were considered to be selective when applied to maize by post-emergence (POST) moments.

The following treatments were used in the experiment:

V1. Untreated - control;
V2. Principal Forte WG 606 g/kg (62.475 g nicosulfuron + 31.25 g rimsulfuron + 510.42 g dicamba + 31.25 g Isoxadifen) – 0.48 kg/ha;
V3. Click Trio EC 490 g/l (75 g mesotrione + 375 g terbutilazin + 40 g clomazona) – 2 kg/ha;
V4. Click Pro EC 376 g/l (50 g mesotrione + 326 g terbutilazin) – 2.3 kg/ha;
V5.Pyxides WG 562.5 g/kg + Adigor ADJ (312.5 g dicamba + 150 g mesotrione + 100 g nicosulfuron + 47% rapeseed methylate oil) – 0.4 kg/ha + 1 l/ha;
V6. SAE 053 H/01 + Nico 40 OD (80 mesotrione +30 nicosulfuron + 40 nicosulfuron) – 1.2 l/ha + 0.5 l/ha;
V7. SAE 053 H/01 + Baracuda (80 mesotrione +30 nicosulfuron + 100 g mesotrione) – 1.2 l/ha + 0.5 l/ha;

According with the recommendations the herbicides Click Pro and Click Trio were sprayed post-emergent in BBCH 12-14, when maize had 2-4 leaves, while the herbicides Principal Forte, Pyxides+ Adigor, SAE 053 H/01 + Nico 40 OD, SAE 053 H/01 + Baracuda were applied post-emergent in BBCH 14-16 when maize had 4-6 leaves. There were used 400 l/ha solution applied using a back sprayer equipped with fan nozzles, a gasoline engine, and a 25 L tank. Weed species and densities were assessed before the trials were set up. The weed species, development stages, and quantity of each weed species in the covered area (m^2) were realized using a 1 m^2 frame in the trial area randomly replaced.

Therefore, prior to spraying, the following species were the focus of the initial assessment of the weed spectrum: *Hibiscus trionum* (HIBTR), *Digitaria sanguinalis* (DIGSA), *Solanum nigrum* (SOLNI), *Atriplex patula* (ATRPL), *Convolvulus arvensis* (CONAR), *Portulaca oleracea* (POROL), *Xantium strumarium* (XANTIST), and *Amaranthus retroflexus* (AMARE), *Cirsium arvense* (CIRAR).

The critical period of crop-weed competition is determined by the density and periodicity of weed population emergence. Consequently, the following formula was used to determine each species' densities:

$$\text{Density (plants/m}^2\text{)} = B/m,$$

where "m" stands for the total number of meters and "B" for the total number of individual plants in the samples (Odum and Barrett, 1971).

Additionally, the species density was calculated using the scale proposed by Üstüner and Güncan (2002) (Table 1).

Table 1. The scale used for assess weeds density

Scale	Density level	Density (plants/m^2)
A	High dense	10+
B	Dense	1-10
C	Middle dense	0.1-1
D	Low dense	0.01-1
E	Rare	Less than 0.01

At four regular intervals of on the 7th, 14th, 21th and on the 28th day after spraying was made the assessment of herbicides efficacy on weed population and weed species. For each assessment was calculated the percentage decrease in the weed population by comparing the treated plots with the weedy control plot. The phenology of the weeds and their impacts are detailed in each evaluation.

The impacts on weeds at the species level and the effects on all weeds were calculated using the Abbott formula (Snedecor et al., 1967):

$$HPE = (CWN - TWN) \times 100/CWN$$

Where: "HPE" indicates Herbicide Percentage Effect, "CWN" indicates Number of Weeds in Control, "TWN" indicates Number of Weeds in Treatments.

The 9-score EWRS scale, as outlined by Zhelyazkov et al. (2017), was used to assess the herbicides' selectivity (score 0 indicates there are not damages on the crop, and score 9 indicates the crop is fully damaged). ANOVA and the mathematical features of Microsoft Office Excel 2013 were used to conduct a statistical analysis of the data that was gathered. Also, the Newman-Keuls complementary test for multiple comparisons was used for significant statistical differences ($p<0.05$).

RESULTS AND DISCUSSIONS

All over the world weeds have been showed their unfavorable impact on maize plants development in terms of their competition for light, water and nutrients with negative consequences on grain yield (Acharya et al., 2022; Reddy et al., 2022; Sharma and Rayamajhi, 2022). Therefore, a good strategy to reduce the negative consequences of weeds on maize output is to combine all mechanical, chemical, and cultural control strategies into a Weeds Integrated Management System.

The expected effect of the herbicides sprayed throughout the trial was different depending on the weed species and the herbicide's active components. A large amount of weeds will be damaged when pre-emergence and post-emergence herbicides are used together to target both annual and perennial weeds. Furthermore, the efficacy of herbicides differed depending on the evaluation periods. Also, the effectiveness and selectivity of herbicides in maize have been the subject of numerous prior research (Alptekin et al., 2023; Brankov et al., 2021; Grzanka et al., 2022; Iqbal et al., 2020; Jagła et al., 2020; Sairam et al., 2023; Sălceanu et al., 2024; Soare et al, 2010b).

However, weeds may develop a resistance problem if a single herbicide or herbicides with the same mechanism of action are used continuously. As a result, new herbicides are required to manage the mixed weed flora in maize. Thus, the use of these new realised dual purposes herbicides offers the opportunity for a new mode of action for weed management in maize, especially on grasses, broadleaved weeds and rhizomatous perennial temperate weeds (Kakade et al., 2020; Şerban et al., 2021). They initially impact meristemic tissues, whose growth stops shortly after spraying, quickly developing chlorosis and necrosis and it takes an additional three to four weeks for the mature plant portions to dieback. One of the most important advantages of these new dual purposes herbicides is that they act at very low dose reducing the environmental concern, which is a very important issue currently. The most common weed species in maize crops are: monocotyledons (*Setaria* sp., *Echinochloa crusgalli*, , *Elymus repens*, *Sorghum halepense* (seed and rhizomes), *Eriochloa villosa* and dicotyledons: *Amaranthus retroflexus*, *Solanum nigrum*, *Raphanus raphanistrum*, *Thlaspi arvensis*, *Datura stramonium*, *Cirsium arvense*, *Convolvulus arvensis*, *Chenopodium album*, *Sinapis arvensis*, *Stellaria media*, *Hibiscus trionum*, *Abutilon theophrasti*, *Sonchus arvensis* (Popescu et al, 2009).With a mono-to-dicotyledonous weed ratio of 6:94, the complex weed structure in the maize field trail at ARDS Caracal resulted in a 97% infestation degree.

The assessment results showed that depending on the previous crop and pedo-climatic conditions, the highest percentage of the weeds were dicotyledonous plants either annual or perennial. Among annual monocotyledonous only *Digitaria sanguinalis* (DIGSA) (6%) was present, while no perennial monocotyledonous was noticed. Among annual dicotyledonous different percentages were assessed depending on weed species, such as *Solanum nigrum* (SOLNI) – 49%, *Atriplex patula* (ATRPL) – 22%, *Portulaca oleracea* (POROL) – 16%, *Hibiscus trionum* (HIBTR) – 3%, *Xantium strumarium* (XANTIST) – 1%, *Amaranthus retroflexus* (AMARE) – 0%, while among perennial dicotyledonous only *Convolvulus arvensis* (CONAR) recorded 4% and *Cirsium arvense* (CIRAR) 0% (Figure 1).

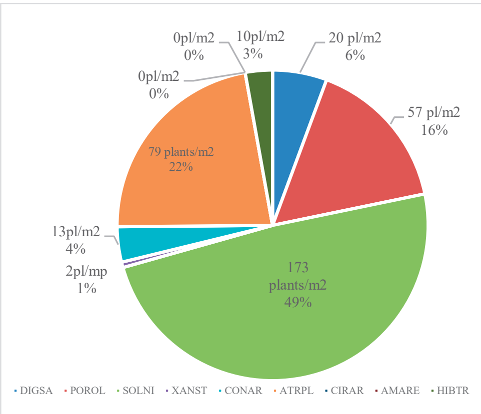

Figure 1. The weed species assessed in maize crop in 2023 year in ARDS Caracal

The highest density of weed species were found as *Solanum nigrum* (SOLNI) (49%), *Atriplex patula* (ATRPL) (22%) and *Portulaca oleracea* (POROL) (16%). Using the scale proposed by Üstüner and Güncan (2002) in the weeds evaluation it was noticed that weed density (weeds/m^2) varied between Dense (B) and High-Dense (A).

It was noticed that excepting the variant 3 (Click Trio Ec 490 g/l (75 g mesotrione + 375 g terbutilazin + 40 g clomazona – 2 kg/ha - B), targeted weeds density was high (A) for all variants at 7 days after treatments application. When the herbicides Click Trio Ec 490 g/l (75 g mesotrione + 375 g terbutilazin + 40 g clomazona – 2 kg/ha), Click Pro Ec 376 g/l (50 g mesotrione + 326 g terbutilazin – 2.3 kg/ha), Pyxides WG 562.5 g/kg + Adigor ADJ (312.5 g dicamba + 150 g mesotrione + 100 g nicosulfuron + 47% rapeseed methylate oil –0.4 kg/ha + 1l/ha) and SAE 053 H/01 + Nico 40 OD (80 mesotrione +30 nicosulfuron + 40 nicosulfuron – 1.2 l/ha + 0.5 l/ha) were applied it was noticed a lower weeds density (B) at 14 days after treatments. When the herbicide Principal Forte WG 606 g/kg (62.475 g nicosulfuron + 31.25 g rimsulfuron + 510.42 g dicamba + 31.25 g Isoxadifen – 0.48 g/ha) was applied it was observed that at 21 days after treatment weeds density was diminished for all targeted weeds, but still high for variant 2, comparatively with the control (V1).

At 28 days after treatments the weeds density decreased for all treated variants (B) and all herbicides formulations showed high efficacy comparatively with the untreated control variant for both annual and perennial weed species (Figure 2).

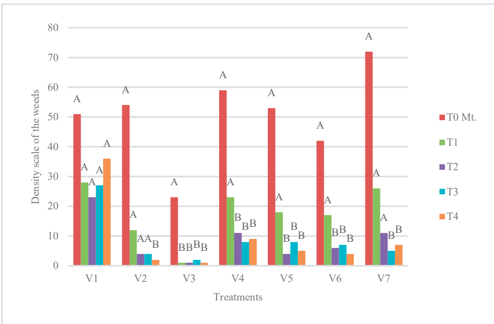

Figure 2. The impact of herbicides on weeds density at 7, 14, 21 and 28 days after treatment

In accordance with selectivity, application and evaluation timing, weed stage, infestation level, and climate, the herbicides' efficacy (HPE, or Herbicide Percentage Effect) varied from 0% to 100%.
Figure 3 shows the average efficacy results (%) noticed in weeds density after the application of Principal Forte WG 606 g/kg (62.475 g nicosulfuron + 31.25 g rimsulfuron + 510.42 g dicamba + 31.25 g Isoxadifen) – 0.48 g/ha (V2).

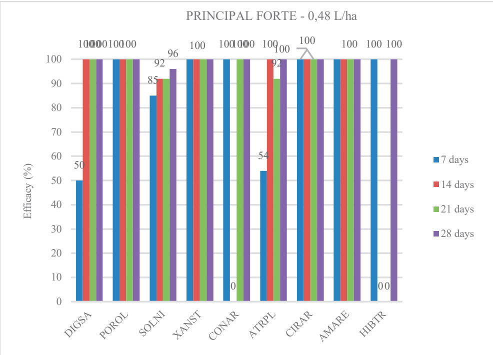

Figure 3. The Efficacy (%) at 7, 14, 21, 28 days after treatment with Principal Forte WG 606 postemergently applied at the stage BBCH 14-16

It was noticed a good efficacy of 85-100% at 14, 21 and 21 days after treatments in controlling annual dicotyledons (*Portulaca oleracea* (POROL), *Xantium strumarium* (XANTIST), *Amaranthus retroflexus* (AMARE), *Solanum nigrum* (SOLNI), *Atriplex patula* (ATRPL) and perennial dicotyledons *Cirsium arvense* (CIRAR), while the lowest efficacy (50%) was observed in the annual monocotyledons *Digitaria sanguinalis* (DIGSA).
The best effect in controlling weeds (100%) at 7 days after treatment was noticed when the herbicide Click Trio EC 490 g/l (75 g mesotrione + 375 g terbutilazin + 40 g clomazona) – 2 kg/ha was applied, excepting the annual monocotyledons *Digitaria sanguinalis* (DIGSA) in which case the herbicide efficacy was 67% at 7 and 14 days after spraying. Even al 14 days after treatment the herbicide efficacy Was 100% for all weeds targeted, excepting annual dicotyledons (*Portulaca oleracea* (POROL), in which case was of 80%.
At 21 and 28 days after treatment the herbicide efficacy was 100% excepting *Convolvulus arvensis* (CONAR – 50% efficacy) (Figure 4). Click Trio EC 490 g/l it contains three active substances (mesotrione + 375 g terbutilazin + 40 g clomazona) that have systemic and residual action high selectivity due to the controlled release of clomazone.

Figure 4. The Efficacy (%) at 7, 14, 21, 28 days after treatment with Click Trio EC 490 g/l postemergently applied at the stage BBCH 12-14

The results emphasized that efficacy ranged between 0% to 100% when the herbicide Click Pro EC 376 g/l (50 g mesotrione + 326 g terbutilazin) – 2.3 kg/ha was applied (Figure 5) depending on the assessment moment. Thus, at 7 and 28 days after treatment the control of *Convolvulus arvensis* (CONAR) and *Hibiscus trionum* (HIBTR) was 0%.

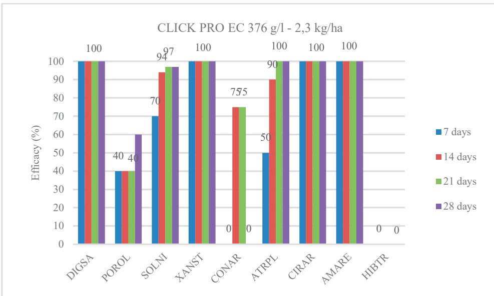

Figure 5. The Efficacy (%) at 7, 14, 21, 28 days after treatment with Click Pro EC 376 g/l applied postemergently at the stage BBCH 12-14

Among all targeted weeds only *Hibiscus trionum* (HIBTR) had no response to Click Pro EC 376 treatment, while for controlling *Convolvulus arvensis* (CONAR) it was noticed an efficacy of 75% at 14 and 21 days after treatment. In case of the annual dicotyledons (*Portulaca oleracea* (POROL) it was noticed an efficacy of 40% after 7, 14 and 21 days after treatment and 60% after 28 days after treatment.

A good efficiency was observed in controlling the aannual dicotyledonous *Solanum nigrum* (SOLNI) of 70% at 7 days after treatment and over 94% at 14, 21 and 28 days after spraying. The greatest efficacy 100% of Click Pro EC 376 with observed in controlling the annual monocotyledons *Digitaria sanguinalis* (DIGSA), annual dicotyledonous *Amaranthus retroflexus* (AMARE) and *Xantium strumarium* (XANTIST) and perennial dicotyledonous *Cirsium arvense* (CIRAR).

Adigor is a blend of surfactant and methylated canola oil for use with a wide range of crop protection products to improve the reliability of weed control, being one of the most effective adjuvant to enhance the efficacy of dual purposes herbicides respectively. Additionally, it lessens the negative effects of abiotic constraints, like vigor losses from herbicide treatments and a quicker recovery of vegetative growth. The average effectiveness outcomes of the herbicide combination Pyxides WG 562.5 g/kg + Adigor ADJ (312.5 g dicamba + 150 g mesotrione + 100 g nicosulfuron + 47% rapeseed methylate oil) –0.4 kg/ha + 1 l/ha was observed especially at 14, 21 and 28 days after treatments, when ranged between 70-100%. Thus, all the targeted weeds showed a high degrees of control that ranged between75% to 100%, excepting the annual dicotyledons *Hibiscus trionum* (HIBTR) that was 100% controlled only after 7 days after treatment. The efficacy of the herbicide Pyxides WG 562,5 g/kg + Adigor ADJ ranged between 60-70% at 7 days after treatment in case of the annual dicotyledons (*Portulaca oleracea* (POROL) and annual dicotyledons *Atriplex patula* (ATRPL) and *Solanum nigrum* (SOLNI) (Figure 6).

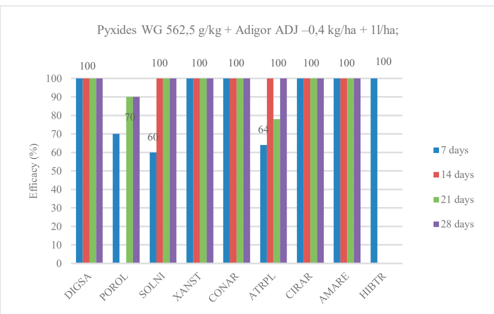

Figure 6. The Efficacy (%) at 7, 14, 21, 28 days after treatment with Pyxides WG 562.5 g/kg + Adigor ADJ applied postemergently at the stage BBCH 14-16

The herbicide Pyxides WG 562.5 g/kg + Adigor ADJ emphasized 100% efficacy in controlling *Cirsium arvense* (CIRAR), *Xanthium strumarium* (XANST), *Digitaria sanguinalis* (DIGSA), *Convolvulus arvensis* (CONAR) and *Amaranthus retroflexus* (AMARE) at 7, 14, 21 and 28 days after treatment. The highest efficacy degree 100% was noticed also when the herbicide SAE 053 H/01 – 1.2 l/ha was mixed with + Nico 40 OD – 0.5 l/ha in controlling weeds such as *Digitaria sanguinalis* (DIGSA), *Amaranthus retroflexus* (AMARE) and *Cirsium arvense* (CIRAR) at 7, 14, 21 and 28 days after treatment. In case of *Atriplex patula* (ATRPL) and *Solanum nigrum* (SOLNI) this combination showed lower efficacy that ranged of 55% to 76% at 7 days after treatment. No efficacy was noticed in *Convolvulus arvensis* (CONAR) at 14, 21 and 28 days after treatment, but a complete control (100%) was noticed at 7 days after treatment.

In case of *Portulaca oleracea* (POROL), *Atriplex patula* (ATRPL) and *Solanum nigrum* (SOLNI), the efficacy of the the herbicide formulation SAE 053 H/01 + Nico 40 OD applied postemergently (BBCH 14-16) at 7 days of treatment ranged between 55-80%, but it was 100% at 14, 21 and 28 days after treatment. This herbicide combination had no efficacy *Hibiscus trionum* (HIBTR) (Figure 7).

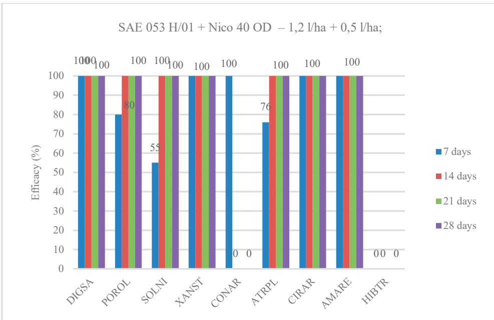

Figure 7. The Efficacy (%) at 7, 14, 21, 28 days after treatment with SAE 053 H/01 + Nico 40 OD applied postemergently at the stage BBCH 14-16

The mixture between the herbicides SAE 053 H/01 and Baracuda showed an efficacy that ranged from 13% to 100% at 7 days after treatment. This mix efficacy increased significantly over 95% at 14, 21 and 28 days after treatment in controlling weeds such as

Digitaria sanguinalis (DIGSA), *Solanum nigrum* SOLNI), *Xanthium strumarium* (XANST), *Atriplex patula* (ATRPL), (*Cirsium arvense* (CIRAR), *Amaranthus retroflexus* (AMARE), *Portulaca oleracea* (POROL). The lowest efficacy of this herbicides mixture was observed in *Hibiscus trionum* (HIBTR) and *Convolvulus arvensis* (CONAR) (Figure 8).

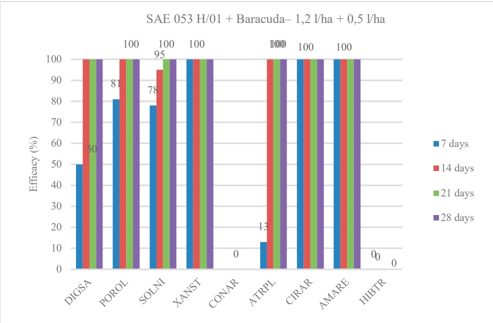

Figure 8. The Efficacy (%) at 7, 14, 21, 28 days after treatment with SAE 053 H/01 + Baracuda applied postemergently at the stage BBCH 14-16

All herbicides treatments tested in the trial showed no phytotoxic effects (EWRS scale = 0) (Table 2).

Table 2. The selectivity (%) of herbicide treatments applied at the maize crop 2023

<table>
<tr><th rowspan="2">Var.</th><th rowspan="2">Treatments</th><th rowspan="2">Dose</th><th rowspan="2">Time *</th><th colspan="4">Selectivity %</th></tr>
<tr><th>7</th><th>14</th><th>21</th><th>28</th></tr>
<tr><td>1</td><td>Untreated – control</td><td>-</td><td>-</td><td colspan="4" rowspan="7">No phytotoxic effects**</td></tr>
<tr><td>2</td><td>Principal Forte</td><td>0.48 kg/ha</td><td>P-EM</td></tr>
<tr><td>3</td><td>Click Trio EC</td><td>2 kg/ha</td><td>P-EM</td></tr>
<tr><td>4</td><td>Click Pro EC</td><td>2.3 kg/ha</td><td>P-EM</td></tr>
<tr><td>5</td><td>Pyxides WG + Adigor ADJ</td><td>0.4 kg/ha + 1 l/ha</td><td>P-EM</td></tr>
<tr><td>6</td><td>SAE 053 H/01 + Nico 40 OD</td><td>1.2 l/ha + 0.5 l/ha</td><td>P-EM</td></tr>
<tr><td>7</td><td>SAE 053 H/01 + Baracuda</td><td>1.2 l/ha + 0.5 l/ha</td><td>P-EM</td></tr>
</table>

*P-EM = Post-Emergent in BBCH 14-16, when maize had 4-6 leaves
** (EWRS scale = 0, where 0 means not damages on the crop, and score 9 means the crop is fully damaged).

The experiment's findings demonstrated that by keeping weeds below the threshold level, herbicides provide an economical and effective way to manage weed populations before crop-weed conflict arises.

CONCLUSIONS

Today, high-yielding agriculture heavily depends on herbicides, as they constitute a vital and integral component of weed management practices. Therefore, using herbicides to reduce weeds before, during, and after emergence will be the most cost-effective and efficient way to manage weeds in maize.

All of the herbicide treatments that were utilized in the experiment showed no phytotoxic effects and had good selectivity for the maize plant.

The experiment's findings showed that all weed control treatments had a substantial impact on weed density at 21 and 28 days after sowing (DAS), excepting V4 (Click Pro EC 376 g/l (50 g mesotrione + 326 g terbutilazin) – 2.3 kg/ha) that showed low control of the annual dicotyledons (*Portulaca oleracea* (POROL), the herbicide efficacy ranging between 40% to 60%.

Among all assessed weeds the most difficult to control were *Hibiscus trionum* (HIBTR) and *Convolvulus arvensis* (CONAR). Despite of this, the best control of *Convolvulus arvensis* (CONAR) was done by Pyxides WG 562.5 g/kg + Adigor ADJ –0.4 kg/ha + 1l/ha, while the best control of *Hibiscus trionum* (HIBTR) was done by Click Trio EC 490 g/l– 2 kg/ha, at 7, 14, 21 and 28 days after treatment.

All herbicides formulations showed 100% efficacy in controlling *Xanthium strumarium* (XANST), *Amaranthus retroflexus* (AMARE) and (*Cirsium arvense* (CIRAR) for all assessed moments. Among all tested herbicide formulations Click Trio EC 490 g/l (75 g mesotrione + 375 g terbutilazin + 40 g clomazona) – 2 kg/ha emphasized 100% efficacy in controlling of all targeted weeds to 7 days after treatment, excepting (*Digitaria sanguinalis* (DIGSA) when the efficacy was only 67%.

The best control of targeted weeds from maize crop was assured by the new herbicides formulations Click Trio EC 490 g/l– 2 kg/ha and Pyxides WG 562.5 g/kg + Adigor ADJ–0.4 kg/ha + 1l/ha. Also, the combination SAE 0.53 H/01+ Baracuda (1.2 l/ha + 0.5 L/ha) proved 100% efficacy in controlling targeted weeds, especially at 14, 21 and 28 days after treatment, excepting *Hibiscus trionum* (HIBTR) and *Convolvulus arvensis* (CONAR).

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ADAPTATION OF SPRING FIELD CROP TECHNOLOGY TO CHANGING CLIMATE CONDITIONS

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Abstract

Trends of changing climatic conditions in recent years have created serious challenges for the cultivation of the region`s traditional spring crops. Analysis of agro-climatic conditions shows a shift towards prolonged rainless periods combined with extremely high temperatures leading to heat and water stress. This worsens the hydrothermal conditions during the vegetative and generative stages. The succession of warm winters and the resulting unhardened plants are stressed, and the significant temperature amplitudes after the resumption of vegetation further deteriorate the phytopathological environment. Conditions of permanent soil and atmospheric drought are observed during all phenological phases. All this creates unfavourable conditions for the development of spring crops. They are particularly dangerous at the generative stage of plant development, which, combined with water deficit conditions, results in the impossibility of growing high-yielding mid- to late-season hybrids under non-flooded conditions. The analysis of conditions in recent years shows that optimisation and balance of the standard technological regimes of crop cultivation should be sought on the one hand and a differentiated approach applied to the different soil types, rainfall and temperature zones on the other.

Key words: *climate change, agrometeorological conditions, spring crops, abiotic stress, drought.*

INTRODUCTION

Agriculture is among the most vulnerable sectors of the economy to climate change. In recent years, fluctuations in agrometeorological conditions have posed challenges for Bulgarian farmers, necessitating adjustments in agronomic practices. The increasing trend in air temperatures, changes in precipitation distribution, and the heightened frequency and intensity of extreme weather events - such as floods, droughts, and thermal and hydric stress - have led to variability and reductions in yields of major agricultural crops.

The Plovdiv region is one of the most favorable areas for agriculture in Bulgaria, with a long-standing tradition of cultivating cereal crops, which occupy up to 50% of the arable land in the region (Agrostatistics, No. 418 - November 2022). Over the past three decades, there has been a deterioration in climatic conditions, consistent with the overall trend of rising air temperatures and seasonal shifts in precipitation distribution, albeit without a clear pattern for Bulgaria (WMO, 2021, 2022). The country's mean annual temperature increased by 0.8°C during the period 1991-2020 compared to 1961-1990 (Marinova, 2023). Positive annual and seasonal anomalies are most pronounced in Northern Bulgaria. Although the total annual precipitation amount has not changed significantly in the period 1991–2020 relative to the previous period, substantial reductions (up to 30%) have been observed in mountainous areas, whereas in Northeastern Bulgaria, local increases of up to 40% have been recorded. The same study (Marinova, 2023) identifies shifts in precipitation patterns post-1990, with an increasing contribution of heavy rainfall events (≥30 mm/24 h) to total annual precipitation, while the contribution of light (≤5 mm/24 h) and moderate (5–15 mm/24 h) rainfall events has declined, following the general regional trend (Alpert et al., 2002).

In a separate study, Malcheva and Bocheva (2023) demonstrated that during the period 1991-2020, significant changes occurred in the distribution of the main climate subtypes according to the Köppen classification. The transition from colder to warmer and/or drier climatic conditions has affected approximately 36% of Bulgaria's territory.

As a result, agrometeorological conditions have also undergone transformations. Between 1986 and 2015, an extension of the potential growing season but a shortening of the actual growing season has been observed, along with an increase in potential evapotranspiration, a decline in precipitation totals over certain periods (particularly in Southern Bulgaria), a decrease in soil moisture reserves, and an increase in soil water deficit during the growing season (Georgieva et al., 2022).

Given the pressure of climatic factors on the economic viability of agricultural production and food security, the global scientific community has been developing strategies and measures to facilitate the adaptation of agriculture to the changing environmental conditions (Olesen et al., 2011; Grigorieva et al., 2023). These measures are formulated at national, regional, and local levels based on risk and vulnerability assessments for specific regions.

Advancements in agricultural technologies in recent years offer opportunities for transforming agri-food systems towards climate-resilient and environmentally sustainable practices.

With the objective of selecting suitable adaptation measures for agriculture under changing environmental conditions, the present study aims to assess the agroclimatic resources of the Plovdiv region for the cultivation of spring crops.

MATERIALS AND METHODS

Study area

The studied area is part of Central Southern Bulgaria. It falls within the climatic region of Central Eastern Bulgaria, classified under the transitional-continental climatic subregion and the European-continental climatic zone (Sabev et al., 1959).

The climatic region of Central Eastern Bulgaria is characterized by relatively mild winters. The mean annual temperatures in the area range between 12-13°C, and precipitation is relatively evenly distributed throughout the year, with the majority occurring in spring and autumn. These conditions are particularly favorable for the cultivation of wheat and barley, which are among the primary cereal crops in the region.

Winter temperatures in Plovdiv are moderate, with a low risk of prolonged frost and freezing events. The average winter temperatures range from -1 to 5°C, which is suitable for winter wheat and barley varieties that are tolerant to lower temperatures. Summer temperatures frequently reach 35-40°C, creating favorable conditions for the growth of spring cereal crops such as maize. The accumulation of heat during the grain ripening period is crucial for the formation of dry matter in the kernels.

The average annual precipitation is approximately 500-600 mm, which is a moderate amount but often insufficient for certain crops without irrigation. Due to the relatively mild winter, spring arrives early, with temperatures consistently exceeding 5°C by late February to early March.

Data

The study utilizes data on key meteorological variables, including air temperature (minimum, maximum, and daily mean [°C]), daily precipitation sum [mm], relative air humidity [%], wind speed [m/s], and wind duration [h]. The study period covers 1991–2020, with data sourced from two stations: the Plovdiv synoptic station and the Sadovo climate station, both part of the meteorological network of the National Institute of Meteorology and Hydrology (NIMH).

Indices

Key agrometeorological indices were analyzed, including:

- Dates of permanent air temperature transitions through 5°C (early) and 10°C (mid-early);
- Potential vegetation season duration;
- Accumulated active temperature sums;
- Rainfall sums during main periods – IV-X; X-III; IV-VI; VII-VIII;
- Potential evapotranspiration (ETp) during the periods – year, IV-X; IV-VI; VII-VIII ;
- Temperature stress was assessed by consecutive days with air temperature above 28°C and 35°C;
- Water stress was assessed by consecutive days without rain >1 mm.

Data on the minimum and maximum temperature, relative air humidity, wind speed, and duration of solar radiation were used to calculate the values of potential evapotranspiration (PET) using the Penman–Monteith equation.

$$ET_o = \frac{0.408\Delta(R_n - G) + \gamma\left(\frac{C_n}{(T+273.16)}\right)u_2(e_s - e_a)}{\Delta + \gamma(1 + C_d u_2)} \quad (1)$$

where ET_o is the reference crop evapotranspiration for short (ETos) or tall (ETrs) reference crops ($mm \cdot d^{-1}$ for daily time step or $mm \cdot h^{-1}$ for hourly time step), R_n is the net radiation at the crop surface ($MJ \cdot m^{-2} \cdot d^{-1}$ for daily time step or $MJ \cdot m^{-2} \cdot h^{-1}$ for hourly time step), u_2 is the mean daily or hourly wind speed at 2 m height ($m \cdot s^{-1}$), T is the mean daily or hourly air temperature at 2 m height (˚C), G is the soil heat flux density at the soil surface ($MJ \cdot m^{-2} \cdot d^{-1}$ for daily time step or $MJ \cdot m^{-2} \cdot h^{-1}$ for hourly time step), e_s is the daily or hourly saturation vapor pressure (kPa), e_a is the daily or hourly mean actual vapor pressure (kPa), Δ is the slope of the saturation vapor pressure-temperature curve ($kPa \cdot {}^{\circ}C^{-1}$), γ is the psychrometric constant ($kPa \cdot {}^{\circ}C^{-1}$), C_n and C_d are constants, which vary according to the time step, the reference crop type (bulk surface resistance, and aerodynamic roughness of the surface) and daytime/nighttime ratio.

The annual sums of precipitation and potential evapotranspiration were calculated. By their ratio, we calculated the values of the aridity index (AI) (2) and Balance atmospheric humidification (BAH) [mm] (3).

$$AI = \sum r / \sum PET \quad (2)$$

$$BAH = \sum r - \sum PET \quad (3)$$

All data, used in the study are from the meteorological and agrometeorological archive of the National Institute of Meteorology and hydrology (NIMH).

RESULTS AND DISCUSSIONS

The long-term mean monthly air temperatures in the Plovdiv region, based on data from two stations—the Plovdiv synoptic station and the Sadovo climate station—are consistently positive (Figure 1).

During the winter months (December, January, and February), temperatures range between 0.9°C (January, Sadovo) and 3.3°C (March, Plovdiv). In Sadovo, temperatures tend to be lower than in Plovdiv from November through February, with differences varying from 0.1°C (November) to -0.3°C (January).

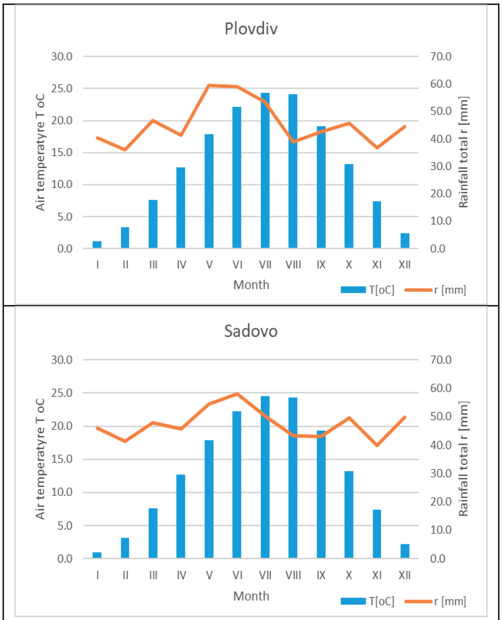

Figure 1. Long-term mean monthly air temperatures and total precipitation sums for the period 1991-2020

During the spring months, the average monthly air temperatures range from 7.6°C in March to 17.9°C in May. The temperatures in March and April are similar across both stations, while in May, Sadovo is 0.1°C warmer than Plovdiv.

In the summer months (June, July, and August), temperatures fluctuate between 22.1°C in June and 24.4°C in July (Plovdiv) and August (Sadovo). During this period, Sadovo is slightly warmer, with differences of 0.1°C in June and 0.3°C in August.

During autumn (September, October, and November), air temperatures vary between 19.1°C in September and 7.4°C in November. In the first two months of autumn, Sadovo recorded higher temperatures than Plovdiv by 0.2°C and 0.1°C, respectively.

The mean annual air temperature in the period 1991-2020 increased by approximately 0.9°C compared to 1961-1990, aligning with global warming trends. Compared to the reference period (1961-1990), long-term monthly mean air temperatures have increased at both stations, except in December (Figure 2). The most significant positive deviations were recorded during the summer months. In August, temperatures increased by 2.1°C in Plovdiv and 1.9°C in Sadovo, while in June and July, the rise was 1.3°C at both stations.

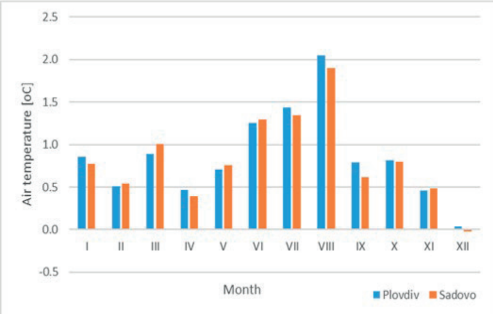

Figure 2. Deviations of average multiannual monthly air temperature values during 1991-2020 period compare with 1961-1990

For the remaining months, positive temperature anomalies ranged between 0.5°C and 1.0°C, with significant increases observed in January, March, September, and October.

During the summer, temperatures frequently exceed 35°C, posing a risk to cereal crop cultivation, particularly during critical growth stages such as flowering and grain filling.

The observed temperature increase in the study area is consistent with the global warming trend (FAO, 2001; Shirley et al., 2001) and the regional warming pattern in the Eastern Mediterranean, where a general warming trend has been evident since the 1980s, primarily driven by rising summer temperatures.

Under these thermal conditions, agrometeorological indicators such as transition dates through 5°C and 10°C, the duration of the potential growing season, and cumulative temperature sums suggest favorable conditions for cereal crop cultivation (Table 1).

The spring transition through 5°C occurs relatively early, in the second half of February, while the autumn decline below 5°C is recorded in late November. This results in a prolonged period with air temperatures above 5°C - 285 days - during which accumulated temperature sums range between 4286°C and 4213°C.

The spring transition through 10°C occurs in the last third of March, while the autumn decrease below 10°C happens in mid-November. This defines a relatively long potential growing season of 238 days, with accumulated temperature sums ranging between 3971°C and 4009°C.

Table 1. Mean multiannual dates of permanent transition of air temperature above and below 5 and 10°C during spring and autumn

Stations	T > 5°C	T < 5°C	Duration/ days/
Plovdiv	18.II	29.XI	285
Sadovo	18.II	30.XI	285
	T > 10°C	T < 10°C	
Plovdiv	19.III	12.XI	238
Sadovo	20.III	13.XI	238

Despite the increased potential vegetation period, the established trends of rising air temperatures, especially during the summer months in the Plovdiv region, and the significant decrease in summer precipitation deteriorate the agrometeorological conditions in the area. Critical risks for the region include extremely low soil moisture reserves and low atmospheric humidity. These factors create conditions for heat stress, particularly for spring crops. The reduction or absence of rainfall during the summer months, combined with extreme high temperatures, hampers pollination in crops with a spring sowing period, such as maize and sunflower, and subsequently affects grain filling.

The high values of accumulated temperature sums during the potential vegetation season indicate that the thermal resources of the region are suitable for cultivating heat-loving crops. Due to the increasing trend of heat waves in recent years (Bocheva et al., 2024) and the fact that these sums do not account for temperatures exceeding the upper optimum threshold for plant growth and development, the values of air temperatures above the optimum have been calculated using the Heat Stress Unit (HSU) for both locations - Plovdiv and Sadovo. The upper limit of the optimum was set at T>28°C and T>35°C (Figure 3, 4). The average sum of maximum air temperature values exceeding 28°C is 350°C in Plovdiv and 370°C in Sadovo, while in the years 2000, 2007, 2012, and 2017, temperatures above 35°C reached 60-80°C. These results indicate that agricultural crops in the studied area are exposed to heat stress.

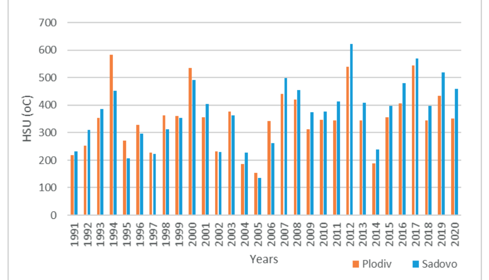

Figure 3. HSU >28°C during 1991-2020

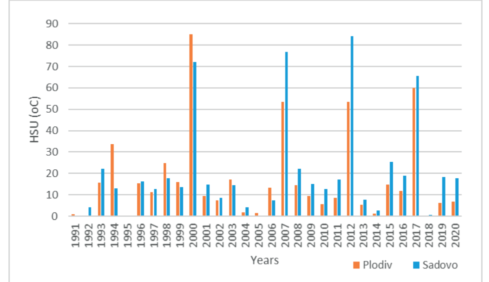

Figure 4. HSU >35°C during 1991-2020

The moisture conditions are directly related to the amount of precipitation in the region. The annual sum is 590 mm in Plovdiv and 618 mm in Sadovo. The annual precipitation distribution has a continental character, with a maximum in April-May, as shown in Figure 1. The distribution of precipitation during the vegetation period of the main crop types and outside the vegetation period is shown in Figure 5.

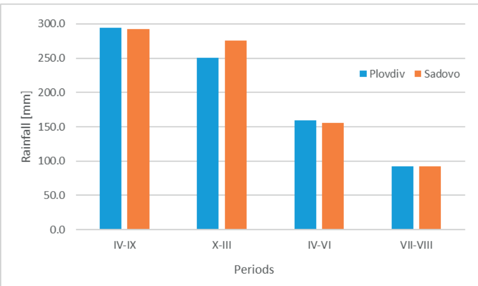

Figure 5. Distribution of multiannual rainfall totals during the periods

During the actual vegetation period from April to October, the average multi-year total precipitation in the Plovdiv region is 340 mm. There are no significant differences in precipitation totals between the two analyzed stations across the periods. An exception is observed during the autumn-winter moisture accumulation period when the total precipitation in Sadovo exceeds that in Plovdiv by 28 mm. It varies between 250 and 275 mm, ensuring good soil moisture availability at the start of the spring vegetation season.

During the spring vegetation period of winter cereal crops (April-June), the multi-year precipitation totals are 160 mm in Plovdiv and 158 mm in Sadovo, close to 150 mm, which is sufficient for achieving good yields of winter cereals.

In July-August, during the generative phase of maize development, the average multi-year precipitation total is around 100 mm-94 mm in Sadovo and 90 mm in Plovdiv. Probability assessments indicate that in a moderately dry year (i.e., once every four years), the precipitation total is 35-40 mm, which is insufficient to compensate for water loss from soil and plants due to evapotranspiration.

The comparison of precipitation totals during the vegetation and non-vegetation periods across the two periods 1961-1990 and the reference period 1991-2020 shows no significant change, except for the total in Plovdiv during the entire vegetation period, which has increased by 40 mm in the current period.

Given the established trends of increasing heavy, potentially hazardous precipitation events (≥ 30 mm/24 h) contributing to the total annual precipitation (Marinova et al., 2023), precipitation totals alone are not sufficient to characterize moisture conditions. Therefore, for both locations, the duration of dry periods from June to October was determined over a twenty-year period. A total of 52 and 54 cases were recorded in Plovdiv and Sadovo, respectively, with an average duration of 27 days (Figure 9). At the Plovdiv station, the maximum duration of this period reached 79 days, while in Sadovo, it was 69 days. A dry period of 22 days occurs with a probability of once every two years at both stations.

The annual total of potential evapotranspiration (ETp) and the totals for different periods are presented in Table 2. The annual total in the Plovdiv region is close to 1000 mm. During the vegetation period (April-October), it varies between 826 and 861 mm. Water consumption during the spring vegetation period is close to 50 mm, while in July-August, it ranges between 317 and 329 mm.

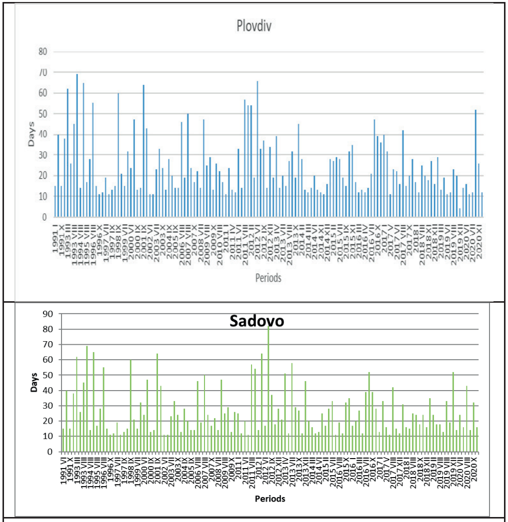

Figure 6. Number of consecutive days wyth rainfall <1 mm

The totals for these periods have increased in both stations compared to the previous period (1961–1990), as shown in Figure 7. The annual total in Sadovo has increased significantly by 160 mm, while in Plovdiv, the increase is 53 mm. All three subperiods also show an upward trend. For the entire vegetation period, the increase in Sadovo is again significant, reaching 125 mm, whereas in Plovdiv, it is 40 mm.

To assess water availability in the Plovdiv region, the atmospheric moisture deficit index and the drought index have been used.

The absolute values of the water deficit (Table 2) indicate that throughout the entire vegetation period, ETp exceeds precipitation by 483 mm in Sadovo and 520 mm in Plovdiv. By subperiods, the deficit is 201 mm and 211 mm for April-June, and 223 mm and 237 mm for July-August, respectively.

Table 2. Balance of atmospheric humidification and Aridity index

Station	Balance of atmospheric humidification, mm		
	IV-X	IV-VI	VII-VIII
Plovdiv	-520	-211	-237
Sadovo	-483	-201	-223
	Aridity index		
Plovdiv	0.4	0.4	0.3
Sadovo	0.4	0.4	0.3

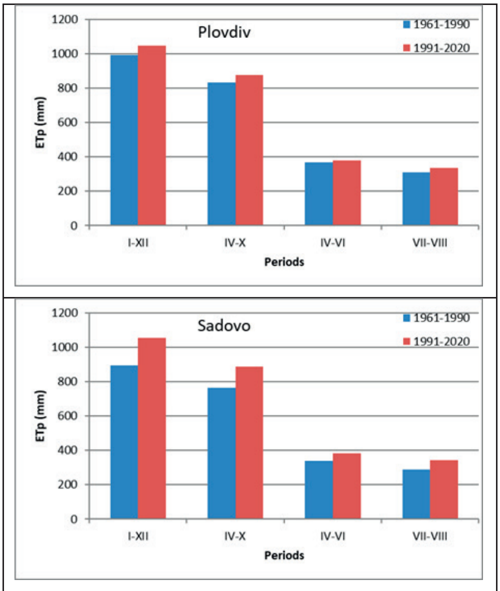

Figure 7. ETp sums during different periods for 1961-1990 г. and 1991-2020 г

A drought index value below 0.5 is considered to classify a region as dry. During both subperiods of the vegetation period, the Plovdiv region is classified as dry (Table 2). In April-June, precipitation compensates for only one-fourth of the losses (ETp), while in July-August, it covers just one-third.

The analysis of agro-meteorological conditions in the Plovdiv region indicates that the generative stage of spring crops occurs under heat and water stress. However, the overall conditions in Central Southern Bulgaria are still considered potentially suitable for growing spring crops. The observed trends of rising temperatures, decreasing precipitation, and the fact that ETp exceeds precipitation at both studied locations (Plovdiv and Sadovo), combined with the early onset of temperatures meeting the biological requirements of spring crops, suggest that sowing dates should be moved as early as possible potentially by 10-15 days, depending on the specific year.

Although some maize hybrids from the new Bulgarian selection in the FAO 500 group exhibit a generative stage around June 20, we recommend earlier FAO hybrids as more suitable for the region. Early spring sowing of sunflower would also ensure development during a more moisture-secured period with lower temperatures, reducing heat stress in the hottest summer months. However, late spring frosts significantly limit these possibilities in some years. The average date of the last spring frost in Plovdiv is April 1. With a probability of once every 10 years, frost can occur on April 22 in Plovdiv and April 23 in Sadovo. The latest recorded frost dates are May 8 in Plovdiv and May 9 in Sadovo. This indicates that early sowing is not always feasible.

These trends in agro-meteorological changes over recent years highlight the relative unsuitability of growing spring crops under rainfed conditions.

CONCLUSIONS

The analysis of agro-meteorological conditions and their changes compared to the previous period shows rising temperatures and worsening moisture conditions.

The increase in temperatures and the earlier stable transition through biological thresholds for different crop types in spring allow for earlier sowing, utilizing the soil moisture reserves available in spring.

The unfavorable temperature regime, combined with prolonged droughts typical for the region, creates adverse conditions for spring crop development, especially during the generative stage. Heat and water stress make it practically impossible to grow high-yielding mid-late and late hybrids under rainfed conditions.

The primary risk factor in the Plovdiv region is summer droughts, which shorten the actual vegetation period, especially for rainfed spring crops.

Successful adaptation to changing agro-climatic conditions requires a comprehensive approach, including the implementation of new technologies and a differentiated approach based on soil types, precipitation, and temperature zones.

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EFFECT OF PRE-SOWING ELECTROMAGNETIC TREATMENT OF TRITICALE (×*Triticosecale* Wittm.) SEEDS AND METEOROLOGICAL CONDITIONS ON GRAIN YIELDS

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Abstract

The study examined the effects of electromagnetic stimulation on seeds of two triticale varieties under different climatic conditions. A three-factor field trial was conducted in 2017-2018 and 2018–2019 at the Institute of Field Crops, Bulgaria. Before sowing, seeds were electromagnetically treated using controllable factors: voltage (U, kV), seed exposure time (τ, s), and stay period (T, days). The values for the electromagnetic options: E0-untreated seeds; E1 (U = 5.2 kV, τ = 24 s, T = 14 days); E2 (U = 5.0 kV, τ = 50 s, T = 7 days). During vegetation, organic and mineral fertilizers were incorporated. Agrometeorological conditions were assessed using Ivanov`s coefficient and De Martonne index. Grain yield was higher for the Boomerang variety in both years. The variety and fertilization had an average effect in both periods. Electromagnetic stimulation showed a greater effect during the semi-humid conditions of 2018. In 2019, based on the impact of the interaction of the electromagnetic stimulation and the variety, an increase in grain yield for the Boomerang variety for E2 was 6.0%. In 2018, for the E1 option, a higher yield of 23.7% was reported for the Boomerang variety.

Key words: *electromagnetic seed treatment, triticale, grain yields, mineral fertilization, organic fertilization.*

INTRODUCTION

The traditional cultivation of cultural plants includes the use of fertilizers and agrochemicals, which increase plant production, but at the same time lead to negative effects, resulting in disruption of relationships between biotic components in agroecosystems. In modern agriculture, ecological methods are sought to increase yields. The quality of the seeds largely determines future yield. Pre-sowing treatment of seeds has economic importance to increase sowing and productive qualities by improving their germination. For pre-sowing preparation, physical methods can be used, for example, laser irradiation, ultrasonic impact, microwave electromagnetic radiation, magnetic field influence, gamma radiation (Aladjadjiyan, 2007), and plasma (Sohan et al., 2021). It is possible for these methods to ensure increased yields from the respective culture at the expense of small, oftentimes minor costs. They are based on the fact that they increase the amount of energy through internal energy transformation, regardless of its origin, in electricity and increase the electropotential of the cell membrane (Vasilevski, 2003). Modern research found that the application of electric and magnetic fields on seeds improves germination, increases root and sprout length, fresh and dry biomass, fruit yield, leaf area, chlorophyll content, accumulation of various ions, improves stomatal conductivity, leads to an increase in the content of photosynthetic pigment, accelerates cell division, and increases the absorption of water and nutrients (Dannehl, 2018; Sarraf et al., 2020).

Triticale is a hybrid species that combines the genomes of wheat (*Triticum* ssp.) and rye (*Secale cereale* L.). Triticale has a beneficial effect on the human body, can be fractionated to yield a variety of components, including starch, dietary fiber, and protein, which can be used in both food and non-food applications (Kamanova et al., 2023). The production of the modern hexaploid varieties of triticale proves that triticale is a good alternative to traditional Polish cereals (Georgieva, 2009).

In Bulgaria and abroad, laboratory tests in the field of pre-sowing treatment with an electromagnetic field of cotton, tomato, pepper, triticale, and corn seeds showed that it is possible to increase their laboratory germination and to stimulate their development (Antonova-

Karacheva & Sirakov, 2020; Ganeva et al., 2015; Koleva & Radevska, 2021a; Koleva & Radevska, 2021b; Sirakov & Mihaylov, 2022). It was reported for a positive effect on germination indices and some parameters, characteristic of the growth and development of triticale seedlings with laser light (Możdżeń et al., 2020) and magnetic treatment (Hussain et al., 2020). It has been noted that the efficiency of seed irradiation from wheat and barley with electromagnetic waves increases with seed treatment with lower sowing qualities (Nizharadze, 2004).

Most of the experiments are limited to laboratory studies. No results have been reported from field experiments conducted with electromagnetically treated triticale seeds. The results of surveys will be valuable regarding increasing grain yields based on the inclusion of the ecological part of the cultivation technology.

In our previous research, we established the limits of controllable factors of electromagnetic impact, through which it is possible to increase laboratory germination and germination energy for two varieties of triticale (Sirakov et al., 2018; Sirakov et al., 2019; Sirakov et al., 2021) and an expected increase in grain yields.

The purpose of the present study was to establish an influence and an effect of pre-sowing electromagnetic seed stimulation for triticale in two climatically different years on grain yield.

MATERIALS AND METHODS

At the experimental field of the Field Crops Institute in Chirpan at the Agricultural Academy during the period 2017-2019, a three-factor experiment was conducted. The applied factors are variety, fertilization, and electromagnetic seed treatment (EMT). The soil is Pellic vertisols. Triticale seeds were sown after the predecessor of sunflower on November 9, 2017 and November 1, 2018. The experimental plot was 18 m^2 in size, and the sowing rate was 550 seeds per m^2. The Bulgarian triticale varieties Boomerang and Colorit were tested. During the growing season, standard cultivation technology for cereals was applied.

60 kg/ha phosphoric fertilizer (P_2O_5) and organic fertilizer at a rate of 2200 kg/ha were incorporated with the main tillage in autumn. In the spring, nitrogen fertilizer (NH_4NO_3) at a rate of 120 kg/ha was spread manually on the plots. The organic fertilizer (Lumbrical) is a product from the processing of manure and other organic waste from red Californian worms and contains: organic substance 45-60%; ammonium nitrogen (NH_4-N) – 33.0 ppm; nitric nitrogen (NO_3-N) – 30.5 ppm; P_2O_5 – 1410 ppm; K_2O – 1910 ppm.

A pre-sowing stimulation device (Terziev et al., 1994) was used to treat the seeds with an electromagnetic field. The controllable factors are voltage between electrode spaces (U, kV), seed exposure time (τ, s), and stay time from treatment to sowing (T, days). The values for the options: E0-untreated seeds; E1 (U = 5.2 kV, τ = 24 s, T = 14 days); E2 (U = 5.0 kV, τ = 50 s, T = 7 days).

Grain yields was reported from four replicates and recalculated in kg/ha.

Climatic data were provided by the synoptic station located at the Institute's experimental field (42°12'58"N, 25°17'0"E). Agroclimatic assessment of conditions was performed. The coefficients of humidification by Ivanov (K_I) and dryness index of the De Marton index (I_{DM}) were established. The Ivanov coefficient estimates the degree of aridity for each month and was calculated based on monthly averages daily temperature and relative humidity, and sum of the precipitation (Ivanov, 1941). The De Martonne dryness index is presented for the triticale growing season and uses the average daily months temperatures and sum of the precipitation. This indicator characterizes the humidification conditions of a given territory, i.e., what type of climate it is in relation to the availability of water (De Martonne, 1926).

Results were subjected to an analysis of variance by applying the Biostat statistical program (Penchev et al., 1989-1991) to detect differences between the mean yields. Means were compared using the LSD test at probably p = 1.0%, p = 0.1% and p = 5.0%.

RESULTS AND DISCUSSIONS

According to the data in Table 1, the monthly temperature sum was 314.5°C and 176.4°C higher than the climatic average, respectively, during the first and second growing seasons. The amounts of precipitation during 2017-2018 were 103.0 mm more, and in the period 2018-2019, they were 54.7 mm less. According to De

Martonne index, the conditions during the period 2017-2018 refer to a semi-humid type of climate, and in 2018-2019 they are characterized as semi-dry.
During the first growing season, emergence of the crop was registered after 15 days at the sum of the active temperature of 143.9°C, the average temperature of 9.6°C, and 16.5 mm of precipitation. According to established Ivanov coefficients, conditions for crop development were favorable for most of the winter period. During the months of February and March, overwetting is observed, which corresponds to the tillering period, according to Table 2. A drought was established in April during the phase of stem elongation. This period of development is deciding on the future production because the segments of the future spikelets are different.

Table 1. Temperature and precipitation sum during the triticale vegetation periods

Period	Months									Σ
	X	XI	XII	I	II	III	IV	V	VI	
Temperature sums (°C)										
1991/21	*406.7*	*214.3*	*54.8*	*9.5*	*74.6*	*215.4*	*363.4*	*532.2*	*642.3*	*2513.2*
2017/18	388.0	244.3	125.6	65.2	97.8	200.5	471.0	584.8	646.9	2824.1
2018/19	434.0	225.3	20.7	53.8	113.1	292.6	335.2	533.4	681.5	2692.6
Precipitation (mm)										
1991/21	*46.9*	*40.7*	*70.0*	*44.4*	*38.4*	*46.3*	*43.8*	*57.3*	*50.8*	*438.6*
2017/18	80.0	48.2	38.9	23.3	109.0	83.4	8.7	62.2	87.9	541.6
±	+33.1	+7.5	-31.2	-21.1	+70.6	+37.1	-35.1	-4.9	+37.1	+103.0
2018/19	25.4	82.3	23.5	28.9	24.5	3.3	51.4	21.4	123.2	383.9
±	-21.5	+41.6	-46.5	-15.5	-13.9	-43.0	+7.6	-35.9	+72.4	-54.7
Period	Coefficient by Ivanov									De Martonne index
2017/18	1.4	1.6	1.7	1.0	6.1	2.6	0.1	0.7	0.8	26.7
2018/19	0.3	2.5	1.3	1.3	0.5	0.0	0.7	0.2	1.0	19.3

Table 2. Dates of occurrence of the main phenological phases for 2017-2019

Varieties / Phases	Colorit		Boomerang	
	2017-2018	2018-2019	2017-2018	2018-2019
Emergence	24.11.17	5.12.18	24.11.17	5.12.18
Tillering	8.03.18	28.02.19	8.03.18	28.02.19
Stem elongation	10.04.18	9.04.19	14.04.18	12.04.19
Heading	20.04.18	30.04.19	2.05.18	3.05.19
Flowering	2.05.18	13.05.19	9.05.18	17.05.19

In the second year, emergence was recorded 24 days after sowing, with a total active temperature of 211.4°C, 82.3 mm of precipitation, and an average temperature of 8.8°C. Droughts were found in the months of March and May, when the plants were going through the phases of stem elongation, heading, and flowering. The insufficient moisture in the soil during flowering reduces the possibility of fertilization and results in a poorly seeded spike.
During the period from April to June, which characterizes the conditions of moisture supply for the yield of winter crops, drought was found in both years. Therefore, the conditions were favorable for the initial development of the crop in 2017, and the drought in April negatively affected grain yield. The late emergence in 2018 retarded the development of triticale during the winter months, the flowering and fertilization occurred at an unfavorable time, which is a possible reason for the decrease in grain yield.
These statements support the data in Table 3, where it can be seen that the average yields are 2957.2 and 2392.1 kg/ha, respectively, in 2018 and 2019.
In 2018, the biggest increase in yields for both varieties of triticale was observed for the E0 + NP option, which is 26.3 and 51.3% more, respectively, for Colorit and Boomerang compared to grain yields obtained from the untreated seeds.

For options of electromagnetically stimulated seeds and fertilization, the best result was obtained for the E1+NP option and the Colorit variety: 20.3%.

Table 3. Influence of variety, EMT and fertilization on grain yields for 2018 and 2019

Year	Options	kg/ha	% Control	Year	Options	kg/ha	% Control
2018	Colorit			2019	Colorit		
	E0	2668.1	100.0		E0	2375.0	100.0
	E0 + L	2998.8^{ns}	112.4		E0 + L	2361.8^{ns}	99.4
	E0 + NP	3370.1*	126.3		E0 + NP	2532.7^{ns}	106.6
	E1	2412.5^{ns}	90.4		E1	2199.3^{ns}	92.6
	E1 + L	2760.4^{ns}	103.5		E1 + L	1931.3^{00}	81.3
	E1 + NP	3209.7^{ns}	120.3		E1 + NP	2426.4^{ns}	102.1
	E2	2289.6^{ns}	85.8		E2	1843.8^{000}	77.6
	E2 + L	2240.3^{ns}	84.0		E2 + L	1986.2^{00}	83.6
	E2 + NP	2763.2^{ns}	103.6		E2 + NP	2350.0^{ns}	98.8
	Boomerang				Boomerang		
	E0	3052.1^{ns}	114.4		E0	2358.4^{ns}	99.3
	E0 + L	3073.0^{ns}	115.2		E0 + L	2493.1^{ns}	105.0
	E0 + NP	4036.8***	151.3		E0 + NP	2934.8***	123.6
	E_1	3300.7*	123.7		E1	2575.0^{ns}	108.4
	E1 + L	3104.9^{ns}	116.4		E1 + L	2290.3	96.4
	E1 + NP	3263.2*	122.3		E1 + NP	2655.6*	111.8
	E2	2947.9^{ns}	110.5		E2	2450.7^{ns}	103.2
	E2 + L	2760.4^{ns}	103.5		E2 + L	2408.3^{ns}	101.4
	E2 + NP	3046.6^{ns}	114.2		E2 + NP	2848.8***	121.5
	Average	2957.2			Average	2392.1	
	St. Error	214.8			St. Error	189.7	
	LSD %				LSD %		
	5.0	553.1	20.7		5.0	268.9	11.3
	1.0	736.6	27.6		1.0	358.1	15.1
	0.1	960.1	34.0		0.1	466.8	19.7

L-Lumbrical; N-nitrogen fertilizer; P-phosphorus fertilizer; L-organic fertilizer Lumbrical; N-nitrogen fertilizer; P-phosphorus fertilizer; *significance at p = 5.0%; *** significance at p = 0.1%; ^{0}significance at $p < 0.05$; ^{00}significance at $p < 0.01$; ^{000} significance at $p < 0.001$; ^{ns} no significance.

For the Boomerang variety for several options, the values were significantly higher compared to the control: E1 and E1 + NP, 23.7 and 22.3%, respectively. Erohin (2018) has reported increase in yield over control for barley (9.7%) and spring wheat (8.4%).

In 2019, the values for varieties for the E0 + NP option demonstrated the highest grain yield by 6.6 and 23.6% more than the control, respectively, for Colorit and Boomerang. Compared to the control, the yield values for Boomerang and for the E1 + NP and E2 + NP options are significantly higher by 11.8 and 21.5%, respectively. The yields for Boomerang variety for E1 and E2 options exceeded the control by 8.4 and 3.2%, respectively. This gives reason to note that the EMO showed a different effect depending on the conditions of the year. Bezpalko et al. (2021) have reported positive and significant results for grain yield in wheat microwave field treated seeds, and yield depends on the conditions of the year. Rye and triticale varieties showed a varied response to pre-sowing treatment with red light, and higher results were obtained in triticale (Dziwulska-Hunek et al., 2022).

The results in Table 4 show that the productivity of the Boomerang variety is significantly higher by 15.7 and 15.1% compared to Colorit in the two harvest years. In 2018, the reported grain production was 3176.2 kg/ha, higher than in 2019 (2561.2 kg/ha). A previous study found higher values of yield related traits for the Boomerang variety compared to Colorit (Muhova et al., 2021).

Table 4. Influence of variety on triticale on grain yields for 2018 and 2019

Year	Options	kg/ha	% Control	Year	Options	kg/ha	% Control
2018	Colorit	2745.9	100.0	2019	Colorit	2222.9	100.0
	Boomerang	3176.2^{***}	115.7		Boomerang	2561.2^{***}	115.2
	LSD %				LSD %		
	5.0	184.4	6.7		5.0	89.6	4.03
	1.0	245.5	8.9		1.0	119.4	5.4
	0.1	320.0	11.6		0.1	155.6	7.0

***significance at $p = 0.1\%$.

The values for the electromagnetically treated variants show a progressive decrease in direction from E0 to E1 and E2 and ranged from 94.6 to 83.0% in 2018 and from 93.5 to 92.5% in 2019, according to Table 5. For option E2, a significantly lowest value was reported in 2019 compared to the control (2320.6 kg/ha). They have reported positive results regarding grain yield in wheat after seed irradiation with red light. The results showed higher values in two of the varieties tested (Szymanek et al., 2020).

Table 5. Influence of EMT on triticale on grain yields for 2018 and 2019

Year	Options	kg/ha	% Control	Year	Options	kg/ha	% Control
2018	E0	3199.8	100.0	2019	E0	2509.3	100.0
	E1	3008.6^{ns}	94.6		E1	2346.3^{ns}	93.5
	E2	2674.7^{ns}	83.6		E2	2320.6^{00}	92.5
	LSD %				LSD %		
	5.0	225.8	7.1		5.0	109.8	4.4
	1.0	300.7	9.4		1.0	146.2	5.8
	0.1	392.0	12.3		0.1	190.6	7.6

^{ns} no significance; ^{00}significance at $p < 0.01$.

In contrast to EMO, fertilization increased grain yield from 3.6 to 20.8% in 2018 and by 14.4% in 2019, as can be seen in Table 6. Organic and synthetic fertilizer had a better impact on yield during the semi-humid conditions of 2018. It is known that good moisture security in the soil improves the assimilation of nitrogenous mineral fertilizers. This has been confirmed by other authors (Zhang et al., 2023; Wang et al., 2023; Boudjabi et al., 2023). Drought can affect nutrient uptake and disrupt acropetal translocation of some nutrients (Hu & Schmidhalter, 2005).

Table 6. Influence of fertilization on grain yields for 2018 and 2019

Year	Options	kg/ha	% Control	Year	Options	kg/ha	% Control
2018	0	2737.3	100.0	2019	0	2300.0	100.0
	L	2841.6^{ns}	103.6		L	2245.2^{ns}	97.6
	NP	3292.7^{***}	120.8		$N_{120}P_{60}$	2630.7^{***}	114.4
	LSD %				LSD %		
	5.0	124.3	4.5		5.0	109.8	4.8
	1.0	165.5	6.0		1.0	146.2	6.4
	0.1	215.8	7.9		0.1	190.6	8.3

L-Lumbrical; N-nitrogen fertilizer; P-phosphorus fertilizer; *** significance at $p = 0.1\%$; ^{ns} no significance.

The effect of EMO on the cultivars is shown in Table 7. E2 was particularly depressing for the Colorit variety. In both years the yields were significantly under the control option - 80.7 and 85.0%. Similarly, in 2019, the grain yield obtained after electromagnetic treatment the seeds with E1 option was lower and confirmed at $p < 0.01$. It was reported an insignificantly increase in yield for the Boomerang variety, by 7.0 and 3.5% in 2018 for E1 option, as well as 6.5% for E2 option in 2019. These results correlate with those presented in Table 10, where a low effect is seen but with good reliability of the factor interaction variety and EMO (4.87%). This means that EMO affects varieties differently in different years. Despite these increases, the highest results for varieties within seasons were obtained for E0 option. Larionov et al. (2021), according to the results of other authors, indicated that the average increase in the yield of cereals (wheat, rye, barley, oats, corn) after electromagnetic treatment of seeds amount to 10-12%, but better results were also reported by 18-26%.

Table 7. Influence of variety and EMT on grain yields for 2018 and 2019

Year	Options	kg/ha	% Control	Year	Options	kg/ha	% Control
2018	Colorit			2019	Colorit		
	E0	3012.3	100.0		E0	2423.1	100.0
	E1	2794.2^{ns}	92.8		E1	2185.7^{00}	90.2
	E2	2431.0^{000}	80.7		E2	2060.0^{000}	85.0
	Boomerang				Boomerang		
	E0	3387.3^{*}	112.4		E0	2595.4^{*}	107.1
	E1	3222.9^{ns}	107.0		E1	2507.0^{ns}	103.5
	E2	2918.3^{ns}	96.9		E2	2581.3^{*}	106.5
	LSD %				LSD %		
	5.0	319.3	10.6		5.0	155.3	6.4
	1.0	425.3	14.1		1.0	206.8	8.5
	0.1	554.3	18.4		0.1	269.5	11.1

*significance at $p = 5.0\%$; ns no significance; 00 significance at $p < 0.01$; 000significance at $p < 0.001$.

According to Table 8, under the influence of organic and mineral fertilizer, the grain yield for Colorit ranged from 8.5 to 26.8%, confirmed higher to the control option in 2018. Next year, 13.9% more yield was reported. Fertilizers applied led to a greater increase in both tested seasons for Boomerang compared to Colorit. In 2018, the Boomerang variety realized a larger production from 21.3 to 40.4% compared to control, and in 2019, from 12.1 to 32.0%.

Table 8. Influence of variety and fertilization on grain yields for 2018 and 2019

Year	Options	kg/ha	% Control	Year	Options	kg/ha	% Control
2018	Colorit			2019	Colorit		
	0	2456.7	100.0		0	2139.4	100.0
	L	2666.5^{*}	108.5		L	2093.1^{ns}	97.8
	NP	3114.4^{***}	126.8		NP	2436.3^{***}	113.9
	Boomerang				Boomerang		
	0	3097.8^{***}	126.1		0	2581.3^{***}	120.7
	L	2979.4^{***}	121.3		L	2397.2^{**}	112.1
	NP	3448.9^{***}	140.4		NP	2825.0^{***}	132.0
	LSD %				LSD %		
	5.0	175.8	7.2		5.0	155.3	7.3
	1.0	234.1	9.5		1.0	206.8	9.7
	0.1	305.1	12.4		0.1	269.5	12.6

L-Lumbrical; N-nitrogen fertilizer; P-phosphorus fertilizer; *significance at $p = 5.0\%$; ** significance at $p = 1.0\%$; *** significance at $p = 0.1\%$; ns no significance.

From Table 9, it can be found that the application of mineral fertilizer increased the yields for all EMO options.

The options for E0 with applied mineral fertilizer in both years showed the highest and most significant values of 29.5 and 15.5%, respectively. In 2018, the yields for E1 and E2 were 99.9 and 91.6%, respectively, to E0. Similarly, in the second harvest year for E1 and E2, yields of 100.9 and 90.7%, respectively, were reported to the control. An insignificant 2.5% increase in yield was observed after organic fertilization in 2018. Talanov (2018) have obtained the highest yield of winter rye (384 t/ha) for the pre-sowing electromagnetic treatment of seeds and application of $N_{87}P_{119}K_{75}$. Nizharadze (2010) has reported that after the treatment of wheat seeds with electromagnetic waves with a wavelength of 7.1 mm was obtained a higher grain yield of 7.4 to 13.7%. Menshova & Nizharadze (2012) have found that pre-sowing exposure to an electromagnetic field and a pulsed magnetic field of barley seeds reduced and increased the biological yield by 2.7 and 17.2 %, respectively. Field experiments have found that grain yield after electrophysical treatment of seeds from wheat is practically not lower than that of an option with applied mineral fertilizers (NPK) and exceeded the control indicators by 3.7 and 3.6% (Tibirkov et al., 2012). In maize, grain yields increase by 10 and 15% under the influence of low-frequency electromagnetic waves (Imbrea et al., 2011).

Table 9. Influence of EMT and fertilization on grain yields for 2018 and 2019

	Options	kg/ha	% Control		Options	kg/ha	% Control
2018	E0			2019	E0		
	0	2860.1	100.0		0	2366.4	100.0
	L	3035.9^{ns}	106.1		L	2427.4^{ns}	102.6
	NP	3703.5***	129.5		NP	2733.7***	115.5
	E1				E1		
	0	2856.6^{ns}	99.9		0	2387.2^{ns}	100.9
	L	2932.6^{ns}	102.5		L	2110.8^{00}	89.2
	NP	3236.5^{ns}	113.2		NP	2541.0^{ns}	107.4
	E2				E2		
	0	2618.8^{ns}	91.6		0	2147.2^{0}	90.7
	L	2500.3^{ns}	87.4		L	2197.2^{ns}	92.8
	NP	2904.9^{ns}	101.6		NP	2617.4*	110.6
	LSD %						
	5.0	391.1	13.7		5.0	190.0	8.0
	1.0	520.9	18.2		1.0	253.2	10.7
	0.1	678.9	23.7		0.1	330.1	13.9

L-organic fertilizer Lumbrical; N-nitrogen fertilizer; P-phosphorus fertilizer; *significance at p=5.0%; *** significance at p = 0.1%; ^{0}significance at $p < 0.05$; ^{00}significance at $p < 0.01$; ^{ns} no significance.

Table 10. Influence of factors variety (A), EMT (B), fertilization (C) on grain yields

Source of variation	df	SS	η (%)	MS	F	P value
2018						
A	1	3215104	21.48***	3215104	69.71	0.0000
B	2	2569984	17.17***	1284992	27.86	0.0000
C	2	4183424	27.95***	2091712	45.35	0.0000
A×B	2	194368	1.30	2091712	2.11	0.12954
A×C	2	172416	1.15	97184	1.87	0.16223
B×C	4	1056512	7.06***	86208	5.73	0.00091
A×B×C	4	1086336	7.26***	271584	5.89	0.00077
Options	17	1.247814E+07	83.36***	734008.5	15.91	0.00000
Error	54	2490688	16.64	46123.9	-	-
2019						
A	1	2060032	27.25***	2060032	57.25	0.0000
B	2	502528	6.65**	251264	6.98	0.00237
C	2	2086464	27.60***	1043232	28.9906	0.00000
A×B	2	368064	4.87**	184032	5.114105	0.00931
A×C	2	23776	0.31	11888	0.3303582	0.72487
B×C	4	33977	4.76	89944	2.499473	0.05243
A×B×C	4	217024	2.87	54256	1.507732	0.21193
Options	17	5617664	74.30	330450.8	9.182968	0.0000
Error	54	1943200	25.70	35985.18	-	-

The effect of the factors, as a result of the analysis of variance carried out, is shown in Table 10. According to the values of η, the variety and fertilization showed confirmed average effects during the two periods, which are expressed in proven differences in yields values between varieties and based on fertilization (Table 3 and Table 6). In 2018, the influence of the interaction of the three factors

A×B×C was low, but confirmed (7.26%). As shown in Table 10, a low interaction between EMO and fertilization (7.06%) was found in 2018, and according to Table 9, this refers to some of the options with organic and mineral fertilizers. In 2019, the interaction between variety and EMO was low (4.87%). The influence of EMO was higher in 2018 (17.17%) than in 2019 (6.65%). This is also confirmed by the data in Table 3, where an increase in yields can be seen for some variants of EMO compared to the control and 2019 data.

CONCLUSIONS

Based on the applied factors and statistical analysis of the data, it can be concluded that certain parameters of the electromagnetic field for seed stimulation had a positive influence on the yields of the two triticale varieties, expressed as an increase in the grain yield. The influence of the selected electromagnetic processing parameters generally showed a tendency towards lower yields. The influence of electromagnetic treatment is also determined by the conditions of the years. Variety and fertilization showed medium effects in both periods. Additional field studies with other levels of electromagnetic parameters are required to refine those that will increase grain yield. Pre-sowing electromagnetic seeds treatment can increase grain yield and contribute to the sustainability of agroecosystems.

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THE SOWING DENSITY INFLUENCE ON YIELD OF THE MAIZE HYBRID P0217

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Abstract

The study aims at determining the extent to which increasing plant density in maize crops is a means of increasing yield per unit area, and the objective is to determine the level of plant tolerance in terms of plant density per unit area with no consequences on the size of productivity elements. As a consequence of downsizing the nutrition area from 0.15 m² (for the 70 cm sown variant) to 0.076 m² (for the 35 cm sown variant), the vegetative growth was diminished with implications on the size of the intake area at the level of each plant, but with an increase in the leaf area index values due to the high density covering the soil surface much better. The yield obtained was 10.16 tons of grains/hectare in the case of the equally spaced row seeding scheme at 70 cm clearance and 7.33 tons of grains/hectare in the equidistant row seeding scheme at 35 cm spacing between rows.

Key words*: foliar surface, phenophase, productivity elements.*

INTRODUCTION

Out of the total area cultivated with cereals worldwide, maize occupies 27.3% whereas out of the total area cultivated with cereals in the European Union, maize occupies 17.7%. The optimum plant density for maximum maize yield per unit area differs from one maize hybrid to another due to interactions between the hybrid and different densities (Luca and Tabără, 2011). Density increase is not always accompanied by the yield increase because stress occurs due to nutrient area depletion, but yield increases when other factors are provided at optimal requirements (Winans et al., 2021). However, in situations where not all environmental factors can be provided at the optimal level (water), it is found that better results are obtained in case of lower plant densities (Tokatlidis et al., 2022).
Given the limited land resources, increasing plant density is considered an efficient method of increasing maize production. (Adnan et al., 2021). Against the background of human population growth, increasing meat and milk consumption and biofuel demand, the results of several studies have shown that global production needs to double by 2050 as regards four key crops - maize, rice, wheat and soybeans (Ray et al., 2013). This output boost can be achieved by increasing density per unit area; research has shown that the contribution of plant density to maize yield growth has ranged from 8.5% to 17% (Assefa et al., 2018), but the optimal density should be decided based on a detailed analysis of the interaction between genotype and environment (Assefa et al., 2016). However, according to research, increasing plant density per unit area leads to competition between plants and decreases plant growth and productivity (Liu et al., 2022), and the recommendation to use higher or lower densities is closely related to the provision of the required plant moisture during the growing season (Haarhoff and Swanepoel, 2022).
Studies conducted on sweet corn by way of using different densities have shown that modern hybrids allow the use of high densities. Nevertheless, with densities of 79,000 plants/hectare the yield per plant has remained unchanged over time regardless of the density level (Daljeet et al., 2021).
Some corn hybrids have the ability to adapt to lower light intensity. Even under these conditions, a high photosynthetic efficiency is found, with beneficial consequences on yield (Yunshan et al., 2021).
At the same time, it was noticed that an increase in planting density significantly increased the leaf area index and the amount of photosynthetically active radiation intercepted, thus favoring plant growth and crop productivity (Yuanhong et al., 2021).

In case of using high densities, competition for nutrients among plants increases with a negative impact on plant productivity growth, but the application of nitrogen and growth regulators clearly have improved the enzymatic activities of carbon and nitrogen metabolism in leaves (Xiaoming et al., 2022). Despite that, there are maize hybrids that react differently to shading stress caused by increasing plant density (Federico et al, 2022).

When using high plant densities (90,000 plants/hectare), the application of nitrogen fertilizer has significantly improved the average kernel filling rate and has optimized the transport system of photosynthetically synthesized substances (Hong et al., 2021) and in semi-arid areas high density combined with high nitrogen fertilization improves soil water utilization and specifically water that is prone to evaporation (Yang et al., 2022).

Higher seeding densities and reduced nitrogen application rates should be considered for yield increase in order to stimulate improved soil nitrogen utilization (Cailong et al., 2017), although using higher amounts of nitrogen fertilizers has resulted in higher amounts of synthesized substances in the plant which facilitates flower development and increases the number of grains in the corncob (Hong et al., 2022).

It is recommended that the choice of hybrids be made under conditions of stress and high plant density because under such conditions, the rate of water loss from the grain increases (Pin et al., 2022). Research has been conducted in maize by growing alternating low-density rows with high-density rows and it has been found that diversified management approaches can provide both economic and environmental benefits of the cropping system (Amanda et al., 2022). A high density of up to 10.5 plants/square metre can lead to a significant increase in yield only when combined with narrow row spacing, as it guarantees greater equal distancing among plants (Giulio et al., 2016). In dry areas, increasing plant density can effectively improve maize yield, but more irrigation water is needed to obtain higher yields. (Guoqiang et al., 2022).

There is a need for new improvement strategies on adaptation of maize plants to stress using novel breeding strategies for alleles harvesting which govern physiological adaptive traits. (Welcker et al., 2022)

Using a low or medium density in maize by alternating low- and high-density rows leads to an optimization of economic production and yield (Amanda and Kemania, 2022) and by using intercropping maize-soybean it was found that the decrease in maize yield is generated by the aboveground intraspecific competition which has reduced biomass accumulation without changing the number of grains per cob and therefore intercropping is not a solution to increase yield (Bing et al., 2022). Yield potential can be increased by extending the flowering and physiological maturity period with constant maintenance of the growing season by complete interception of radiation during the flowering period (Capristo et al., 2007).

Hybrids with low leaf area have tolerated higher plant density better (Lambert et al., 2014).

Increasing plant density is one way to increase leaf area, but brightness decreases proportionally with leaf system development and leaf position.

Following the investigation undertaken, the research hypothesis that we started from was to determine how much the decrease in plant nutrient surface area influences the size of productivity elements in maize crops.

The aim of this paper is to examine the extent to which increasing plant density is a means of increasing production per unit area, and the objective is to determine what level of plant density is tolerable without any consequences on the size of the productivity elements.

MATERIALS AND METHODS

The biological material tested was the hybrid P0217, a medium-late hybrid (FAO group 420), characterized by stability and productivity and very good drought tolerance, recommended for arid and semi-arid lowland areas in the south and west of the country.

The crop establishment was performed on a typical chernozem soil, on April 26th 2021, in Suțu village, Braila county, with two experimental varieties: a variety seeded in equidistant rows at a distance of 70 cm between rows and a variety seeded in equidistant rows at a distance of 35 cm bandwidth, each variety with 3 repetitions, randomly placed.

The soil where the maize crop was established is a typical chernozem with physical and chemical properties favourable to the growth and development of maize plants, the area is characterized by a semi-arid steppe climate, the main restricting factor being water. Soil agrochemical determinations (soil reaction, humus content, mobile phosphorus and mobile potassium) were carried out on soil samples collected up to a depth of 30 cm, the determination methods used being according to the National Research-Development Institute for Pedology, Agrochemistry and Environmental Protection Bucharest. The determination of soil reaction was done by the potentiometric method in aqueous suspension in a ratio of 1:2.5, humus content was determined by the wet oxidation method and titrimetric determination (Walkley - Black), whereas the mobile phosphorus was determined by the Egner-Riehm-Domingo method in ammonium acetate lactate solution and the mobile phosphorus was determined by the photometric method in Egner-Riehm-Domingo extract.

During the growing season, the sequence of growth and development phenotypes of the plants was monitored and biometric measurements (average stem height, average height of cob insertion and stem diameter) were taken on May 14th, June 10th, July 01st and August 28th 2021 using a measuring tape and ruler. Among the productivity elements, the weight of grains per cob (grams) was determined by weighing and the yield obtained (tons/hectare) at harvest time.

Determination of plant leaf area was done by measuring leaf width and leaf length on several plants determining the average leaf area and then the average leaf area per plant, and the leaf area index was estimated using the formula (Dugje, 1992) cited by (Albert Berdjou et. al, 2020) LAI = (P × L × A)/(GA), where, LAI = Leaf area index, P = Plant population/ground area (ha), L = Number of fully expanded green leaves/plant, A = Single leaf area (cm^2), GA = Ground area or hectares.

RESULTS AND DISCUSSIONS

The results of the agrochemical determinations carried out show that the pH values of the analysed soil of 7.5 are slightly alkaline, which reveals the presence of carbonates in the soil and ensures good growth and development conditions for maize, whose soil reaction requirements lie between pH values of 6.5-7.5.

The humus content of the soil was 2.5% showing a medium humus supply, the results regarding the mobile phosphorus content was 42 ppm showing a good mobile phosphorus supply of the soil and for potassium supply it was 198 ppm mobile potassium showing a good potassium supply of the soil as well.

From a climatic point of view, during the vegetative period, rainfall was below the multiannual monthly average, except in June, when rainfall was above the multiannual monthly average. From a thermal point of view during the whole growing season the average monthly temperatures were above the multiannual monthly mean.

The preplant was also maize, considering that maize is a plant with modest requirements in relation to the preplant, but maize monoculture practised for more than 2-3 years leads to crop losses directly proportional to the duration and the technology applied, resulting in the so-called phenomenon of “soil exhaustion”. Rotations that also involve fodder crops are much more sustainable compared to current short-term rotations (Trenton and Lauerb, 2008).

Seedbed preparation was done by a disc harrow pass followed by tillage with a seedbed cultivator at a right angle to the direction of sowing, on the day before sowing.

Sowing was achieved on 26 April 2021, the start of the sowing was on 04 May 2021, the density at sprouting was 6.7 plants/m² in the case of sowing in equidistant rows at a distance of 70 cm and 13.1 plants/m² in the case of sowing in equidistant rows at a distance of 35 cm between rows.

Complex fertilizers with a ratio of 20:20:20 at a rate of 200 kg/hectare were also applied with sowing.

After sowing, before sprouting, TENDER 1.5 l/hectare was applied as herbicide for a superior and long-lasting control of grass weeds and some dicotyledonous weeds.

On May 24th 2021, herbicide Fornet extra 0,7l/hectare was sprayed for the control of annual grass weeds but also for *Sorghum halepense* (Johnson grass) from rhizomes. The harvesting was carried out on October 02nd 2021.

The results on phenophase sequence are presented in Tables 1 and 2.

Table 1. Phenophase succession and biometric measurements for the distance between rows of 35 cm

Date	Phenophase	Medium stem height (cm)	The average insertion height of the cob (cm)	Stem diameter (cm)
14.05. 2021	four fully formed leaves	17.50	-	-
10.06. 2021	six fully formed leaves, seven in the cornet	45.80	-	-
01.07. 2021	ten fully formed leaves, leaf 11 in cone	189.00	-	-
26.08. 2021	fourteen fully formed leaves	291.00	128.00	2.11

Table 2. Phenophase succession and biometric measurements for the distance between rows of 70 cm

Date	Phenophase	Medium stem height (cm)	The average insertion height of the cob (cm)	Stem diameter (cm)
14.05. 2021	four fully formed leaves	17.06	-	-
10.06. 2021	six fully formed leaves, seven in the cornet	43.50	-	-
01.07. 2021	ten fully formed leaves, leaf 11 in cone	180.30	-	-
26.08. 2021	fourteen fully formed leaves	278.20	124.80	2.73

Looking into the results presented in Table 1 and 2, one can see that there are differences related to both stem height and the corncob insertion height.

Thus for the 70 cm clearance sown variety, the stem height is 278.20 cm as compared to the 291.00 cm for the 35 cm range sown variety, while the corncob insertion height is 128.00 cm in the 35 cm range sown variant, compared to 124.8 cm in the 70 cm range sown variant. The conclusion is that the higher plant stem and corncob insertion height for the denser sown variant can be explained by the competition for light of the plants which causes a slight increase in both height and corncob insertion height.

For the two sowing variants analysed, the stem diameter was also determined and it was observed that in the case of the variant sown at 35 cm distance, the stem diameter was smaller compared to the variant sown at 70 cm by 0.62 cm, which also resulted in a decrease in the resistance of the stem to fall and breakage.

In the phenophase of ten fully formed leaves, the leaf area of the plants was determined and resulted in a leaf area/plant of 4,888.64 cm² for the variety sown at 35 cm range and 5,144.33 cm² for the variety sown at 70 cm, range. Based on these findings, the leaf area index was also determined, which for the variety sown at 70 cm distance has values of 3.44 and for the variety sown at 35 cm distance has values of 4.60.

As a result, the reduction of the nutrient area from 0.15m² (in the 70 cm-weighed variant) to 0.076 m² (in the 35 cm-weighed variant) resulted in a reduction of the vegetative growth with implications on the size of the uptake area for each plant, but with an increase of the leaf area index values due to the higher density of the soil covering the soil surface.

By increasing plant density, the total leaf area expands and the land cover is accelerated, allowing a better use of light energy, although the light intensity inside the field decreases with the development of the foliar system, consequently increasing the shading of the lower leaves.

Among the elements of productivity, the weight of the kernels per cob was determined, which was 150 g for the variety sown at a distance of 70 cm and 55.73 g for the variety sown at a distance of 35 cm.

The weight of the kernels on the cob varies within quite large limits, so that the difference in this element of productivity for the two variants is 94.27 g per cob in favour of the variant planted at 70 cm, which is also shown by the difference in terms of size of the yields obtained for the two variants under analysis.

In order to see whether the differences recorded in terms of yields between the variants analysed are statistically reliable, we have used the JASP statistical analysis programme, the analysis of the variants being carried out using the ANOVA test, the results of which are shown in Table 3.

Table 3 ANOVA - Production

Cases	Sum of Squares	df	Mean Square	F	p
Variant	11.760	1	11.760	48.329	0.002
Residuals	0.973	4	0.243		

Note. Type III Sum of Squares

Since p = 0.002 is below the significance threshold of 0.05, the difference is highly significant between the averages of the two variants examined, the values of the coefficient of variation appear in Table 4.

Table 4. Descriptives - Production

Variant	N	Mean	SD	SE	Coefficient of Variation
Rows dist 35 cm	3	7.367	0.379	0.219	0.051
Rows dist 70 cm	3	10.167	0.586	0.338	0.058

Figure 1 shows the yields of each repetition (tons) in the experimental variants considered.
In order to check the homogeneity of the dispersion, Levene's test was performed, and Figure 2 is a graphical representation of the homogeneity of the dispersion showing that there is no systematic deviation from the straight line.

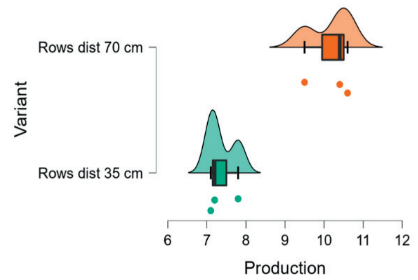

Figure 1. Yield per each repetition of the variants analysed (tons/hectare)

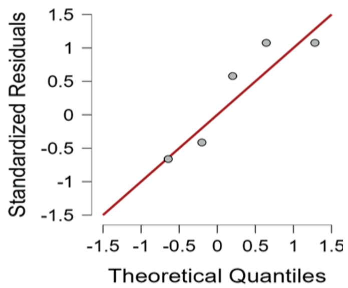

Figure 2. Dispersion homogeneity

Table 5. Post Hoc Comparisons – Variant

			95% CI for Mean Difference					
		Mean Difference	Lower	Upper	SE	t	p_{tukey}	p_{bonf}
Rows dist 35 cm	Rows dist 70 cm	-2.800	-3.918	-1.682	0.403	-6.952	0.002	0.002

CONCLUSIONS

The results obtained indicate that increasing the density causes a reduction in yield by influencing the size of the productivity elements due to a reduction in the uptake surface for each plant.
However, the increase in production can only be achieved by increasing the density, but on condition that the photosynthetic activity is maintained at a high level, and this can be achieved with the help of hybrids whose leaves are positioned closer to the vertical, which will be suitable for higher densities with consequences on the increase in the solar energy conversion coefficient.
Following the determinations made and the production results obtained, it appears that doubling the density per unit area results in a decrease in production, because the reduction in productivity of a plant cannot be compensated by an increase in the number of plants/hectare, so that when sown in equidistant rows at a distance of 35 cm, a yield of 7.33 tons/hectare has been obtained, 2.8 tons/hectare less than when sown in equidistant rows at a distance of 70 cm where a yield of 10.16 tons/hectare has been achieved.
Therefore, the increase in plant density does not result in an yield increase, because a density threshold is reached. Above this threshold, the yield does not increase, on the contrary, it starts to decrease, as light becomes a limiting factor and the rate of nutrients absorption decreases. This fact influences the number of plants per hectare corresponding to the optimal density of the maize crop.

According to the results obtained, it is recommended to increase the density especially in hybrids that are suitable for higher densities but with the maintenance of high photosynthetic activity.

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33-YEAR OLD GRAIN YIELD FROM *Triticum durum* Desf. AFFECTED BY MINERAL FERTILIZATION WITH NITROGEN AND PHOSPHORUS

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Abstract

The long-term 33-year study was aimed at clarifying the impact of mineral fertilizers under the influence of different weather conditions. The experiment was conducted at the Field Crops Institute, Chirpan, Bulgaria. The data presented in this article are for the period 1990-2022 (33 consecutive years). The following nitrogen (N) and phosphorus (P) rates were applied: 40, 80, 120 and 160 kg ha^{-1}. Each fertilizer rate was introduced alone and in combinations (25 variants). All fertilization rates were compared to non-fertilized variant (N_0P_0). The results showed that the conditions of the year (47.87% of the total variation) and fertilization (33.40% of the total variation) have a significant influence on the productivity of durum wheat. Furthermore, we reported that nitrogen fertilization had a stronger effect than phosphorus, but combined fertilization at the $N_{120}P_{120}$ rate had the highest grain yield. The correlation coefficient (R=0.897) and the coefficient of determination (R^2=0.805) were proven. Through second-order polynomial equations, it was found that the optimum fertilization rate was in the range of 120-130 kg N ha^{-1}.

Key words*: grain yield, nitrogen, phosphorus, triticum durum.*

INTRODUCTION

For millennia, durum wheat has been an indispensible part of people's daily diet. The written history of wheat science began 2500 years ago when Greek botanist Theophratus (371-287 BC) wrote the study "Enquiry into Plant's" (Xynias et al., 2020). Wheat was introduced into the Balkan Peninsula through migrations from Anatolia in the Neolitic area (Velimirovic et al., 2023). In Bulgaria it is a traditional crop that serves as a material for the production of pasta and is dispensable in this respect from common wheat (Dragov, 2022). In the past (unit 1943) in Bulgaria, the production of durum wheat grain amounted to 550-850,000 tons. However, farmers' interest in this crop is declining with the development of high-yield varieties of bread wheat, and the cultivation of durum wheat remains in the background. At the end of the 1980s, there was grain an increase in interest and areas for cultivation. According to data from the Ministry of Agriculture and Food (2022) the total area sown with durum wheat in Bulgaria in 2022 was 8,962 ha, while the area sown with bread wheat was 1,208,457 ha. Wheat crops occupy about 218 million acres and account for 1/3 of the worlds' cereal production, with 771 million tons of production per year that satisfies the demand of 21% to 36% of the world's population (Ltaief & Krouma, 2023). While globally minor, accounting for less than 7% of the total wheat produced worldwide, it is concentrated in relatively small geographic regions where it can be considered as a main cereal crop, contributing significantly to food production and agricultural income (Martinez-Moreno et al., 2022).

The need to study agrocenosis as a system with interacting components to identify function relationship and find the way to manage them led to the appearance of long-term field experiments more than 175 years ago (Romanenkov et al., 2020). Most of the older experiments were started to provide information on the amounts of nutrients and forms of fertilizer to use to increase crop yield (Jhonston & Poulton, 2018). These field trials provide valuable information on long-term crop production in a particular climate zone. Long-term experiments provide of the means to measure sustainable management systems of agriculture (Patel et al., 2021). Challenges unique to a long-term experiment are discussed, including maintaining relevance to current farming issue, human error, and how changes have been implemented while maintaining the historic experiment treatments (Brooker et al., 2020). In recent decades, have attempted to developed various methods to optimize the application rate of chemical fertilizer to solve the associated economic and environmental problems caused by improper application (Hu et al., 2023). Results obtain from long-term experiments have already substantially improved our knowledge on changes in soil productivity with various fertilization practices (Cai & Qin, 2006). Also, long-term field experiments with different fertilization regimes can provide a good way to separate the impact of climate and soil properties and crop yields (Wei et al., 2021).

In the natural environment, plants often face unfavorable factors affecting their growth and production (Zuluaga et al., 2023). Ambient temperatures and precipitation are some of these factors that cannot be adjusted. The main purpose of nutrient application is to increase the level of soil fertility and crop yield (Azeem et al., 2023). Furthermore, farmers have prioritized grain yield over grain quality, with large amounts of chemical fertilizers being used to increase grain production (Song et al., 2022). Nitrogen (N) is one of the most important essential nutrients in regular in crop physiological processes and determining grain yield (Li et al., 2022). Nitrogen is an element that has greatest influence on the vegetative growth of the plant, photosynthetic capacity and yield (Lalevic et al., 2019). The world applies more than 120 million tons of nitrogen fertilizer every year (Zhao et al., 2023). Given that 15 kg N ha^{-1} is lost at optimal N levels, farmers are probably used to spreading more N fertilizer than necessary to increase grain yield (Sarker et al., 2023). However Xing et al. (2023) warn that excessive blind application of N will fail to achieve the expected yield, and it will increase cultivation costs and decrease grower incomes. One of the main reasons for limited yield and grain quality is the lack of knowledge in nitrogen fertilization practice (Boulelouah et al., 2022). Valuable information can be derived from long-term field experiments with different fertilization rates on the impact of synthetic fertilizers, with some of the most important issues being related to soil microbiota, groundwater and CO_2 emissions. Of course, for scientists in the field of crop production, the question of grain production in quantitative terms and the optimal nitrogen rate remains the most important.

The production of crops for food requires an adequate supply of phosphorus (P) in soil (Khan et al., 2018). P is vital for the growth of crops from seed to the harvest (Rehim et al., 2018). The authors add that it is essential for the transformation of energy and plays an important role in different metabolic processes in plants. Thus, in fear for P deficiency, excess P fertilizers are routinely applied by farmers for producing wheat (Mihoub et al., 2019). The authors add that, however, wheat plants take up and use a small amount of applied phosphorus. Indeed, the greater part of P remains in insoluble form in soil and, hence, unavailable for the plant (Cherchali et al., 2019). Moreover, Sanchez-Rodriguez et al. (2021) estimated that $<20\%$ of all added P remained available to plants. For this reason, improvement in phosphorus management is of high priority (Kominko et al., 2019). Long-term experiment, rather than short-term ones are valuable for accurately assessing P-use efficiency (Khan et al., 2018). Unlike nitrogen, which can be restored by air fixation, phosphorus cannot be replenished without external sources (Arsad et al., 2022). For this the optimum rate of phosphorus is important for grain yield improving (Lalevic et al., 2019).

The excess of the unused nutrients gets into ground and surface water as well as to the atmosphere (Piwowar, 2021). So increasing the use of fertilizers will cause more and more problems for the environment in the world in future (Ejraei, 2021). Agricultural policies aiming at lowering environmental impacts by limiting nutrient impost are a main reason for the stagnating yield increase in Europe countries (Kirchmann et al., 2020). Therefore, our long-term 33-year study was aimed at clarifying the impact of mineral fertilizers under the influence of different weather conditions, as well as comparing different fertilizer rates of nitrogen and phosphorus applied alone and in combinations.

MATERIALS AND METHODS

Experimental location and experimental design

The study was started as a multi-year fertilizer experiment in 1966 at the Field Crops Institute, Chirpan, Bulgaria (42°11'58"N, 25°19'27"E). The data presented in this article was from 1990-2022 (33 consecutive years). The experiment was a complete randomized block design with 4 replications. Each plot was 10 m^2 in size and was separated from the neighboring one by concrete slabs in the soil at a depth of two meters to prevent the passage of the imported mineral fertilizers. The experiment included the following rates of nitrogen (N) and phosphorus (P): 40, 80, 120 and 160 kg ha^{-1}. Each fertilizer rate was applied individually and in combinations as follows: $N_{40}P_{40}$, $N_{40}P_{80}$, $N_{40}P_{120}$, $N_{40}P_{160}$, $N_{80}P_{40}$, $N_{80}P_{80}$, $N_{80}P_{120}$, $N_{80}P_{160}$, $N_{120}P_{40}$, $N_{120}P_{80}$, $N_{120}P_{120}$, $N_{120}P_{160}$, $N_{160}P_{40}$, $N_{160}P_{80}$, $N_{160}P_{120}$, and $N_{160}P_{160}$. All fertilization rates were compared with a non-fertilized variant (N_0P_0). Phosphorous fertilizer (triple superphosphate) was incorporated with the last discing of the soil before sowing durum wheat. Nitrogen (ammonium nitrate) was applied in the tillering phase as an early spring top dressing.

Soil analysis

The soil type was *Pellic Vertisols*. In a depth of up to 20 cm there was a high content of humus - 3.85%, and it decreased to 1.9% in the 80-100 cm layer. Total carbonates were below 5% in the deep layers. The salt content was negligible. This shows that the soil have good drainage and there is no danger of a negative impact on the wheat. The soil reaction varies from 6.5 to 7.4 pH and allows the introduction of all types of mineral fertilizers. At a depth of 30 cm, the soil had the following characteristics regarding macronutrients: well stocked with total nitrogen, as in the form of $N\text{-}NH_4^+$ it was 3.2-3.6 kg ha^{-1}, and in the form of $N\text{-}NO_3^-$ – 2.8-3.2 kg ha^{-1}; the amount of P_2O_5 was 6.1 mg/100 g soil, with a large part of phosphorus bound in the form of primary phosphorus minerals, hardly soluble and poorly available to plants. The potassium content was significant at 24.1 mg/100 g soil.

Meteorological data sources

Bulgaria is divided into five climate zones, and the place of the experiment is qualified for the transitional-continental climate zone. Regarding temperature totals during the wheat growing season, seven of the observed years were very warm (2007, 2009, 2013, 2016, 2019, 2020, and 2022), five years were warm, eight years were moderately warm, seven years were normal, three years were moderately cool and three years (1993, 1996 and 2006) were cold (Figure 1). According to the amount of precipitation, the years studied

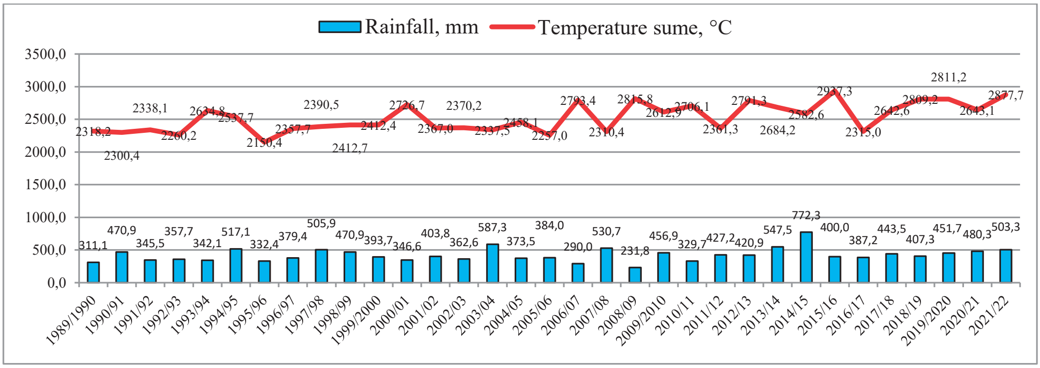

Figure 1. Temperature sum (°C) and precipitation (mm) during wheat vegetation for period 1990-2022

were: very wet (2004, 2015 and 2022); wet (1998, 2008, 2014 and 2018); moderately wet (1991, 1999, 2010, 2012, 2013 and 2017); moderately wet (1995, 2019, 2020 and 2021); moderately dry (1997, 2000, 2002, 2005, 2006 and 2016); dry (1992, 1993, 1994, 1996, 2001 and 2003); very dry (1990, 2007, 2009 and 2011).

Statistical analysis

A one-way ANOVA was used to analyze the differences between fertilization rates and combinations on average over the years of the study. For this purpose, each year was treated as a repetition. Comparisons were performed using least significant difference (LSD) ($P \leq 0.01$). The regression model was done using Statistica 13.

RESULTS AND DISCUSSIONS

The total variance presented in Table 1 revealed a statistically significant influence of both mineral fertilization and year conditions on grain yield (GY) formation. Moreover, the influence of weather conditions has a stronger effect (47.87% of the total variation) than mineral fertilization (33.40% of the total variation). However, year and fertilization were equally statistically significant at $p \leq 0.001$.

On average, over a 33-year period, durum wheat realized a grain yield of 2,346 kg ha^{-1} without fertilization (Figure 2). When nitrogen fertilization was included, there was a tendency to increase the values up to the rate of 120 kg N ha^{-1} (3,987 kg ha^{-1}). Increasing N to 160 kg ha^{-1} resulted in lower GY – 3,795 kg ha^{-1}, respectively. Phosphorous fertilization had a weak and unproven effect on the formation of grain yield. Moreover, when P_{40} was applied, GY was lower than the control.

Table 1. Analysis of variance for durum wheat grain yield under NP fertilization, 1990-2022

Source of variation	df	Sum of squares	Sum of squares, %	Mean squares	F
Total	824	1.037,193E+09	100		
Year	32	4.964,956E+08	47.87***	1.551,549E+07	61.33
Mineral fertilization	24	3.464,038E+08	33.40***	1.443,349E+07	57.05
Error	768	1.942,938E+08	18.73	252,986.7	

, * – significant at p≤ 0.01 and p≤ 0.001 level of probability, respectively

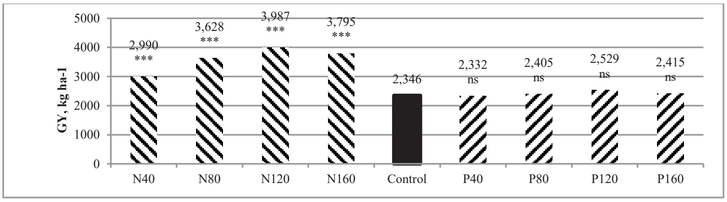

Figure 2. Durum wheat grain yield under nitrogen-phosphorus fertilization, 1990-2022

The GY data showed that all N and P combinations tested had a statistically confirmed effect (Table 2). Analysis of 33 years of durum wheat fertilization showed that phosphorus had no significant effect even in combination with N. Therefore, nitrogen fertilization plays a major role in grain formation. The lowest GY was reported when fertilizing with $N_{40}P_{160}$ – 3,100 kg ha^{-1}. An increase in grain was observed with increasing N dose up to 120 kg ha^{-1} and with the strongest effect on GY formation was fertilization with $N_{120}P_{120}$ – 4,229 kg ha^{-1}. Exceeding this fertilization threshold resulted in a decrease in values.

Since nitrogen fertilization had a major effect, the variation of GY under the influence of N was described by second-order polynomial equations (Figure 3). The correlation coefficient (R = 0.897) and the coefficient of determination (R^2 = 0.805) were proven. The GY curve showed that the optimum fertilization rate was in the range of 120-130 kg N ha^{-1}.

Table 2. Durum wheat grain yield (kg ha^{-1}) at NP fertilization, 1990-2022

Fertilizer rate	N_{40}	N_{80}	N_{120}	N_{160}
P_{40}	3,143***	3,835***	4,100***	3,968***
P_{80}	3,144***	3,926***	4,187***	4,081***
P_{120}	3,192***	3,939***	4,229***	4,001***
P_{160}	3,100***	3,893***	4,061***	4,002***
LSD 5%, 1%, 0.1% = 243.1; 319.8; 409.0				

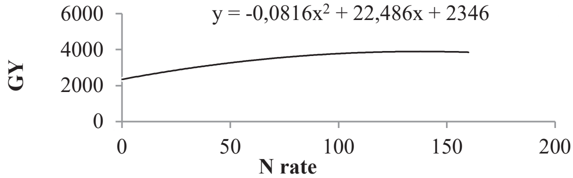

Figure 3. Relationship between wheat grain yield and nitrogen rate

Climate change is a major challenge for the world economy, especially for the agricultural sector, where climate conditions strongly and directly influence crop yields (Rapacz et al., 2022). This statement fully coincides with the results of Table 1 of the total variance for a reliable and strong influence of years on GY, as well as the results obtained by Mangini et al. (2021). The authors reported that ANOVA confirmed the influence of year conditions. Zhang et al. (2018) reported that there was a large interannual variability within the same treatment, which was mainly due to rainfall during the growing season. Application of mineral fertilizers acted as a stabilizing element that reduced the degree of yield inter-annual variability (Hlisnikovsky et al., 2023). This explains the high reliability of applied mineral fertilization and the high percentage of impact from the total variance. In a long-term fertilization experiment started in 2007, a strong effect of year (**) was observed, but fertilization had a stronger effect (***) (Zhang et al., 2019), which was contrary to the results of this research.

Efficient nitrogen fertilizer management is critical for wheat production and the long-term protection of the environment (Tedone et al., 2018). The results obtained in this study for a decrease in GY with increasing N rate (Figure 2) were also reported in other studies. For example, Abera et al. (2021) reported that bread wheat GY was highest when fertilized with 92 kg N ha^{-1} and increases above this rate indicated lower yield. Chen et al. (2022) obtained 1,933 kg ha^{-1} GY from N_{65} fertilization, but increasing N to 135 kg ha^{-1} reduced GY to 1,779 kg ha^{-1}. From 20 years of fertilization, Wei et al. (2021) reported that wheat yield showed significantly decreasing trends with N alone treatment. According to Kizilgeci et al. (2021) this may be due to the higher amount of N application causing more vegetative growth (more succulent leaves and taller strems) which delayed the reproductive growth and ultimately led to suboptimal reproductive yield attributes. Shuklina et al. (2021) even reported the highest GY under the influence of the low nitrogen rate studied (N_{60}) – 3.70 t ha^{-1}, while at the highest dose included in the study (N_{150}) GY decreased to 3.62 t ha^{-1}. However, this trend does not necessarily hold. For example, Mondal et al. (2022) observed that increasing the N rate from 0 to 140 kg ha^{-1} resulted in an increase in GY and Ayadi et al. (2022) confirm the increase in values. Moreover, the authors reported that GY under the influence of N_{75} was significantly different from N_{150}, 3.4 t ha^{-1} and 5.7 t ha^{-1}, respectively. In other cases, as in the study by Tedone et al. (2018), increasing nitrogen rate increased GY, but the difference in grain between low and high fertilization rates was small. Staugaitis et al. (2022) reported that high nitrogen fertilizer rates (N_{180} and N_{240}) had no difference in terms of GY, 7.39 t ha^{-1} and 7.31 t ha^{-1}, respectively. This confirms the assertion of Guerrero et al. (2021) that plant response to N is limited by a threshold, N fertilization above this level does not further increase yield.

After nitrogen stress, phosphorus is the second most widely occurring nutrient deficiency in cereal systems around the world (Awulachew, 2019). In the study conducted, P fertilization had a weak and insignificant effect (Figure 2). This result confirms the claim of Gao & Grant (2012) that there was no statistically significant effect on grain yield of different P fertilization rates. Similar results can be found in the studies of Lakew (2019) and Chen et al. (2020) in bread wheat. In contrast to these results, Arsad et al. (2022) reported that P fertilization increased GY values proportionally with increasing rate. From the unfertilized control, the authors obtained a grain yield of 2,220 kg ha^{-1}, and at the highest P dose tested (60 kg ha^{-1}) GY was 3,523 kg ha^{-1}. Chen et al. (2019) from a 3-year study on the influence of phosphorus self-application obtained similar results. Arbacauskas et al. (2021) reported that 49 years of P fertilization resulted in soil P accumulation. Continuous application of inorganic P-fertilizer leads to significant accumulation of available P in the soil (Vishwanath et al., 2020). This accumulation may account for inefficient P fertilization. If sufficient resources are available in the soil, the effect of applying a given nutrient cannot be accounted for.

The data from table 2 clearly show that the formation of grain yield was influenced to the greatest extent by nitrogen fertilization. Both in single and combined fertilization, the dose of 120 kg N ha^{-1} has the strongest impact. Above this rate, grain yield decreases. Eshetu et al. (2022) observed the same response in bread wheat. The authors reported that GY increased up to $N_{46}P_{40}$ (7,018.6 kg ha^{-1}), but when fertilization increased to $N_{69}P_{50}$, GY was lower by 1,355.2 kg ha^{-1} (5,663.4 kg ha^{-1}). This result was also confirmed by the mathematical model in Figure 3 from the regression equation, where it can be seen that the rate of 120-130 kg N ha^{-1} was optimal. Although P fertilization did not significantly affect GY in this study, Tudor et al. (2023) reported that phosphorus fertilizers aided plant rooting by increasing N efficiency. This statement was in sync and fully explained the results of this study. However, as a one of the essential nutrient element in the process of plant growth and development, nitrogen contributes 40-50% to the final yield (Chen et al., 2022).

CONCLUSIONS

In this study, special attention was paid to durum wheat grain yield affected by the long-term application of mineral nitrogen and phosphorus fertilizers (33 consecutive years). The results showed that the conditions of the year (47.87% of the total variation) and fertilization (33.40% of the total variation) had a significant influence on the productivity of wheat. Furthermore, we reported that nitrogen fertilization had a stronger effect than phosphorus, but combined fertilization at the $N_{120}P_{120}$ (4,229 kg ha^{-1}) rate had the highest grain yield. The regression model showed that the optimal fertilization rate was in the range of 120-130 kg N ha^{-1}.

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CORN RESPONSE TO FOLIAR FERTILIZER APPLICATION

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Abstract

A field experiment was conducted with five maize hybrids during the period 2022-2024. The parameters of the yield of green mass, of the additional yield formed under the influence of foliar fertilization, have been established. The application of the organic liquid fertilizer Aminosol, the single-component inorganic fertilizers Boron, Zinc and Nutriplant 36 contributed to an increase in biomass by 20608.4 to 26799.0 kg/ha, on average over the study period. After treatment with the organo-mineral fertilizer Kinsidro Grow and the nitrogen stabilizer N-Lock, an increase in the productivity of the hybrids was again established. The greatest increase was registered at LG 31,390, respectively with 26068.8 kg/ha. In total, for hybrids and variants for the period of the field study, an increase of 41.4% was recorded after foliar fertilization with the products of the first technology. A strong correlation was established between the indicators of plant height and green mass yield for Premeo (R^2 = 0.9284) and Pioneer P9889 (R^2 = 0.9043). A positive correlation dependence, with a coefficient of determination R^2 = 0.8998 for DKC 4416.

***Key words**: corn, yield, biomass, foliar fertilization, regressions.*

INTRODUCTION

Global climate change, prolonged droughts, insufficient water resources and last but not least interrupting the growing population of the planet raises the question of the need to produce more output per unit of area. An increase in average temperature leads to variability in the productivity of all agricultural crops, Tigchelaar et al. (2018) prove in their research. This is a serious challenge to crop production and agricultural science.

Management and adaptation can greatly reduce the potential impacts of climate change and climate variability on crop yields and farmers' incomes (Mineva, 2020; Popova et al., 2022). It is necessary to study the changes induced by the elements of climate on the growing seasons and yields of agricultural crops (Cojocaru, 2022; Song et al., 2023; Bijnens et al., 2024).

According to world grain production data in 2022/2023, maize has become the third most important grain crop in the world after wheat and rice (Shahbandeh, 2024).

For optimal nutrient supply, ecological, sustainable and innovative strategies need to be developed to be implemented as effective tools to maintain productivity and increase the tolerance of maize to drought stress (Ghiyasi et al., 2023; Moitazedi et al., 2023; Zarea et al., 2023).

The assimilation of nutrients throughout the growing season is linked in a number of agronomic practices that contribute to optimizing the process and guaranteeing high yields. These practices include structuring the soil, having enough readily available moisture for plants, combating water deficit and nutrient deficiency. Balanced fertilization is a key factor in the development and productivity of plants. Mineral fertilizers are crucial for increasing yields, but their unrestricted use leads to deterioration of the quality of plant production and environmental pollution (Yan et al., 2012; Zhao et al., 2013; Mu et al., 2015; Mustafa et al., 2021; Harish et al., 2022). According to research by Marschner (2012), after nitrogen fertilization, an increase in plant biomass was observed. The effect of nitrogen sources on the biomass and quality of silage maize has been the focus of a number of studies (Amin, 2011; Safdarian et al., 2014). An increase in the photosynthesizing surface of the leaves was also reported by Leghari et al. (2022).

Application of foliar fertilizers after soil fertilization is an effective method for increasing crop micronutrient content and crop yield and improving soil environment (Niu et al., 2021;

Stoyanov et al., 2024). According to Ramesh et al. (2018) foliar fertilization with organic fertilizers had a positive effect on growth and yield of maize (*Zea mays* L.). The authors reported increases in plant height, leaf area index, dry matter production, cob length and diameter, number of cob kernels, and grain yield. Asare et al. (2023) also reported an increase in leaf area and productivity in maize after foliar application of micronutrient-rich fertilizers.

Additionally, micronutrient fertilization is an effective strategy to reduce the harmful effects of water stress in plants (Solanki, 2021; Umar et al., 2022; Stoyanova et al., 2024). Foliar nutrition of plants, including application of the main nutrients (N, P and K) alone and in different combinations, leads to increased yields even under water stress conditions, reported Kakar et al. (2014). The authors found taller plants (221 cm), heavier grains (229 g/1000 grains) and prolongation of physiological maturity after foliar fertilization. Grain yield increased (2896 ha^{-1}) and harvest index (28.2%) compared to the control plots (no foliar nutrients were applied).

The objective of the present study was to investigate the effect of foliar fertilizers on biomass productivity in five maize hybrids.

MATERIALS AND METHODS

The study was conducted in the Stara Zagora region, Bulgaria during the period 2022-2024. The field experiment involved five maize hybrids from the FAO early and medium-early group. The hybrids tested were DKC 4416, Pioneer P9889, LG 31.390, Premeo and Knezha-461. They are of a different genetic background. LG 31.390 (FAO 390) is a representative of Hydraneo technology. This is a new generation of hybrids for managing drought risk DKC4416 (FAO 330) is a hybrid of the FieldShield hybrids and is characterized by high resistance to abiotic stress.

Pioneer P9889 (FAO 360) is a hybrid of the Optimum® AQUAmax® product line, a selection of hybrids adapted to drought. Premeo (FAO 400) is an Artesian technology representative. Knezha-461 (FAO 400-500) is a representative of the Bulgarian selection of hybrids. The study was carried out using the method of fractional plots, in 4 replications, with the size of the experimental area of 15 m^2. Variations of the experience are the following: 1. Control - Soil fertilization with N_{14} (without irrigation); 2. Soil fertilization with N_{14}; 3. Soil fertilizing + foliar fertilizing with Aminosol + Lebozol B + Lebosol Zn, Nutriplant 36; 4. Soil fertilization + Kinsidro Grow, N-Lock.

In the control variant, soil fertilization of the crop was carried out. In variants 2-4, irrigation was carried out using a drip irrigation system. The distance between the drippers was 0.15 m, in order to create a continuous moistened strip in the soil horizon. With the artificial supply of irrigation, conditions are created for optimizing the water regime in the soil and increasing the moisture in the ground air layer. The realized irrigation rate is 30 mm when the soil moisture reaches below 75-80% marginal field moisture capacity (FMC).

The tested hybrids were fed with foliar fertilizers during the growing season. The first technology included the organic liquid fertilizer Aminosol (2.0 l/ha), the single-component inorganic fertilizers Lebozol B (2.0 l/ha), Lebosol Zn (1.0 l/ha), and Nutriplant 36 (10.0 l/ha). According to the second technology, foliar fertilizing was carried out with the organo-mineral fertilizer Kinsidro Grow (150 g/ha), in the form of granules and is the nitrogen stabilizer N-Lock (2.50 l/ha).

RESULTS AND DISCUSSIONS

In the development of the crop, the soil-climatic features of the region where the experimental field is located are important. Of the elements of climate, temperature and rainfall are the factors that most influence the growth and development of agricultural crops.

Figure 1 presents the dynamics of the monthly average daily temperature for the three-year study period. Significant differences in temperature values have been reported when compared to the multi-year norm (for the period 1930-2024).

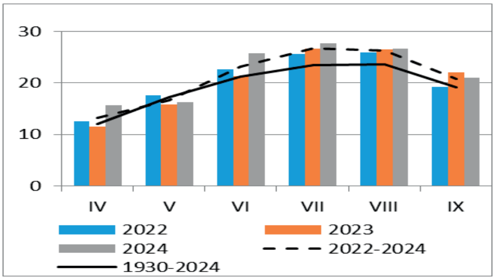

Figure 1. Dynamics of average daytime temperatures, by months, for the period 2022-2024

The analysis of the values shows that the average day-night temperature is above the norm in the three years, by 5.7%, 6.0% and 13.9%, respectively, and on average for the period by 8.5%. The extremely high temperatures registered in the second ten-day period of July 2024 make an impression. The increase in this month is 4.2ºC, or 13.4% compared to the climate norm. On average for the period, positive differences have been established compared to the temperatures for a multi-year period. According to Wang (2021) higher temperatures accelerate plant development, which shortens the length of reproductive phases.

In terms of the amount and distribution of precipitation, Figure 2 shows that the humidity conditions are different in the three years. In 2022, the amount of precipitation recorded during the vegetation period was 254.9 mm, which is 14.1% less than the norm for the period. In the second year, precipitation was 7.1% less, while in 2024 a decrease of 1.2% was found. It should be noted that in the last year of the field experiment, 98.5 mm were measured in September, when corn had completed its vegetation and was in the process of harvesting. The reported amount and distribution of precipitation during the corn growing season is extremely uneven. As a result, there is a deficiency of sufficiently readily available moisture, necessary for the growth and development processes of the studied corn hybrids.

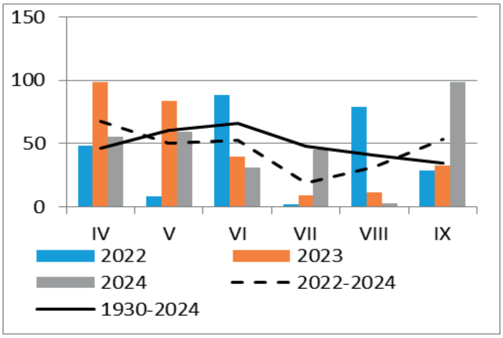

Figure 2. Amount and distribution of rainfall, during the period 2022-2024

The productivity of the culture and the stability of the yields depend not only on the level of agricultural technology, but also on the biological potential and adaptability of the respective hybrids. In the present study, the productivity of five maize hybrids of different genetic origins was examined. In natural, non-irrigated conditions, and under optimization of humidity conditions, the productivity under the influence of foliar nutrition of plants was established (Figure 3).

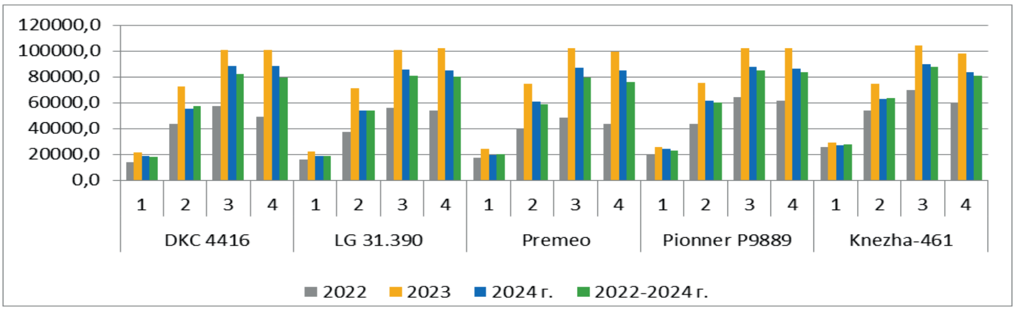

Figure 3. Green mass yield, for the period 2022-2024, kg/ha

The experimental results show that with natural moisture supply, yields in the range of 18100.0 kg/ha (for DKC 4416) to 27500.0 kg/ha (for Knezha-461) were established in all three years (Table 1). When optimizing the water factor, yields increased by 131.7% (Knezha-461), 159.9% (Pionner P9889), 182.4% (LG 31.390), 190.2% (Premeo) to 216% (DKC 4416). During the study, the nature of the dependence between yield and irrigation rate, the productivity of irrigation water and the additional yield was established (Stoyanova, 2018).

The experimental results show that with natural moisture supply, yields in the range of 18100.0 kg/ha (for DKC 4416) to 27500.0 kg/ha (for Knezha-461) were established in all three years (Table 1). When optimizing the water factor, yields increased by 131.7% (Knezha-461), 159.9% (Pionner P9889), 182.4% (LG 31.390), 190.2% (Premeo) to 216% (DKC 4416). During the study, the nature of the dependence between yield and irrigation rate, the productivity of irrigation water and the additional yield was established (Stoyanova (2018).

Table 1. Production of green mass and additional production, average for the period 2022-2024, kg/ha

Corn hybrids	Variant	Average yield for the period 2022-2024	Additional yield	%
DKC 4416	1	18100,0	0,0	100,0
	2	57254,8	39154,8	316,3
	3	82089,4	63989,4	453,5
	4	79327,3	61227,3	438,3
LG 31.390	1	19133,3	0,0	100,0
	2	54028,5	34895,2	282,4
	3	80827,5	61694,2	422,4
	4	80097,3	60963,9	418,6
Premeo	1	20166,7	0,0	100,0
	2	58531,7	38365,1	290,2
	3	79140,1	58973,4	392,4
	4	75995,7	55829,0	376,8
Pionner P9889	1	23100,0	0,0	100,0
	2	60037,8	36937,8	259,9
	3	84690,7	61590,7	366,6
	4	83350,2	60250,2	360,8
Knezha -461	1	27500,0	0,0	100,0
	2	63708,1	36208,1	231,7
	3	87816,0	60316,0	319,3
	4	80545,7	53045,7	292,9

The analysis of the results reports an increase in green mass yield under the influence of foliar application of fertilizers during the vegetation of the corn hybrids. The obtained data are based on the optimization of the water reserve in the soil horizon.

Foliar fertilization of plants with the organic liquid fertilizer Aminosol, single-component inorganic fertilizers Boron, Zinc and Nutriplant 36 contributed to increase the biomass by 20608.4 to 26799.0 kg/ha, on average over the study period. The increase over unfertilized variants was 35.2% for Premeo, 37.8% for Knezha-461, 41.1% for Pionner P9889, 43.4% for DKC 4416 and 49.6% for LG 31.390. After treatment with the organo-mineral fertilizer Kinsidro Grow and the nitrogen stabilizer N-Lock, an increase in the productivity of the hybrids was found again. The greatest increase was registered at LG 31,390, respectively with 26068.8 kg/ha.

In total, across hybrids and variants for the field study period, an increase of 41.4% was reported after foliar fertilization with the products from the first technology. After applying the second technology, the average increase over the period was 36.4% for the five hybrids. Therefore, Zhuk (2022) also recommends that when growing corn for green table, foliar treatment is applied to increase yields.

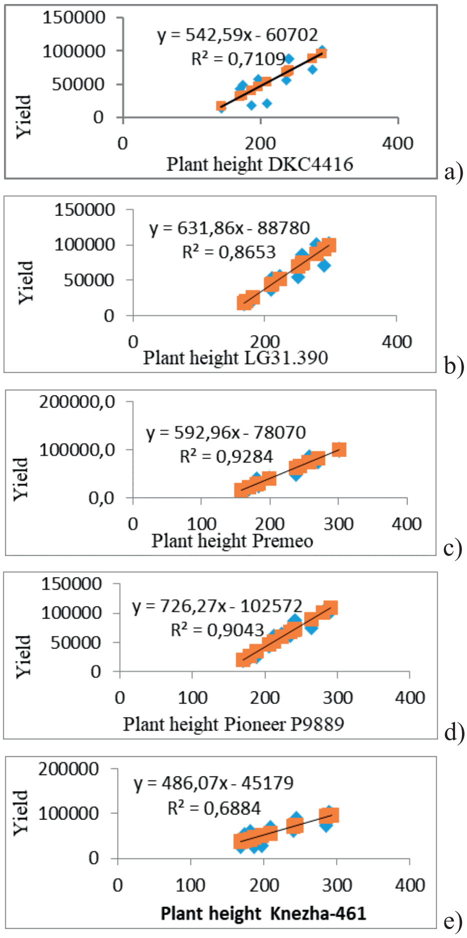

Figure 4. Linear regression model between plant height and yield of green mass

On average over the three-year test period, LG 31.390 stood out as the most responsive to foliar fertilizers. The application of the organic fertilizers Aminosol + Lebozol B + Lebosol Zn and Nutriplant 36 help to increase productivity by 49.6%, and after treatment with the products Kinsidro Grow and N-Lock, an increase of 48.2 5 was found. In the other hybrids, productivity increased by 35.2% (Premeo), by 37.8% (Knezha-461), by 41.1% (Pioneer P9889) and by 43.4 (Pioneer P9889) after application of Aminosol + Lebozol B + Lebosol Zn and Nutriplant 36. The increase was by 26.4 %, 29.8 %, 38.6 %, 38.8 %, respectively, in Knezha-461, Premeo, DKC 4416 and Pioneer P9889.
The developed linear regression equations show the nature of the relationship between the studied biometric indicators and yield of green mass. A strong positive correlation (R^2) was found. The determination coefficients were calculated for the five hybrids. A strong correlation was found between the parameters of plant height and green mass yield in Premeo (R^2 = 0.9284) and Pioneer P9889 (R^2 = 0.9043) (Figure 4).
In the remaining three hybrids, the relationship is a little less pronounced, respectively, in LG 31.390 the coefficient of determination is R^2 = 0.8653, in DKC 4416 it is R^2 = 0.7109 and in Knezha-461 R^2 = 0.6884. The coefficient of determination shows what percentage of the variance in the outcome variable is explained by the action of the factor variable. In the calculated values of for the five hybrids means that from 69 to 93 % of the yield depends on the plant height.
When investigating the nature of the interrelationships between the number of leaves per plant and green mass yield, the regression equations developed show that there are strong correlational dependencies in them as well (Figure 5).
A strong positive correlation was found between the indicators number of leaves and yield of green mass, with a coefficient of determination R^2 = 0.8998 for DKC 4416. A strong positive relationship was also found for Knezha-461 (R^2 = 0.8867), Premeo (R^2 = 0.7768), Pioneer P9889 (R^2 = 0.7607) and LG 31.390 (R^2=0.7106). The results show that from 71 to 89% green mass yield depends on the number of leaves per plant.

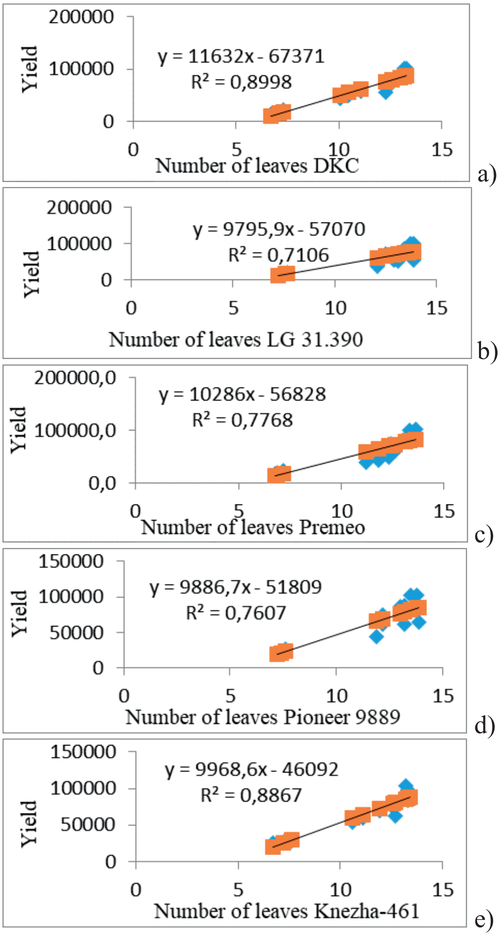

Figure 5. Linear regression model between number of leaves per plant and yield of green mass

CONCLUSIONS

Based on the field experience, several main conclusions were made.
A positive trend towards an increase in green mass yield after foliar fertilization was established. The application of the organic liquid fertilizer Aminosol, the single-component inorganic fertilizers Boron, Zinc and Nutriplant 36 contributed to an increase in biomass by 20608.4 to 26799.0 kg/ha, on average for the study period. The increase compared to the unfertilized variants was highest in LG 31.390, by 49.6%. After treatment with the organo-mineral fertilizer Kinsidro Grow and the nitrogen stabilizer N-Lock, an increase in the productivity of the hybrids has also been found.

The greatest increase was registered at LG 31,390, respectively with 26068.8 kg/ha.

In total, across hybrids and variants for the Polish study period, an increase of 41.4% was reported after foliar fertilization with the products from the first technology. After applying the second technology, the average increase over the period was 36.4% for the five hybrids.

A strong correlation was found between the parameters of plant height and green mass yield in Premeo (R2 = 0.9284) and Pioneer P9889 (R^2 = 0.9043).

A positive correlation dependence, with a coefficient of determination R^2 = 0.8998 in DKC 4416. A strong positive relationship was also established in Knezha-461 (R^2 = 0.8867).

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MAXIMIZING RAPESEED YIELD AND QUALITY THROUGH ADJUSTMENTS IN SOWING DATE AND ROW SPACING

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Abstract

To explore optimal agronomic practices for maximizing rapeseed productivity and quality under specific environmental conditions, a field experiment was conducted during the 2022-2023 agricultural year in Jucu, Cluj County. The study aimed to evaluate the effects of two critical factors influencing crop performance (sowing date and row spacing) on the yield and quality of the rapeseed hybrid PT 275. Using a randomized split-plot design with three replications, the experiment tested three sowing dates (August 20, August 30, and September 10) and three row spacings (25 cm, 37.5 cm, and 50 cm). Yield data and quality indicators were assessed at the end of the growing season to determine the influence of the experimental variables, using standardized laboratory methods. The results revealed that sowing date significantly impacted yield, with August 30 emerging as the most favorable, achieving the highest yield of 3900 kg/ha, while narrower row spacings (25 cm and 37.5 cm) significantly outperformed wider spacing (50 cm). Delayed sowing (September 10) increased oil content but reduced yield, highlighting a trade-off between productivity and quality. While the genetic characteristics of the hybrid were the primary determinant of quality, the study highlighted significant variations in quality indicators associated with the technological factors examined. These findings provide actionable insights for optimizing rapeseed cultivation under temperate continental climates.

Key words*: rapeseed, row spacings, sowing date, seed quality, yield optimization.*

INTRODUCTION

Rapeseed (*Brassica napus* L.) is the third-most cultivated oilseed crop globally, valued for its versatile applications in the edible oil production, protein sources (Raboanatahiry et al., 2021) and biodiesel (Wang and Yin, 2014). It has emerged as a significant crop in Romania in recent years, occupying approximately one-third of the total oilseed crop area (Ion et al., 2024) and is highly valued by apiarists. Additionally, rapeseed is extensively studied for its medicinal properties, including its anti-inflammatory, antiviral, antidiabetic, anticancer, and antioxidant effects (Tileuberdi et al., 2022). As with other species, rapeseed productivity is influenced not only by genetic factors but also by variations in environmental conditions such as climatic factors (temperature, precipitation, photoperiod duration and abiotic stress), soil type and agricultural practices including sowing time, pest, weed, and disease management (Rajković et al., 2022; Wu et al., 2020). Crop yield losses can range between 3% and 73% due to these factors (Lipianu et al., 2023).

In the context of climate change, rapeseed sowing protocols are continuously adjusted, to maximize both yield and quality (Wang et al., 2011). Identifying the optimal sowing time is a crucial step to ensuring high and stable yields, as significant deviations in temperature and water availability can negatively impact crop production (Turhan et al., 2011). When agronomic practices are implemented appropriately and field conditions are ideal, temperature and soil moisture are the primary factors that govern seed germination and seedling emergence (Marjanović-Jeromela et al., 2019).

For optimal rapeseed development, it is critical to sow at the appropriate time, as spring climatic conditions can strongly influence crop performance. Early-flowering plants risk frost damage, while late-flowering plants are more susceptible to heat stress and water deficit (Butkevičienė et al., 2021). Delayed sowing can substantially reduce the number of primary branches and flowers per plant (Balodis and Gaile, 2016), resulting in lower yields and compromised seed quality. Seed filling is particularly vulnerable to environmental stressors, especially temperature and precipitation patterns (Rajković et al., 2022).

The optimal inter-row spacing used for most cultivated species is not only intended to maximize yields but also to facilitate weed control. Weeds present an inherent challenge in crop production and therefore, row spacing is often adjusted to enable mechanical weed control, sometimes prioritizing this over yield optimization (Grimes et al., 2019).

Rapeseed oil is crucial for human nutrition due to its high unsaturated fatty acid content (Karunarathna et al., 2020). As a result, improving seed yield and oil production remains a key objective for rapeseed growers worldwide (Ren et al., 2022).

Studying the carotenoid content in rapeseeds, despite its relative low levels, offers potential for genetic improvements and breeding strategies to increase carotenoid concentrations, enhancing the nutritional value of rapeseed oil and animal feed; as the demand for natural carotenoids rises, rapeseeds could become a more sustainable source, providing an eco-friendly alternative to synthetic carotenoids. (Franke et al., 2010; Gao et al., 2007).

In the context of climate change and the specific agronomic requirements of autumn rapeseed cultivation, this study primarily aimed to evaluate the impact of sowing time and row spacing on yield and some quality parameters of the PT 275 hybrid under temperate continental conditions; the findings aim to refine agronomic protocols for climate-resilient rapeseed production.

MATERIALS AND METHODS

The trial was conducted in 2022-2023 agricultural year, at the Jucu VEGETAL farm (46°51′N, 23°48′E, part of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca (Figure 1), on a slightly acidic, humus-rich, argic-stagnic feoziom soil, with high fertility levels except for phosphorus. The experimental design followed a split-plot design with 3 replications (Figure 2).

Figure 1. Aspect from experimental field (original)

Figure 2. Experimental field design (original)

The experimental factors and their graduations are as follows:

- Factor S – sowing date with 3 levels:
  - S1 – August 20 (control);
  - S2 – August 30;
  - S3 – September 10.
- Factor D – row spacing with 3 levels:
  - D1 – 25 cm (control);
  - D2 – 37.5 cm;
  - D3 – 50 cm.

Two technological factors were evaluated: three sowing dates (S) and three row spacing distances (D). Yield for each experimental variant was determined using the following formula: average number of plants per square meter, multiplied by the average number of siliques per plant, multiplied by the average number of seeds per silique, and by the 1000-seed weight (TKW), all divided by 100 (Buzdugan and Năstase, 2013).

Representative samples from each variant were collected for assessing the several quality issues: the determination of fat content was accomplished using Soxhlet extraction, protein content by Kjeldahl method with mineralization in a Turbotherm TT 265 unit (Gerhardt, Koenigswinter, Germany), ash content by heating at 550°C for 5 hours in a Nabertherm B180 (Nabertherm GmbH, Lilienthal, Germany) muffle furnace and total carotenoids through VIS spectrophotometry using a UV/VIS T80+ (PG Instruments Ltd, Leicestershire, UK) instrument (Latimer, 2012).

Data analysis was performed using Past4 for cluster analysis, visualized through heatmaps. Technological variants were characterized and assessed using the Bray-Curtis distance, incorporating the minimum and maximum values obtained from descriptive statistical analysis. This approach enabled the graphical representation of experimental results through a color gradient, with dark blue indicating lower values and red representing higher values.

RESULTS AND DISCUSSIONS

Rapeseed's temperature requirements for seed and oil production are high, ranging from 2,100-2,500°C for autumn genotypes. During the study period (August 2022 - July 2023), at Jucu Herghelie, Cluj-Napoca County, the sum of precipitation was 642.47 mm, and the average temperature recorded in the 2022-2023 agricultural year was 11.37°C, with a positive deviation of 1.37°C compared to the 50-year multi-annual average (Figure 3).

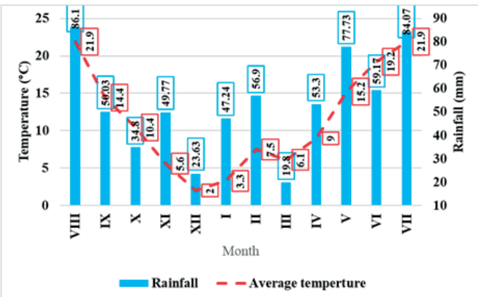

Figure 3. Thermal and Precipitation Regime at Jucu from August 2022 to July 2023

The yield of the PT 275 rapeseed hybrid ranged between 3,297 kg/ha and 4,053 kg/ha. The lowest yield occurred under late sowing (September 10), with a row spacing of 50 cm, underscoring the critical role of timely field preparation for autumn rapeseed (Suveț et al., 2021). Delaying field operations until the end of August results in yield losses of up to 794 kg/ha, losses that can be avoided by sowing the crop at the optimal time under suitable conditions. Conversely, the highest yield of 4,053 kg/ha was recorded when the crop was planted at the optimal time (August 20) and 25 cm row spacing. Overall, the results indicate a reduction in yield associated with delayed sowing and increased row spacing (Figure 4). These results align with studies showing that climatic factors during flowering directly impact rapeseed branching, pod formation and seed yield (Ozer, 2003).

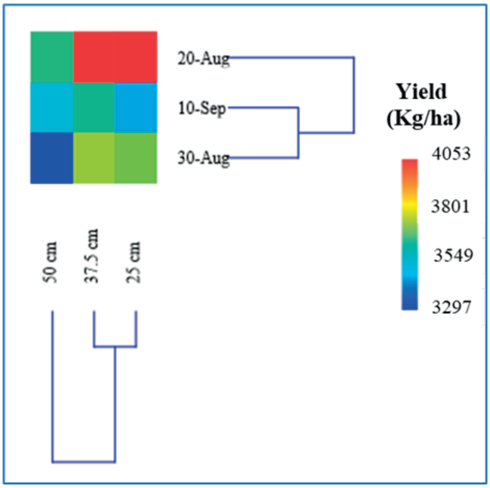

Figure 4. Yield of the PT 275 rapeseed hybrid by sowing date and row spacing

The oil content exhibited considerable variation throughout the experiment (4.5%). Higher oil content values were recorded in the variants where the rapeseed was sown later, specifically on September 10, with a row spacing of 50 cm, as well as on August 30, with a row spacing of 25 cm. These suggest that delayed sowing enhances oil accumulation in seeds, as air temperature, radiation, and precipitation during the flowering period generally play a significant role in determining the oil content of rapeseed seeds (Faraji, 2012). In terms of row spacing, the results showed greater variability. Although the highest average oil content was not observed at the 37.5 cm row spacing, this spacing consistently produced good results. The highest and lowest oil content values in the experiment were obtained at the other two row spacings, each associated with different sowing dates. Kirkegaard et al. (2016) found in their studies that as the sowing date was delayed, stress caused by low temperatures and other factors reduced rapeseed yield and oil content. Precipitation around the August 20 sowing date ensured rapid and uniform emergence, while later dates faced drought, causing uneven emergence and negatively development of plants impacting yield and quality. These findings highlight the importance of both sowing time and row spacing as agronomic practices that can influence the oil content of rapeseed, and they suggest that further research could explore the underlying mechanisms driving these responses.

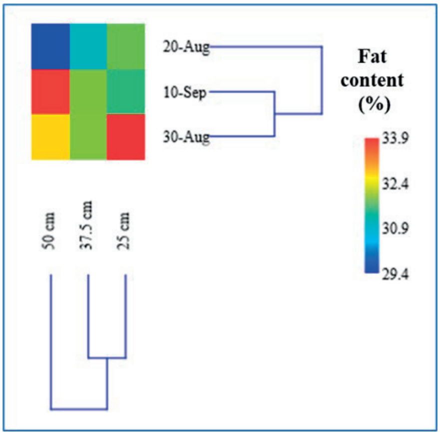

Figure 5. Fat content of the PT 275 rapeseed hybrid by sowing date and row spacing

The protein content ranged from 15.3% to 16.5%, with the lowest value under optimal sowing (August 20, 37.5 cm row spacing), and the highest under late sowing (September 10, 37.5 cm spacing). This divergent response at identical spacing suggests a non-linear response of protein synthesis to the combined effects of sowing date and plant density (Șuveț et al., 2024). These results suggest that the protein content is influenced by the interaction between these two agronomic factors, with both the minimum and maximum values occurring at a row spacing of 37.5 cm (Figure 6). Research conducted by Yang (2003) showed that early-sown rapeseed had higher protein, fat, and trace element content compared to late-sown rapeseed.

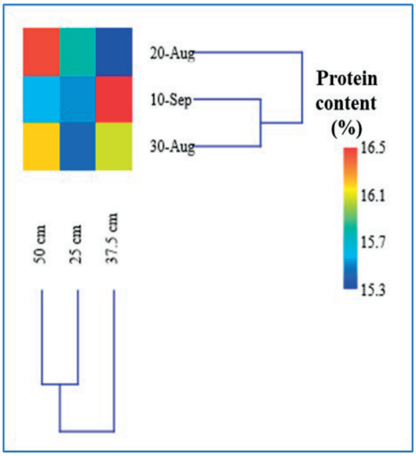

Figure 6. Protein content of the PT 275 rapeseed hybrid by sowing date and row spacing

A notable increase in ash content was observed when rapeseed sowing was delayed until August 30. In this experimental variant, the highest ash content of 3.57% was recorded when rapeseed was sown at a row spacing of 50 cm, while the other two row spacings also produced average or even favorable ash content values. In contrast, optimal sowing (August 20) consistently yielded the lowest ash content, regardless of the row spacing. This trend may reflect that sowing date plays a significant role in influencing ash accumulation, with delayed sowing tending to increase ash content in rapeseed (Figure 7).

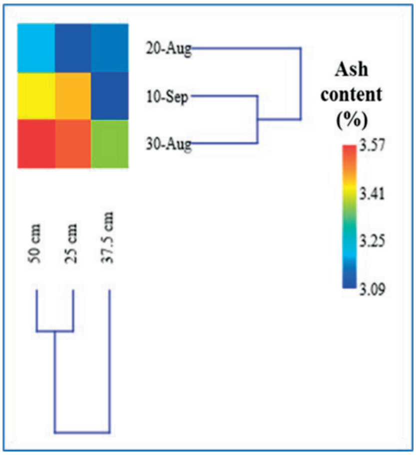

Figure 7. Ash content of the PT 275 rapeseed hybrid by sowing date and row spacing

The total carotenoid content was significantly influenced by both the sowing date and the row spacing, as illustrated in Figure 8. Across all row spacing variations, the highest average carotenoid levels were observed when the rapeseed was sown on September 10. In contrast, for the other two sowing dates, the lowest carotenoid values were recorded at a row spacing of 37.5 cm. The highest carotenoid content (21 mg/kg), was found when sowing occurred on August 30 at a row spacing of 25 cm. For the 50 cm row spacing, carotenoid values remained relatively consistent, showing similar results regardless of the sowing date. These findings suggest that both sowing time and row spacing affect carotenoid content, although their interaction patterns are not linear and warrant further investigation (Figure 8).

Small improvements in carotenoid content could have a significant impact on crop value and market competitiveness; additionally, research into rapeseed carotenoids provides valuable insights into plant metabolism and offers broader benefits for crop improvement and addressing global nutritional needs (Ning et al., 2024; SzydłOwska-Czerniak, 2013). Carotenoids, primarily β-carotene and xanthophylls in rapeseed seeds, showed pronounced sensitivity to agronomic practices (Shen et al., 2023).

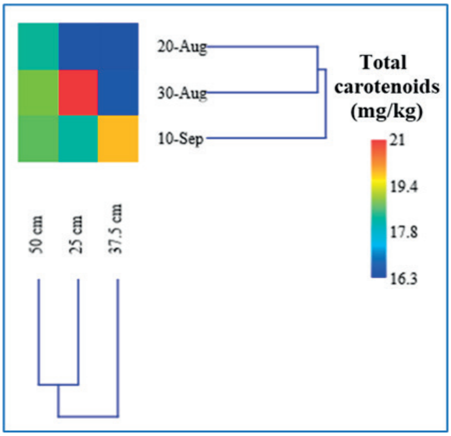

Figure 8. Total carotenoids content of the PT 275 rapeseed hybrid by sowing date and row spacing

An important factor is identifying technological solutions that can enhance both production and oil yield. A key inverse relationship emerged between oil content and yield: delayed sowing increased oil content but reduced yield (Figure 9).

When calculating the amount of oil obtained per hectare based on yield, it becomes evident that, on average, the hybrid in question yielded 1175 kg of oil per hectare. The highest yield of 1210 kg/ha was achieved when the rapeseed was sown on August 20, which corresponds to the optimal sowing period. As expected, the relationship between yield and oil production in this case appears to reverse and take on a positive correlation. An increase in production is linked to a corresponding rise in the amount of oil obtained per hectare.

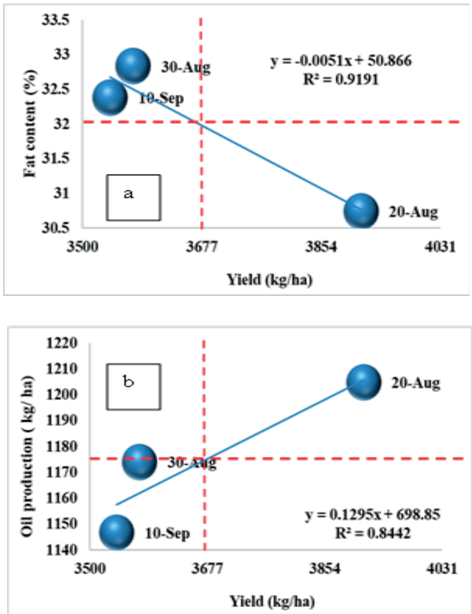

Figure 9. Relationship between yield vs. oil content (a) and yield vs. oil production per hectare in the PT 275 rapeseed hybrid sown on three different dates

When studying the relationship between production and oil content, as well as the correlation between production and total oil yield based on row spacing, a strong positive correlation emerges in both cases. The most favorable results are observed when the distance between rows was 25 cm. In both instances, this row spacing leads to the highest values for both production and oil yield, suggesting that optimal row spacing plays a crucial role in enhancing both the quantity of the harvested product and the amount of oil obtained per hectare. By adjusting the row distance to 25 cm, it is possible to maximize the benefits in terms of both oil content and overall yield (Figure 10).

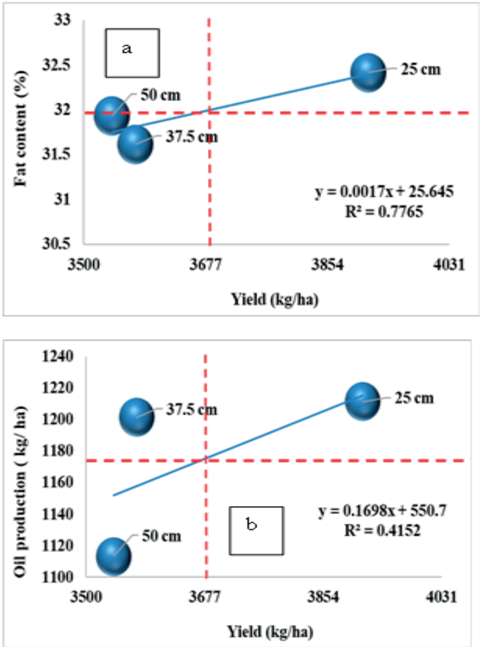

Figure 10. Impact of row spacing on yield, oil content and oil production per hectare in the PT 275 rapeseed hybrid sown on three different dates

CONCLUSIONS

This study emphasizes the importance of both sowing date and row spacing as key agronomic factors influencing various quality parameters and yields in rapeseed. Further multi-year trials and molecular analysis are needed to validate the repeatability of quality traits under variable climatic conditions.

The results highlight that optimizing these practices can improve oil content, protein levels, ash content, and carotenoid accumulation, ultimately leading to more productive and higher-quality rapeseed crops.

The yield of the PT 275 rapeseed hybrid showed a clear relationship with both sowing time and row spacing.

Optimal sowing (August 20) and 25 cm row spacing maximized yield (4,053 kg/ha) and oil production (1,210 kg/ha), while delayed sowing (September 10) reduced yield, emphasizing the importance of timely sowing in maximizing crop productivity. The oil content of rapeseed varied significantly across different sowing dates and row spacings, with the highest values recorded in delayed sowing, specifically on September 10 and August 30, at varying row spacings, highlighting that delayed sowing may increase oil content. Furthermore, row spacing played an important role, with the 37.5 cm consistently producing good results, although the highest and lowest oil content values were observed at the other row spacings.

The protein content of rapeseed showed a range of 15.3% to 16.5%, with the highest protein content observed in the variant sown on September 10, at a row spacing of 37.5 cm.

A significant increase in ash content was observed when sowing was delayed until August 30, particularly at a row spacing of 50 cm. The highest ash content of 3.57% was recorded in this variant. In contrast, sowing at the optimal time (August 20) consistently resulted in the lowest ash content, regardless of row spacing.

The total carotenoid content was also influenced by sowing date and row spacing, with the highest carotenoid levels observed when sowing occurred on September 10. However, the highest carotenoid content of 21 mg/kg was recorded when sowing took place on August 30 at a row spacing of 25 cm. This suggests that both sowing time and spatial arrangements interact to affect carotenoid accumulation in rapeseed. These findings underscore the need for adaptive agronomic practices to optimize rapeseed productivity and nutritional value under changing climatic conditions. Further research is needed to explore the underlying mechanisms and refine these agronomic strategies.

Sowing rapeseed on August 20 yields the highest production, though it may result in slightly lower oil content (%).

Delaying sowing increases oil content but reduces overall yield, making it less efficient for maximizing total oil production per hectare Despite the reduced yield from delayed sowing, when considering oil yield per hectare, it becomes clear that higher yields correspond to greater oil production. On average, the rapeseed hybrid yielded 1175 kg of oil per hectare, with the highest oil yield observed at the optimal sowing date of August 20, an increasing yield being positive correlated with increased oil per hectare.

A 25 cm row spacing yields the best results, improving both yield and oil content, highlighting the significance of optimizing row distance in rapeseed cultivation.

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DETERMINATION OF THE DEPENDENCE OF ECONOMICALLY VALUABLE INDICATORS OF MOLDAVIAN DRAGONHEAD ON GROWING CONDITIONS

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Abstract

The study highlights the results of research into the phases of growth and development of the Moldavian dragonhead (Dracocephalum moldavica) depending on the timing of sowing and moisture conditions. The research was conducted in the agro-ecological conditions of the Central Forest-Steppe of Ukraine. The emergence of seedlings was noted on the 6th - 7th day, depending on the time of sowing. After 20 days from the beginning of the appearance of seedlings, the tillering phase was noted in plants of the first sowing period, and in the case of the second sowing period, after 28 days. In the phase of mass flowering, the height of plants reached 90-95 cm with indicators of above-ground mass of 200-240 g per plant. The mass fraction of essential oil in experiments in the phase of mass flowering varied from 0.08% to 0.16%. The yield of essential oil was the highest during the first sowing period with moderate moisture. Based on the results of the obtained values, a model of economically valuable indicators of the Moldavian dragonhead was built.

Key words: *dracocephalum moldavica; sowing time; aboveground mass; fraction of essential oil; model of economically valuable indicators.*

INTRODUCTION

The importance of essential oils and medicinal plants as sources of biologically active substances is growing. The increasing demand for these substances can be met by expanding the raw material base of medicinal and essential oil plants through the search for and inclusion of new crops and potential areas for their cultivation. The successful introduction of promising plant species into cultivation is only possible if their developmental biology is understood, the processes of formation and accumulation of essential oil are studied, and the features of their cultivation and productivity are investigated to determine the feasibility of industrial cultivation (Ovechko, 2003).

One of the valuable and promising essential oil crops is *Dracocephalum moldavica* L. The native habitat of *Dracocephalum moldavica* is considered to be Southern Siberia and China, although it grows wild in Central Asia and Eastern Europe. This plant is cultivated in European and Asian countries both as an essential oil crop and as a valuable honey plant (Kotiuk, 2013; Boiko, 2023). *Dracocephalum moldavica* is an annual herbaceous plant of the Lamiaceae Martynov family. The root is thin and taprooted. The stem is upright, four-angled, and branched. The leaves are short-petioled, opposite, oblong-ovate or oblong-lanceolate, serrated at the edges, and dark green. Large essential oil glands are located on the underside of the leaf blade. The flowers are pale purple or white, collected in racemose inflorescences (Kotiuk, 2013).

The herb *Dracocephalum moldavica* is commonly consumed both as a food product and as a medicinal preparation due to its known therapeutic properties (Dastmalchi et al., 2007). The plant material of *Dracocephalum moldavica* contains essential oil, flavonoids, phenols, phenylpropanoids, lignans, terpenoids, vitamins, lipids, proteins, sugars, tannins, macro- and microelements, and other chemical components (Meng et al., 2024). Plants of this species have significant antioxidant and antimicrobial effects (Simea et al., 2023). It is used for medicinal purposes as an anti-inflammatory and sedative remedy, for treating colds, headaches, joint pain, neuralgia, rheumatism, tachycardia, hypertension, and insomnia (Yang et al., 2014). The above-ground part of *Dracocephalum moldavica* is used in the confectionery industry, for the production of absinthe, vermouth, kvass, compote, flavoring tea and vinegar, and as a seasoning for meat, fish, and vegetable dishes (Svidenko et al., 2018). *Dracocephalum moldavica* is a valuable honey and fodder plant (Shtakal, 2023). It has been shown that extracts of *D. moldavica* act as an effective and biologically safe insect repellent for food storage (Hussein et al., 2015).

The essential oil of *Dracocephalum moldavica* is used in the food industry, pharmaceuticals, perfumery, and cosmetology (Kotiuk, 2021). The widespread use of *Dracocephalum moldavica* herb necessitates the study of its agrobiological fundamentals for cultivation in the potential zones of Ukraine. One of the challenges of adapting plants to local agroclimatic conditions is determining the optimal levels of heat and moisture (Moiseyenko, 2019). The plant is resistant to low temperatures and prefers moisture (Kotiuk & Rakhmetov, 2017). Climate and agrotechnical measures during cultivation have a significant impact on the height of *Dracocephalum moldavica* plants and the size of the above-ground biomass (Moldovan et al., 2022). Due to climate change, particularly global warming and the unusual lack of rainfall, there has been a decline in biological and economically valuable indicators in agricultural crops (Khaleghnezhad et al., 2021). This has led to the necessity of artificial irrigation, even in regions where it was previously solely dependent on natural sources. Scientific literature provides data on the positive impact of irrigation on the yield of essential oil from plants, as observed in other regions of Ukraine and other essential oil crops (Kovalenko & Stebliichenko, 2020).

The aim of our work is to study the growth and development phases, as well as the formation of economically valuable traits of *Dracocephalum moldavica*, depending on the sowing dates and moisture conditions.

MATERIALS AND METHODS

The research was conducted in 2024 under the conditions of the Central Forest-Steppe of Ukraine, in the Lysyansky district of the Cherkasy region. The district is located on the Dnieper Upland of the Eastern European Plain, within the basin of the Hnylyi Tykich River. The soil at the study site is typical chernozem. According to its granulometric composition, the soil is heavy loam. The humus content in the plow layer is 4.58%. The material used for the research was a sample of *Dracocephalum moldavica*, variety "Yuvileinyi". The seeds of this sample were sown in two terms, with a row spacing of 50 cm. The first sowing was carried out in the third decade of April. Ten days later, the second sowing was carried out in the first decade of May. Irrigation of the crops was performed in three variants: intensive drip irrigation, moderate drip irrigation, and natural moisture.

Phenological observations were carried out on the crops according to established methods (Tkachyk, 2015; Tkachyk, 2016). The following phases were recorded: seedling emergence, tillering, bud formation, flowering (beginning, mass, end), and seed-setting (beginning, mass, end). Biometric measurements were taken every two weeks throughout the plant's growing season. Plant height and diameter were measured. During the plant's growing season, the aboveground biomass of the plant material was determined every two weeks. The mass fraction of essential oil in the plant material was determined according to the plant development phases using the Ginsberg method on a Clevenger apparatus and was calculated on a dry weight basis (Rabotyagov & Svidenko, 2010). Essential oil distillation was carried out 4-5 days after irrigation.

RESULTS AND DISCUSSIONS

Dracocephalum moldavica reproduces by seed. Under laboratory conditions, the seeds begin to germinate on the third day, and by the seventh day, the germination rate reaches 95%.

Under field conditions, during the first sowing (third decade of April), seedling emergence was recorded on the 6th day in both irrigation variants (intensive and moderate) and in the non-irrigated variant. This can be explained by the fact that during this period, the soil contained sufficient moisture for normal seed germination. During the second sowing (first decade of May), in the intensive and moderate irrigation variants, seedlings began to emerge on the 6th day, while in the non-irrigated variant emergence occurred on the 7th day. This difference is due to the reduced soil moisture reserves, which led to a lower seed germination rate in the non-irrigated variant.

The plants (seedlings) of *Dracocephalum moldavica* initially grew slowly. After 20 days from the emergence of the seedlings, the tillering phase was observed in the plants of the first sowing date. In the case of the second sowing date, the tillering phase was observed 28 days after the emergence of the seedlings. The onset of the bud formation phase was observed 44 days (in the first decade of July) after the beginning of the previous phase in the plants of the first sowing date. In the plants of the second sowing date, the onset of bud formation occurred earlier, after 34 days. The duration of the interphase period between bud formation and the beginning of flowering in the plants of the second sowing date was also shorter by 7 days. The beginning of flowering for both sowing dates was recorded in the second decade of July. The plants of the first sowing date flowered simultaneously across all watering treatments. The phase of end of flowering–beginning/start of seed-setting occurred at the beginning of the second decade of August. In the plants of the second sowing date, the duration of the developmental phases was 7-8 days shorter compared to those of the first sowing date.

Studying the dynamics of growth and development in *Dracocephalum moldavica*, we determine that the plants initially grew very slowly for both sowing dates. A noticeable acceleration in plant growth occurred during the tillering phase. In this phase, the height of the plants for the first sowing date ranged from 10 to 16 cm, with a diameter ranging from 9.5 to 11 cm. The above-ground biomass of a single plant ranged from 1.5 to 8 g. After two weeks, the height of the plants ranged from 26 to 50 cm, with a diameter ranging from 20 to 50 cm.

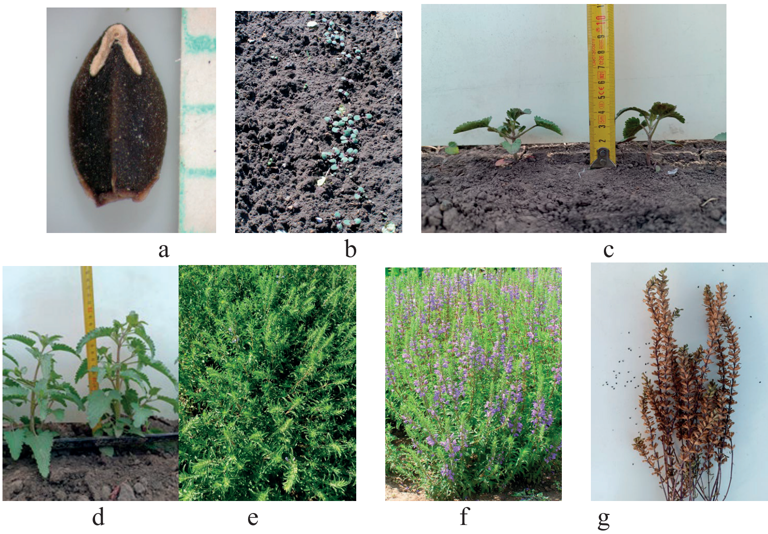

Figure 1. Development stages of *Dracocephalum moldavica*: a - seed; b - seedling emergence; c - seedling (two true leaves stage); d - tillering (formation of lateral shoots); e - budding; f - flowering; g - seed-setting

The above-ground biomass also increased, ranging from 22 to 138 g. In the bud formation–early flowering phase, the height of the plants ranged from 69 to 95 cm, with a diameter ranging from 44 to 56 cm. The above-ground biomass ranged from 55.3 to 197 g. The plant height further increased in the mass flowering phase, ranging from 75 to 95 cm, with a diameter ranging from 50 to 80 cm. In this phase, the above-ground biomass ranged from 140 to 240 g per bush. For the first sowing date, under both intensive and moderate watering conditions, the plants exhibited nearly identical measurements for plant height and above-ground biomass.

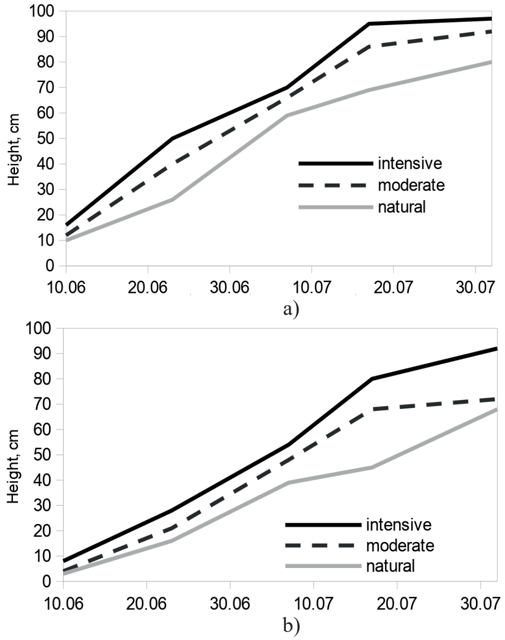

Figure 2. Growth dynamics of *Dracocephalum moldavica* plants under different irrigation variants: a - first sowing (third decade of April), b - second sowing (first decade of May)

During the development of the plants for the second sowing date, a gradual increase in both height and diameter of the plants was observed, continuing until the mass flowering phase. In the mass flowering phase, the plants for the second sowing date had a height ranging from 65 to 90 cm, with a diameter ranging from 35 to 55 cm. The above-ground biomass ranged from 60 to 200 g per bush. The highest values for the second sowing date were observed in plants that received intensive soil irrigation. The lowest values for above-ground biomass were found in plants that grew without irrigation. In these plants, during the mass flowering phase, a noticeable decrease in leaf turgor was observed, indicating insufficient soil moisture.

We conducted a study on the dynamics of essential oil accumulation across the developmental phases of *Dracocephalum moldavica* plants, depending on the intensity of drip irrigation and natural moisture (no irrigation). According to our findings, throughout the growing season, the essential oil content in the plant material gradually increased, reaching its peak during the mass flowering phase in all experimental variants.

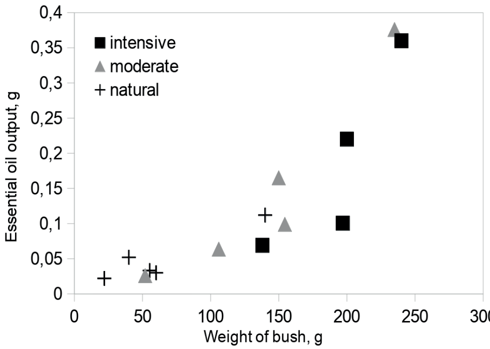

Figure 3. Comparison of wet mass of single bush and essential oil output per bush under diffferent irrigation conditions during growth of plants

We conducted a study on the dynamics of essential oil accumulation across the developmental phases of *Dracocephalum moldavica* plants, depending on the intensity of drip irrigation and natural moisture (no irrigation). According to our findings, throughout the growing season, the essential oil content in the plant material gradually increased, reaching its peak during the mass flowering phase in all experimental variants.

Considering all experimental variants, the mass fraction of essential oil in plants during the mass flowering phase ranged from 0.08% to 0.16%. The highest mass fraction of essential oil in the current year was obtained in plants from the first sowing date under the moderate irrigation variant. In contrast, plants with intensive irrigation, both for the first and second sowing dates, exhibited less mass fraction values of essential oil.

The total essential oil yield per plant (productivity) was highest for the first sowing date with moderate moisture, amounting to 0.38 g. This can be explained by the fact that plants from the first sowing date with moderate moisture exhibited high aboveground biomass and average essential oil content, which together contributed to higher plant productivity.

Based on one year of observations, an essential oil output can be described by simple equation:

$$Oil = e^{1.118 \cdot ln(W) - 7,699}$$

where: *Oil* - output of essential oil, gramm; *W* - weight of single bush, gramm.

Described model is suited for full valuable range of plant grows stages and have $R^2 = 0.79$ with p-value of coeficients on level less than 0.001. It should be noted that the described model are primitive and take into account only data by single year. In the future it is planned to take into account various plant growth factors, which will allow for a full assessment of the dynamics of bush growth along with the yield of essential oil.

CONCLUSIONS

We investigated the dynamics of growth and development, as well as the formation of economically valuable traits in *Dracocephalum moldavica* plants under the conditions of the Central Forest-Steppe of Ukraine, with two sowing dates and three irrigation treatments: intensive drip irrigation, moderate drip irrigation, and natural moisture (no irrigation). The greatest height and diameter were observed in plants under the first sowing date and intensive drip irrigation. The smallest height was recorded in plants under the second sowing date and natural moisture (no irrigation). The maximum aboveground biomass values were obtained for the first sowing date with intensive irrigation, while the minimum values were noted for the second sowing date with moderate moisture. The highest essential oil content per plant in production ready plant development stages was found in plants from the first sowing date under moderate irrigation, while the lowest was observed for plants from the second sowing date with intensive drip irrigation. Collected data give an opportunity to build models of economically valuable indicators of *Dracocephalum moldavica* and spread its cultivation in the Central Forest-Steppe of Ukraine.

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COMPARATIVE CHARACTERISTICS OF SELECTED SAMPLES OF *Hyssopus officinalis* L. BY BIOMORPHOLOGICAL INDICATORS IN THE CONDITIONS OF THE SOUTHERN STEPPE OF UKRAINE

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Abstract

Hyssopus officinalis L has recently attracted particular attention as a valuable raw material for pharmaceuticals. To identify the biomorphological features of Hyssopus officinalis L., a field experiment was initiated in 2020 in the Kherson region, Ukraine. Samples of Hyssopus officinalis L of the local ecotype No. 108 were selected for the research. It was noted that seedlings in the flowering phase differ in terms of the timing of the beginning of flowering and flower colour. Plants of Sample 1-20 (pink-flowered) bloom first. After 3-4 days, the beginning of flowering is noted in sample 3-20 (blue-flowered), and after another 2 days, Sample 2-20 (white-flowered) begins to bloom. The height of first year plants ranged from 50 to 60 cm. The diameter of the bushes varied from 20 to 35 cm. The central shoot consisted of 17 to 38 first-order shoots. The largest size of the inflorescence of the first and second order shoots is in sample 3-20. Sample 2-20 has slightly higher average values of the length of the inflorescence of the first order shoots than in Sample 1-20.

***Key words**: Hyssopus officinalis L., essential oil plants, biomorphological indicators, introduction, medicinal plants.*

INTRODUCTION

Due to climate change, significant damage and irreversible losses are observed in natural ecosystems. The acute situation in food security is becoming increasingly unstable for various reasons, such as the increase in the incidence of natural disasters, military conflicts between countries, economic crises, global epidemiological diseases, etc. (Chaika et al., 2021; Habibullah et al., 2022; Muluneh et al., 2021).
The increase in average temperatures and the uneven distribution of precipitation caused by global climate change may lead to a significant transformation of the majority of climatic and agricultural zones of Ukraine (Chaika et al., 2021). It is known that the most noticeable consequence of climate change will not be so much gradual warming, but an increase in the number and intensity of extreme weather events: droughts, floods, and the number of extremely hot days in summer (Habibullah et al., 2022; Muluneh et al., 2021).
This situation requires agricultural producers to introduce more drought-resistant plant species, among which essential oil crops occupy a special place. Essential oil crops are mostly resistant to soil and air drought compared to other traditional agricultural crops for the region.
These plants are valuable raw materials for many industries. Natural essential oils are in demand on the domestic and international markets, as they have high antimicrobial activity (Vlase et al., 2014; Tahir et al., 2018). Many types of essential oil crops are used as non-food industrial products, pharmaceuticals, phytoproducts, cosmetics, plant protection products, etc. (Rabotyagov et al., 2003; Fathiazad et al., 2011; Hussein et al., 2015; Atazhanova et al., 2024).
One of the many species of essential oil plants, the products of which are widely used in the national economy, is *Hyssopus officinalis* L. (Stan et al., 2019; Tkachova et al., 2022).
H. officinalis L. is a valuable essential oil plant, a typical xerophyte, which is well adapted to drought and unpretentious in cultivation (Kovalenko et al., 2019; Dobrovolskyi et al., 2021).
H. officinalis L. (Family: *Lamiaceae* alt. *Labiatae*) is a perennial herbaceous, semievergreen shrub or subshrub and has a well-developed woody and twiggy taproot that penetrates to a depth of 2-2.5 m. *H. officinalis* L.

is native to Mediterranean and Western Europe and Central Asia (Chrysargyris et al., 2022; Preedy, 2015; Zawiślak, 2013). It is cultivated as a medicinal, ornamental, and aromatic plant in Ukraine, Moldova, Central Asia, and other countries (Druțu et al., 2014; Jangi et al., 2022; Lubbe et al., 2011; Riabchun et al., 2019).

The tops of stems up to 20 cm long, collected during flowering, are used. The yield of dry raw materials is 18-20%. The herb contains essential oil (0.6-2.0%) with a peculiar strong turpentine-camphor odour, tannins and bitter substances, resins, dyes, triterpenic acids, glycoside issopine, flavonoids (0.9-1.0%) pigments, vitamin C, macroelements K, Ca, Mg, Fe, microelements Mn, Cu, Zn, Co, Mo, Se, Ba (Ghanbari-Odivi et al., 2024; Mohammadi et al., 2020; Naderi et al., 2023).

H. officinalis L. is one of the most important pharmaceutical herbs widely cultivated in Romania. Romanian scientists studying the antioxidant and antimicrobial activity of *H. officinalis* L., note that it is a typical xerophyte and well adapted to drought and low humidity conditions. Their results confirm that *H. officinalis* L. can be considered a potential source of polyphenols with antioxidant and antimicrobial properties and emphasize further research on this crop with the aim of growing and preparing high-value natural pharmaceutical products (Aqeel et al., 2023).

According to literature data, 40% ethanol extract of *H. officinalis* L. grown in the conditions of Polissya (Ukraine) had high biological activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, which are pathogenic against other organisms (Kotiuk et al., 2015).

Scientists have found that *H. officinalis* L. essential oil has high antioxidant, antifungal and fungicidal effects (Zawiślak, 2013; Vlase et al., 2014; Tahir et al., 2018).

H. officinalis L. is a valuable honey plant. The honey belongs to the best varieties. This plant has a long flowering period and can be used to close non-harvesting periods during the beekeeping season (Fathiazad et al., 2011; Riabchun et al., 2019; Tkachova et al., 2022).

Despite the significant importance of this plant for various sectors of the national economy, its cultivation has not yet reached significant scales. Currently, scientists from different countries are constantly improving the *H. officinalis* L. growing technology by optimizing individual technological measures and studying their impact on product quality (Gholamreza et al., 2015; Amini et al., 2019; Kovalenko et al., 2019; Naderi et al., 2023).

Today, the production of raw materials for essential oil crops, in particular, *H. officinalis* L., does not fully meet the needs of consumers of essential oil products in Ukraine.

To introduce a particular crop into production, it is first necessary to study its biological characteristics in a given region. Therefore, scientists introduce plant species, select promising samples and on this basis create varieties that would be promising for cultivation in new conditions (Riabchun et al., 2019; Svyrydovskyi et al., 2024).

MATERIALS AND METHODS

The research was conducted in the Kherson region on the basis of the State Enterprise Research Farm "Novokakhovske" of the Institute of Climate Smart Agriculture of NAAS of Ukraine, in 2020-2022.

The soil and climatic conditions of the Kherson region contribute to the cultivation of introduced essential oil and aromatic plants, especially those species that are suitable for cultivation in conditions of insufficient soil moisture and air humidity in southern Ukraine (Dobrovolskyi et al., 2021; Kovalenko et al., 2019; Svyrydovskyi et al., 2024). The experimental site is located in the first, northern agroclimatic region of the Kherson region, which is characterized by a temperate continental climate with a short spring, and relatively long hot and dry summer, and a mild winter with frequent thaws. The sum of temperatures above 10°C is 3200-3300°C, the amount of precipitation during this period is 215-220 mm, the annual amount of precipitation is 380-430 mm, and the hydrothermal coefficient is 0.7. The average duration of the period with positive temperatures is 175-180 days, the vegetation period is 215-225 days. Spring frosts stop mostly in the third decade of April. The average time of the onset of autumn frosts is the second decade of October, occasionally at the end of September. The Kherson region is characterized by annual droughts, 40% of which are very intense.

The plot where the experiments were carried out was located on chernozem light loamy soils with a humus layer thickness of 76 cm and a humus content in the arable layer of 1.33%.

The material for the research was samples of *Hyssopus officinalis* L., selected from seeds of the local ecotype №108. Ecological and phenological observations and biometric measurements were carried out on the plants according to generally accepted methods (Kyienko et al., 2015; Kyienko et al., 2016). All measurements were carried out for the mass flowering phase.

RESULTS AND DISCUSSIONS

It is well known that *H. officinalis* L. is well propagated by seeds, lignified and green cuttings and division of the shrub. In early spring (first decade of April) we sowed seeds of *H. officinalis* L. ecotype No. 108 (seeds of local reproduction).

We received shoots after 12 days. At first, the plants grew slowly, but after 45-50 days the growth noticeably accelerated and in the first decade of August, the plants of the first year of development began to bloom. The maximum growth of plants was observed in the budding phase and in the phase of the beginning of flowering.

It was noted that seedlings in the flowering phase had differences in terms of flowering onset and flower color. Samples with white, pink, and purple flower colors were selected (Figures 1, 2).

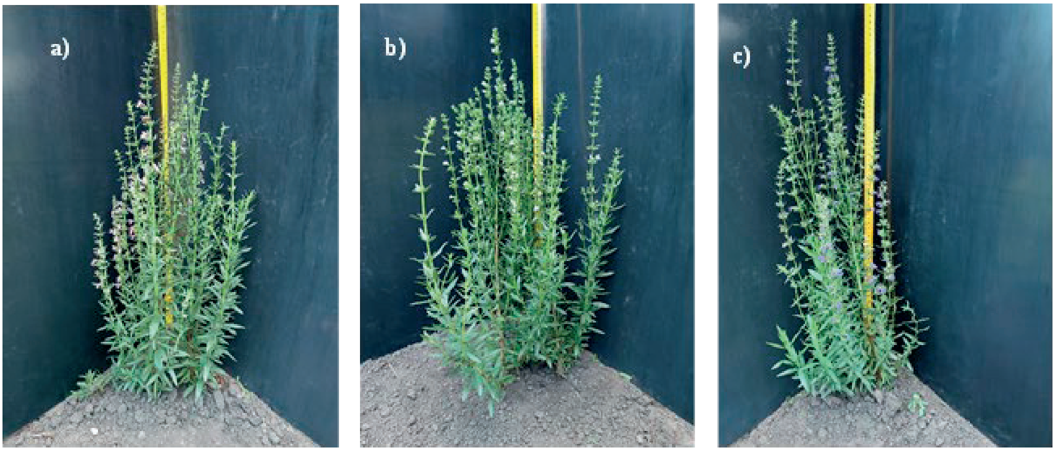

Figure 1. Selected samples of *Hyssopus officinalis* L. (plants of the first year of development in the phase of the beginning of flowering): a - Sample 1-20 (pink-flowered form); b - Sample 2-20 (white-flowered form); c - Sample 3-20 (blue-flowered form)

Figure 2. Inflorescence coloration of *Hyssopus officinalis* L. samples: 1-20 (pink-flowered form); 2-20 (white-flowered form); 3-20 (blue-flowered form)

The plants of Sample 1-20 (pink-flowered) bloomed first. After 3-4 days, the beginning of flowering was noted for Sample 3-20 (blue-flowered), and after another 2 days for Sample 2-20 (white-flowered). In addition to the diverse color of flowers, the selected samples also had differences in the growth habit (Table 1).

The first-year development plants reached 50-60 cm tall a height of 56-60 cm. At the same time, the plants of Sample 3-20 (blue-flowered form) reached the greatest height (60 cm) and were per 1.67% higher than the plants of Sample 2-20 (white-flowered form) and per 6.67% higher than Sample 1-20 (pink-flowered form).

The diameter of these plants varied from 20 to 35 cm. The largest plant diameter was achieved by the plants of Sample 2-20 (white-flowered form). The plant diameter of Sample 3-20 (blue-flowered form) was per 6.25% smaller, and the plant diameter of Sample 1-20 (pink-flowered form) was per 25.00% smaller than the plant diameter of Sample 2-20 (white-flowered form). That is, the plants of Sample 1-20 on the first year of development formed a smaller growth habit. The first-year development plants of Sample 2-20 (white-flowered form) and Sample 3-20 (blue-flowered form) had insignificant differences in the growth habit.

Table 1. Habit of the bushes and characteristics of vegetative-generative shoots of *Hyssopus officinalis* L.

Indicators	Sample 1-20 (pink-flowered form)			Sample 2-20 (white-flowered form)			Sample 3-20 (blue-flowered form)		
	Year of development								
	I	II	III	I	II	III	I	II	III
Plant height, cm	56	75	80	59	78	85	60	79	87
Plant diameter, cm	24	85	88	32	95	98	30	90	95
Number of first-order shoots	13	85	96	14	105	123	13	94	115
Number of second-order shoots	6	170	220	8	220	240	8	251	260
Length of first-order shoots, cm	19	72	79	28	77	77	32	79	82
Length of second-order shoots, cm	12	25	30	17	27	35	24	36	45

In addition to the growth habit, the plants of the selected samples of *H. officinalis* L. on the first year of development had differences in the number and length of the first and second order shoots. Despite the fact that the plants formed almost the same number (13-14) of the first-order shoots on the central shoot, their length differed depending on the biological characteristics of the samples. Thus, the greatest length of the first-order shoots (32 cm) on the first year of development was formed by plants of the Sample 3-20 (blue-flowered form). The length of the first-order shoots of Sample 2-20 (white-flowered form) was per 12.50% less, and for Sample 1-20 (pink-flowered form) it was per 40.63% less than the similar indicator of Sample 2-20 (white-flowered form).

H. officinalis L. plants on the first year of development had 6-8 second-order shoots, which also differed in length depending on the biological characteristics of the samples. Thus, the plants of Sample 3-20 (blue-flowered form) had the greatest length (24 cm) of the second-order shoots. The plants of Sample 2-20 (white-flowered form) had the length of the second-order shoots per 29.17% less, and plants of Sample 1-20 (pink-flowered form) had the length of the second-order shoots per 50.00% less than the similar indicator of Sample 2-20 (white-flowered form).

The vegetation of *H. officinalis* L. plants on second year of development begins in second or third decade of March. The beginning of budding was observed in first half of June. The beginning of flowering was in late June.

Due to the presence of numerous lateral shoots of the first and second order, the plants of selected samples on second year of development took on the appearance of a sprawling bush, the height of which varied, on average, from 75 to 79 cm, and their diameter varied, on average from 85 to 95 cm. The number of first-order shoots varied from 85 to 105 pieces. The number of the second-order shoots had significantly increased, which ranged from 170 to 251 shoots (Table 1).

At the same time, the smallest plant sizes (height – 75 cm, diameter – 85 cm) were in exemplaries of Sample 1-20 (pink-flowered form). Plants of this sample also formed the smallest number of the first-order shoots (85 pcs.) and second-order shoots (170 pcs.). The length of shoots of both first and second order of this sample was also the smallest in the experiment equal to 72 and 25 cm, respectively.

Plants of Sample 3-20 (blue-flowered form) formed the greatest height (79 cm), and had the greatest number of second-order shoots (251 pcs) with the greatest length (36 cm). The length of the first-order shoots in plants of this sample was also the greatest in the experiment and was 79 cm. Plants of Sample 2-20 (white-flowered form) were per 1.27% lower than plants of Sample 3-20 (blue-flowered form), but at the same time they had a 5.27% larger diameter due to a 10.41% larger number of first-order shoots.

There were also changes in the size of the hyssop plants on the third year of development. Compared to the second-year plants, the height of the plants and the number of shoots increased. In the third year of development, the plants were 80-87 cm high and had diameter of 88-98 cm. The number of second-order shoots varied from 220 to 260 shoots (Table 1).

Mass flowering of the plants on second and third years of development lasted from the second decade of July to the first decade of August. Flowering began from the central peduncle, then the lateral peduncles bloomed. The flowering end was on the third decade of August – the first decade of September. The total duration of flowering was two months.

According to literature data, the leaves and inflorescences of *H. officinalis* L. are the most important organs, because they accumulate the largest amount of biologically active substances (Fathiazad et al., 2011; Atazhanova et al., 2024).

During the growing season of *H. officinalis* L., there was a gradual increase in the number of leaves due to the elongation of shoots.

The size of the leaves can be evaluated by the measurement results (Table 2).

The largest indicators of the length (3.7 cm) and width (0.9 cm) of the leaf blade were in Sample 3-20 (blue-flowered form). Plants of Sample 1-20 (pink-flowered form) had the smallest values of the length (3.5 cm) of the leaf blade.

Table 2. Morphometric characteristics of leaves and inflorescences of *Hyssopus officinalis* L. samples on the second year of development

Indicators	Sample 1-20 (pink-flowered form)	Sample 2-20 (white-flowered form)	Sample 3-20 (blue-flowered form)
Leaf length, cm	3.5	3.6	3.7
Leaf width, cm	0.9	0.8	0.9
Length of the first-order shoot inflorescence, cm	28	29	30
Length of the second-order shoot inflorescence, cm	23	22	24
Number of rings of the first-order shoot inflorescence, pcs	17	18	17
Number of rings of the second-order shoot inflorescence, pcs	15	16	16
Number of flowers in a semi-ring of the first-order shoot inflorescence, pcs.	10,5	10	9
Number of flowers in a semi-ring of the second-order shoot inflorescence, pcs.	9	9	8

The studied samples differed not only in leaf size, but also in the length of the inflorescence of the first and second order shoots (Table 2).

The largest inflorescences of the first -order shoots (30 cm) and second-order shoots (24 cm) had Sample 3-20. Sample 2-20 had slightly higher average values of the length of the first-order shoots inflorescence than Sample 1-20. On the contrary, the inflorescence length of the second-order shoots was higher in Sample 1-20. It was noted that the selected samples of *H. officinalis* L. differed in the number of whorls in the inflorescence and flowers in the semi-whorls in the shoots of the first and second orders.

The same number of whorls of the first-order shoots was recorded on samples 1-20 and 3-20. The smallest number (8) of flowers in the semi-whorl in the shoots of the first and second orders was noted on Sample 3-20 (blue-flowered form).

CONCLUSIONS

The samples selected from seed generation of *H. officinalis* L. (biotype № 108) had differences in biomorphological indicators (plant size, number and length of shoots, inflorescence color) and timing of phenological phases of development.

Sample 1-20 had early flowering and pink flower color. The most height of the plant and blue flower color were characteristic of Sample 3-20. The maximum plant diameter, white flower color and the latest flowering date were characteristic of Sample 2-20. Sample 2-20 had the largest number of first-order shoots, and Sample 3-20 has the largest number of second-order shoots. Differences between samples of *H. officinalis* L. were also in the size of the leaf and inflorescence. The maximum indicators of leaf length and inflorescence length were recorded in Sample 3-20.

The plants developed well and can be successfully grown in the agroclimatic conditions of the Southern Steppe of Ukraine, which will allow to satisfy the country's needs in the corresponding medicinal raw materials and contribute to the preservation of the region's biodiversity.

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ANALYSIS OF THE EFFICACY OF MCPA HERBICIDE IN THE CONTROL OF *Convolvulus arvensis* and *Chenopodium album* IN FLAX CROPS

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Abstract

Linum usitatissimum is a valuable crop with food and industrial significance, yet its production is often reduced by biotic and abiotic factors, particularly weed competition. This study focused on the chemical control of Convolvulus arvensis and Chenopodium album in flax crops, aiming to determine the optimal dose of MCPA 750%. Four doses (0.16, 0.21, 0.27, and 0.33 l/ha) were tested in Western Romania, with evaluations conducted 14, 28 and 42 days after application (DAT). The results of this study showed that the two species had similar responses to the action of the MCPA herbicide. The population reduction of the two species, from the flax agroecosystem, was influenced by the applied dose and the time interval. The MCPA herbicide had maximum efficacy at 28 DAT. Good effectiveness was registered in the variants treated with MCPA 0.27 l/ha and 0.33l/ha. As a result of the relatively small differences between the two doses, as well as the phytotoxicity observed in the plots treated with the 0.33 l/ha dose, it is recommended to use the 0.27 l/ha MCPA.

***Key words**: Convolvulus arvensis, Chenopodium album, MCPA, Linum usitatsimum, chemical control.*

INTRODUCTION

Linum usitatissimum L. (flax) remains an agricultural crop of global interest due to its use for both seed production and fibre. Flax has numerous applications in the food, textile, pharmaceutical industries. Flaxseeds are recognized as a functional ingredient, rich in omega-3 fatty acids, lignans and dietary fibre, offering nutritional and health benefits (Popa et al., 2012), and flaxseed fibres are appreciated for their stability in use, the comfort they provide to textiles and their high biodegradability, contributing to the development of "green" value chains in the European Union (Nowak et al., 2023; Stavropoulos et al., 2023, Chishty, S. and Monika, 2016).

According to FAOSTAT data, the harvested area of flax for seed exceeded 3-4 million ha worldwide in the last decade, with an increasing trend in 2018-2021, with the main producing countries being Canada, Russia, Kazakhstan, China and some European states (Yaşar and Yetişshin, 2023).

Romania has historically played a significant role in flax production, reaching in the last decades of the twentieth century about 70-80 thousand ha, supported by a national fibre processing industry. However, after 1990, economic reset, the loss of industrial capacity, the orientation of farmers towards more profitable crops and the decline in domestic demand led to a drastic reduction in cultivated areas. The statistical data available for the period 1990-2008 confirm this contraction (Panaitescu et al., 2010), and after 2015 flax is no longer reported separately in the official statistics of the INS (https://insse.ro/cms/ro/tags/comunicat-productia-vegetala-la-principalele-culturi), which indicates the maintenance of the crop at a marginal, niche or experimental level. Paradoxically, the revival of global interest in natural fibres, bioindustries and premium vegetable oils could offer Romania strategic advantages, given its agricultural tradition, technological experience, favourable pedoclimatic conditions and potential for integration into European green value chains.

In this context, the reanalysis of the agronomic, economic and technological importance of flax, as well as the identification of the necessary conditions for the revitalization of this crop in

Romania, become relevant objectives for contemporary agricultural research, rural development and the transition to sustainable agri-food systems.

Under these circumstances, global germplasm collections highlight considerable genetic diversity that can support the adaptation of the crop to varied pedoclimatic conditions, including in Central and Eastern Europe (Diederichsen and Richards, 2003; Kaur et al., 2023).

However, the productive potential of flax is rarely achieved under production conditions, due to the complex interaction between abiotic factors (drought, extreme temperatures, inadequate fertilization) and biotic factors, among which weeds represent one of the most persistent and costly constraints (Zimdahl, 2018; Harker and O'Donovan, 2013). Flax is recognized as a crop that is poorly competitive with weeds, especially in the early stages of vegetation, due to its slow growth rate, erect architecture, and narrow leaves, which allows weeds to intercept a large proportion of light, water, and nutrient resources (Stevenson et al., 1996; Mühleisen, 2000; Stavropoulos et al., 2023). Recent studies indicate that weed interference can reduce flax production for seed by about 10-30% or even more, depending on the density and composition of the weed flora, cultivation technology, and local climatic conditions (Friesen, 1986, cited by Kurtenbach et al., 2019; Çiğnitaş, 2023).

In temperate regions of Europe, including the flax-cultivated area of western Romania, the species *Convolvulus arvensis* L. (bindweed) and *Chenopodium album* L. (lamb's quarter) are among the most problematic dicotyledonous weeds of arable land (Ștef et al., 2013, Ștef et al., 2017). Weaver and Riley (1982) described *Convolvulus arvensis* as a deeply rooted perennial species, capable of developing perennial root networks, with a remarkable vegetative regeneration capacity and a high longevity of seed reserves in the soil, which explains its persistence in crop rotations and the difficulty of mechanical control (Weaver and Riley, 1982). In addition, voluble stems twist around crop plants (Culhavi and Manea, 2011), reduce photosynthetic efficiency, favour plant fall, and make harvesting difficult. *Chenopodium album* is a phenotypically highly plastic annual species with a prolonged emergence period, high seed production potential, and rapid growth, which gives it a considerable ecological advantage in spring crops (Grundy et al., 2003). Studies frequently indicate *Chenopodium album* among the dominant species, in flax cultivation, associated with significant production losses and decreased fibre quality (Çiğnitaş, 2023; Ozer et al., 2004).

In the context of these pressures, effective weed management is an essential condition for the stability of flax production. Although agronomic measures (increasing seeding density, narrow rows, appropriate rotations) can improve crop competitiveness, in practice, weed control in flax fields relies heavily on herbicides, given the relatively short window in which mechanical interventions are effective and the high risk of mechanical plant damage (Stevenson et al., 1996; Mühleisen, 2000). However, compared to other crops (e.g. rapeseed, sunflower), flax has a limited number of approved herbicides, reflecting the high sensitivity of the crop and narrow margins of selectivity (Mühleisen, 2000; Kurtenbach et al., 2019; Morton et al., 2020). This situation is particularly problematic for the control of perennial or highly competitive species, such as *Convolvulus arvensis* and *Chenopodium album*, for which incomplete control in a single season can lead to rapid re-infestations.

In cereal and crop-based production systems, phenoxycarboxylic herbicides (synthetic auxins) have been a central component of dicotyledonous weed control for more than five decades (Harker and O'Donovan, 2013). 2-methyl-4-chlorphenoxyacetic acid (MCPA) is one of the most used representatives of this group, being applied post-emergent in numerous crops to control broadleaf weeds (Morton et al., 2019; Ștef et al., 2024). MCPA acts as a synthetic auxin, inducing profound growth disorders through uncontrolled elongation of cells, disruption of auxin transport, and changes in vascular tissues, ultimately leading to physiological collapse and death of sensitive weeds (Johnson et al., 2023; Morton et al., 2020). At the same time, registration labels and tolerance studies emphasize that flax has a relatively higher sensitivity to MCPA compared to other crops, and exceeding certain doses or applying at advanced stages of development can induce phytotoxicity (yellowing, delayed ripening, reduced production) (Greenbook, 2018; https://www.saskflax.com/quadrant/media/Pdfs/Flax%20on%20the%20Farm/180427Fla xon_the_Farm-April_2018_Final.pdf).

In addition to selectivity considerations, the use of MCPA is under increasing pressure from an environmental regulatory perspective. The European Union's Farm to Fork Strategy foresees a 50% reduction in the use and risk associated with chemical pesticides by 2030, as well as a 50% reduction in the use of more hazardous pesticides, which implies a strict optimisation of doses and treatment programmes in all crops, including minority crops, such as flax (European Commission, 2020). At the same time, recent reports on weed resistance to auxin herbicides already indicate more than 40 species with confirmed resistance to this group, and the global analysis of resistance cases highlights a gradual increase in the frequency of tolerant biotypes (Heap, 2024; Ghanizadeh and Harrington, 2017, https://www.weedscience.org/summary/MOA.aspx?MOAID=12&utm_source.com). This context underlines the need to define minimum effective doses ('low-input') that ensure a high level of control, while reducing the impact on the crop and the environment.

Although the efficacy of MCPA has been extensively studied in cereal crops and other dicotyledonous systems (e.g., rapeseed, maize in tank-mix mixes), data on the use of this herbicide in flax agroecosystems are relatively scarce, fragmented, and often limited to the conditions of specific regions (Vasilakoglou et al., 2001; Mühleisen, 2000; Morton et al., 2019; Yaşar and Yetişsin, 2023). More importantly, the comparative response of the species *Convolvulus arvensis* and *Chenopodium album* to different doses of MCPA in flax crops, under the pedoclimatic conditions of Central and Eastern Europe, is insufficiently documented, despite the high frequency and economic relevance of these weeds in the region. This knowledge gap limits the development of scientifically recommendations for farmers and advisors, especially in areas where the number of herbicides available is already restrictive.

In this context, the present study aimed to evaluate the efficacy of the herbicide MCPA 750 g L^{-1} applied post-emergently, in four doses (0.16; 0.21; 0.27; 0.33 L ha^{-1}), on the species *Convolvulus arvensis* and *Chenopodium album* in flax culture in western Romania (Carani, Timiş County). Weed control was analysed at 14, 28 and 42 DAA to characterise the temporal dynamics of efficacy and to identify the minimum effective dose that ensures maximum infestation reduction, while minimising risks of phytotoxicity to the crop. The results obtained have the potential to contribute to the optimization of weed management in flax production and to the formulation of strategies for the usability of phenoxy-carboxylic herbicides in modern agroecosystems.

MATERIALS AND METHODS

Site description

The study was carried out in the 2022 growing season in Carani locality - Sânandrei commune, Timiş county (Western Romania).

The altitude of the experimental site was at 117 m, located at the geographical coordinates 45°54'50.2"N 21°09'15.7"E, on a chernozem-type soil (Figure 1).

Figure 1. Geographical location of the experimental field (Carani - Western Romania) (Google Maps, 2025)

The area is characterized by a temperate-continental climate, with an average annual temperature of 12.4ºC and an average annual rainfall of 717 mm, the conditions being favorable for the cultivation of flax for fibers. The average values of precipitation and temperatures during the study period (2022) are shown in Figure 2.

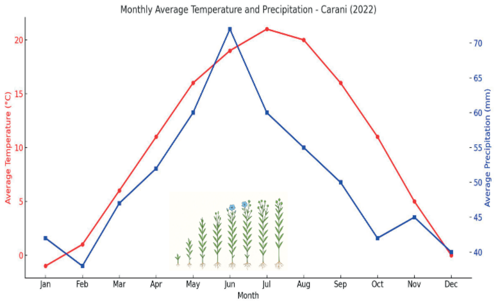


Figure 2. Monthly averages of temperature and precipitation recorded in Carami (Timiș county – Western Romania) in 2022

Biological material

In the present study, the flax variety (*Linum usitatissimum* L.) Attila. Drilling was performed on 02.05.2022, with a sowing norm of 55 kg/ha.

For the development and protection of the flax crop for fibre, ammonium nitrate fertiliser was applied (on May 1, 2022), and to limit pest attack, cypermethrin 100 g L^{-1} (on May 26, 2022) was applied. The cultivation technology was identical for all variants, the differences being generated exclusively by the herbicide regime.

Experimental design

The six experimental variants were organized into randomized blocks, including one untreated control and five herbicide-treated variants, each variant having four replicates. The experimental version had an area of 30 m2 (10 m * 3 m). Between replicates, 1 m paths were made to avoid cross-contamination.

Treatments

The herbicides were applied post-emergence, when flax plants were in BBCH growth stage 13-14 (02.06.2022).

The application was carried out with the back-pack sprayer using compressed air, the volume of water used in the experiment was 300 L^{ha-1}.

The following variants have been tested:

V1 – Untreated Control (CHK) (T0)

V2 – Dicopur M (MCPA 750 g L^{-1}) – 0.16 L ha^{-1}(T1)

V3 – Dicopur M (MCPA 750 g L^{-1}) – 0.21 L ha^{-1} (T2)

V4 – Dicopur M (MCPA 750 g L^{-1}) – 0.27 L ha^{-1} (T3)

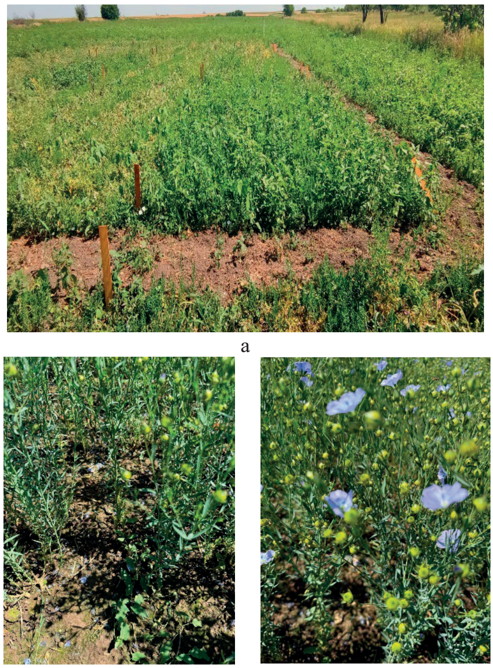
V5 – Dicopur M (MCPA 750 g L^{-1}) – 0.33 L ha^{-1} (T4)

V6 – Cerlite (fluroxipyr-meptyl 250 g L^{-1}) – 1.0 L ha^{-1} (T5)

Determination of the floristic composition and the degree of weeding

The weeding was determined by the quantitative numerical method (a metric frame of 0.25 m^2 was used, it was randomly placed at four points in each variant), the metric frame was randomly placed at four points.

The degree of weeding was determined in: the day before the application of the treatments (01.06.2022), 14 days after application (16.06.2022), 28 days after application (30.06.2022) and 42 days after application (14.07.2022). Thus determining the floristic composition (Figure 3), the abundance and the percentage of soil cover (%).



b c

Figure 3. (a) Weeding in the untreated control version (Photo: Ramona Ștef, 2022); (b-c) The appearance of flax culture in the flowering growth stage (Photo: Alin Cărăbeț, 2022)

The efficacy of herbicides was determined using the Abbott formula:

Efficacy after Abbott %= $\left(\frac{C_a - C_t}{C_a}\right) x\ 100$

where:
C_a percentage of infestation is under control,
C_t percentage of infestation in the treated version.

Assessment of phytotoxicity on flax

The phytotoxicity of the products on the flax crop was assessed visually, at the same time intervals (14, 28 and 42 DAA), using **the EWRS scale 1-9**, in which 1 indicates the absence of symptoms, and 9 - severe damage. The following were followed: chlorosis and necrosis of the leaves, growth retardation, deformations of the vegetative organs and reduction of crop cover.

Statistical analysis

For the weed control percentages, factorial ANOVA was applied, with two factors: treatment (T0-T5) and time of evaluation after application (14, 28 and 42 DAA). Each treatment-time combination included four experimental repetitions.

In order to highlight the response of the two species to the applied treatments, ANOVA was used with three factors: the species, the treatment variant and the number of days after application, and the interactions between them were also tested. The analyses were performed based on the sums of squares of type III, and the effect was considered significant at $p < 0.05$.

After identifying the significant differences, the comparison of the means was carried out by the Tukey HSD post-hoc test. All statistical processing was performed using IBM SPSS Statistics software.

RESULTS AND DISCUSSIONS

The mapping carried out on the day before the application of the treatments revealed a spontaneous flora dominated by: *Chenopodium album*, *Convolvulus arvensis, Amaranthus retroflexus*, *Sorghum haelepense*, *Datura stramonium, Xanthium strumarium, Echinochloa crus-galli, Abutilon theophrasti, Polygonum convolvulus*, these being the species with the highest frequency and density in flax cultivation (Table 1).

Table 1 shows the total weed density in the flax crop of 43 plants/m2, which indicates the high weed pressure and a significant competitive potential on the crop. The percentage of participation shows that three species: *Chenopodium album* (32.56%), followed by *Amaranthus retroflexus* (18.60%) and *Convolvulus arvensis* (13.95%) account for over 65% of the total species present in the control version. The monocotyledonous species present in the flax crop were represented by the species *Sorghum halepense* and *Echinochloa crus-galli* with a participation percentage of 9.30%. The species of the annual dicotyledonous class (*Chenopodium album, Amarathus retroflexus, Datura stramonium*, *Xanthium strumarium, Abutilon theophrasti, Fallopia convolvulus*) were dominant, totaling a participation of ≈ 72% of the total.

Table 1. Composition of spontaneous flora from flax culture in Carani locality (Timiș county)

Species	Botanical family	Number of plants/m^2	Percentage of participation (%)	The notary class
Chenopodium album	Amaranthaceae	14	32,56	D.A.
Convolvulus arvensis	*Convolvulaceae*	6	13,95	D.P
Amaranthus retroflexus	*Amaranthaceae*	8	18,60	D.A.
Sorghum haelepense	*Poaceae*	2	4,65	M.P
Datura Stramonium	*Solanaceae*	2	4,65	D.A.
Xanthium strumarium,	*Asteraceae*	1	2,33	D.A.
Echinochloa crus-galli	*Poaceae*	2	4,65	M.A.
Abutilon theophrasti	*Malvaceae*	5	11,63	D.A.
Polygonum convolvulus/ Fallopia convolvulus	*Polygonaceae*	3	6,98	D.A.
	Total	43	100	

The presence of *Sorghum haelepense* and *Convolvulus arvensis*, both perennial species, indicates a potential long-term persistence in the absence of adequate control measures.

The floristic composition confirms the need to apply herbicides, especially against dominant and competitive – aggressive species such as *Chenopodium album*, *Amaranthus retroflexus* and *Convolvulus arvensis*, which can significantly reduce production if not properly managed.

The results obtained following treatments applied in the flax crop (Figure 4), located in Carani, were oriented on two target species, namely *Convolvulus arvensis* and *Chenopodium album*. Also, the impact of the treatments on the two target species was evaluated at three time intervals after their application, respectively at 14, 28 and 42 days.

Figure 4. Visual comparison between herbicidal variants and untreated control in flax crop at 28 DAA

The ANOVA analysis applied to the obtained results showed the existence of statistically significant differences between the variants, the main effect being very strong and significant ($F (35, 108) = 327.35$, $p < .001$; Eta2 = 0.99, 95% CI [0.99, 1.00]).
Focussing at this result, post hoc Tukey comparisons were performed to identify differences between treatment–species–endpoint combinations.
Figure 5 illustrates the temporal dynamics of control over the species *Convolvulus arvensis* in the six experimental plots, while Figure 5 shows the corresponding response of the species *Chenopodium album*. In both species, weed mortality increased significantly between 14 and 28 days after application, followed by a stabilization or slight improvement at 42 days. However, the rate and amplitude of response varied depending on the treatment.

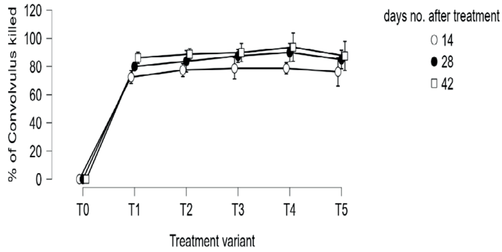


Figure 5. Descriptive plot of the response of *Convolvulus arvensis* to herbicide treatments in three time intervals after the treatment application (error bars are displayed)

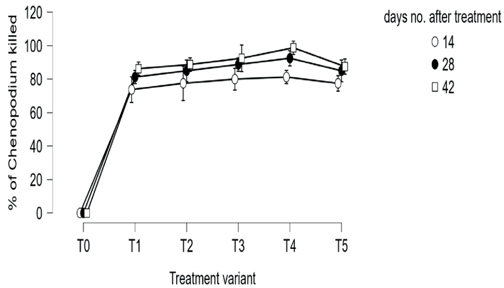


Figure 6. Descriptive plot of the response of *Chenopodium album* to herbicide treatments in three time intervals after the treatment application (error bars are displayed)

Table 2 shows the mean weed control values (± ES) for each treatment and time of assessment.

Efficacy of herbicides in controlling *Convolvulus arvensis species*

The first evaluation (14 DAA) of the efficacy of herbicides in combating the species *Convolvulus arvensis* showed that all treatments (T1-T5) ensured a level of protection for the flax crop between 72.5-78.75%, significantly higher than the control (untreated) variant. The post-hoc Tukey analysis confirmed very large statistical differences between the control and the herbicidal variants, with mean differences between - 72.5 and -78.75 ($p < 0.001$).

The control of *Convolvulus arvensis* in the herbicidal variants with MCPA (0.16-0.33 L ha^{-1}) (72.50-78.75%) was better compared to that obtained in the variant treated with fluroxypyr-meptyl (1.0 L ha^{-1}) 76.25%, but the differences did not present statistical assurance. MCPA 750 g $^{L-1}$ 0.33 L ha^{-1} (maximum dose) significantly exceeded the variant treated with minimum dose 0.16 L $^{ha-1}$ (T1), with a difference of 6.25% ($p = 0.033$; 95% CI: −12.02, −0.48).

The other treatments (MCPA 750 g L^{-1} – 0.21 L ha^{-1}, MCPA 750 g L^{-1} – 0.33 L ha^{-1}, fluroxypyr-meptyl 250 g L^{-1} – 1.0 L ha^{-1}) showed no statistical differences (Tukey test, $p > 0.05$).

At 28 DAA, the efficacy of herbicides in reducing the population of *Convolvulus arvensis* in flax crop increased slightly, ranging from 80–90% for all treatments. ANOVA indicates a significant effect of the "time after treatment" factor, and comparisons 14 vs. 28 DAA show significant increases in the percentage of destroyed plants ($p < 0.001$). These differences reflect the typical dynamics of auxin herbicides, in which symptoms gradually set in (epinasty, necrosis) in the weeks following application (Grossmann, 2010).

At 42 DAA, the efficacy of herbicides in the control of bindweed reached the maximum experimental values (86.25-93.75%), with the highest efficacy for T4 (0.33 L ha^{-1} MCPA) and very close values for T3 (0.21 L ha^{-1} MCPA) and T5 (fluroxypyr-meptyl 250 g L^{-1} – 1.0 L ha^{-1}). The treated variants showed strongly significant differences compared to the control ($p < 0.001$), but the differences between treatments were relatively small. However, Tukey's post-hoc analysis showed that T4 (0.33 L ha^{-1} MCPA) had superior efficacy over T5 (fluroxipyr-meptyl 250 g L^{-1} – 1.0 L ha^{-1}) in several treatment-time combinations, especially in the assessment of 42 DAA, the differences ranged from 6.25 to 8.75% ($p = 0.004 – 0.040$). For example, T4_d42 was higher than T5_d42 ($\Delta = 6.25$ p.p., $p = 0.0402$) and T5_d28 ($\Delta = 8.75$ p.p., $p = 0.004$). At the same time, T5 showed a significant increase in efficacy over time, from 14 to 42 DAA ($\Delta = 11.25$ p.p., $p < 0.001$), which confirms the progressive nature of the herbicide's action in this variant.

Table 2. Mean weed control (%) for *Convolvulus arvensis* and *Chenopodium album* across treatments and assessment timing

Treatment	Days after application treatment	Mean *Convolvulus arvensis* control (%)	Mean *Chenopodium album* control (%)
T0 (untreated control)	14	0.000^{l}	0.000^{l}
	28	0.000^{l}	0.000^{l}
	42	0.000^{l}	0.000^{l}
T1 (MCPA 750 g L^{-1} – 0.16 L ha^{-1})	14	72,500^{k}	73.750^{jk}
	28	80,000^{ghi}	81,250^{fghi}
	42	86.250^{def}	86.250^{def}
T2 (MCPA 750 g L^{-1} – 0.21 L ha^{-1})	14	77,500^{ijk}	77,500^{ijk}
	28	83.750^{efgh}	85,000^{defg}
	42	88,750^{bcde}	88,750^{bcde}
T3 (MCPA 750 g L^{-1} – 0.27 L ha^{-1})	14	78,750^{hij}	80,000^{ghi}
	28	87,500^{cde}	88,750^{bcde}
	42	90,000^{bcd}	92,500^{bc}
T4 (MCPA 750 g L^{-1} – 0.33 L ha^{-1})	14	78,750^{hij}	81,250^{fghi}
	28	90,000^{bcd}	92,500^{bc}
	42	93,750^{ab}	98,750^{a}
T5 (fluroxipyr-meptyl 250 g L^{-1} – 1,0 L ha^{-1})	14	76,250^{ijk}	77,500^{ijk}
	28	85,000^{defg}	85,000^{defg}
	42	87,500^{cde}	87,500^{cde}

*Different letters indicate statistically significant differences (Tukey HSD, $p < 0.05$).

Overall, the results indicate that *Convolvulus arvensis* is sensitive to MCPA and fluroxipyr-meptyl, but a very clear separation between doses of MCPA and fluroxipyr is not statistically achieved – all treated variants are in a high efficacy "plateau" (86,250-93,750%). These results are in agreement with the literature, which shows that hormonal herbicides, including phenoxyacidides (MCPA, 2,4-D) and their combinations with other active substances, are commonly recommended for the control of perennial dicotyledons, such as *Convolvulus arvensis*. Previous studies have reported high efficacy of post-emergent hormonal treatments on perennial weeds (Schroeder et al., 1990; Robinson et al., 2015; Soltani et al., 2012), and the use of combinations from different groups can increase combat performance (Sosnoskie and Hanson, 2016; Bayat and Zargar, 2020). *Convolvulus arvensis* is a perennial weed with important reserves in deep roots, which makes it difficult to completely destroy the vegetative apparatus with a single treatment, chemical control usually only ensures a short-term reduction of aerial parts, which is why programs with repeated applications of systemic herbicides are required (Bayat and Zargar, 2020).

Efficacy of herbicides in controlling the species *Chenopodium album*

For *Chenopodium album*, the response to herbicides was higher.

At 14 days after the application of the treatments, the comparative analysis revealed very wide and statistically significant differences between the control variant (T0) and all treatments applied to *Chenopodium album*. The values of the mean differences ranged from −73.75 (T1; p < 0.001.95% CI: −79.04... −68.46) and −81.25 (T4; p < 0.001.95% CI: −87.27... −75,23), indicating effective and consistent weed control. The other variants showed similar differences: T2 (−77.5; p < 0.001, 95% CI: −83.27... −71.73), T3 (−80; p < 0.001, 95% CI: −85.98... −74.02) and T5 (−77.5; p < 0.001, 95% CI: −83.19... −71,81). Relatively narrow and overlapping confidence intervals demonstrate homogeneity of herbicide response, suggesting that at this early stage, all active substances acted effectively and reduced infestation by more than 73,750–81,250% compared to the control.

At 14 DAA, the efficacy of herbicides in controlling *Chenopodium album* ranged from 73.750% to 81.250% for the T1–T5 variants. Although the values show a slight upward trend with the dose of MCPA (T4 0.33 L $^{ha-1}$ ≈ T3 MCPA 750 g $^{L-1}$ 0.27 L $^{ha-1}$ > T2 0.21 L $^{ha-1}$ > T1 0.16 L $^{ha-1}$), the differences between treatments were not statistically significant ($p > 0.05$). The largest numerical differences were around 6.25 percentage points between Q3/Q4 and Q1, but these variations were not statistically validated. Fluroxipyr (T5) was at a similar level to the average doses of MCPA, with no significant differences.

At 28 DAA, the population of *Chenopodium album* was reduced by 81.250-92.500%, and at 42 DAA values of 86.250-98.750% were reached, with the maximum value in the T4 variant (0.33 L ha^{-1} MCPA), the differences recorded compared to the low doses T1 (Δ = 12.5, $p < 0.001$, 95% CI [6.45; 18.55), T2 (Δ = 10.0, $p < 0.001$, 95% CI [4.23; 15.77]) and fluroxipyr (T5) being significant.

Interspecific comparison between *Chenopodium album* and *Convolvulus arvensis*

The comparative analysis demonstrated the existence of consistent differences in sensitivity between the two dominant weeds. In general, *Chenopodium album* was more sensitive to the treatments applied compared to *Convolvulus arvensis*, this difference increased proportionally with the increase in the time interval after application.

At 14 DAA, the responses of the two species to the 5 treatments were almost similar (1–3 percentage points), with no statistically relevant differences.

At 28 DAA, interspecific separation was already detectable, but with small differences (≈2-4 percentage points) and only statistically significant in isolation, suggesting that after 3-4 weeks different responses on the sensitivity of the two species occur, when systemic translocation of herbicides is complete.

At 42 DAA the interspecific differences were the most obvious. For the same variant, the efficacy on *Chenopodium album* exceeded that recorded in *Convolvulus arvensis* by 8.75-11.25 percentage points, the differences being statistically significant ($p < 0.01$).

Treatment 4 (0.33 L ha^{-1} MCPA) reached 98.75% in *Chenopodium album* compared to

93.75% in *Convolvulus arvensis* (Δ = 11.25 p.p.; 95% CI [5.34; 17.16]), and similar differences were identified for T2 and T3 (Δ = 10.00 and Δ = 8.75 p.p.).

Overall, all treatments (T1-T5) were very effective compared to the control for both species ($p < 0.001$), but the response dynamics were distinct.

Chenopodium album showed rapid increases in efficacy between 14 and 28 DAA, followed by almost complete stabilization at 42 DAA (where T4 reached 98.75%). In *Convolvulus arvensis*, the final values were high (85-93.750%), but the differences between the doses were more attenuated, and the upper T4 variant statistically distanced itself from the low doses only in the late evaluations.

The results of the study showed that *Chenopodium album* is more sensitive to auxin herbicides as an annual species without a perennial structure; in contrast, *Convolvulus arvensis*, a perennial species, can diminish the effects of herbicides due to the underground reserves and cerate cuticle, which explains both the relatively lower efficacy and the need for higher doses for optimal results. This confirms that, for bindweed, the maximum effectiveness depends on the number of days after the application of the treatments (28-42) and the use of high-dose variants (T4). On the other hand, in *Chenopodium album*, the response to herbicides occurs more quickly, and the differences between doses are more clearly delineated statistically, the maximum control thresholds being reached from the first 28 days

.

Table 3. Phytotoxicity and selectivity of herbicides (MCPA and fluroxipyr-meptyl) applied in flax culture

Treatment variant	Phytotoxicity				Mean (%)
	I	II	III	IV	
T0 (untreated control)	0	0	0	0	0
T1 (MCPA 750 g L^{-1} – 0.16 L ha^{-1})	0	0	0	0	0
T2 (MCPA 750 g L^{-1} – 0.21 L ha^{-1})	0	0	0	0	0
T3 (MCPA 750 g L^{-1} – 0.27 L ha^{-1})	0	0	0	0	0
T4 (MCPA 750 g L^{-1} – 0.33 L ha^{-1})	6	6	5	5	5.5
T5 (fluroxipyr-meptyl 250 g L^{-1} – 1,0 L ha^{-1})	7	7	6	7	6.75

The phytotoxicity assessment (Table 3) revealed clear differences in selectivity between the variants tested. In T1-T3 treatments (0.16-0.27 L ha^{-1} MCPA), flax culture did not show any visible symptoms of stress, all repetitions being evaluated with a score of 0. These results confirm the high tolerance of flax to low and medium doses of MCPA, a characteristic previously reported in other dicotyledonous crops, where MCPA is considered a herbicide with a low risk of phytotoxicity at approved doses.

In the high-dose MCPA variant (T4, 0.33 L ha^{-1}), phytotoxicity scores ranged from 5 to 6, corresponding to moderate chlorosis and minor foliar lesions, with no anticipated impact on production. These symptoms reflect the proximity of the dose to the upper limit of physiological tolerance of the plant, but the reduced severity suggests that the detoxification mechanisms of the crop are still functional.

The weakest selectivity was observed in fluroxypyr-meptyl (T5) treatment, where scores between 6 and 7 indicate a high level of stress: accentuated chlorosis, leaf deformities and a potential negative effect on production. Unlike MCPA, fluroxypyr causes a more aggressive hormonal flow and rapid redistribution of synthetic auxins, which may exceed the physiological compensating capacity of flax culture, especially at high doses.

These results underline that although efficacy against weeds increases with dose, herbicide selectivity may decrease and the trade-off between control and safety needs to be carefully assessed in integrated weed management programmes.

Discussions

The results of the study demonstrate a clear dependence of herbicide efficacy on both the treatment variant and the timing of evaluation, with weed control steadily increasing between 14 and 42 days after application. This progressive efficacy is characteristic of post-emergent systemic auxin herbicides, whose absorption, phloemic mobility, and metabolic activation require several weeks to reach maximum biological expression (Grossmann, 2010). In this case, the significant increases detected by the Tukey HSD test confirm a sustained herbicide action, which intensifies over time as the active substance accumulates in the meristematic tissues.

Of the treatments tested, T4 (0.33 L ha^{-1} MCPA) provided the most consistent and high level of control for both species, with efficacy reaching 93.75% for *Convolvulus arvensis* and 98.75% for *Chenopodium album* at 42 DAA. The statistical superiority of T4 over lower doses of MCPA (T1-T3) and fluroxipyr (T5) at assessment III (Δ = 10-12.5 p.p., $p < 0.001$) suggests either a higher systemic load or a more effective physiological disturbance. T3 was also in the upper efficacy group, representing a robust agronomic option in situations where the use of higher doses is limited by the authorisation label or economic considerations. In contrast, T1 showed the weakest control, especially in the early stages, indicating insufficient auxin stimulus at the minimum dose.

The interspecific comparison revealed marked differences in sensitivity, relevant for integrated weed management. *Chenopodium album* was consistently more sensitive than *Convolvulus arvensis*, and this difference became more pronounced in late evaluations. At 42 DAA, *Chenopodium album* outperformed *Convolvulus arvensis* by 8.75-11.25 percentage points in MCPA treatments ($p < 0.01$; 95% confidence intervals not including zero). These contrasts correspond to the distinct morphological and anatomical features of the species: *Chenopodium album* has a thinner cuticle, high leaf permeability, and a rapid rate of tissue growth and renewal processes, which favours the absorption of auxin herbicides (Pannacci and Covarelli, 2015). In contrast, *Convolvulus arvensis*, a species with an extensive network of rhizomes, exhibits superior tolerance due to abundant epicuticular waxes, rosette architecture, and well-documented metabolic detoxification pathways in numerous auxin-tolerant perennial weeds (Westra et al., 1992). Consequently, even if MCPA in increased doses produces numerical improvements in control, these increases are physiologically limited and do not exceed the threshold necessary to become statistically significant.

The herbicide–time interaction also differed between species. *Chenopodium album* was controlled at maximum after 28 DAA (92-93%), while *Convolvulus arvensis* required 42 DAA to reach similar levels. This result highlights a rapid loss of vegetative functions in the annual species, while in the perennial species the reduction in viability is gradual, reflecting the physiological tolerance conferred by the deep root system - an observation frequently reported in the literature on auxin responses of annual versus perennial weeds (Westra et al., 1992).

Regarding the selectivity towards flax cultivation, marked differences between treatments were highlighted. Low and medium doses of MCPA (T1-T3) produced no visible symptoms of phytotoxicity, consistent with the well-documented high selectivity for MCPA in dicotyledonous fibre and oil cultures (Hartzler and Anderson, 2016). These doses are within the physiological ability of the plant to rapidly detoxify the compound, mainly by conjugation with carbohydrates and amino acids. In contrast, the high dose (T4) induced moderate chlorosis and leaf deformations (5.5%), suggesting partial exceeding the detoxification capacity. Fluroxipyr (T5) produced the highest level of phytotoxicity (6.75%), corresponding to its higher auxin potency and rapid phloemic mobility. Similar effects are reported in flax and other sensitive cultures, where excessive auxin stimulation compromises vascular integrity and hormonal balance (Grossmann, 2010; Sosnoskie and Culpepper, 2014).

From an agronomic perspective, the high efficacy observed at 42 DAA underlines the significant potential for reducing weed competition during the critical period for the formation of flax production. However, the modest levels of control at 14 DAA suggest that in fields with high early infestations, additional cultural or mechanical measures may be required. The highlighted interspecific differences suggest a specific adaptation of strategies: annual weeds such as *Chenopodium album* can be effectively controlled with moderate doses of MCPA, while perennial weeds such as *Convolvulus arvensis* may require higher doses or mixtures of active substances to limit regeneration in underground organs.

Finally, integrating these results into a long-term approach highlights the need to rotate herbicides with different modes of action and avoid the repeated use of high doses of auxins, given the increasing number of auxin-resistant weed biotypes reported globally (Heap, 2024).

CONCLUSIONS

MCPA-based treatments tested at doses of 0.16-0.27 L ha^{-1} (T1-T3) demonstrated effective control of dominant weeds, with an efficacy of 80–93% between 28 and 42 DAA, without inducing symptoms of phytotoxicity.

The higher dose of MCPA (0.33 L ha^{-1}, T4), although generating the highest efficacy under experimental conditions, resulted in moderate symptoms of phytotoxicity, indicating the need for further studies prior to any recommendation for use in flax culture.

Fluroxypyr-meptyl (T5) treatment achieved high levels of control, but induced the most pronounced phytotoxicity (6-7%) and is not approved for flax, which limits practical use. Its results must be considered strictly exploratory.

Chenopodium album was the most sensitive species, being 90-99% sensitive to MCPA, which confirms the vulnerability of this taxon to auxin herbicides.

Convolvulus arvensis showed a higher tolerance, with maximum efficacy at 42 DAA. The need for longer intervals is reflected by the fact that it is perennial with regenerative capacity.

Selectivity was outstanding for T1–T3 (no symptoms). In contrast, T4 and T5 generated stress reactions that can limit their application in practice, regardless of their effectiveness on weeds.

From an agronomic point of view, the optimal strategies remain those based on MCPA doses (T1-T3), and the high doses tested (T4) or non-approved alternatives (T5) require additional studies and crop safety evaluations before use.

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STUDY ON OBTAINING POTATO MINITUBERS BY USING VARIOUS CULTURE SUBSTRATE

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Abstract

The modernization of the production of planting material for potato cultivation and especially the production of minitubers on industrial substrate represents a highly topical research direction worldwide, in line with the major objective of upgrading potato cultivation in Romania. The Research Laboratory for Plant Tissue Cultures, of the National Institute of Research and Development for Potatoes and Sugar Beet Braşov evaluated the minituberization process was on two culture substrates for four genotypes to observe whether the use of industrial substrate is more efficient than soil. The biological material used consisted of virus-free in vitro plants, starting from the culture of meristems. In vitro potato plants were planted on the two types of substrates. Determinations of number and weight of the minitubers were made. The industrial substrate favoured the production of a higher average number of minitubers (7.82 minitubers) with a distinctly significant positive difference, compared to the conventional substrate (6.94); also on industrial substrate the average weight of minitubers was higher than on peat

***Key words**: potato, in vitro plants, variety, culture substrate, minitubers.*

INTRODUCTION

Food security has become a crucial issue due to climate change, reducing arable land areas, population growth and the frequent occurrence of natural disasters. To deal with this situation, increasing the food supply has become a priority.

The soilless cultivation technique facilitates many socio-economic benefits, including the ability to address growing global food challenges, environmental changes aimed at alleviating malnutrition, and the efficient management and use of natural resources.

The potato is a versatile and affordable staple food that plays a significant role in global food security, and its mild flavour and diverse culinary applications make it popular worldwide.

The development of new technology for food production is used to sustain the transformation of agriculture in response to population growth and resource demands (Gebreegziher, 2023). One emerging and promising technique to overcome current threats facing in soil-based dryland vegetable farming is soilless culture.

In the global horticultural production, vegetable crops 'without soil' had begun already gain a leading position. These unconventional systems of culture are great interest both for researchers and for those who practice in order to achieve products for human consumption.

Expanded perlite is a substrate of culture that completely replaces soil (Drăghici et al., 2013). Perlite which is less expensive than rockwool has been used as soilless culture substrate around the world for successful production of vegetables, in the greenhouse (Jerca, 2015). Perlite is a sterile medium free of bush and pathogens and excellent medium for germination (Alkhateeb et al., 2019). Perlite is a material resulting from heating silicon volcanic rocks from 900 to 1000 degrees Celsius (Faleh, 2023). This heating results in countless air gaps that absorb water by 430% of their volume. Perlite has a moderate pH and is light in weight. It is used as a carrier for fertilizers, herbicides, and pesticides (Faleh, 2023). Perlite is widely preferred as it encourages faster root development, reduces risk of damping off, avoids water logging and provides an optimum balance of air and water (Asaduzzaman, 2013). In general, it has a closed cellular structure, with the majority of water being retained superficially and released slowly at a relatively low tension, providing

excellent drainage of the medium and aeration of rhizosphere (Markoska et al., 2018).
Artificial growing system provides plants with mechanical support, water and mineral nutrient for higher growth and development (Asaduzzaman et al., 2015).
Substrates are formulated from various inorganic and organic components to provide suitable physical and chemical properties as required by the specific crop and growing conditions (Markoska et al., 2018).
Use of media type possibly is the most intensive culture system utilizing all the resources efficiently for maximizing yield of crops and the most intense form of agricultural enterprises for commercial production of greenhouse crops (Makau et al., 2021). They are considered as important technologies for better water use efficiency as well as high good quality and quantity products. Number of organic and inorganic materials such as gravel, sand, peat, sawdust, pumice, tuff, coir, vermiculite, perlite, and rock wool pure or in mixture are used as solid growing media in addition to hydroponics (Makau et al., 2021).
In the conditions of our country, in recent years there has been an interest increase of unconventional culture technologies, which open attractive perspectives for professional growers (Atanasiu, 2007).
In Romania, concerns regarding the use of soilless methods are materializing especially in the research sector, with no trends for their implementation at national level for now.
At National Institute of Research and Development for Potato and Sugar Beet Brasov, within the Research Laboratory for Plant Tissue Cultures, various studies were conducted on the behaviour of potato varieties created by the specialized staff of the Research Laboratory for Genetic Breeding and Plant Selection.
Well-trained personnel are needed to carefully monitor fertigation and the main factors: the pH of the nutrient solution and the electroconductivity value, which for potatoes is not recommended to exceed 2 ms/S.
The minituberization process was evaluated on different hydroponic systems with and without automation and on different culture substrates: perlite, clay (Tican, 2018; Tican et al., 2025). In 2016, the Castrum variety recorded higher values for the number of minitubers/plant (11.0), followed by the Marvis variety (10.20 minitubers/plant) (Tican et al., 2018).

MATERIALS AND METHODS

In the year 2024, within the Research Laboratory for Plant Tissue Cultures, of the National Institute of Research and Development for Potatoes and Sugar Beet Brasov, the minituberization process was evaluated on different culture substrates, in a bifactorial experience, of the 2*4 type, in which factor a was the culture substrate: with two gradations: a_1 – perlite industrial substrate; a_2 – conventional substrate consisting of a mixture of peat and perlite, and factor b: the variety, with four gradations: Azaria, Brasovia, Cosiana and Cezarina. The statistical analysis was performed by the ANOVA program.
The biological material used consisted of virus-free *in vitro* plants, starting from the culture of meristems.

Figure 1. *In vitro* potato plants

At the beginning of May 2024, *in vitro* plants (Figure 1) were transferred to an “insect proof” protected area and planted on the two types of substrates, to obtain minitubers (these are being the first link in the national seed potato production system).
Figure 2 shows aspects of the development of potato plants on perlite substrate. For the plants that were grown on the substrate with perlite, a nutrient solution (prepared in the laboratory) was administered, based on: nitrogen, phosphorus, potassium, magnesium, iron, manganese, copper, zinc, boron, molybdenum, and the electroconductivity was 2 mS/cm. In October minitubers (Figure 3) were harvested and determinations of the number and weight of the minitubers obtained were made.

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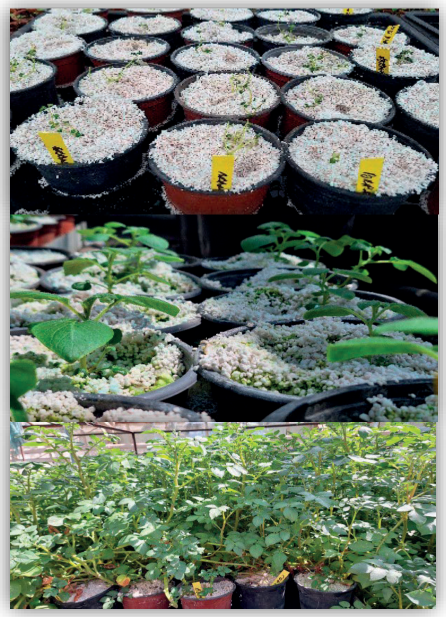

Figure 2. Plant development on industrial substrate

Figure 2 shows aspects of the development of potato plants on perlite substrate. For the plants that were grown on the substrate with perlite, a nutrient solution (prepared in the laboratory) was administered, based on: nitrogen, phosphorus, potassium, magnesium, iron, manganese, copper, zinc, boron, molybdenum, and the electroconductivity was 2 mS/cm. In October minitubers (Figure 3) were harvested and determinations of the number and weight of the minitubers obtained were made.

The production of potato minitubers is the classic intermediate step and represents connections between the biological material obtained *in vitro* and tubers produced in the clonal field. The techniques used for the production of minitubers are diverse, but they are mainly based on the propagation of microplants on a classic substrate or on an industrial substrate.

Figure 3. Minitubers obtained on industrial substrate

The scheme for producing minitubers starting from the meristem is shown in Figure 4.

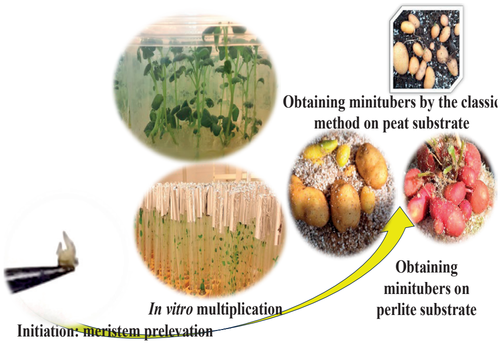

Figure 4. the steps for obtaining minitubers (potato pre-base material) starting from the virus-free meristematic explant

RESULTS AND DISCUSSIONS

Table 1 shows the significant influence of the culture substrate, the variety on number of minitubers obtained. The cultivar studied and the culture substrate interaction with cultivar had a significant influence on the weight of minitubers.

Table 1. Analysis of variance

Source of variation	Sum of squares	DF	The mean square	F
Analysis of variance for minitubers number/plant				
Culture substrate (a)	4.65521	1	4.65521	107.718 **(18.51; 98.50)
Variety (b)	104.99610	3	34.99868	11.616 **(3.49; 5.95)
Culture substrate*Variety	27.74687	3	9.24896	3.070 ns (3.49; 5.95)
Analysis of variance for minituber weight/plant				
Culture substrate (a)	2833.15800	1	2833.15800	4.221 ns (18.51; 98.50)
Variety (b)	22125.38000	3	7375.12700	5.359 *(3.49; 5.95).
Culture substrate*Variety	24996.15000	3	8332.05200	6.054 **(3.49; 5.95)

df, degrees of freedom.
*Significant at the 0.05 probability level.
**Significant at the 0.01 probability level.
ns - not significant

From the analysis of the influence of the culture substrate on the number of minitubers, a distinctly significant positive difference can be observed when using the perlite industrial substrate. Regarding the weight of the minitubers obtained, the difference is not significant, but positive when using the perlite substrate, compared to the conventional substrate (Table 2).

Table 2. The culture substrate influence on minitubers number of and their weight (g)/plant

Culture substrate (a)	Minitubers number	Diff./ Sign.	Minitubers weight (g)	Diff. (g)/Sign.
Industrial substrate (a_1)	7.82 A	0.88 **	127.75 A	21.73 ns
Peat-perlite (a_2) (Ct)	6.94 B	-	106.02 A	-

LSD 5% = 0.36; 1% = 0.84; 0.1% = 2.68 — LSD 5% = 45.48; 1% = 105.03; 0.1% = 334.23

Means found in the same columns followed by the same letters are not significant according to the Duncan test (p≤0.05).

The analysis of variety influence highlights for the first element analyzed (Table 3), number of minitubers, the superiority of Cezarina variety, which registers the highest number of minitubers (9.31), followed by the Azaria and Brașovia varieties (9.03 and 7.11 minitubers), without to differ significantly (according to Duncan test). By studying variety influence on mean weight of the minitubers/plant, it is observed that the varieties Azaria and Brașovia determined the achievement of statistically assured results, with distinctly significant positive differences (77.67 and 68.66 g), compared to the control variety (Cezarina).

Table 3. The variety influence on minitubers number of and their weight (g)/plant

Variety (b)	Minitubers number	Diff./ Sign.	Minitubers weight (g)	Diff. (g)/Sig.
Azaria (b_1)	9.03 A	-0.27 ns	148.33 A	77.67 **
Brașovia (b_2)	7.11 A	-2.20 o	139.32 A	68.66 **
Cosiana (b_3)	4.07 B	-5.24 ooo	109.21 AB	38.55 ns
Cezarina (b_4) (Ct)	9.31 A	-	70.66 B	-

LSD 5% = 2.18; 1% = 3.07; 0.1% = 4.33 — LSD 5% = 46.69; 1% = 65.54; 0.1% = 92.53

Means found in the same columns followed by the same letters are not significant according to the Duncan test (p≤0.05).

For Cosiana variety, the industrial substrate had a negative influence, leading to the lowest number of minitubers (2.80), with a very significant negative difference compared to the control variety. The best behaviour of the cultivars regarding minitubers formation was presented by Azaria and Cezarina cultivars, which obtained a high number of minitubers (10.07 and 9.70), without significant differences. On mixture peat-perlite substrate, Cezarina variety obtains the highest number of minitubers (8.92), but this number is inferior to the same variety on the industrial substrate (9.70), degerming a positive difference, but insignificant between the two types of substrates, for the control variety (0.78 g). For the Brașovia variety, there is a significant difference (3.21 minitubers), positive, between industrial substrate and conventional substrate (Table 4).

Table 4. Combined influence of culture substrate and cultivar on mean number of minitubers/plant

Culture system/ Variety	Industrial substrate (a_1)		Peat-perlite (a_2)		a_1-a_2 Sign.	
	Minitub. number	Diff. Sign.	Minitub. number	Diff. Sign.		
Azaria	10.07	0.37 ns	8.0	-0.92 ns	2.07	ns
Brașovia	8.81	-0.99 ns	5.50	-3.42 o	3.21	*
Cosiana	2.80	-6.90 ooo	5.33	-3.58 o	-2.53	ns
Cezarina (Ct)	9.70	-	8.92	-	0.78	ns

LSD 5% = 3.09; 1% = 4,34; 0,1% = 6.12. — LSD 5%= 2.69; 1% = 3.80; 0.1% = 5.74

On industrial substrate, the superior capacity of Azaria and Brașovia varieties is observed (Table 5) to form minitubers with a higher mean weight/plant, compared to the control variety, and significant positive differences

(78.82 and 71.04 g). On conventional substrate, Cosiana variety obtains a distinctly significant positive difference (111.88 g), compared to the Cezarina variety. When analysing the mean weight of minitubers obtained, between the two types of substrates, for Cezarina variety, a significant negative difference was obtained (90.03); the other varieties obtained positive, but insignificant differences on the industrial substrate compared to the classical substrate.

Table 5. Combined influence of culture substrate and cultivar on mean weight of minitubers (g)/plant

Culture system/ Variety	Industrial substrate (a_1)		Peat-perlite (a_2)		a_1-a_2 Sign.	
	Minitub. weight (g)	Diff. (g) Sign.	Minitub. weight (g)	Diff. (g) Sign.		
Azaria	177.79	78.82 *	118.87	76.52 *	58.93	ns
Brașovia	140.02	71.04 *	108.63	66.28 *	61.39	ns
Cosiana	64.20	-34.78 ns	154.23	111.88 **	-90.03	o
Cezarina (Ct)	98.98	-	42.35	-	56.63	ns

LSD 5% = 66.03;
1% = 92,69;
0,1% = 130.85.

LSD 5%= 70.04;
1% = 113.72;
0,1% = 230.09.

CONCLUSIONS

The industrial substrate favoured production of a higher mean number of minitubers (7.82 minitubers), compared to the conventional substrate (6.94 minitubers). Varieties Cezarina, Azaria and Brasovia recorded higher values of minitubers number (9.31; 9.03; 7.11), significantly different from the variety Cosiana (4.07). Variety Cezarina, although it had the ability to form a large number of minitubers, their weight was low.

Azaria and Cezarina varieties showed the best behaviour regarding minitubers formation on industrial substrate, which obtained a high number of minitubers (10.07 and 9.70), without significant differences.

On the peat-perlite substrate, Cezarina variety obtained the highest number of minitubers (8.92), but this number is inferior to the industrial substrate (9.70).

For Brașovia variety, there is a significant difference (3.21 minitubers), positive, between the industrial substrate and control substrate.

On industrial substrate, the superior capacity of Azaria and Brașovia varieties to form minitubers with high average weight/plant (177.79 and 140.02 g) can be observed.

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STUDIES ON THE INFLUENCE OF INPUT APPLICATION ON THE PRODUCTIVITY OF ROMANIAN WHEAT VARIETIES, AT ARDS CARACAL

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Abstract

Nutrients play a vital role in wheat production, both macro- and micronutrients being necessary for plants. Each nutrient has its own character and is involved in various metabolic processes of the life of wheat plants, that is why the influence of each cannot be clearly delineated. The purpose of the research is to present the yield results obtained at ARDS Caracal in the 2023-2024 agricultural year, for three Romanian wheat varieties (Glosa, Otilia and Carom), cultivated after different preceding crops (rape, peas, sunflower), using four different fertilization schemes. The obtained yields highlight the fact that each of the links in the technological scheme influences, to some extent, the quantity and quality of wheat production. Amidst prolonged soil droughts both in the fall of 2023 and in the spring-summer of 2024, the application of gradual-release inputs, doubled by biostimulants, managed to provide production increases of up to 22%, compared to the control variants. The results confirm that inputs play an essential role in increasing soil fertility, in full correlation with the fertilizers type, but also with the timing of their application.

***Key words**: wheat crop, inputs, macronutrients, micronutrients, yields.*

INTRODUCTION

Nutrient deficiency and toxicity conditions inhibit normal plant growth and exhibit characteristic symptoms (Gomaa et al., 2015). For optimal growth, development and production, plants need all the necessary nutrients in balance. A balanced application of primary nutrients (N, P, K), secondary nutrients (S, Mg) and other micronutrients (Zn, B) is necessary to improve wheat yield (Pandey et al., 2020). Soil and crop nutrient requirement tests should be conducted periodically, in order to identify the recommended amount of fertilizer for the wheat crop in that year (Saquee et al., 2023).

A study conducted on clay soils in Bangladesh shows that wheat yield was significantly affected by combinations of inputs with secondary macronutrients, S and Mg, but also micronutrients Zn and B, together with the recommended dose of NPK – 100 kg N + 30 kg P + 70 kg K/ha, expressed in active substance (Azad et al., 2021). Plant height and the number of tillers per plant had significantly higher values than the control in each of the fertilization variants proposed for testing. Similar research has been carried out in Romania, in several areas of the country, by researchers such as Bacanu et al. (2019), Berca et al. (2019), Cernat et al. (2020), Cioineag & Cristea (2015), Horoias et al. (2013), Mihalache et al. (2014) and many others.

An efficient plant nutrition system can also be defined as that interaction between the different agrochemical, biochemical, technological and managerial measures that lead, with minimum costs and maximum yields and quality, to satisfying the farmers' requirements, but also of the environment and society (Berca, 2011).

Although new nutritional products are constantly emerging, the most difficult task is to test their practical effectiveness, through research over several years, so that the long-term influence they have on the soil (Bajgiran, 2013) and the crops they target becomes clear.

An example of such a product is the biofertilizer Rom-Agrobiofertil NP, a fertilizer based on three bacterial strains (*Azospirillium lipoferum*, *Azotobacter chroococcum* and *Bacillus megaterium*), which, following testing in an organic wheat crop, led to average production increases of 350 kg/ha (Toader et al., 2019). Good results were also obtained in other field crops (rapeseed, sunflower). Following the evaluation of the pedo-climatic parameters of the ecosystem, the biometric data of the crops and the production differences, major positive differences were identified in favor of the bacterial biopreparations, in the soil-plant-production system (Toader et al., 2020).

Another study (Ali et al., 2022) presents the results of *T. harzianum* application in combination with foliar applied zinc and iron, which significantly positively influences wheat plant height, yield, number of grains/ear and harvest index. The current study can be successfully used for bread wheat development programs (Rosculete et al., 2023).

The effect of plant growth biostimulants results from the synergy of several components, in different concentrations, by increasing the absorption of minerals from the soil by plants and by improving the efficiency of the use of these nutrients. Considering the multi-elemental composition of the amino acid hydrolysates tested by Popko et al. (2018) - small amounts of macroelements: 2.8-3.5% N, 0.8-1.1% P_2O_5, 3.9-4.5% K_2O and microelements –, it is suggested that their function is to increase the absorption of nutrients by plants from the environment.

At the same time, meat and bone meal contains appreciable amounts of total nitrogen (8%), phosphorus (5%) and calcium (10%). Therefore, it can be a useful fertilizer for various crops, including wheat (Jeng et al., 2006). Similar effects are obtained with composts from sewage sludge, also sources of macro and micronutrients that can successfully contribute to crop fertilization (Safta & Ilie, 2022).

As a conclusion of the results obtained, in the mentioned research, different types of inputs have been shown to be beneficial in improving nitrogen use efficiency and crop yield under low nitrogen applications (Li et al., 2023), which provides them with an economic advantage.

Starting from the examples identified in the specialized literature, the purpose of this research was established, namely to exemplify how the application of different inputs influences the productivity of different wheat varieties, depending on the preceding crop.

MATERIALS AND METHODS

The experiments were carried out on the farms of ARDS Caracal, located in the southern part of Romania, in Olt County, on chernozem-type soils. Romanian wheat varieties were tested.

The main objective of the paper is represented by the study of the influence of some technological factors (variety, the previous crop and the type of fertilization with chemical fertilizers and biostimulators) on the elements of productivity and production, in the wheat crop.

Regarding the evolution of the wheat crop, for each of the test variants the following parameters were periodically monitored (Picture 1, a and b):

- number of plants/sqm;
- number of tillers/plants;
- plants height (cm);
- number of ears/sqm;
- ears length (cm);
- number of spikelet's/ears;
- number of grain/ears;
- grain weight/ear (g).

Photo 1. Evaluation of the development stage of wheat plants (original photos from 21.03.2024): a - counting plants/sqm; b - number of tillers/plants

The climatic conditions of the 2023-2024 agricultural year weren't mentioned, since all the research plots benefited from the same environmental conditions. However, being an area with prolonged droughts that repeat annually, as were those recorded in the fall of 2023 and in the spring-summer of 2024, the application of correct fertilization schemes is even more important for the good development of the wheat crop. A second aspect is the availability of plant inputs, amid the acute lack of water in the soil.
As control, the variant without inputs in autumn, at the establishment of the crop, followed by a single application of inputs in spring, at the resumption of vegetation – 250 kg/ha ammonium nitrogen, was chosen. The factors included in the technological scheme of the research are detailed in Table 1.

Table 1. Factors used to draw up the technological scheme of the research

Wheat variety (A)	Previous crop (B)	Fertilization scheme (C)
A1 – Glosa A2 – Otilia A3 – Carom	B1 – peas B2 – rapeseed B3 – sunflower	**C1** – *unfertilized in autumn + ammonium nitrate 250 kg/ha in spring (control)*
		C2 – *fertilized in autumn with NPK 250 kg/ha + ammonium nitrate 250 kg/ha in spring in a single application*
		C3 – *fertilized in autumn with NPK 250 kg/ha + ammonium nitrate 250 kg/ha in spring, applied in two fractions*
		C4 – *fertilized with NUTRI TOP80 250 kg/ha in autumn + UREA NG with gradual release 250 kg/ha*

Source: own data

For data accuracy, three 5 sqm microplots (three repetitions) were delimited from each test plot, by excluding the influence of the edges. The harvesting was carried out with small-sized equipment, dedicated to research activities. The harvested quantity of grains was weighed, its humidity was determined, in order to calculate the production per hectare at standard humidity (14%). In the present case, the emphasis was placed on the quantitative yield of the analysed plots, even if the qualitative evaluation of the production was also carried out.
The data related to yields were managed in Excel, in complex tables, and were later processed using the Anova program, in order to perform statistical analysis in interaction.

RESULTS AND DISCUSSIONS

Starting from the productions of each research plot, average productions per hectare were calculated, by relating them to 14% humidity, for uniformity. The results were entered into the Anova statistical program and processed for a 5% reference interval, with the aim of identifying factors that exceed it, either at the lower or upper limit.
We started from the first factor (A – variety), for which the average data from Table 2 were obtained. The general average of the entire experiment was chosen as control, against which the productions of the three Romanian varieties – Glosa, Otilia and Carom – were compared.

Table 2. One-way analysis for factor A – analysis of variance for the tested wheat varieties

Variety	Average yield (q/ha)	Ratio to the control (%)	Difference to the control (q/ha)	Statistical influence
A1 – Glosa	48.80	98.34	-0.82	
A2 – Otilia	53.31	107.43	3.69	*
A3 – Carom	46.75	94.22	-2.86	
General average	49.62	100.00	–	Control
Limit difference (LD) 0.1%				5.2111
Limit difference (LD) 1%				4.0172
Limit difference (LD) 5%				3.0250
Limit difference (LD) 10%				2.5289
Fisher factor (F)				9.7752
Corrected dispersion (S2)				404.9494
Error of corrected dispersion				41.4263
Correlation ratio (r^2)				0.4672
Correlation coefficient (r)				0.6835

Source: own data

The statistical results obtained from the variance analysis program highlight the fact that there is a significant difference between the productions obtained by the three wheat varieties in the 2023-2024 agricultural year. Carom is the variety with the weakest performance, namely 46.75 q/ha, followed by Glosa, with 48.80 q/ha. The difference between the two isn't significant. However, the Otilia variety is noteworthy, with 53.31 q/ha, which places it at the top of the hierarchy, with very significant positive differences, compared to the other two.
The correlation coefficient (r) is medium, which confirms the accuracy of the data. The entire analysis shows that the choice of variety is essential for achieving the highest possible yields, in a similar technological scheme.

The graph in Figure 1 exemplifies the 5% confidence interval (LD5%), outside of which only the Otilia variety is found, as well as the function that was the basis of the obtained curve.

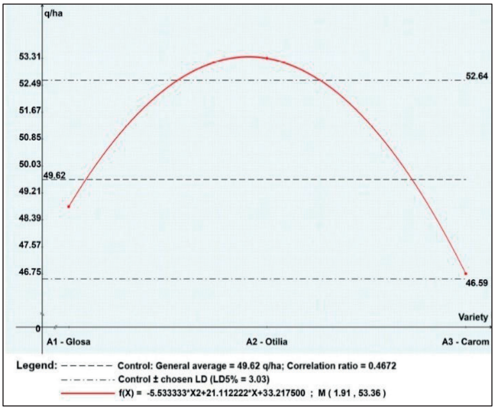

Figure 1. Graphical representation of the unifactorial analysis for the varieties included in the research (original)

A similar approach was used for the other two factors. Regarding the second one (B – preceding crop), the average data from Table 3 were obtained. The general average of the entire experiment was also chosen as the control, although the variants were subsequently compared with each other – wheat after peas, rapeseed and sunflower.

Table 3. One-way analysis of factor B – analysis of variance for the tested preceding crops

Preceding crop	**Average yield (q/ha)**	**Ratio to the control (%)**	**Difference to the control (q/ha)**	**Statistical influence**
B1 – peas	52.14	105.08	2.52	
B2 – rapeseed	50.10	100.97	0.48	
B3 – sunflower	46.62	93.95	-2.99	o
General average	49.62	100.00	–	Control
Limit difference (LD) 0.1%				4.9523
Limit difference (LD) 1%				3.8176
Limit difference (LD) 5%				2.8748
Limit difference (LD) 10%				2.4033
Fisher factor (F)				7.4952
Corrected dispersion (S2)				280.4196
Error of corrected dispersion				37.4133
Correlation ratio (r^2)				0.4200
Correlation coefficient (r)				0.6481

Source: own data

Wheat yields aren't significantly different when the crop comes after peas or rapeseed, both known to be good precursors for wheat, with high capacity to enrich the soil with nitrogen through biological processes. It is confirmed that both peas and rapeseed are good precursors for wheat, with significantly positive differences compared to the plots grown after sunflower: +5.52 q/ha after peas, +3.48 q/ha after rapeseed. Compared to the general average (control), it's observed that it's much higher than the productions offered by the two favorable preceding crops (peas and rapeseed), and sunflower stands out as being significantly negative. This aspect is also observed in the graph in Figure 2, where the function used to calculate the variance analysis leads to an almost linear evolution, starting from sunflower at the bottom, continuing with rapeseed and then with peas, at the opposite pole.

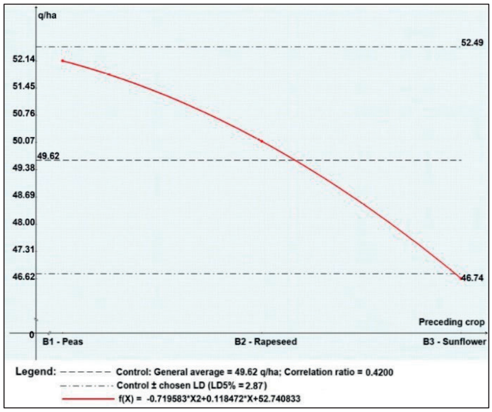

Figure 2. Graphical representation of the unifactorial analysis for the preceding crops included in the research (original)

Main part of the research is the one that targets fertilization systems, in this case the control being C1 (Table 4), namely the application of a single dose of fertilizers in spring.

From the detailed analysis of yields, it can be concluded that in the case of interactions between factors, the control variant, without fertilization in the autumn, when establishing the wheat crop, is sometimes beneficial and offers multiple advantages – when wheat is grown after peas, the lack of fertilizer application in the autumn leads to increased productivity, while reducing input costs. In this case, autumn fertilization can induce the loss of the amount of nitrogen that is already in the soil.

Table 4. One-way analysis of factor C - analysis of variance for the tested fertilization systems

Fertilization system	Average yield (q/ha)	Ratio to the control (%)	Difference to the control (q/ha)	Statistical influence
C1	46.11	100.00	–	Control
C2	46.54	100.94	0.43	
C3	49.68	107.74	3.57	*
C4	56.15	121.79	10.05	***
Limit difference (LD) 0.1%				4.8318
Limit difference (LD) 1%				3.7314
Limit difference (LD) 5%				2.8134
Limit difference (LD) 10%				2.3551
Fisher factor (F)				21.5002
Corrected dispersion (S2)				580.7156
Error of corrected dispersion				27.0098
Correlation ratio (r^2)				0.6728
Correlation coefficient (r)				0.8202

Source: own data

When averaging the varieties and preceding wheat crops, it turns out that the difference between C1 and C2 is one without statistical significance. Slightly significant increases in production are generated by the C3 system, with fertilization both in autumn and in spring, in the form of two graduations. The C4 system is the only one that, at the level of a rotation like the one studied, leads to very significant increases, of 10.05 q/ha, which represents an increase of almost 22%.
By plotting the data in Table 4, Figure 3 was obtained, which highlights that the C3 and C4 fertilization systems exceed the confidence interval provided by the 5% limit differences. The function underlying the graph is supported by a correlation ratio (r^2) of 0.6728, and therefore by a correlation coefficient of high value (r = 0.8202).

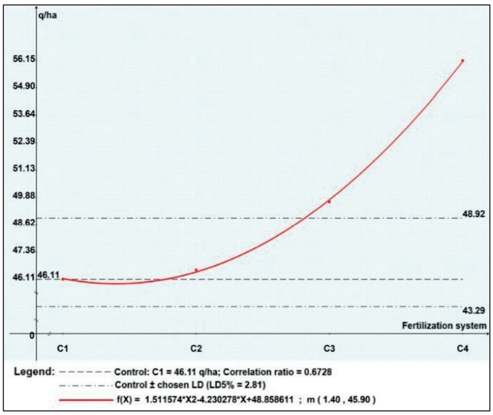

Figure 3. Graphical representation of the unifactorial analysis for the fertilization systems included in the research (original)

The continuation of the statistical analysis is carried out through the interactions of each two factors, and finally of the three factors, in random order, depending on what is desired to be highlighted. For this point in the research, the three-factor analysis is much too detailed, generating a graph for each intersection of the factors.
Unifactorial data processing led to the conclusion that the fertilization system (factor C) and the preceding crop (factor B) are the ones that most significantly influence wheat yields. As a result, the bifactorial C x B analysis was chosen as representative, in which the control was fertilization C1 and the preceding crop B3 (Table 5).

Table 5. Two-way analysis for factors C and B - analysis of variance for wheat yields in 2023-2024

Fertilization	Preceding crop	Average yield (q/ha)	Ratio to the control (%)	Difference to the control (q/ha)	Statistical influence
	B1	51.02	127.37	10.96	***
C1	B2	47.25	117.98	7.20	**
	B3	40.05	100.00	–	Control
	B1	48.11	120.11	8.05	**
C2	B2	45.97	114.78	5.92	*
	B3	45.54	113.69	5.48	*
	B1	51.36	128.22	11.30	***
C3	B2	49.75	124.20	9.69	***
	B3	47.93	119.66	7.87	**
	B1	58.08	145.00	18.03	***
C4	B2	57.42	143.37	17.37	***
	B3	52.96	132.23	12.91	***
Limit difference (LD) 0.1%					9.3048
Limit difference (LD) 1%					7.1961
Limit difference (LD) 5%					5.4319
Limit difference (LD) 10%					4.5458
Fisher factor (F)					6.8251
Corrected dispersion (S2)					229.7583
Error of corrected dispersion					33.6637
Correlation ratio (r^2)					0.6785
Correlation coefficient (r)					0.8237

Source: own data

By comparison with the chosen control – applying fertilization only in spring, in a single dose (C1), after sunflower (B3) –, it is observed that all other variants offer statistically significant increases in yield. Very significant are the production increases offered by the C4 fertilization system (Nutri Top80 in autumn + urea NG with gradual release, in spring), regardless of the preceding crop. Although at a great distance, in second place is the C3 system, with a single application of inputs in autumn and fractionated in spring (2 applications), whose effect seems to be to standardize productions, but at an average level.

In addition, for wheat grown after peas and rapeseed, the C4 system is the only effective one, with the other three yields remaining constant. Especially for the preceding peas, but also for rapeseed, autumn fertilization leads to moderate yield decreases or brings no benefit (Figure 4).

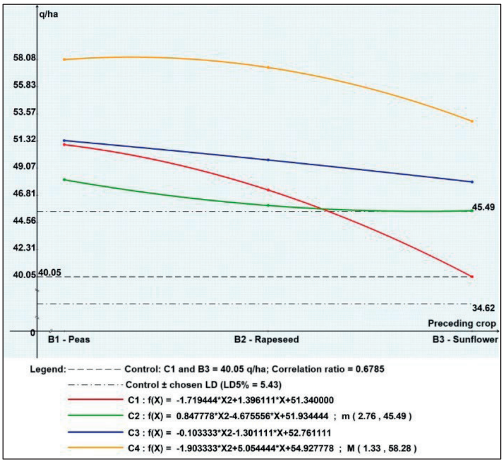

Figure 4. Graphical representation of the bifactorial analysis for factors C and B of the research (original)

On the other hand, for the case where the previous crop is sunflower, the application of autumn fertilization is crucial, providing a production increase of at least 500 kg/ha.

CONCLUSIONS

Over time, research conducted on various crops has proven that excessive use of fertilizers ends up causing losses of nutrients from the soil, which become inaccessible to plants and pollute the environment, without bringing economic benefits. According to the presented data, it can be concluded that fertilization should be established according to the preceding crop, which would increase the production level of the wheat crop and reduce expenses at the farm level.

If the classic C3 fertilization system brings production, regardless of variety and preceding crop, to an average level, the C4 system, with new, gradual-release inputs, applied both in autumn and spring, offers increases in yield levels of up to 22%, equivalent to 10 q/ha, very significantly positive compared to the C1 control.

Our own results confirm that inputs play an essential role in increasing soil fertility, in full correlation with the type of fertilizers and the timing of their application, as well as the fact that each of the technological links plays an essential role in the level of production obtained.

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ASSESSING POTATO MORPHOLOGICAL AND PHYSIOLOGICAL TRAITS UNDER FERTILIZATION DURING THE FIRST YEAR OF FIELD ADAPTATION

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Abstract

*Potato, Solanum tuberosum L. represent one of the most important crop worldwide and from Romania. The general aim of the study was represented by the overall assessment of potato growth and development under specific field condition respectively Râşca county. Therefore, were chosen six potato germplasm (Red Fantasy, Bella Rosa, Dutch Red, Mauve, Captiva and Elfe), free of pest and diseased and the morpho-physiological features were evaluated. Different vegetative phenophases were assessed according to BBCH scale (**B**iologische **B**undesanstalt, Bundessortenamt und **CH**emische Industrie) with and without fertilization, productivity, chlorophyll content and chemical distribution of the most important chemical elements from potatoes. The results were differentiated between different principal growth stages pointed and also depending on potato variety. Râşca still remains one of the main location for obtaining high potato productivity.*

Key words: *BBCH scale, chemical distribution, chlorophyll content, Energy dispersive X-ray spectroscopy, Scanning electron microscopy, Solanum tuberosum L.*

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most widely grown crops in the world (Birch et al., 2012), with a high contribution to food security (George et al., 2017). At the same time, it is an affordable crop (Zaheer & Akhtar, 2016) with high yields and can have a wide range of uses: as a source of food contribute to global food security (Birch et al., 2012), up to the industrial-scale production of by-products such as starch, alcohol and bioethanol (Thatoi et al., 2016).

Globally, potato production peaked in the last two decades in 2021 at around 376 million tons (FAO, 2022). The top potato producers worldwide are China, India and Ukraine (FAO, 2023). In Romania, in the year 2023, about 80000 ha were cultivated with potatoes, with a total production of more than one million tons (INSSE, 2024). More than 40% of the potato farms in the European Union come from Romania, and the annual national per capita potato consumption was about 36 kg/year in 2021 (Sterie et al., 2022). However, potato production and consumption has decreased notably in Romania since 2014 (Sterie et al., 2022). The underlying causes of this trend are represented by the increasing crop requirements for fertilizers and irrigation, especially in the context of climate change.

The use of fertilizers plays an important role in obtaining quality potato crops (Alva, 2004; Khan et al., 2012; Blecharczyk et al., 2023). The plant may have high fertilizer requirements due to its shallow root system (Iwama, 2008). Adequate fertilization of the potato crop requires a balanced supply of essential macro- and micronutrients, which play an important role in the physiological processes taking place in all organs of the plant (Tolesa, 2021). Potato fertilization can be achieved both by mineral or chemical fertilizers and by organic fertilization (manure) (Baniuniene & Zekaite, 2008). While manure increases soil quality in the long term (Edmeades, 2003), mineral fertilization is associated with a rapid supply of nutrients (Harris, 1992). Mineral fertilizers based on NPK represent the macronutrients needed by the potato plant (Tulung et al., 2021), nitrogen being responsible for the vegetative growth of the plant (Jenkins & Mahmood, 2003), phosphorus is associated with tuber initiation (Ekelöf, 2007), and potassium is involved in the crop resistance to diseases (Zörb et al., 2014). Iron should not be neglected as it has a pivotal role in chlorophyll biosynthesis (Zhang et al., 2022). Magnesium and calcium play an important role in tuber starch content and cell wall structure resistance (El-Hadidi et al., 2017).

As an alternative to chemical formulas, the use of organic fertilizers, which gradually release nutrients (Shaji et al., 2021), improves soil structure and can positively influence the soil microbiome (Li et al, 2021). On the other hand, the use of mineral fertilizers in quantities that are not adapted to the crop and land area type can have undesirable effects on the soil by excess accumulation of salts, installing salinity, an increasingly common problem that endangers the quality of the substrate (Trușcă et al., 2023). The use of balanced fertilization is the long-term assurance of soil quality (Shah & Wu, 2019) and, implicitly, of plant health through the proper functioning of physiological processes associated with good crop yields and quality products (Brevik, 2015).

The aspects mentioned above require special attention, and testing the morpho-physiological responses of plants to different types of fertilization is an important step in establishing appropriate crop technologies for the needs of each plant and variety.

Advanced analytical methods, such as SEM-EDX (Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy), provide a detailed understanding of nutrient-plant interactions, allowing an accurate assessment of morphological and physiological changes following the application of different types of fertilization. This analytical technique not only measures the chemical composition of plant tissues, but also helps to identify variations in cell structure, which may be essential for understanding the processes of plant adaptation to environmental conditions (Bollavarapu et al., 2020).

This study focuses on the assessment of morphological and physiological traits of potatoes in the first year of field adaptation under the influence of different fertilization regimes, by using SEM+EDX as the main method of analysis and chlorophyll content. Through this research, we aim to contribute to a better understanding of the factors influencing potato development and to provide relevant information for the optimization of agricultural practices.

For this purpose, an experiment was proposed and carried out in which different parameters of six potato varieties were assessed in the presence of mineral and organic fertilizers. The objectives of the study were to monitor the chlorophyll content during three BBCH stages and to evaluate the levels of some chemical elements in the leaves. The yield for each variety was also determined according to the type of fertilization used.

MATERIALS AND METHODS

The experiment took place in the field in Dealu-Mare village, Rîsca, Cluj county, 46.7289772, 23.1306339. The testing started in 20 April 2024 and the harvest was collected in 25 September 2024. The experimental design consisted in six potato varieties respectively Red Fantasy (Rf), Bella Rosa (Br), Red Olanda (Ro), Purple (Mv), Captiva (Cap), Elfe (El) and two fertilization regime with required fertilization and without any fertilization. The potato for sowing was purchased from the Europlant SRL Research Center, in Avrig, Sibiu County and was stored between 15.10.2023-20.02.2024 in raschel net bags, in a place protected from humidity with a temperature between 8-10°C.

In the field with no fertilization, the seed potatoes were planted manually in nests with a depth of approximately 15-20 cm, at an air temperature at planting of 16-18°C.

The field with fertilization was previously plowed at 20-25 cm, with manure and chicken droppings applied. Afterwards the soil was milled one week before planting. On the planting day was also applied to the soil ammonium nitrate (NH_4NO_3) purchased from Azomures Producer (400 kg/ha). The seed potatoes were planted mechanically in nests approximately 15-20 cm deep. The pests and disease treatments were done at 3 weeks after planting on 17.04.2024, and an additionaly weed treatment was applied.

As for the foliar treatment, Polyfeed 20-20-20 from the manufacturer Haifa was used, 2 times before flowering, and 1 time after flowering, in a quantity of 4 kg/ha.

The experimental field consisted from 9000 m^2, to which a nested design was applied for the monitoring of physiological parameters. The registered parameters for all analysis consisted in three replications, and each parameter was extracted from a $5m^2$ plot, randomly chosen from the experimental field.

Potato leaf chlorophyll content was recorded in three representative BBCH stage intervals for the crop. Therefore, the first assessments of the parameter were performed during the first interval, from leaf growth and development to tuber formation (C1-BBCH 1-4). The second set of assessments (C2) was performed from inflorescence emergence (BBCH 5) to fruit development (BBCH 7). Finally, chlorophyll content was also recorded for the late vegetative stages, i.e. in the interval BBCH 8 and 9 from fruit ripening to seedling senescence (C3). The parameter was assessed by a non-destructive method with the chlorophyll measurement instrument in SPAD units, i.e. chlorophyll meter MC100C from Apogee Instruments.

To determine the chemical composition of the leaves, the Scanning Electron Microscopy method was used with the Energy Dispersive X-Ray detector, using the equipment provided by the TermoFischer Scientific company.

To introduce the samples into the analysis chamber, they were placed on an aluminium foil on which a carbon tape was applied. The analysis was performed at different magnifications 400-1000 555X, using a Low Vacum Detector, the pressure in the analysis chamber was 100 Pa and the voltage varied between 15.00-25.00 kV, with a spot size of 4.5-5. The chemical analysis was performed using the EDX detector together with the Pathfinder application. The leaves subjected to SEM+EDX analysis were sampled on 15.07.2024 and then left at room temperature (21-23°C) for a period of 3 weeks.

RESULTS AND DISCUSSIONS

The relative chlorophyll content differed significantly depending on assessed phenophase C1-C3, betand based on the specific interaction of each potato varieties with fertilization regimes. Two-way ANOVA was performed for each individual assessment and highlighted significant differences in relative chlorophyll content between potato varieties (Figure 1).

In the phenophase of leaf development-tuber formation BBCH 1-4, the highest value vas registered at purple potato variety (Mv) of 542±26.19 (SE-standard error) SPAD units from the control treatment. This value was significantly higher than the values obtained for Rf, with and without fertilization, and Mv with fertilization. The technology applied on potato only impacted significantly Mv variety, fertilization reduced significantly the relative chlorophyll content. This could be because of variety sensitivity to common potato technology. This sensitivity comes from physiological processes that happen especially in the vacuole where anthocyanins are present. Coloured potatoes contain anthocyanins in all the plant tissues including in tubers. The fertilization status due to osmosis process could alter anthocyanidin metabolism and biosynthesis (Zhang et al., 2024). In the control treatment with a low nitrogen dose, the relative chlorophyll content of purple potato was higher compared to the fertilized treatment where it appears that nitrogen presence inhibited anthocyanin synthesis process and relative chlorophyll content.

In the C2 assessment when the potatoes were in BBCH 5-7 inflorescence emergence-development of fruit, the only significant differences in relative chlorophyll content were registered at the varieties Br and Ro. The values were higher in the control treatment without fertilization 409±18.31SPAD units for Br and 416±11.65 SPAD units for Ro. The highest value of relative chlorophyll content registered in the second assessment was at Mv from the control treatment (449±20.65 SPAD units) with only 41.77 SPAD units more compared with the fertilized treatment. The Br variety registered the lowest value of relative chlorophyll content under fertilization of 233±24.23 SPAD units. Overall the relative chlorophyll content was higher in the treatment that lack fertilization. The only exception was El variety which registered similar value in both variants around 396 SPAD units at the second assessment.

In the last set of assessments (C3), corresponding to the final phenophases under evaluation, (fruit ripening and seed senescence - BBCH 8-9) (Figure 2), only in one potato variety a significant difference in relative chlorophyll content was observed with the application of fertilizers in the cultivation technology. The treatment produced a halving of the parameter value, precisely in purple potato (Mv), where the maximum of 488±53.10 SPAD units was recorded in the control. The lowest leaf relative chlorophyll content was recorded for the Cap variety in the presence of fertilizers (205±15.26 SPAD units), a value with 110 SPAD units lower than the control. Altogether, the relative chlorophyll content evolution maintains the trend highlighted in C2, the values of the analysed parameter being higher in the absence of fertilizers.

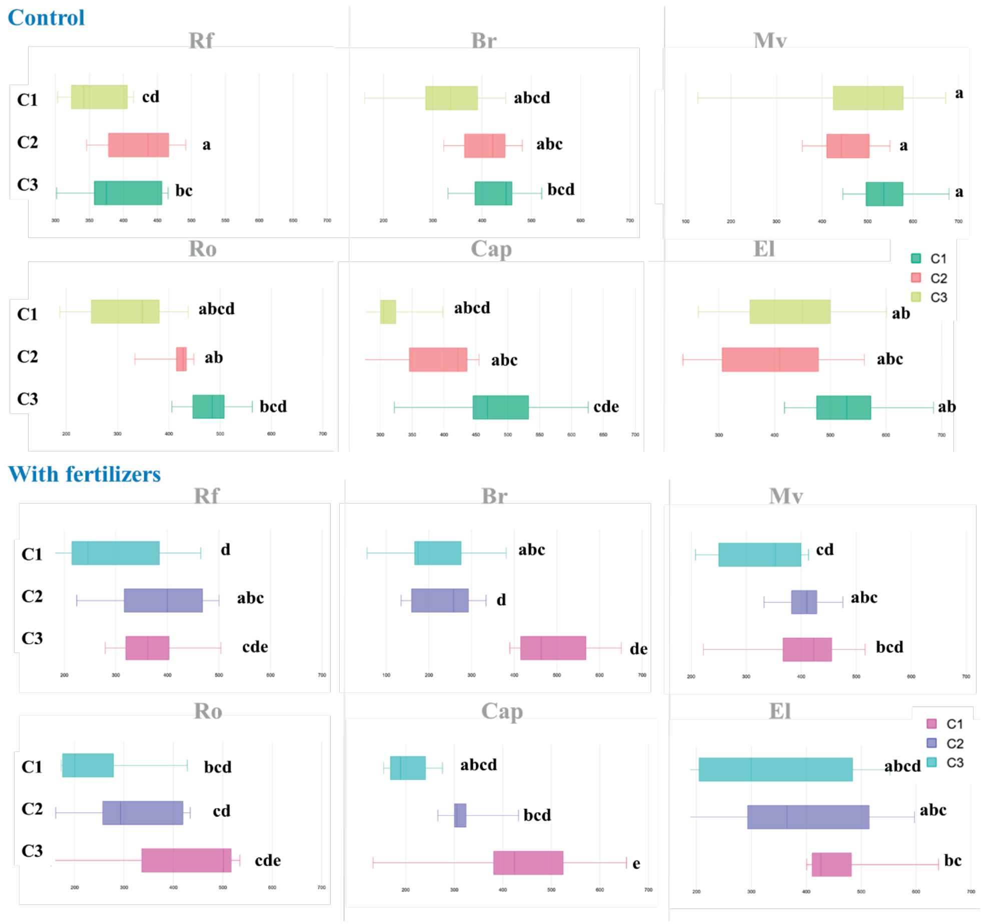

Figure 1. Chlorophyll content according BBCH scale C1 – BBCH 1-4 (leaf development-tuber formation), C2 – BBCH 5-7 (inflorescence emergence- development of fruit), C3- BBCH 8-9 (ripening of fruit and seed-senescence). Where ANOVA C1 var (StatF=3.16, p val=0.011), F (StatF=6.87, p val=0.010), var:F (StatF=2.31, p val=0.050), ANOVA C2 var (StatF=4.34, p val=0.001), F (StatF=21.43, p val<0.001), var:F (StatF=2.75, p val=0.023), ANOVA C3 var (StatF=7.70, p val<0.001), F (StatF=31.32, p val<0.001), var:F (StatF=0.51, p val=0.769), different letters from LSD test represent significant differences at p<0.05.

Figure 2. Potato (*Solanum tuberosum* L.) BBCH 1-4 (leaf development-tuber formation), BBCH 5-7 (inflorescence emergence- development of fruit) and BBCH 8-9 (ripening of fruit and seed-senescence).

The mass percentage analysis of the chemical elements of interest in the potato crop differed significantly depending on the potato variety and the chemical element evaluated (Table 1). The presence of leaf oxygen content did not vary with fertilizer application for all the varieties. However, except for Ro and El, no significant decreases in the mass percentage of oxygen were recorded for all other potato varieties. The highest value was recorded for Cap where the cultivation technology insignificantly increased the leaf oxygen proportion by 4%. Ro and El showed the opposite aspects to those observed for the other varieties. In the presence of fertilizers, the mass percentage of oxygen in the leaves decreased compared to the control values. The minimum percentages of oxygen were visible in Rf and Ro where decreases of 5.36 and 5.83% percent were recorded compared to the maximum in Cap.

Table 1. Leaves chemical properties of all six potato varieties studied with SEM+EDX (average±SE-standard error)

Var	F	O	C	K	Ca	Si	Mg	P
Rf	CE	50.79±0.60 **b**	40.42±1.10 **ab**	4.56±0.75 **abc**	1.71±0.26 **bc**	0.92±0.22 **ab**	0.44±0.09 **bcd**	0.2±0.05 **ab**
	F	54.22±0.85 **ab**	39.45±1.18 **ab**	2.59±0.38 **c**	1.37±0.04 **bc**	0.34±0.07 **b**	0.90±0.10 **ab**	0.13±0.01 **b**
Br	CE	52.07±0.74 **ab**	43.05±0.85 **a**	2.87±0.31 **c**	0.53±0.07 **bc**	0.36±0.09 **b**	0.09±0.01 **d**	0.14±0.03 **b**
	F	53.43±1.07 **ab**	38.94±1.23 **abc**	3.53±0.34 **bc**	1.96±0.13 **bc**	0.14±0.04 **b**	1.11±0.09 **a**	0.19±0.02 **ab**
Mv	CE	54.37±0.89 **ab**	40.06±1.08 **ab**	3.26±0.20 bc	0.91±0.10 **bc**	0.54±0.18 **ab**	0.35±0.06 **cd**	0.11±0.02 **b**
	F	54.67±2.56 **ab**	39.7±2.60 **ab**	3.44±0.37 **bc**	0±0 **c**	0.37±0.07 **b**	0.35±0.06 **cd**	0.11±0.01 **b**
Ro	CE	54.34±2.04 **ab**	26.79±5.16 **c**	10.35±1.93 **a**	2.19±0.44 **bc**	2.58±1.85 **a**	0.49±0.18 **bcd**	0.09±0.03 **b**
	F	50.38±1.36 **b**	43.86±1.45 **a**	1.98±0.1 **c**	1.70±0.33 **bc**	0.39±0.06 **b**	1.10±0.02 **a**	0.20±0.04 **ab**
Cap	CE	52.1±0.26 **ab**	40.01±0.08 **ab**	4.48±0.25 **abc**	1.76±0.03 **bc**	0.53±0.02 **ab**	0.51±0.08 **bcd**	0.20±0.01 **ab**
	F	56.15±1.05 **a**	29.06±7.56 **bc**	9.55±4.95 **ab**	2.97±1.80 **ab**	0.31±0.12 **b**	0.49±0.13 **bcd**	0.16±0.03 **ab**
El	CE	53.59±1.32 **ab**	38.82±1.85 **abc**	4.92±1.17 **abc**	1.22±0.19 **bc**	0.52±0.37 **ab**	0.30±0.11 **cd**	0.16±0.03 **ab**
	F	52.52±1.15 **ab**	29.07±3.40 **bc**	10.62±1.43 **a**	4.75±1.16 **a**	0.41±0.07 **ab**	0.62±0.27 **bc**	0.27±0.05 **a**
Var	StatF	0.94	2.11	3.05	3.75	1.28	3.42	2.62
	p val	***0.471***	***0.099***	***0.029***	***0.012***	***0.305***	***0.018***	***0.050***
F	StatF	0.83	0.72	0.05	3.93	3.29	32.37	2.25
	p val	***0.372***	***0.403***	***0.826***	***0.059***	***0.082***	***p<0.001***	***0.146***
Var:	StatF	2.59	5.40	4.84	3.32	1.06	5.31	2.83
	p val	***0.052***	***0.002***	***0.003***	***0.020***	***0.405***	***0.002***	***0.038***

Note: Var-potato variety; C- control field without fertilization, F-field with fertilization; O-oxygen, C-carbon, K-potassium, Ca-calcium, Si-silicon, Mg-Magnesium, P-phosphorus, different letters from LSD test show significant differences at $p<0.05$

Leaf carbon mass percentage can be associated with the carbon accumulation degree in the leaf. It is clearly visible that with the application of fertilizers, this parameter decreases but not significantly. There is a particular aspect in Ro in the presence of fertilizers, where the carbon mass percentage increases with about 17% compared to control. The mass percentage of leaf potassium varies both with changing cropping technology and potato variety. Its value more than doubles as a result of fertilizer application for Cap and El potato varieties. On the other hand, in Ro variety, where the minimum K percentage was recorded, the same cropping technology had an opposite effect on the parameter, its value being significantly reduced by 5 times compared to the control. An interesting aspect was observed for purple potato, where the application of fertilizers did not influence the potassium mass percentage. For varieties Rf and Br, the values of the parameter varied insignificantly in the range 2.59-4.56, which can be concluded that fertilizer application does not noticeably influence the K percentage of leaf dry mass for the both varieties.

For half of the potato varieties tested, the calcium mass percentage decreases negligibly with the application of fertilizer cultivation technology. The Ca content in the plant is usually between 0.5 and 3% (Vâtcă, 2020). The Ro potato has the closest value within this normal range, however El has the highest value of 4.75% in the fertilization field, significant compared with the Ca content from other varieties and treatments. The lowest Ca content was recorded in purple potato, where fertilizer application totally reduced the Ca content in the leaf.

The mass percentage analysis of the chemical elements in the leaves shows insignificant decreases for silicon in the tested variants in the presence of fertilizers, with only one exception for Ro potato. This potato variety recorded the maximum value of Si in the control. Also in the control, but in the Rf potato, the value is almost double the maximum content of 0.5% Si that can enter in the composition of dicotyledonous and leguminous plants (Table 1).

The mass percentage of magnesium increased significantly in the presence of fertilizers in leaves of Br and Ro potatoes, where the maximum was recorded. In Br potato, the use of cultivation technology was associated with a 12-times increase in Mg content. Further, but not significant, increases were observed in Rf and El potatoes, where the element mass percentages doubled. On the other hand, two other potato cultivars, Mv and Cap, maintained a constant magnesium content in leaves, regardless of the cultivation technology, with values in the range 0.35-0.51%.

Within the same potato variety, fertilizer application does not significantly influence the mass percentage of phosphorus. Only two varieties, Rf and Cap, showed reduced decreases of the parameter in response to the cultivation technology. The maximum value of the parameter was recorded in El fertilized potatoes. At the opposite, the lowest percentage of this element was recorded in Ro control, however, the application of fertilizers to this potato variety was associated with a doubled value of the parameter. The cropping technology did not produce differences in the leaf phosphorus mass percentage in purple potato, maintaining the value of the parameter at the same level as that recorded in the control (Table 1).

Table 2. Average yield with standard error for all potato varieties from the field (t/ha)

No.	Var	C	F
1.	Br	7.67±0.33 **f**	36.67±0.88 **c**
2.	Cap	9.67±1.20 **f**	47.67±1.45 **a**
3.	El	7.00±1.15 **fg**	22.33±0.88 **d**
4.	Mv	3.33±0.33 **g**	16.33±1.45 **e**
5.	Rf	10.00±1.15 **f**	42.33±1.45 **b**
6.	Ro	6.33±0.33 **fg**	25.33±0.33 **d**

Note: Var-potato variety studied, C- control field without fertilization, F-field with fertilization, different letters from LSD test show significant differences at $p<0.05$

The average production recorded the highest values in the fertilized field for all potato varieties (Table 2). Although from a physiological point of view and relative chlorophyll content, the plants reported good functioning in the unfertilized field, in terms of production this was 5 times higher in Br, Cap, Mv, 4 times higher in Rf and Ro and 3 times higher in El. The maximum yield was registered to Cap potato variety from the fertilized field and significantly higher compared with all other yield values. In previous studies, Cap was classified as medium yielding sort with a medium-fast growing period around 92-94 days and also with around 17-27 t/ha productivity. On the other hand, El was set in the high yielding sort with medium growing time of around 95-97 days with a relative yield in the range of 12-17 t/ha (Eyvazov, 2025).

From the unfertilized field, Rf had the higher yield value significantly higher compared to Br, Cap and Rf.

Analyzing the correlation coefficients of the monitored parameters, significant correlations (*p<0.05*) are observed between different mass percentages of the chemical elements based on SEM+EDX assessment (Figure 3). A share of 36% from the leaves C mass percentage is negatively strong correlated with the oxygen. This could be explained by the biochemical reactions of amino-acids, water and other compound with oxygen in order to allocate carbon in the leaves (Figure 3).

Potassium has a weak positive correlation with oxygen, a share of 12% of K is influenced by O_2 and a negative strong correlation with carbon from leaves, a share of 88% of K is influenced by leaf compounds containing C. Potassium represent the principal microelement from the plants with the highest share (Vâtcă, 2020). It's role in growth and development of plants is was established. Furthermore, K have an important in the formation of bio-colloids and ensures their hydration, ensures good activity of plant enzymes. The plant leaves contain potassium around 10-15 mg/g dry matter (Vâtcă, 2020).

About 55% of calcium level is due the strong negative correlation with the leaves carbon content, and 64% of the level is sustained by its strong positive correlation with K. The presence of Si in the potato leaves is weak negatively correlated with C content (Figure 3). A share of 12% and 15% of P is based on the weak negatively correlation with O and the weak positively correlation with Mg. Phosphorus ester, phytin, is the Ca or Mg salt of phytic acid and represents 2% of the total phosphorus in the green organs of plants (Vâtcă, 2020). Phosphorus is a very important element for the composition of cellular structures with the role of energy carrier (Maciá, 2005; Pandey, 2018; Vâtcă, 2020).

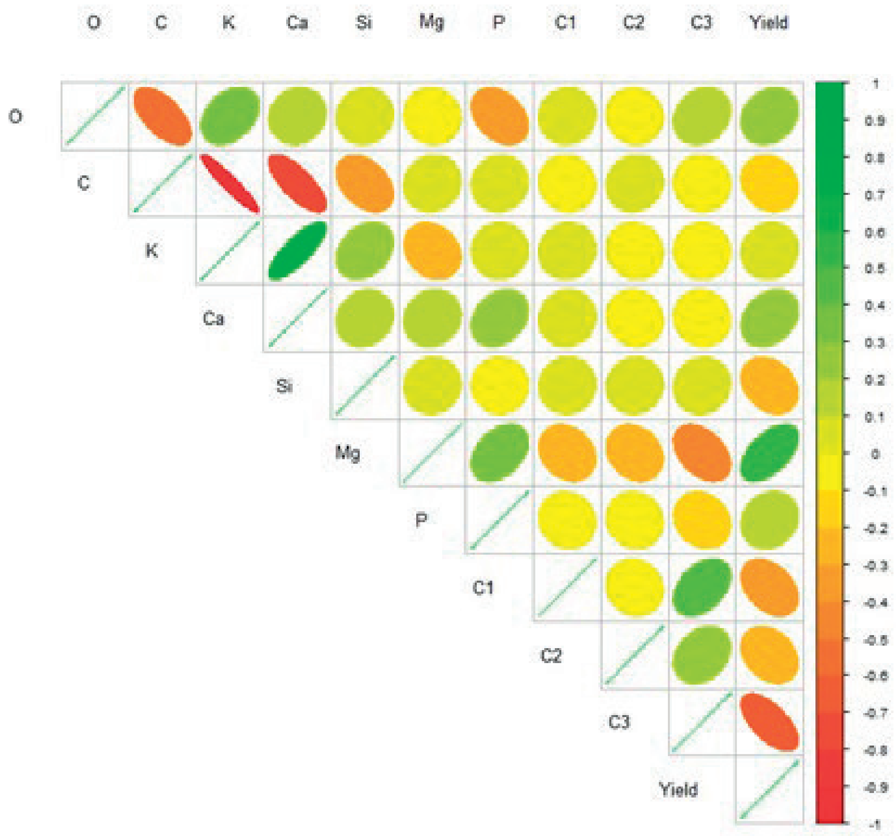

Figure 3. Correlation chart between all tested parameters physio-chemical and yield of six potato varieties

The values of relative chlorophyll content from the third assessments are weak negatively correlated with Mg in leaves content and weak positively correlated with the plant development during first assessment (Figure 3).

The obtained potato yield correlates positively around 30% with Mg content from the leaves, negatively weak determined by the relative chlorophyll content of potato leaves at the first assessment C1 (15%) and negatively strong determined (40%) by the relative chlorophyll content from the third assessment. A high chlorophyll content in the fertilized treatment could be explained by the fact that potato prolongs the vegetation period and invest again in the growth to the detriment of yield accumulation (Figure 3).

CONCLUSIONS

The relative chlorophyll content was higher at potato varieties from the field without fertilization.

The purple potato registered the highest relative chlorophyll content at all assessments.

The variety Cap has the lowest chlorophyll content at the end of the vegetation period.

The registered physiological parameters of potato are negatively correlated with the application of high fertilizer regimes for the majority of varieties tested.

The elements from potato leaves presented both convergent and divergent directions of correlations, indicating a variety specific response to treatment.

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EFFICIENCY OF SUNFLOWER SEEDS INOCULATION WITH DIFFERENT MICROORGANISMS

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Abstract

The aim of the study was to determine the effectiveness of sunflower seeds inoculation with various microorganisms and their impact on enhancing plant resistance to negative environmental factors. The study on the reaction of sunflower hybrids to different growing conditions was conducted at two locations: 'Location 1', which imposed limiting conditions, and 'Location 2', which imposed optimal conditions. The study was conducted over the period 2023-2024. In the optimal conditions, the highest weight of 1000 seeds (70.44 g) was observed in the variant with $N_{40}P_{60}$, while the highest oil content of 50.3% and the actual yield of 2763 kg/ha were achieved in the variant with $N_{20}P_{30}$ and multicomponent inoculation. In the context of limiting conditions, the variant with $N_{40}P_{60}$ demonstrated the highest oil content (42.3%) and actual yield (1167 kg/ha). Conversely, the variant with $N_{20}P_{30}$ and multicomponent inoculation exhibited the highest weight of 1000 seeds (47.01 g).

Key words: *sunflower, fertilizer, inoculation, yield, oil content.*

INTRODUCTION

Sunflower is the main crop in Ukraine and accounts for 70% of the oilseeds sown and 85% of the gross harvest. High demand for oil and meal both in Ukraine and in other countries encourages producers to grow sunflower as a highly profitable crop, which is one of the main sources of their income (Melikh & Pasmenko, 2015; Hladni, 2016). The value of sunflower seeds relates to the fact that they contain essential vitamins and are a source of vitamin D, as well as B vitamins such as thiamine, niacin and folate, including vitamin E, which is a powerful antioxidant. The presence of vitamins helps to normalize the acid-alkaline balance of a person and strengthens their skin. In addition, sunflower seeds are rich in minerals such as magnesium, phosphorus, selenium and copper (Malunjkar et al., 2024).

However, the expansion of sunflower cultivation in Ukraine leads to increasing degradation of ecosystem, including soils, which will have very negative consequences in the future. Therefore, to meet the growing global demand for food in the context of climate change and reduce the degree of ecosystem degradation, it is necessary to maintain and increase agricultural productivity while reducing resources, especially those harmful to the environment (mineral fertilizers, pesticides, etc.), by enhancing the role of microorganisms in crop cultivation technologies (Tyshchenko et al., 2020). Beneficial soil microorganisms provide plants with nutrients, increase their resistance to abiotic and biotic stresses, and improve their growth and development, as well as increase yields (Nanjundappa et al., 2019; Jamaluddin, 2019; Enebe & Babalola, 2018).

Plant growth promoting rhizobacteria (PGPR) is a group of bacteria that colonies the rhizosphere and produce substances that improve plant growth and development and enhance their resilience to adverse factors (Miransari, 2011; Vozhehova et al., 2022). The increase in various agronomic yields by PGPR was due to the production of phytohormones, phosphate mobilization, production of siderophores and antibiotics, inhibition of ethylene synthesis in plants and induction of systemic plant resistance to pathogens. Among PGPRs, nitrogen-fixing bacteria (NFB) and phosphorus-solubilizing bacteria (PSB) are important for crops as they increase nitrogen (N) and phosphorus (P) (Singh et al., 2011) uptake and, consequently, increase crop yields (Daiss et al., 2008; Javaid, 2009; Zarabi et al., 2011).

Among different microorganisms that colonies the rhizosphere, arbuscular mycorrhizal fungi (AMF) are unique because they are partly inside the root and partly outside the root, thus affecting other soil microorganisms, as well as plant growth and development (Redecker et al., 2000; Tyshchenko et al., 2023). The association of AMF and plants is one of the oldest symbiotic relationships in the biological world (Rimington et al., 2018). AMF, forming a symbiotic association with higher plants, promotes the absorption of plant nutrients, which is usually limited by diffusion, such as phosphorus, zinc, copper etc. (Bagyaraj et al., 2015). AMF inoculation improves crop nutrition, growth and yields (Desai et al., 2016), especially AMF improves phosphorus nutrition of plants, to the extent of saving 50% of P fertilizer application, without adverse effects on their growth, development and yield (Jyothi & Bagyaraj, 2018; Thilagar et al., 2016). In addition, AMF also improves plant resilience mechanisms against biotic and abiotic stresses by stimulating the synthesis of secondary metabolites such as phenolic compounds, phytoalexins and peroxidases (Mathimaran et al., 2017; Muthukumar et al., 2019; Gholinezhad & Darvishzadeh, 2021; Kabir et al., 2020; Meddich et al., 2015). Several researchers have reported the role of AMF in improving water availability in various host plants such as chicory, grain corn, chickpea, sesame and tomato (Leventis et al., 2021; Langeroodi et al., 2020; Hashem et al., 2019; Ren et al., 2019), due to hyphae, which increase the area of the plant root system in the rhizosphere and improve water absorption (Langeroodi et al., 2021).

MATERIALS AND METHODS

The study on the reaction of eight sunflower hybrids to different growing conditions was conducted at 'Location 1' - Odesa State Agricultural Station of the ICSA NAAS, Khlibodarske village, Odesa region, Odesa district (46°29.05''N; 30°35.31''E) and at 'Location 2' - State Farm 'Pioneer' in Lyubymivka village, Kherson region, Beryslav district (47°23.34''N; 33°43.00''E) during 2023-2024.

The hybrids were tested on first-order plots of 275 m^2 and second-order plots of 25 m^2 in three replications using the randomized replication method (blocks), with the seeding rate adjusted to 55,000 viable seeds per ha. The research was carried out according to the generally accepted methodology, the number of chemical treatments was adjusted with accordance to the growing conditions and the presence of weeds, diseases and pests (Table 1).

Table 1. Experimental design

Hybrids of sunflower	Fertilization and inoculation
Niagara (102-106 days[1]) *Hysun 158 IT* (108-112 days[1]) *Hysun 280* (114-117 days[1]) *Bella* (100-105 days[1]) *P64LE136* (112-116 days[1]) *Generalis* (108-110 days[1]) *Hysun 232 IT H0* (112-118 days[1]) *P63LE113* (104-108 days[1])	Control (without fertilizer and inoculation)
	$N_{40}P_{60}$
	$N_{20}P_{30}$
	Inoculation with nitrogen-fixing bacteria (NFB)
	Inoculation with phosphorus-solubilizing bacteria (PSB)
	Inoculation with arbuscular mycorrhizal fungi (AMF)
	Inoculation with NFB + PSB + AMF
	$N_{20}P_{30}$ + Inoculation with nitrogen-fixing bacteria (NFB)
	$N_{20}P_{30}$ + Inoculation with phosphorus-solubilizing bacteria (PSB)
	$N_{20}P_{30}$ + Inoculation with arbuscular mycorrhizal fungi (AMF)
	$N_{20}P_{30}$ + Inoculation with NFB + PSB + AMF

[1] - Period from germination to harvest

Location 1 is represented by a southern heavy-loamy, residually slightly saline black soil. The arable layer contains 2.1% humus; the content of mineral nitrogen is 2.7 mg per 100 g of soil, mobile phosphorus content is 3.7, and exchangeable potassium content reaches 39 mg per 100 g of soil, respectively; the pH of water extract is 6.8-7.1; the equilibrium bulk density is 1.45 g/cm^3, soil porosity is 44.1%, and water permeability is 1.16 mm/min. The fore-crop is sunflower. Location 2 is represented by a southern heavy-loamy black soil. The topsoil contains 2.5% humus; the content of mineral nitrogen is 3.8 mg per 100 g of soil, mobile

phosphorus content is 5.2, and exchangeable potassium content is 54 mg per 100 g of soil; the pH of water extract is 6.9-7.3; the equilibrium bulk density is 1.37 g/cm^3, soil porosity is 50.2%, and water permeability is 1.24 mm/min. The fore-crop is winter wheat.
Weather conditions during the years of the study were unstable and differed in terms of both the amount and distribution of precipitation and temperature.
The mean temperatures, precipitation and relative humidity for all experimental seasons are represented in Table 2, along with the long-term mean values.

Table 2. Weather conditions during the research

Location 1									
Month, period	Long-term mean			2023			2024		
	T (°C)	P (mm)	φ, %	T (°C)	P (mm)	φ, %	T (°C)	P (mm)	φ, %
April	10.0	28.0	79	10.0	116.0	81	14.6	70.0	67
May	15.9	33.2	62	15.9	7.0	57	15.8	35.0	62
June	20.8	48.5	65	20.8	32.0	61	22.7	77.0	66
July	23.4	45.0	61	23.4	48.0	64	27.3	18.0	48
August	23.1	29.4	59	23.1	15.0	62	25.0	20.0	52
September	17.7	2.0	55	17.7	0.0	57	21.8	164.0	62
April - August	18.6	184.1	65	18.6	218.0	65	21.1	220.0	59
April - September	18.5	186.1	63	18.5	218.0	64	21.2	384.0	59
Location 2									
Month, period	Long-term mean			2023			2024		
	T (°C)	P (mm)	φ, %	T (°C)	P (mm)	φ, %	T (°C)	P (mm)	φ, %
April	10.7	32.0	76	10.3	72.0	84	15.1	37.0	82
May	17.1	30.0	69	16.4	12.3	66	16.2	58.0	71
June	21.3	40.0	65	21.0	35.0	65	24.0	47.8	68
July	23.7	45.0	60	24.0	50.0	63	25.6	0.4	62
August	23.3	20.0	59	25.0	52.0	62	26.7	1.6	59
September	17.3	10.0	68	20.3	0.1	56	22.1	15.0	61
April - August	19.2	167.0	66	19.3	221.3	68	21.5	144.8	68
April - September	18.9	177.0	66	19.5	221.4	66	21.6	159.8	67

RESULTS AND DISCUSSIONS

In the control variant (without fertilization and inoculation), the average diameter of the head under optimal conditions was 17.6 cm, the number of seeds in the head was 748 seeds with the weight of 1000 seeds of 63.56 g, oil content of 46.8%, biological yield of 2379 kg/ha and actual yield of 2213 kg/ha, while under limiting conditions - 10.8 cm, 446 seeds, 42.16 g, 37.7%, 939 kg/ha and 832 kg/ha, respectively. The highest yields among the hybrids under optimal conditions were obtained in *Niagara* - 2451 and *Hysun 232 IT H0* - 2406 kg/ha, under limiting conditions *P63LE113* - 906, *Hysun 158 IT* - 891 and *Bella* - 874 kg/ha (Table 3).
The use of mono-inoculation contributed to the increase in the crop structure constituents and, accordingly, both biological and actual yields. Thus, under inoculation with nitrogen-fixing bacteria, the average diameter of the head under optimal conditions was 20.1 cm, the number of seeds in the head was 801 seeds with the weight of 1000 seeds of 66.72 g, the oil content of 47.8%, the biological yield of 2671 kg/ha and the actual yield of 2366 kg/ha, while under limiting conditions - 11.5 cm, 457 seeds, 43.97 g, 38.7%, 1005 kg/ha and 934 kg/ha, respectively. Inoculation with phosphorus-solubilizing bacteria increased the diameter of the head compared to the control under optimal conditions by 1.3 cm, the number of seeds in the head by 30 seeds, the weight of 1000 seeds by 1.7 g, the oil content by 0.6%, the biological yield by 159 kg/ha and the actual yield by 112 kg/ha, while under limiting conditions - by 0.3 cm, 9 seeds, 0.59 g, 0.9%, 33 kg/ha and 62 kg/ha, respectively. Inoculation with arbuscular mycorrhizal fungi also increased the diameter of the head compared to the control under optimal conditions by 1.2 cm, the number of seeds in the head by 26 seeds, the weight of 1000 seeds by 1.56 g, the oil content by 0.6%, the biological yield by 140 kg/ha and the actual yield by 86 kg/ha, while under limiting conditions - by 0.5 cm, 10 seeds, 0.71 g, 0.8%, 38 kg/ha and 43 kg/ha, respectively.
However, compared to mono-inoculation, multicomponent inoculation (nitrogen-fixing + phosphorus-solubilizing bacteria + arbuscular

mycorrhizal fungi) was more effective. Thus, the diameter of the head under optimal conditions was 19.8 cm, the number of seeds in the head was 798 seeds with the weight of 1000 seeds of 66.59 g, the oil content of 48.5%, the biological yield of 2657 kg/ha and the actual yield of 2403 kg/ha, while under limiting conditions - 11.9 cm, 466 seeds, 44.15 g, 39.4%, 1030 kg/ha and 954 kg/ha, respectively.

The use of fertilizer at the rate of $N_{20}P_{30}$ increased, compared to the control, the indicators of the crop structure and yield elements both under optimal and limiting conditions: the head diameter by 2.3 cm and 1.7 cm, number of seeds in the head by 56 and 31 seeds, the weight of 1000 seeds by 3.37 and 3.08 g, the oil content by 1.8 and 2.6%, the biological yield by 312 and 140 kg/ha and the actual yield by 142 and 153 kg/ha, respectively; it also slightly increased the indicators of complex inoculation excepting the actual yield under optimal conditions. Thus, the diameter of the head under optimal conditions increased by 0.1 cm, under limiting conditions - by 0.6 cm, the number of seeds in the head - by 6 and 11 seeds, the weight of 1000 seeds by 0.34 and 1.09 g, the oil content by 0.1 and 0.7%, the biological yield by 34 and 49 kg/ha, respectively, and the actual yield under limiting conditions by 31 kg/ha, while the actual yield under optimal conditions was lower by 48 kg/ha.

The combination of mono-inoculation and fertilizer at the rate of $N_{20}P_{30}$ exceeded not only the control, but also the application of fertilizer at the rate of $N_{20}P_{30}$. The use of poly-component inoculation and $N_{20}P_{30}$ not only increased the crop structure constituents and sunflower seed yield compared to mono-inoculation and $N_{20}P_{30}$, but was almost at the same level as the variant with fertilizer application at the rate of $N_{40}P_{60}$, and even exceeded it by some indicators. Under $N_{40}P_{60}$ application, the diameter of the head under optimal conditions was 22.6 cm, under limiting conditions - 13.5 cm, which surpassed the control by 5.0 and 2.7 cm, respectively, the number of seeds in the head was 869 and 500 seeds, which was 121 and 54 seeds higher than in the control, the weight of 1000 seeds was 70.44 and 46.93 g, which was 6.88 and 4.77 g higher, respectively, the oil content - 49.8 and 42.3%, which surpassed the control by 3.0 and 4.6%, respectively, the biological yield - 3060 and 1173 kg/ha, which surpassed the control by 681 and 234 kg/ha, respectively, and the actual yield - 2657 and 1167 kg/ha, which surpassed the control variant by 444 and 335 kg/ha, respectively.

Instead, in the combination of poly-component inoculation and $N_{20}P_{30}$, the diameter of the head was smaller than that at $N_{40}P_{60}$ by 0.2 cm, both under optimal conditions and under limiting conditions, the number of seeds in the head was 10 seeds less under optimal and 12 seeds less under limiting conditions, the weight of 1000 seeds was 0.67 g higher under optimal conditions, and 0.08 g higher under limiting conditions. The oil content in the variant with poly-component inoculation and $N_{20}P_{30}$ under optimal conditions was 0.5% higher than that at $N_{40}P_{60}$, while under limiting conditions it was 1.2% lower. The biological yield of poly-component inoculation and $N_{20}P_{30}$ was lower than that at $N_{40}P_{60}$ by 63 kg/ha under optimal conditions and by 25 kg/ha under limiting conditions. However, the actual yield under poly-component inoculation and $N_{20}P_{30}$ was 106 kg/ha higher under optimal conditions, while under limiting conditions it was 54 kg/ha lower.

The largest diameter of the head under optimal conditions was formed by the hybrids *Niagara* - 23.0 cm and *Hysun 232 IT H0* - 23.1 cm in the variant with $N_{40}P_{60}$ and by the hybrid *Niagara* - 23.1 cm in the variant with poly-component inoculation and $N_{20}P_{30}$, while under limiting conditions the hybrid *P63LE113* was characterized with the largest head diameter of 14.3 cm. The greatest number of seeds under the optimal conditions was formed by *Niagara* hybrid - 908 seeds, under limiting conditions – by *P63LE113* - 529 seeds. The highest weight of 1000 seeds under optimal conditions were attributed to the hybrids *Hysun 232 IT H0* - 71.95 g in the variant with $N_{40}P_{60}$ and *Niagara* - 71.93 g under poly-component inoculation and $N_{20}P_{30}$, while under limiting conditions by *P63LE113* - 49.69 g. The highest oil content under optimal conditions was recorded for the hybrid *Hysun 232 IT H0* - 51.5% in the variant with $N_{40}P_{60}$ and 51.4% under poly-component inoculation and $N_{20}P_{30}$, while under limiting conditions the highest oil content was attributed to the hybrid *Bella* - 43.3%

Table 3. Yield structure, oil content, biological and actual yield of sunflower hybrids depending on fertilization and inoculation with bacterial and fungal preparations (2023-2024)

Variant (Factor B)	Hybrid (Factor C)	Head diameter, cm		Number of seeds in the head		Weight of 1000 seeds, g		Oil content, %		Biological yield, kg/ha		Actual yield, kg/ha	
		Location 1 (Factor A)	Location 2 (Factor A)	Location 1 (Factor A)	Location 2 (Factor A)	Location 1 (Factor A)	Location 2 (Factor A)	Location 1 (Factor A)	Location 2 (Factor A)	Location 1 (Factor A)	Location 2 (Factor A)	Location 1 (Factor A)	Location 2 (Factor A)
1	2	3	4	5	6	7	8	9	10	11	12	13	14
Control (without fertilizer and inoculation)	*Niagara*	10.3	18.2	426	799	40.35	65.47	36.1	47.7	860	2617	754	2451
	Hysun 158 IT	11.2	17.0	463	706	43.77	61.43	39.1	46.6	1012	2169	891	1995
	Hysun 280	10.1	17.3	417	723	39.51	62.25	35.3	44.8	825	2252	755	2150
	Bella	11.0	17.2	454	713	42.96	61.98	38.4	45.6	975	2208	874	2027
	P64LE136	10.9	17.6	451	746	42.64	63.49	38.1	46.2	961	2368	855	2167
	Generalis	10.9	17.4	449	729	42.52	62.70	38.0	46.1	955	2285	839	2165
	Hysun 232 IT H0	10.5	18.3	436	792	41.26	66.09	36.9	49.1	899	2616	781	2406
	P63LE113	11.3	18.1	468	780	44.27	65.07	39.6	48.3	1035	2537	906	2347
	Average	**10.8**	**17.6**	**446**	**748**	**42.16**	**63.56**	**37.7**	**46.8**	**939**	**2379**	**832**	**2213**
$N_{40}P_{60}$	*Niagara*	13.4	23.0	496	908	46.59	71.79	41.9	49.7	1155	3260	1098	2881
	Hysun 158 IT	13.5	22.0	499	826	46.87	68.64	42.2	49.7	1169	2834	1203	2413
	Hysun 280	12.6	22.4	466	859	43.80	69.88	39.4	49.0	1021	3000	1056	2640
	Bella	13.8	22.2	512	842	48.09	69.28	43.3	49.6	1231	2918	1234	2479
	P64LE136	13.6	22.8	505	871	47.45	70.91	42.7	49.8	1199	3088	1200	2619
	Generalis	13.6	22.4	504	858	47.32	69.88	42.6	49.0	1192	2999	1177	2639
	Hysun 232 IT H0	13.1	23.1	486	901	45.62	71.95	41.1	51.5	1108	3243	1088	2834
	P63LE113	14.3	22.9	529	884	49.69	71.23	44.7	50.5	1315	3148	1282	2753
	Average	**13.5**	**22.6**	**500**	**869**	**46.93**	**70.44**	**42.3**	**49.8**	**1173**	**3060**	**1167**	**2657**
$N_{20}P_{30}$	*Niagara*	12.2	20.8	465	841	44.11	70.03	39.3	48.7	1025	2946	910	2555
	Hysun 158 IT	12.7	19.1	483	771	45.81	64.15	40.8	48.4	1106	2472	1030	2156
	Hysun 280	12.1	19.3	462	781	43.85	64.99	39.1	47.5	1013	2537	925	2298
	Bella	12.8	19.5	490	789	46.48	65.68	41.4	48.2	1139	2592	1044	2224
	P64LE136	12.6	19.9	480	805	45.51	67.00	40.6	48.5	1092	2697	1008	2317
	Generalis	12.5	19.6	478	794	45.35	66.10	40.4	48.3	1084	2625	988	2337
	Hysun 232 IT H0	12.1	20.5	463	831	43.90	69.14	39.1	50.0	1016	2871	917	2500
	P63LE113	13.0	20.3	495	821	46.95	68.36	41.8	49.5	1162	2807	1060	2450
	Average	**12.5**	**19.9**	**477**	**804**	**45.24**	**66.93**	**40.3**	**48.6**	**1079**	**2691**	**985**	**2355**
Inoculation by nitrogen-fixing bacteria (NFB)	*Niagara*	11.3	20.8	449	829	43.22	69.12	38.0	47.3	971	2866	870	2543
	Hysun 158 IT	11.3	19.2	448	766	43.09	63.88	37.9	47.6	966	2448	945	2165
	Hysun 280	11.1	19.5	440	777	42.35	64.76	37.2	46.8	933	2516	872	2309
	Bella	11.7	19.6	465	781	44.68	65.10	39.3	47.2	1038	2542	980	2221
	P64LE136	11.9	19.9	473	792	45.46	66.01	40.0	47.7	1075	2614	982	2301
	Generalis	11.4	20.4	453	815	43.60	67.91	38.3	49.0	989	2766	926	2420
	Hysun 232 IT H0	11.3	20.5	447	818	43.01	68.20	37.8	48.3	962	2790	877	2486
	P63LE113	12.2	20.7	482	825	46.34	68.80	40.8	48.7	1117	2840	1021	2485
	Average	**11.5**	**20.1**	**457**	**801**	**43.97**	**66.72**	**38.7**	**47.8**	**1005**	**2671**	**934**	**2366**

Continued from Table 3

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Inoculation by phosphorus-solubilizing bacteria (PSB)	*Niagara*	10.8	19.6	442	808	41.54	67.75	37.4	47.3	918	2736	822	2505
	Hysun 158 IT	11.6	18.5	475	762	44.62	63.92	39.7	47.8	1059	2435	963	2177
	Hysun 280	9.8	18.4	402	756	37.78	63.46	36.7	46.0	759	2400	765	2274
	Bella	11.4	18.7	464	769	43.66	64.50	39.3	47.3	1014	2479	941	2212
	P64LE136	11.5	18.7	470	772	44.18	64.77	39.4	47.0	1038	2500	939	2269
	Generalis	11.2	18.6	456	766	42.90	64.26	38.6	47.1	979	2461	896	2302
	Hysun 232 IT H0	10.9	19.4	445	797	41.80	66.91	37.6	48.5	929	2668	838	2451
	P63LE113	11.9	19.2	484	793	45.52	66.49	40.2	48.2	1102	2635	987	2414
	Average	**11.1**	**18.9**	**455**	**778**	**42.75**	**65.26**	**38.6**	**47.4**	**972**	**2538**	**894**	**2325**
Inoculation by arbuscular mycorrhizal fungi (AMF)	*Niagara*	11.0	19.1	444	787	41.81	66.25	37.6	47.1	929	2608	809	2427
	Hysun 158 IT	11.4	18.6	462	765	43.47	64.34	39.1	47.6	1004	2459	917	2171
	Hysun 280	11.1	18.3	448	754	42.15	63.42	37.9	46.5	944	2390	834	2252
	Bella	11.5	18.8	463	773	43.59	65.08	39.2	47.2	1010	2517	919	2212
	P64LE136	11.4	18.8	462	773	43.47	65.06	39.1	47.2	1004	2515	903	2259
	Generalis	11.2	18.6	453	766	42.67	64.50	38.3	47.3	967	2472	871	2290
	Hysun 232 IT H0	11.0	19.2	444	789	41.77	66.38	37.5	48.0	927	2618	818	2410
	P63LE113	11.6	19.0	468	783	44.03	65.90	39.6	47.8	1030	2580	933	2371
	Average	**11.3**	**18.8**	**456**	**774**	**42.87**	**65.12**	**38.5**	**47.4**	**977**	**2519**	**875**	**2299**
Inoculation by NFB + PSB + AMF	*Niagara*	11.6	20.5	455	825	43.07	68.83	38.5	48.2	980	2839	882	2577
	Hysun 158 IT	11.8	19.5	465	787	44.01	65.69	39.3	48.8	1023	2586	982	2266
	Hysun 280	11.7	19.1	458	769	43.38	64.20	38.8	47.3	994	2470	909	2330
	Bella	12.2	19.7	479	794	45.33	66.27	40.5	48.3	1085	2632	1011	2302
	P64LE136	11.9	19.8	469	797	44.39	66.53	39.7	48.5	1041	2653	976	2361
	Generalis	12.0	19.5	471	787	44.63	65.64	39.9	48.3	1052	2582	965	2382
	Hysun 232 IT H0	11.4	20.3	448	818	42.40	68.27	37.9	49.7	950	2794	879	2534
	P63LE113	12.4	20.0	486	807	46.02	67.30	41.1	49.0	1119	2714	1032	2475
	Average	**11.9**	**19.8**	**466**	**798**	**44.15**	**66.59**	**39.4**	**48.5**	**1030**	**2657**	**954**	**2403**
$N_{20}P_{30}$ + Inoculation by nitrogen-fixing bacteria (NFB)	*Niagara*	12.4	22.3	469	872	44.65	71.42	39.7	48.5	1047	3115	971	2802
	Hysun 158 IT	12.4	21.0	471	820	44.87	67.17	39.9	49.4	1058	2755	1064	2427
	Hysun 280	12.3	21.2	467	829	44.44	67.87	39.5	48.4	1037	2813	989	2580
	Bella	13.1	21.2	499	828	47.50	67.78	42.2	49.9	1185	2806	1125	2467
	P64LE136	12.6	21.5	478	837	45.53	68.57	40.5	48.9	1089	2871	1063	2549
	Generalis	12.7	21.4	482	834	45.88	68.25	40.8	49.2	1106	2844	1053	2594
	Hysun 232 IT H0	12.2	22.1	462	864	43.98	70.73	39.1	50.5	1016	3055	969	2750
	P63LE113	13.5	22.0	511	858	48.68	70.27	42.4	50.2	1245	3015	1160	2707
	Average	**12.6**	**21.6**	**480**	**843**	**45.69**	**69.01**	**40.5**	**49.4**	**1097**	**2908**	**1049**	**2610**
$N_{20}P_{30}$ + Inoculation by phosphorus-solubilizing bacteria (PSB)	*Niagara*	12.2	21.6	468	845	44.29	69.85	39.5	48.3	1035	2952	943	2687
	Hysun 158 IT	12.5	20.5	479	803	45.40	66.38	40.5	49.3	1088	2666	1054	2352
	Hysun 280	12.1	20.7	464	812	43.98	67.12	39.3	48.3	1021	2726	959	2502
	Bella	12.9	20.9	492	818	46.59	67.62	41.6	48.7	1145	2767	1081	2413
	P64LE136	12.5	21.1	478	825	45.33	68.21	40.5	49.1	1084	2815	1037	2486
	Generalis	12.3	21.1	472	826	44.75	68.28	39.9	49.2	1057	2821	1006	2545
	Hysun 232 IT H0	12.1	21.8	463	853	43.87	70.45	39.2	50.2	1016	3003	946	2685
	P63LE113	13.1	21.4	503	839	47.61	69.33	42.5	49.9	1196	2909	1111	2619
	Average	**12.5**	**21.1**	**477**	**828**	**45.22**	**68.41**	**40.4**	**49.1**	**1080**	**2831**	**1017**	**2536**

Continued from Table 3

1	2	3	4	5	6	7	8	9	10	11	12	13	14
$N_{20}P_{30}$ + Inoculation by arbuscular mycorrhizal fungi (AMF)	*Niagara*	12.1	21.3	465	847	44.05	69.47	39.3	48.1	1024	2942	923	2669
	Hysun 158 IT	12.3	20.3	474	810	44.86	66.40	40.0	49.3	1062	2688	1025	2350
	Hysun 280	12.2	20.1	467	799	44.25	65.56	39.5	47.7	1033	2620	949	2441
	Bella	12.6	20.3	485	808	45.96	66.31	40.6	48.9	1115	2680	1049	2363
	P64LE136	12.6	20.6	483	820	45.75	67.22	40.4	48.9	1105	2755	1030	2447
	Generalis	12.4	20.5	476	817	45.06	66.97	40.2	48.7	1072	2734	997	2493
	Hysun 232 IT H0	12.0	21.0	461	837	43.68	68.62	39.8	49.9	1007	2871	927	2613
	P63LE113	12.7	20.9	489	832	46.29	68.26	40.9	49.7	1131	2840	1063	2575
	Average	**12.4**	**20.6**	**475**	**821**	**44.99**	**67.35**	**40.1**	**48.9**	**1068**	**2765**	**995**	**2494**
$N_{20}P_{30}$ + Inoculation by NFB + PSB + AMF	*Niagara*	12.8	23.1	472	886	45.40	71.93	39.9	49.4	1070	3185	1018	2955
	Hysun 158 IT	13.3	21.9	491	840	47.30	68.22	41.6	50.3	1162	2865	1156	2582
	Hysun 280	12.9	21.8	477	838	45.90	68.06	40.3	49.2	1094	2852	1053	2710
	Bella	13.5	22.0	496	843	47.78	68.47	41.6	50.0	1186	2886	1167	2610
	P64LE136	13.6	22.5	500	864	48.10	70.19	41.8	50.7	1202	3033	1158	2733
	Generalis	13.2	22.3	488	855	46.94	69.45	41.2	50.2	1144	2970	1111	2765
	Hysun 232 IT H0	12.9	22.8	476	875	45.87	71.10	40.3	51.4	1093	3112	1042	2895
	P63LE113	13.8	22.7	507	871	48.78	70.76	42.0	51.1	1236	3083	1198	2855
	Average	**13.3**	**22.4**	**488**	**859**	**47.01**	**69.77**	**41.1**	**50.3**	**1148**	**2997**	**1113**	**2763**
$LSD_{05}A$		6.2		249		14.51		5.37		548		607	
$LSD_{05}B$		2.7		22		3.92		1.44		36		44	
$LSD_{05}C$		0.8		11		1.62		0.68		14		18	

Table 4. Yield structure, oil content, biological and actual yield of sunflower hybrids on average by the studied variants (2023-2024)

Hybrid (Factor C)	Head diameter, cm		Number of seeds in the head		Weight of 1000 seeds, g		Oil content, %		Biological yield, kg/ha		Actual yield, kg/ha	
	Location 1 (Factor A)	Location 2 (Factor A)	Location 1 (Factor A)	Location 2 (Factor A)	Location 1 (Factor A)	Location 2 (Factor A)	Location 1 (Factor A)	Location 2 (Factor A)	Location 1 (Factor A)	Location 2 (Factor A)	Location 1 (Factor A)	Location 2 (Factor A)
Niagara	20.9	11.8	841	459	69.27	43.55	48.2	38.8	2915	1001	2641	909
Hysun 158 IT	19.8	12.2	787	474	65.47	44.92	48.6	40.0	2580	1064	2278	1021
Hysun 280	19.8	11.6	791	452	65.60	42.85	47.4	38.4	2598	970	2408	915
Bella	20.0	12.4	796	482	66.19	45.69	48.3	40.7	2639	1102	2321	1039
P64LE136	20.3	12.3	809	477	67.09	45.26	48.4	40.2	2719	1081	2410	1014
Generalis	20.2	12.1	804	471	66.72	44.69	48.4	39.8	2687	1054	2448	984
Hysun 232 IT H0	20.8	11.8	834	457	68.89	43.38	49.7	38.7	2876	993	2597	917
P63LE113	20.7	12.7	827	493	68.34	46.74	49.4	41.4	2828	1153	2550	1068
$LSD_{05}A$	6.2		249		14.51		5.37		548		607	
$LSD_{05}C$	0.8		11		1.62		0.68		14		18	

The highest biological yield under optimal conditions was obtained on the variant with $N_{40}P_{60}$ in *Niagara* - 3260 kg/ha and *Hysun 232 IT H0* - 3243 kg/ha, and under limiting conditions in *P63LE113* - 1315 kg/ha. The highest actual yield under the optimal conditions was provided by the hybrid *Niagara* - 2955 kg/ha in the variant with poly-component inoculation and $N_{20}P_{30}$, and under limiting conditions in the hybrid *P63LE113* - 1282 kg/ha in the variant with $N_{40}P_{60}$.

Evaluating average indicators by the studied hybrids, the largest head diameter under the optimal conditions was formed by *Niagara* - 20.9 cm, *Hysun 232 IT H0* - 20.8 cm and *P63LE113* - 20.7 cm, and under limiting conditions by *P63LE113* - 12.7 cm (Table 4).

The largest number of seeds in the head and the biggest weight of 1000 seeds under the optimal conditions was formed by the hybrid *Niagara* - 841 seeds and 69.27 g, respectively, while under limiting conditions they were formed by the hybrid *P63LE113* - 493 seeds and 46.74 g. The highest oil content under optimal conditions was provided by the hybrids *Hysun 232 IT H0* - 49.7% and *P63LE113* - 49.4%, and under limiting conditions – by the hybrid *P63LE113* - 41.4%.

The highest biological and actual yields under the optimal conditions were obtained in the hybrid *Niagara* - 2915 kg/ha and 2641 kg/ha, respectively, slightly lower yields were harvested in the hybrid *Hysun 232 IT H0* - 2876 and 2597 kg/ha and the hybrid *P63LE113* - 2828 and 2550 kg/ha, while under limiting conditions in the hybrid *P63LE113* - 1153 and 1068 kg/ha, respectively.

CONCLUSIONS

Under the optimal conditions, the highest number of seeds in the head of 869 seeds, the weight of 1000 seeds of 70.44 g and the biological yield of 3060 kg/ha were obtained on the variant with $N_{40}P_{60}$, and the highest oil content of 50.3% and the actual yield of 2763 kg/ha were obtained on the variant with $N_{20}P_{30}$ and poly-component inoculation (nitrogen-fixing + phosphorus-solubilizing bacteria + arbuscular mycorrhizal fungi).

Under limiting conditions, the highest number of seeds in the head of 500 seeds, the oil content of 42.3%, the biological and actual yields of 1173 and 1167 kg/ha, respectively, were obtained on the variant with $N_{40}P_{60}$, and the highest weight of 1000 seeds of 47.01 was obtained on the variant with $N_{20}P_{30}$ and poly-component inoculation (nitrogen-fixing + phosphorus-solubilizing bacteria + arbuscular mycorrhizal fungi).

Under the optimal conditions, the highest number of seeds in the head of 841 seeds, the weight of 1000 seeds of 69.27 g, the biological and actual yields of 2915 and 2641 kg/ha, respectively, were obtained in the hybrid *Niagara*, and the highest oil content – in the hybrids *Hysun 232 IT H0* and *P63LE113* - 49.7 and 49.4%, respectively.

Under the limiting conditions, the highest number of seeds in the head of 493 seeds, the weight of 1000 seeds of 46.74 g, the oil content of 41.4%, the biological and actual yields of 1153 and 1068 kg/ha, respectively, were obtained in the hybrid *P63LE113*.

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EVALUATION OF BIOCHEMICAL COMPOSITION AND NUTRITIONAL VALUE OF FODDERS FROM *Lablab purpureus* L.

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Abstract

The identification of alternative forage crops that need less water and produce high yields of proteins is important for animal husbandry and agricultural durability. The aim of this paper was to evaluate the biochemical composition and nutritional value of fodders from Lablab purpureus introduced and grown in monoculture in the experimental plot of the NBGI Chişinău. The results of our research revealed that the dry matter of harvested whole plants contained 176 g/kg CP, 90 g/kg ash, 281 g/kg ADF, 453 g/kg NDF, 44 g/kg ADL, 196 g/kg TSS, 237 g/kg Cel, 171 g/kg HC with 742 g/kg DMD, 709 g/kg DOM, RFV=138, 10.78 MJ/kg ME and 6.80 MJ/kg NEl. The prepared silage was characterized by agreeable olive colour with pleasant smell and the dry matter contained 186g/kg CP, 120 g/kg ash, 307g/kg ADF, 510 g/kg NDF, 37g/kg ADL, 270 g/kg Cel, 203 g/kg HC with 748 g/kg DMD, 686 g/kg DOM, RFV=119, 10.48 MJ/kg ME and 6.50 MJ/kg NEl. It has been found that the prepared hay had 167 g/kg CP, 94 g/kg ash, 45 g/kg ADL, 158 g/kg TSS, 260 g/kg Cel and 198 g/kg HC, 720 g/kg DMD, 664 g/kg DOM, RFV=120, 10.48 MJ/kg ME and 6.22-6.52 MJ/kg NEl. Lablab purpureus contains many nutrients, which make it suitable to be used as fodders for farm animals.

Key words: *biochemical composition, green mass, hay, Lablab purpureus, nutritional value, silage.*

INTRODUCTION

Climate change affects crop production by directly influencing animal growth and productivity. The assessment of the effects of global climate change on agriculture can be helpful to anticipate and adapt farming to maximize the agricultural production more effectively. The identification of alternative forage crops that need less water and produce high yields of proteins is important for animal husbandry and agricultural durability. The plants of the *Fabaceae* family have been gaining attention recently. The introduction and use of new *Fabaceae* plants are considered an essential part of the process of intensification of agricultural production, can help improving the soil fertility, reducing the use of chemical fertilizers, providing for the production of food and fodder rich in protein and essential amino-acids (European Parliament resolution, 2018; ECPGR, 2021).

Regarding various attributes and multiple uses, one of the most versatile domesticated species of the *Fabaceae* family is *Lablab purpureus* (L.) Sweet (syn. *Lablab purpureus* (L.) Sweet*., Dolichos purpureus* L., *Dolichos lablab* L.; *Dolichos bengalensis* Jacq., *Lablab leucocarpos* Savi, *Lablab niger* Medik., *Lablab vulgaris* Savi), which is also known by the common names Hyacinth bean, Egyptian kidney bean, Lablab or Dolichos Lablab. It is an ancient domesticated crop, widely distributed in Africa, the Indian sub-continent and Southeast Asia. It is a summer-growing annual or, occasionally, a short-lived perennial forage legume. It is a twining, climbing, trailing or upright herbaceous plant, which can grow to a length of 3-6 m. Lablab leaves are alternate and trifoliate. The leaflets are rhomboid in shape, 7.5-15 cm long and 1.5-14 cm wide, acute at the apex. The upper surface is smooth while the underside has short hairs. Inflorescences are many-flowered racemes borne on elongated peduncles. The flowers are white to blue or purple in color, about 1.5 cm long. The flowers are typically cross-pollinated. Pods – variable in shape and colour, flat or inflated, 5-20 x 1-5 cm, straight or curved, usually with 3-6 ovoid, laterally compressed seeds, 0.5-1.2 cm long, 0.3-0.9 cm wide, and 0.2-0.7 cm thick, white or cream to light and dark brown, red to black, sometimes mottled of varying colour and size. The weight of 1000 seeds varied from 60 to 1000 g. Germination is epigeal and normally takes 5 days. Lablab is a short-day plant. It requires high temperatures to grow well (18-30°C), and minimum temperature for growth is 3°C. Lablab can be grown on a wide range of soil textures and types, pH regimes ranging from 5.9 to 7.8, varying from deep sands to heavy clays, provided that they are well drained. It has a strong tap root with many lateral and well-developed adventitious roots, capable of extracting soil water from at least 2 metres deep in the soils; therefore, it can make use of residual soil moisture. Lablab is excellent to suppress weeds and provides soil erosion control, besides it is a very good N-fixer with *Bradyrhizobium lablabi* sp. and *Rhizobium leguminosarum*. Also, *Lablab purpureus* is a good candidate for intercropping with grass, corn, sorghum crops. As a multipurpose legume, lablab is used as a pulse crop in the human diet, as a fodder crop for livestock, as a rotational and cover crop, as well as a pioneer species to improve soil fertility and soil organic matter. For livestock production, lablab can be used as a fodder crop, rather provided in form of hay, crop residues, silage, or directly grazed and, can be mixed with other feed (Murphy & Colucci, 1999; Madzonga&Mogotsi, 2014; Heuzé et al., 2016; Maass, 2016; Naeem et al., 2020; Aleme, 2022; Kumsa, 2022; Umesh et al., 2022;).

The main goal of this study was to evaluate the biochemical composition and nutritional value of fodders from *Lablab purpureus* introduced and grown under the soil climatic conditions of the Republic of Moldova

MATERIALS AND METHODS

The introduced species lablab *Lablab purpureus* grown in monoculture on the experimental land of National Botanical Garden (Institute) Chişinău, N 46°58′25.7″ latitude and E 28°52′57.8″ longitude, served as subject of the research, and the local cultivar *'Clavera'* of soybean, *Glycine max* and the local biennial ecotype of white sweet clover *Melilotus alba* were used as controls. The experimental design was a randomised complete block design with four replications, and the experimental plots measured 10 m^2. *Lablab purpureus* and *Glycine max* were sown in early May, at a depth of 4.0 cm, in rows at a distance of 45 cm. The green mass was harvested manually. The lablab and soybean samples were collected in early pod stage, while the white sweetclover sample was collected in full flowering period, in the second year of vegetation. The leaf/stem ratio was determined by separating the leaves from the stem, weighing them separately and establishing the ratios for these quantities (leaves/stems). The prepared hay was dried directly in the field. The silages were prepared from harvested green mass, cut into small pieces and compressed in glass containers. The containers were stored for 45 days, and after that, they were opened and the organoleptic assessment and the determination of the organic acids composition of the persevered forage were done in accordance with the Moldavian standard SM 108. The fresh mass and fermented fodder samples were dehydrated in an oven with forced ventilation at a temperature of 60°C. At the end of the fixation, the biological material was finely ground in a laboratory ball mill. The quality of the fodders was evaluated by analysing such indices as: crude protein (CP), crude fibre (CF), crude ash (CA), total soluble sugars (TSS), acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL), digestible dry matter (DDM), digestible organic matter (DOM) which have been determined by near infrared spectroscopy (NIRS) technique PERTEN DA 7200 of the Research and Development Institute for Grassland Braşov, Romania. The concentration of hemicellulose (HC) and cellulose (Cel), the digestible energy (DE), the metabolizable energy (ME), the net energy for lactation (NEl) and the relative feed value (RFV) were calculated according to standard procedures.

RESULTS AND DISCUSSIONS

Analysing the results of the assessment of bio-morphological peculiarities of the studied legume crops, it can be noted that the harvested *Lablab purpureus* whole plants contained 22.6% dry matter, 68.57% leaves + flowers, while controls leguminous plants *Glycine max* - 26.5% dry matter, 57.8% leaves + flowers

+pods and *Melilotus albus* - 27.8% dry matter and 73.7 % leaves + flowers.

The biochemical composition, nutritive and energy value of the green mass from the studied leguminous plants are presented in Table 1.

We found that the dry matter nutrient content varied and depending on the leguminous plants species: 132-178 g/kg CP, 261-381 g/kg CF, 281-386g/kg ADF, 453-567 g/kg NDF, 44-64 g/kg ADL, 86-196 g/kg TSS, 237-322 g/kg Cel, 171-181g/kg HC, 81-94 g/kg ash, 525-742 g/kg DMD, 449-709 g/kg DOM, RFV= 97-138, 11.66-13.33 MJ/kg DE, 9.57-10.78 MJ/kg ME and 5.59-6.80 MJ/kg NEl. Analysing the results of the biochemical composition of green mass, we would like to mention that the concentrations of crude protein and hemicellulose in the dry matter from *Lablab purpureus* and *Glycine max* do not differ significantly, but are much higher than in *Melilotus albus.*

The *Lablab purpureus* fodder is characterized by lower level of crude fibre, structural carbohydrates and acid detergent lignin, but high amount of total soluble sugars, hemicellulose and digestible matter which have a positive effect on the nutritional value and energy supply of the feed.

Table 1. The biochemical composition and the fodder value of the green mass from the studied crops

Indices	*Lablab purpureus*	*Glycine max*	*Melilotus albus*
Crude protein, g/kg DM	176	178	132
Crude fibre, g/kg DM	261	286	381
Minerals, g/kg DM	90	94	81
Acid detergent fibre, g/kg DM	281	310	386
Neutral detergent fibre, g/kg DM	453	484	567
Acid detergent lignin, g/kg DM	44	49	64
Total soluble sugars, g/kg DM	196	142	86
Cellulose, g/kg DM	237	261	322
Hemicellulose, g/kg DM	171	174	181
Digestible dry matter, g/kg DM	742	686	525
Digestible organic matter, g/kg DM	709	634	449
Relative feed value	138	124	97
Digestible energy, MJ/ kg	13.13	12.73	11.66
Metabolizable energy, MJ/ kg	10.78	10.45	9.57
Net energy for lactation, MJ/ kg	6.80	6.46	5.59

Different results regarding the biochemical composition and the nutritive value of the green mass from *Lablab purpureus* are given in the specialized literature. According to Murphy & Colucci (1999), whole plants contained 17% CP, 27.8% CF, 43.0% NDF, 38.6% ADF and 7.1% ADL. Amodu et al. (2005) remarked that the dry matter from *Lablab purpureus* plants contained 17.5% CP, 1.4% EE, 29.8% CF and 46.3% NFE. Contreras-Govea et al. (2011) reported that *Lablab purpureus* whole plants had 270-272 g/kg DM, 19.0-19.9% CP, 42.1-42.6% NDF, 29.9-30.7% ADF, 875-880 g/kg IVDMD, 665-670 g/kg TDN. Amole et al. (2013) found that the concentrations of nutrients and energy in the leaf dry matter during the growing season of *Lablab purpureus* plants were 20.4-22.6% CP, 5.56-7.56 % EE, 29.2-37.1% CF, 15.0-58.9% NFE, 5.50-6.87% ash and 47.3-67.8 kJ/kg GE, while stem dry matter 18.1-20.4% CP, 5.29-7.04% EE, 30.4-42.2 % CF, 15.9-96.0% NFE, 8.09-8.63 % ash and 53.2-76.2 kJ/kg GE. Hassan et al. (2014) mentioned that the chemical composition of lablab forage was 13.90-15.33% CP, 0.63-0.94% EE, 15.79-16.72% CF, 56.79-58.24% NDF, 19.18-21.46% ADF, 52.49-59.42% NFE, 9.01-9.43% ash, 8.1-8.8 g/kg Ca and 8.9-13.2 g/kg P. Amole et al. (2015) reported that the studied lablab genotypes had 15.3-15.7% CP, 5.55-7.39 % EE, 42.5-47.4% NDF, 31.7-34.1% ADF, 1.20-1.28% ADL and 11.6-12.4% ash. Heuzé et al. (2016) revealed that *Lablab purpureus* aerial part contained 211 g/kg DM, 18.4% CP, 2.6 % EE, 28.2 % CF, 44.6% NDF, 32.0% ADF, 7.2% lignin, 11.1% ash, 13 g/kg Ca and 2.9 g/kg P, 67.0% ODM, 18.2 MJ/kg GE, 11.7 MJ/kg DE, 9.2 MJ/kg ME. González-García et al. (2017) reported that *Lablab purpureus* aerial part contained 258.3 g/kg DM, 18.17% ash, 19.65% CP, 1.16% EE, 45.94% NDF, 29.83% ADF, 8.48% ADL, 16.11% HC, 21.35% Cel, 65.67% DDM, 625.05% TDN, RFV=133, 2.74 Mcal/kg DE, 2.24 Mcal/kg ME. Washaya et al. (2018) remarked that the *Lablab purpureus* forage, harvested in different growth stages, contained 17.51-21.14% CP, 1.87-3.00% EE, 46.51-52.61% NDF, 33.65-39.87% ADF, 12.74-13.61% HC, 10.67-12.67% ash. Omodewu et al. (2019) found that *Lablab purpureus* plants contained 27.36% CP, 19.72% CF, 8.72% ash, 1.55% EE, 27.63% NFE, 51.68% NDF, 41.93% ADF, 4.83 % ADL. Bah et al. (2020) revealed that the nutritive content of the *Lablab* forages harvested at different stages of growth, from week five up to fifteen, was 377.2-884.0 g/kg DM, 9.03-14.90% CP, 5.51-13.34% ash, 9.45-13.14% EE, 11.16-41.23% CF. Adem et al. (2021) found that the nutritive value of lablab was 21.59% CP, 11.82% ash, 40.84% NDF, 28.79% ADF, 4.3 % ADL, 632.3 g/kg TDN, 656.7 g/kg IVDMD. Wangila et al. (2021) found that the concentration of dry matter and nutrients in fresh plants was 168.7 g/kg DM, 22.0% CP, 8.7% ash, 46.6% NDF, 33.6% ADF, 10.5% ADL, 709 g/kg IVDMD. Aleme et al. (2022) remarked that recently released lablab variety had 22.1-23.6% CP, 13.5-15.7% ash, 47.6-49.3% NDF, 33.0-35.7% ADF, 6.3-6.7% ADL, 609-621 g/kg IVDMD and RFV=113.6-117.6. Angadi et al. (2022) reported that the fodder value of harvested mass of lablab at 58 days after sowing was: 29.0-29.1% CP, 22.9-25.6% ADF, 578-593 g/kg IVTDMD, RFQ=265-292, while the harvested mass at 124 days after sowing: 13.0-17.2% CP, 36.6-37.2% ADF, 39.9-43.7% NDF, 769-812 g/kg IVTDMD, RFQ=151-136. Omodewu et al. (2022) reported that the dry matter chemical composition of *Lablab purpureus* whole plants was 127.29-153.25 g/kg CP, 91.25-92.25 g/kg EE, 105.83-115.00 g/kg ash, 216.17-247.29 g/kg NFC, 427.50-442.50 g/kg NDF, 353.33-365.00 g/kg ADF, 143.33-165.00 g/kg ADL, 74.17 -77.50 g/kg HC. Umesh et al. (2022) remarked that green mass quality from pure *Lablab purpureus* was 17.91% CP, 38.21% ADF, 43.75 % NDF, 577.9 g/kg TDN, RFQ=145.0. Ishiaku et al. (2023) remarked that the chemical composition and energy nutritional value of lablab harvested fresh mass was 22.53% CP, 6.37% EE, 22.14% CF, 38.69% NFE, 52.08% NDF, 27.82% ADF, 14.22% ADL, 24.26% HC, 13.60% Cel, 10.32% ash, RFV=120.03, 12.13 MJ/kg ME. Aguerre et al. (2023) reported that fresh plants contained 313 g/kg DM, 18.7% CP, 15.4% ash, 40.0% aNDFom, 25.7% ADFom, 5.35% ADL 7.9% WSC, 731 g/kg IVDMD. Jabessa et al. (2023) mentioned that the investigated genotypes contained 10.8-23.5% CP, 11.2-22.23% NDF, 9.25-21.00% ADF, 3.42-14.75% ADL, 499.3-62.40 g/kg IVDMD, 422.6-500.4 g/kg IVOMD. Munza et al. (2023) reported that the quality of white lablab was 12.87-14.70% CP, 7.05-8.13% EE, 22.14% CF, 35.88-44.37% NFE, 23.93-24.80% NDF, 19.84-22.86% ADF, 6.73-7.75% ADL, 7.17-8.87% HC, 6.53-7.52% Cel, 10.15-11.82% ash. Belete et al. (2024) remarked that the chemical composition and *in vitro* organic matter digestibility of fresh forages from *Lablab purpureus* were 3.8-8.8% ash, 15.0-26.5% CP, 44.0-59.4% NDF, 24.2-47.3% ADF, 1.7-8.3% ADL and 42.2-68.7% IVOMD; while from *Medicago sativa:* 8.0-15.0%ash, 11.3-30.1% CP, 25.8-72.0% NDF, 14.7-49.5% ADF, 1.9-16.7% ADL, 68.9-79.5% IVOMD and from *Vicia* species 6.7-11.2% ash, 10.43-34.6% CP, 29.3-66.4% NDF, 21.6-46.0% ADF, 5.7-18.1% ADL, 64.6-82.0% IVOMD.

The ensiling process has substantial effects on the nutritive value of the prepared feed and animal performance. During the sensorial assessment, it was found that, in terms of colour and smell, the silage from *Lablab purpureus* had homogeneous agreeable olive colour with pleasant smell specific to pickled watermelon, while silage from *Melilotus albus* olive stems with dark green leaves and smell specific to pickled apples. The texture of ensiled mass was preserved well, without mould and mucus. The results regarding the quality of the prepared silages from *Lablab purpureus* and *Melilotus albus* are shown in Table 2. It has been determined that the fermentation profile of the prepared legume silage depending on the species and was: pH = 4.11-4.42, 24.0-56.0 g/kg organic acids, including 0.3-6.9 g/kg free acetic acid, 2.3-7.1 g/kg free lactic acid, 3.1-7.3 g/kg fixed acetic acid, 18.3-34.5g/kg fixed lactic acid, 0-0.2 g/kg fixed butyric. *Lablab purpureus* silage had lower pH value and higher content of organic acids than *Melilotus albus* silage. The concentrations of nutrients, nutritive and energy values of the prepared silages were: 127-186 g/kg CP, 281-414 g/kg CF, 307-407 g/kg ADF, 510-581 g/kg NDF, 37-58 g/kg ADL, 62-107 g/kg TSS, 270-349 g/kg Cel, 174-203 g/kg HC, 99-129 g/kg ash, 509-748 g/kg DMD, 411-686 g/kg DOM, RFV= 92-119, 11.38-12.76 MJ/kgDE, 9.34-10.48 MJ/kg ME and 5.30-6.50 MJ/kg NEl. It was found that during the process of ensiling, the concentrations of minerals, crude fibre, neutral detergent fibre, acid detergent fibre increased, but the level of total soluble sugars and acid detergent lignin decreased in comparison with the initial green mass. In lablab silage the amount of crude protein was high compared with the initial green mass. The silage prepared from *Lablab purpureus* plants has higher concentration of protein, total soluble sugars, hemicellulose and a lower concentration of structural carbohydrates and acid detergent lignin, a higher level of digestibility, metabolizable energy and net energy for lactation.

Several studies have evaluated the quality indices of *Lablab purpureus* silage. Quigley et al. (2000) reported that lablab silage with grain was characterized by 493 g/kg DM, pH 5.0, 11.4 g/kg lactic acid, 4.90 g/kg acetic acid, 0.04 g/kg butyric acid, 21.9 g/kg N, 448 g/kg ADF, 544 g/kg NDF, 550 g/kg IVDMD and 6.8 MJ/kg ME. Contreras-Govea et al. (2011) reported that the silage from *Lablab purpureus* plants had pH=4.56-4.58, 81.2-82.4 g/kg lactic acid, 40.3-42.1 g/kg acetic acid, 248-257 g/kg DM, 20.2-20.6% CP, 39.6-40.2 % NDF, 32.5-32.6 % ADF, 829-834 g/kg IVTDMD, 631-644 g/kg TDN. Wangila et al. (2021) mentioned that *Lablab purpureus* silage was characterized by pH= 4.37-4.81, 427.4g/kg DM, 16.0% CP, 8.9% ash, 42.4% NDF, 28.6% ADF, 7.3% ADL, 754 g/kg IVDMD.

Table 2. The biochemical composition and the nutritive value of the silage fodder from the studied crops

Indices	*Lablab purpureus*	*Melilotus albus*
pH index	4.11	4.42
Organic acids, g/kg DM	56.0	24.0
Free acetic acid, g/kg DM	6.9	0.3
Free butyric acid, g/kg DM	0	0
Free lactic acid, g/kg DM	7.1	2.3
Fixed acetic acid, g/kg DM	7.3	3.1
Fixed butyric acid, g/kg DM	0.2	0
Fixed lactic acid, g/kg DM	34.5	18.3
Total acetic acid, g/kg DM	14.2	3.4
Total butyric acid, g/kg DM	0.2	0
Total lactic acid, g/kg DM	41.6	20.6
Acetic acid, % of organic acids	25.36	14.20
Butyric acid, % of organic acids	0.36	0
Lactic acid, % of organic acids	74.28	85.80
Crude protein, g/kg DM	186	127
Crude fibre, g/kg DM	281	414
Minerals, g/kg DM	129	99
Acid detergent fibre, g/kg DM	307	407
Neutral detergent fibre, g/kg DM	510	581
Acid detergent lignin, g/kg DM	37	58
Total soluble sugars, g/kg DM	107	62
Cellulose, g/kg DM	270	349
Hemicellulose, g/kg DM	203	174
Digestible dry matter, g/kg DM	748	509
Digestible organic matter, g/kg DM	686	416
Relative feed value	119	92
Digestible energy, MJ/ kg	12.76	11.38
Metabolizable energy, MJ/ kg	10.48	9.34
Net energy for lactation, MJ/ kg	6.50	5.30

Table 3. The biochemical composition and the fodder value of the hay from the studied crops

Indices	*Lablab purpureus*	*Glycine max*
Crude protein, g/kg DM	167	173
Crude fibre, g/kg DM	273	303
Minerals, g/kg DM	94	105
Acid detergent fibre, g/kg DM	305	331
Neutral detergent fibre, g/kg DM	503	504
Acid detergent lignin, g/kg DM	45	53
Total soluble sugars, g/kg DM	158	110
Cellulose, g/kg DM	260	278
Hemicellulose, g/kg DM	198	173
Digestible dry matter, g/kg DM	720	646
Digestible organic matter, g/kg DM	664	578
Relative feed value	120	116
Digestible energy, MJ/kg	12.76	12.42
Metabolizable energy, MJ/kg	10.48	10.20
Net energy for lactation, MJ/kg	6.52	6.22

Hay making is one of the oldest techniques used for preserving forage, plays an important role in the livestock feeding system, representing a low-cost and abundant source of nutrients, it is vital to keep animals healthy and productive. The hays prepared from the studied legume crops (Table 3) contained 167-173 g/kg CP, 273-303 g/kg CF, 94-105 g/kg ash, 305-331g/kg ADF, 503-504 g/kg NDF, 45-53 g/kg ADL, 110-158 g/kg TSS, 260-278 g/kg Cel and 173-193 g/kg HC. The nutritive and energy value of studied legume hays were 646-720 g/kg DMD, 578-664 g/kg DOM, RFV=116-120, 12.42-12.76 MJ/kg DE, 10.20-10.48 MJ/kg ME and 6.22-6.52 MJ/kg NEl. We would like to mention that in the hay making process of the studied legume crops, we noticed an increase in the concentration of structural carbohydrates, minerals and a decrease in the content of crude protein, total soluble sugars, digestibility, relative feed value and energy concentration as compared with the initial harvested green mass. The lablab hay is characterized by lower level of minerals, crude fibre, acid detergent fibre, acid detergent lignin, cellulose, but high amount of total soluble sugars and hemicellulose, but lower level of crude fibre, cellulose, which have a positive effect on the nutritive and energy value.

Some authors mentioned various findings about the quality of *Lablab purpureus* hay. Mupangwa et al. (2006) mentioned that dried lablab forage at 8 weeks of growth stage contained 25.1-25.4% CP, 28.2-29.4% ADF, 32.8-37.3% NDF, 8.9-9.6% ADL, 9.5-11.4 % ash, 7.2-7.6 g/kg Ca, 1.1-1.2 g/kg P, while lablab forage at 20 weeks of growth stage contained 16.2-18.3% CP, 35.3-38.6% ADF, 52.2-56.6% NDF, 5.86-7.83% ADL, 6.80-7.74 % ash, 16.5-19.0 g/kg Ca, 1.0-1.2 g/kg P. Heuzé et al. (2016) revealed that lablab *Lablab purpureus* hay contained 16.3% CP, 2.2% EE, 32.0% CF, 45.5% NDF, 31.9% ADF, 6.7% lignin, 10.5% ash, 14.3 g/kg Ca and 2.9 g/kg P, 60.0% ODM, 18.3 MJ/kg GE, 10.3 MJ/kg DE, 8.2 MJ/kg ME. Kumsa et al. (2022) mentioned that the chemical composition of *Lablab purpureus* hay was: 24.00% CP, 9.53 % ash, 44.53% NDF, 24.53% ADF, 4.8 % ADL, but *Vigna unguiculata* hay 18.24% CP, 11.07 % ash, 22.90% NDF, 20.63% ADF, 6.42% ADL. Seid & Animut (2018) remarked that lablab hay had 958 g/kg DM, 89.1% OM, 20.2% CP, 44.3% ADF, 50.1% NDF, 9.7% ADL, while alfalfa hay 962 g/kg DM, 88.3% OM, 23.0% CP, 40.0% ADF, 46.0% NDF, 7.5% ADL. Wangila et al. (2021) reported that that *Lablab purpureus* hay had 834.8 g/kg DM, 17.80% CP, 8.8% ash, 54.2% NDF, 39.7% ADF, 10.4% ADL, 681 g/kg IVDMD. Shibeshi et al. (2022) showed that lablab hay contained 871 g/kg DM, 21.5% CP, 12.0% ash, 42.7% NDF, 32.4% ADF, 6.10% ADL, 10.3% HC, 26.3% Cel. Kumsa (2022) mentioned that lablab hay had 976 g/kg DM, 19.47% CP, 7.68% ash, 38.23% NDF, 22.41% ADF. Tulu et al. (2024) reported that that concentration of nutrients in lablab hay was 22.92-23.46% CP, 43.89-44.57% NDF, 25.34-26.94% ADF, 5.57-5.64% ADL, 10.08-13.46% ash. Belete et al. (2024) remarked that the nutrient content of *Lablab purpureus* hays was 37.9-11.9 % ash, 16.1-

24.0% CP, 41.0-51.3% NDF, 24.4-41.3% ADF, 4.4-9.7% ADL; *Medicago sativa* hays – 12.7-13.1% ash, 13.6-20.1% CP, 46.4-51.1% NDF, 13.7-40.9% ADF, 4.2-9.0% ADL, and from *Vicia* species hays – 8.0-11.8% ash, 14.9-21.1% CP, 36.5-58.4% NDF, 27.1-48.2% ADF, 4.6-16.6% ADL.

CONCLUSIONS

The concentration of nutrients in the dry matter of the *Lablab purpureus* whole plants contained reached 176 g/kg CP, 90 g/kg ash, 281 g/kg ADF, 453 g/kg NDF, 44 g/kg ADL, 196 g/kg TSS, 237 g/kg Cel and 171 g/kg HC with 10.78 MJ/kg ME and 6.80 MJ/kg NEl. The *Lablab purpureus* silage was characterized by pH= 4.11, 56.0 g/kg organic acids and the dry matter contained 186g/kg CP, 120 g/kg ash, 307g/kg ADF, 510 g/kg NDF, 37g/kg ADL, 270 g/kg Cel and 203 g/kg HC, with 10.48 MJ/kg metabolizable energy and 6.50 MJ/kg net energy for lactation.

The *Lablab purpureus* hay had 167 g/kg CP, 94 g/kg ash, 45 g/kg ADL, 158 g/kg TSS, 260 g/kg Cel, 198 g/kg HC, 720 g/kg DMD, 664 g/kg DOM, RFV=120, 12.76 MJ/kg DE, 10.48 MJ/kg ME and 6.52 MJ/kg NEl.

Lablab purpureus plants contains many nutrients, which make it suitable to be used as fodders for farm animals.

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STATUS OF THE MEDICINAL AND AROMATIC PLANTS COLLECTION IN THE NATIONAL GENEBANK OF BULGARIA

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Abstract

Bulgaria is known for its wealth of medicinal plants. The diversity that exists ecologically is a prerequisite for the collection and cultivation of various species and forms with a high content of biologically active substances. The country therefore has centuries-old traditions relating to the collection, cultivation, processing and marketing of medicinal plants. More than 250 species are used in official medicine and are very well accepted in both the domestic and foreign markets. The collection of medicinal and aromatic plants in the National Genebank in IPGR-Sadovo, Bulgaria is represented totally by of 484 specimens of which 210 are annuals, 66 perennials, 26 bulbous flowers and 182 medicinal specimens, mainly wild.

Key words*: medicinal and aromatic plants, conservation, collection, PGR, National Genebank.*

INTRODUCTION

Medicinal and aromatic plants have been used by humans since ancient times. Nowadays the genetic diversity of medicinal and aromatic plant species in nature is under a threat of severe reduction or even extinction, die to the urbanization of the areas of natural habitats, to harvesting of row materiam or to land use changing. The aim of this paper is to give overview for the status of the medicinal and aromatic plants collection in the Bulgarian genebank in IPGR-Sadovo as well as the methods for their conservation.

MATERIALS AND METHODS

Bulgaria is situated in Southeast Europe, at the Balkan peninsula. The land area of Bulgaria is 110,994 square kilometres.

Despite the relatively small area, the relief of Bulgaria varies quite a lot. There are extensive lowlands, plains, hills, low and high mountains, many valleys and deep gorges. The main characteristic of Bulgaria's topography is four alternating bands of high and low terrain that extend east to west across the country. From north to south, those bands, called geomorphological regions, are the Danubian Plain, the Balkan Mountains, the Transitional region and the Rilo-Rhodope region. The easternmost sections near the Black Sea are hilly, but they gradually gain height to the west until the westernmost part of the country is entirely high ground.

The specific geographical position of Bulgaria as the southern border of the Central European flora, the northern border of the Mediterranean flora and the western border of the East Asian flora determines both the rich diversity and the large number of endemic plant species. Of the 3567 known species of vascular plants, about 750 are medicinal. Most (85%) of the medicinal plants are wild and only about 15% have been introduced into culture and are cultivated. Many of the wild species are rare or protected (Popova & Marinkova, 1999; Jordanov, 1976).

The goal of conservation in the PGR is to ensure sustainable development by conserving and using biological resources in a way that does not reduce the world's genetic and species diversity and does not destroy habitats and ecosystems. It includes activities such as collection, propagation, characterization, assessment, conservation and distribution. The conservation of plant genetic resources has long been realised as an integral part of biodiversity conservation. There are two methods of conserving plant genetic resources - *in situ* and *ex situ* conservation. *In situ* conservation is the process of protecting plant species in their natural habitats. *Ex situ* conservation is the conservation of plant species outside their natural habitats. This type of conservation is implemented through seed banks, field gene banks, in vitro conservation, pollen and DNA banks and cryopreservation (Engels & Visser 2003).

RESULTS AND DISCUSSIONS

At the Institute of Plant Genetic Resources - Sadovo medicinal plants are stored in the National Genebank and in a living collection. The conservation of seeds of plant species under controlled conditions at the Sadovo Institute began in 1980. Currently, over 62 000 seed accessions are preserved in the National Genebank. Three types of collections are maintained - long-term storage, active and exchange collections. In long-term storage, seeds are stored at 3-7% moisture (depending on the species) and at -18°C in airtight three-layer aluminium foil packets. Active collections are for medium-term storage, where seeds are stored at 6-7°C and 40-45% relative humidity with free access to air (paper bags). In the exchange collection, seeds are stored at 6-7ºC (Rao et al., 2006).

The collection of medical species in the IPGR - Sadovo includes 80 genera, 28 families, and 173 species. The total number of stored specimens is 6398. The greatest species diversity occurs in the genera *Allium, Brassica* and *Lathyrus*, followed by *Centaurea, Helianthus, Impatiens* and *Vicia*. The remaining genera include between 1 and 3 species. The family *Asteraceae* is represented by 22 genera, *Brassicaceae* by 9, *Lamiaceae* by 8, *Fabaceae* by 7, *Apiaceae* by 6 and *Solanaceae* by 3, C*aryophyllacea*e, *Plumbaginaceae* and *Ranunculaceae* are composed of 2 genera, and the remaining 19 families are represented by a single genus. The medicinal plants of Bulgarian origin are 1059 specimens. They belong to 61 genera, respectively 21 families (Table 1). In the National Genbank are maintained 42 accessions included in the Medicinal Plants Act of Bulgaria (Table 2).

Table. 1. List of the medicinal plant species in the collection of the Natoional Genebank in IPGR - Sadovo

Family	Genera	Number of species	Number of accessions	Number of accessions with origin from Bulgaria
Amaryllidaceae	*Allium*	17	291	75
Apiaceae	*Anethum*	1	5	3
Apiaceae	*Carum*	1	1	1
Apiaceae	*Coriandrum*	1	3	3
Apiaceae	*Foeniculum*	1	8	6
Apiaceae	*Opopanax*	1	1	1
Apiaceae	*Pimpinella*	1	10	2
Asteraceae	*Achillea*	1	1	1
Asteraceae	*Artemisia*	1	1	1
Asteraceae	*Calendula*	2	24	0
Asteraceae	*Carthamus*	1	14	0
Asteraceae	*Centaurea*	5	5	2
Asteraceae	*Cichorium*	2	7	1
Asteraceae	*Cynara*	1	3	2
Asteraceae	*Echinacea*	1	1	0
Asteraceae	*Erigeron*	1	1	0
Asteraceae	*Gaillardia*	1	1	0
Asteraceae	*Gazania*	1	6	0
Asteraceae	*Helianthus*	4	431	47
Asteraceae	*Helichrysum*	3	14	1
Asteraceae	*Heliopsis*	3	4	0
Asteraceae	*Lactuca*	2	627	67
Asteraceae	*Lonas*	1	1	0
Asteraceae	*Madia*	2	4	0
Asteraceae	*Matricaria*	1	1	1
Asteraceae	*Rudbeckia*	3	7	1

Asteraceae	*Serratula*	1	1	0
Asteraceae	*Silybum*	1	1	1
Asteraceae	*Zinnia*	2	25	1
Balsaminaceae	*Impatiens*	4	6	0
Begoniaceae	*Begonia*	1	1	0
Boraginaceae	*Phacelia*	2	2	0
Brassicaceae	*Brassica*	15	746	20
Brassicaceae	*Camelina*	3	58	0
Brassicaceae	*Crambe*	1	20	0
Brassicaceae	*Eruca*	2	4	1
Brassicaceae	*Isatis*	1	1	1
Brassicaceae	*Lepidium*	1	9	2
Brassicaceae	*Sinapis*	2	87	4
Brassicaceae	*Raphanus*	1	2	1
Caprifoliaceae	*Valeriana*	1	1	1
Caryophyllaceae	*Gypsophila*	1	1	1
Caryophyllaceae	*Silene*	1	1	1
Crassulaceae	*Rhodiola*	1	1	1
Droseraceae	*Drosera*	1	1	1
Euphorbiaceae	*Ricinus*	1	20	0
Fabaceae	*Astracantha*	1	1	1
Fabaceae	*Glycine*	1	702	11
Fabaceae	*Lathyrus*	14	335	12
Fabaceae	*Lotus*	1	18	0
Fabaceae	*Melilotus*	1	1	1
Fabaceae	*Trifolium*	2	150	40
Fabaceae	*Vicia*	4	692	254
Gentianaceae	*Gentiana*	1	6	6
Geraniaceae	*Erodium*	1	2	1
Hypericaceae	*Hypericum*	1	2	1
Lamiaceae	*Agastache*	1	1	0
Lamiaceae	*Betonica*	1	1	1
Lamiaceae	*Hyssopus*	1	2	2
Lamiaceae	*Ocimum*	1	1	1
Lamiaceae	*Salvia*	2	4	3
Lamiaceae	*Satureja*	1	6	6
Lamiaceae	*Sideritis*	1	2	2
Lamiaceae	*Linum*	2	1240	14
Onagraceae	*Oenothera*	1	1	0
Papaveraceae	*Papaver*	4	124	9
Pedaliacae	*Sesamum*	1	304	225
Plantaginaceae	*Digitalis*	1	1	1
Plumbaginaceae	*Goniolimon*	4	4	4
Plumbaginaceae	*Limonium*	6	6	6
Polygonaceae	*Rheum*	2	2	1
Ranunculaceae	*Aquilegia*	1	1	1
Ranunculaceae	*Nigella*	2	2	1
Rosaceae	*Alchemilla*	2	2	2
Rutaceae	*Ruta*	1	2	1
Scrophulariaceae	*Verbascum*	5	6	4
Solanaceae	*Atropa*	1	1	1
Solanaceae	*Nicotiana*	1	311	191
Solanaceae	*Valeriana*	1	1	1
Total - 28	***80***	**173**	**6398**	**1059**

Table 2. National GenBank Medicinal Plants Act of Bulgaria

Family	Plant species	Number of accessions from Bulgara
Amaryllidaceae	*Allium schoenoprasum* L.	5
Apiaceae	*Anethum graveolens* L.	3
Apiaceae	*Carum carvi* L.	1
Apiaceae	*Coriandrum sativum* L.	3
Apiaceae	*Foeniculum vulgaris* Mill.	6
Apiaceae	*Opopanax chironium* (L.) Koch. subsp. *bulgaricum* *	1
Asteraceae	*Artemisia annua* L.	1
Asteraceae	*Centaurea cyanus* L.	1
Asteraceae	*Cichorium inthybus* L.	1
Asteraceae	*Lactuca serriola* L.	2
Asteraceae	*Silybum marianum* (L.) Gaerth.	1
Brassicaceae	*Brassica juncea* (L.) Czern.	10
Brassicaceae	*Brassica nigra* (L.) Koch.	1
Brassicaceae	*Lepidium sativum* L.	2
Brassicaceae	*Raphanus raphanistrum* L.	1
Caprifoliaceae	*Valeriana officinalis* L.**	1
Caryophyllaceae	*Gypsophila paniculata* L.	1
Crassulaceae	*Rhodiola rosea* L.*	1
Droseraceae	*Drosera rotundifolia* L.*	1
Fabaceae	*Lathirus pratensis* L.	1
Fabaceae	*Lathyrus sativus* L.	10
Fabaceae	*Melilotus alba* Med.	1
Fabaceae	*Trifolium pratense* L.	13
Fabaceae	*Trifolium repens* L.	34
Fabaceae	*Vicia cracca* L.	1
Fabaceae	*Vicia grandiflora* SCOP.	2
Fabaceae	*Vicia pisiformis* L.	1
Fabaceae	*Vicia sativa* L.	209
Gentianaceae	*Gentiana lutea* L.*	6
Hypericaceae	*Hypericum perforatum* L.	1
Lamiaceae	*Hyssopus officinalis* L.**	2
Lamiaceae	*Salvia officinalis* L.	1
Lamiaceae	*Salvia sclarea* L.	2
Lamiaceae	*Sideritis scardica* Grsb.**	2
Plumbaginaceae	*Limonium vulgare* Mill.*	1
Ranunculaceae	*Nigella damascena* L.	1
Rosaceae	*Alchemilla achtarowii* Pawl.*	1
Rosaceae	*Alchemilla mollis* (Buser) Rothm.*	1
Rutaceae	*Ruta graveolens* L.*	1
Scrophulariaceae	*Verbascum nobile* Vel.	1
Solanaceae	*Atropa bella-donna* L.***	1
Valerianaceae	*Valeriana officinalis* L.**	1
Total	**42**	**337**

*Protected med plants
**Med Plants with special regime of protection and use – collection for trade purposes is forbidden.
***Med Plants with special regime of protection and use – collection for trade purposes can be done only according to annual quota.

In the collection of IRGR - Sadovo are kept 32 accessions of 13 families included in the Red Data Book of Bulgaria. There are 16 critically endangered specimens, 15 endangered specimens and one specimen with extinct status.

Species such as *Achillea thracica* Velen., *Silene caliacrae* D. Jord. & P. Pan., *Alchemilla achtarowii* Pawl. and *Verbascum tzar-borisii* (Davidov ex Stoj.) Stef. - Gat., which are Bulgarian endemics, and *Verbascum*

anisophyllum Murb., *Goniolimon dalmaticum* (C. Presl) Rchb. f. and *Sideritis scardica* Griseb. are Balkan endemics.
All accessions in the collection were collected as a result of expeditions or through exchange with other genebanks.

Management of PGR of medical species in the National Genebank.

The management of seed collections includes storage under optimum conditions, periodic monitoring of seed for viability and quantity, and recovery when the situation warrants. The success of long-term seed storage depends on continuous monitoring of viability and regeneration or re-collection when sample viability falls below a minimum level. The storage conditions for medicinal plant seeds are at -18°C, in airtight AL/PE-film containers (Figure 1), and seed humidity of 3 to 7% (wet basis). Seed storage is monitored regularly. Under these conditions, plant germplasm can be maintained with minimal changes for decades or a hundred years or more.

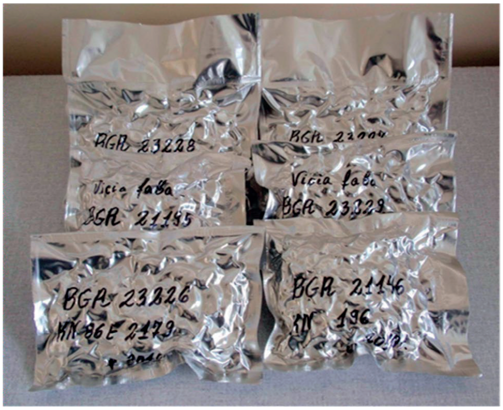

Figure 1. AL/PE-film containers

It is important to anticipate the appropriate frequency of germination control tests in the genebank. Determining the maximum storage period for each seed in the specific conditions of each seed bank is important to minimize the need for reproductions. Reproductions are very labour-intensive and costly activities, which can also have a negative impact due to the likelihood of human error.

Storage of medical species in a living collection /*in vivo*/

The in vivo collection maintained at the IRGR - Sadovo numbers about 444 accessions from 21 families. Most of them belong to the families *Lamiaceae* and *Asteraceae*. Some of them have the status of protected species, included in the Red Data Book of Bulgaria, or are regulated by the Medicinal Plants Act. Figure 2 (Uzundzhalieva, K. et al., 2014)

Figure 2. *In vivo* collection

In vivo conservation is particularly important because it is the last hope for rare and threatened plant species, especially those that are on the brink of extinction. The main role of in vivo conservation and the creation of such collections is more to do with research and education than conservation itself. Conducting research and educating children and young people are very important if we are going to have knowledge of endangered plant populations.

This provides a sufficient basis for their management and the certainty that generations after us will have the knowledge and motivation to conserve them.

CONCLUSIONS

Plant species grown *in vivo* are much more readily available for research purposes and provide many more opportunities, something that is almost impossible to achieve under on-farm conditions where species are dispersed in wild and remote populations. This also provides an opportunity to raise public awareness of rare and endangered species.

With *in vivo* conservation, consistent, easily accessible and understandable databases can be compiled with information on each species - origin, ecology, etc. On this basis, adequate conservation measures can be taken.

Clearly, the existing networks of protected areas in areas of high plant diversity will not be sufficient to ensure the long-term survival of all plant diversity. The application of an integrated approach to the conservation of PGR, combining conservation measures for plants in their natural habitats (*in situ*) with conservation in vivo, is the most effective tool for building a safety net against plant extinction. Furthermore, farmers should also be encouraged to implement such measures.

Applying integrated approaches to conservation of PGR, combining on-farm and *in situ* conservation with in vivo conservation in living collections and gene banks is the most effective way to combat plant species extinction.

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GRAINS YIELD IN SOME CORN HYBRIDS: COMPARATIVE ANALYSIS

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Abstract

The present research tested, in comparative crops, 15 corn hybrids (F8021 to F8025), created within NIARD Fundulea. The field experiments were conducted under the conditions of ARDS Lovrin during the agricultural year 2023-2024. The comparative corn crop was grown in an unfertilized and unirrigated system, to test the genetic potential of the hybrids. The yield recorded values Y = 3387.80±174.57 kg ha^{-1} (hybrid F8035) and Y = 5994.47±174.57 kg ha^{-1} (hybrid F8021). Compared to the mean calculated at the experiment level (Y_m = 4804.61±174.57 kg ha^{-1}), nine hybrids recorded yield above the mean value, with statistical safety the hybrids F8021 and F8023 at the p<0.001 level (***), hybrid F8030 at the p<0.05 level (**) and hybrid F8025 at the p<0.05 level (*).Yield values below the experimental mean were recorded for hybrids F8031 and F8035 at the p<0.001 level (ooo), and for hybrid F8027 at the p<0.01 level (oo). In the case of the other hybrids, the differences did not present statistical certainty. The positive yield increase (ΔY) was between ΔY = 125.71 kg ha^{-1} (hybrid F8033) and ΔY = 1189.86 kg ha^{-1} (hybrid F8021).

Key words*: comparative analysis, genetic potential, maize, positive yield increase, unfertilized comparative crops, yield.*

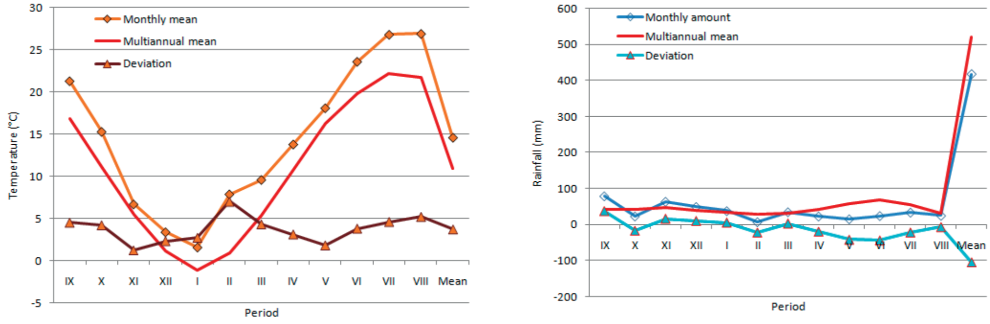
INTRODUCTION

Evaluating corn hybrids based on yield and main quality indices is an important objective in the breeding process, but also for farmers and agricultural practice (Mircea et al., 2023; Vana et al., 2023; Li et al., 2025).
The selection and cultivation of appropriate corn hybrids plays an important role in increasing productivity and yield (Bougma et al., 2024).
The analysis of the influence of genotype, environment, management practices, as well as the interactions of these elements on corn crop yield, has shown interest for agricultural production (farmers) as well as for research directions in breeding programs (Assefa et al., 2017).
Testing corn hybrids in different locations to evaluate yields is important for selecting hybrids with adaptability to certain environmental conditions (Wicaksana et al., 2022). Based on the results recorded, the study authors identified hybrids with high yield and stability, which showed interest for sustainable corn development programs.
The selection of corn hybrids, in relation to stability and yield levels associated with different environmental conditions, requires specific methods, and the "multienvironment" evaluation is an appropriate method (Ruswandi et al., 2022a).
The level of hybrids adaptation to regional (or local) environmental conditions, and the genetic potential for production, are important elements for the choice of hybrids, as they influence crop technologies and yields (Lingua et al., 2023).
The selection of corn genotypes, adapted to the environmental conditions of the area, and correlated with the agricultural system practiced, represents an important decision in the management of the farm and the farmers (Lingua et al., 2023; Bhat et al., 2024).
Climate change requires effective strategies to adopt new genotypes with high adaptability and economic yields (Zhao et al., 2023). Testing corn hybrids in various locations represents a step in future strategies for selecting genotypes with high adaptability (Zhao et al., 2023).
Climate change has significantly reduced corn crop yields, and the selection of hybrids

adaptable to new climatic conditions is of high interest (Kunwar et al., 2024).
The decrease in production and yield in corn has been analyzed and studied in relation to environmental factors that generate "multiple stress" (Konate et al., 2023). Cultivation of tolerant genotypes is important, but the authors considered it difficult to select stable and high-yielding hybrids, precisely as a result of the "genotype x environment" interaction. Therefore, testing a wide range of hybrids under specific cultivation conditions is a necessary and important step for identifying adapted genotypes.
The selection of genotypes suitable for divergent categories of farmer preferences (for corn cultivation) is of interest (Dermail et al., 2022). For this, simultaneous selection methods, in relation to the categories of interest, are necessary to identify appropriate hybrids (Dermail et al., 2022).
Yield potential has been studied in different plants and significant variability has been recorded in relation to cultivation locations (Ostberg et al., 2018; Pobkhunthod et al., 2022; Sjulgård et al., 2023).
Corn yield was analyzed in relation to agronomic traits through multivariate analysis on a collection of hybrids (59 hybrids) in order to describe the contribution of the traits considered to yield formation (Long et al., 2024). Corn yield was analyzed comparatively in relation to different categories of hybrids and agricultural systems (Reisig and Heiniger, 2024). Based on the results, the study authors concluded the need to improve yield in the studied genotypes.
Comparative analysis and selection of corn hybrids based on yield is important and requires appropriate mathematical and statistical analysis methods (Ruswandi et al., 2022a). Hierarchical modeling, within the synthesis analyses in comparisons of corn hybrids, was used to rank different hybrids based on yield (Assefa et al., 2017). Multivariate, non-parametric and parametric analyses were used to evaluate orumb hybrids grown in pure culture (single plant in the crop) and in intercropping (Ruswandi et al., 2022b). The authors selected different hybrids, suitable for the tested cropping systems, with the aim of promoting them in agricultural practice. In other studies, two clusters of corn hybrids and four groups of traits in relation to hybrid performance were identified through multivariate analysis (Long et al., 2024).
This study comparatively evaluated the yield of a collection of fifteen maize hybrids created within NIARD Fundulea, hybrids that were cultivated in comparative crops within ARDS Lovrin, under the pedoclimatic conditions specific to the Western Plain of Romania.

MATERIALS AND METHODS

In accordance with the research and testing protocols of plant genotypes for breeding programs and promotion in agricultural practice for farmers, a collection of fifteen corn hybrids, created at NIARD Fundulea, were tested under the conditions of the Western Plain of Romania. The field research and study were conducted under the specific conditions of ARDS Lovrin. The study period was in the agricultural year 2023-2024. The field experiment was located on an experimental plot with chernozem soil, and in a non-irrigated system. Climatic conditions, in the form of mean monthly temperatures (°C) and rainfall (mm) during the study period are presented in Figure 1.
The biological material was represented by fifteen hybrids, with experimental names F8021 to F8035.
The experiment was organized in comparative crops, and each hybrid was cultivated in repetitions. The crop technology was uniform and consisted of land preparation (plowing, followed by soil harrowing and work with the combiner), and weed control (pre-emergence herbicides, mechanical weeding in vegetation, supplemented with manual work). No fertilization was applied. The sowing was done in the first decade of April 2024. At harvest maturity (Meier, 2001), samples were collected from each variant (hybrid) and repetition to determine yield.
The authors generated a flow chart, which included the phases and work stages, in relation to the purpose of the study (Figure 2).
The recorded experimental data were analyzed in relation to the purpose of the study, to compare the tested hybrids and identify hybrids with generic advantage for yield under the experimental study conditions.



(a) (b)

Figure 1. Climatic conditions during the study period; (a) temperature values, (b) precipitation values

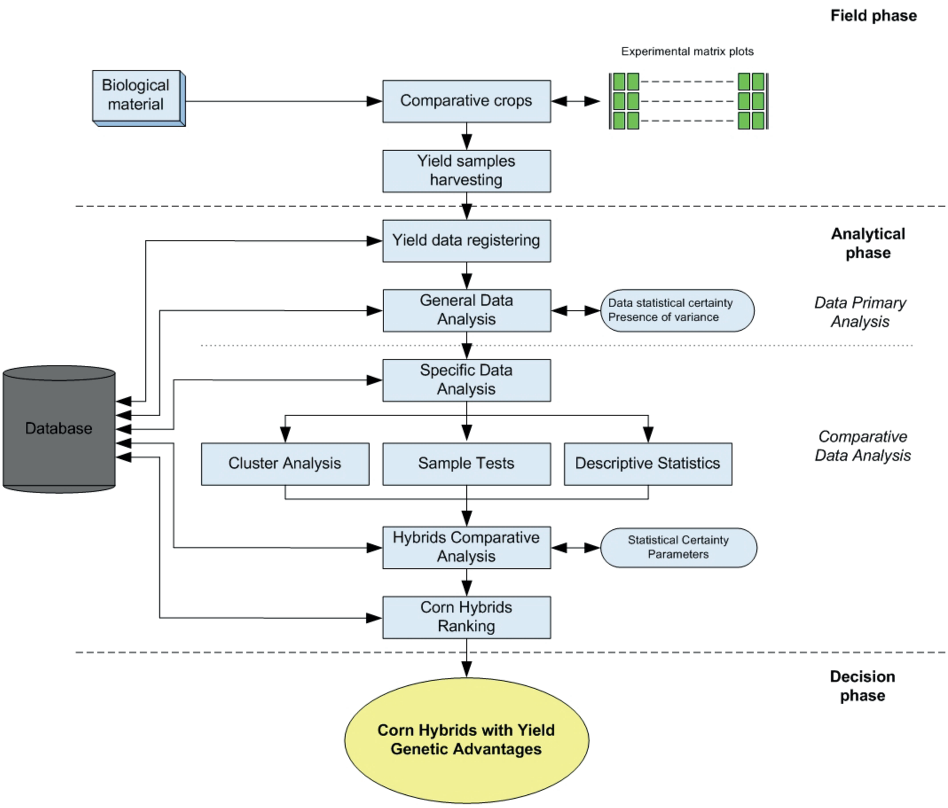


Figure 2. Workflow for identifying valuable corn genotypes

Different methods were applied, Anova Test (data reliability and presence of variance), t Test (comparative analysis), descriptive statistical analysis (definition of quartile thresholds), cluster analysis, ranking analysis. The calculations and statistical processing of the data were done in EXCEL and with the PAST software, which also resulted in graphical models (Hammer et al., 2001).

RESULTS AND DISCUSSIONS

Corn crop yield is the expression of the genetic potential of the cultivated genotypes, in relation to environmental and technological conditions, as an interaction between them.

The present study focused on the genetic potential of the tested corn hybrids. A cultivation technology was applied to ensure uniform conditions for the comparative corn crop, but without fertilization and irrigation, so that the corn hybrids expressed their genetic potential in relation to yield.

Yield values were recorded from Y = 3387.80±174.57 kg ha^{-1} (hybrid F8035), to Y = 5994.47±174.57 kg ha^{-1} (hybrid F8021). The Anova test (Alpha = 0.05) validated the experimental data reliability, and the existence of variance, in the data set (Table 1).

Table 1. Anova Test

Source of variation	SS	df	MS	F	P-value	F crit
Between Groups	25600002	14	1828572	2.3817	0.0141	1.9182
Within Groups	34549596	45	767768.8			
Total	60149598	59				

The calculated mean yield value (Y_m) at the experiment level was Y_m = 4804.61±174.57 kg ha^{-1}. Compared to the calculated mean (Y_m), nine hybrids recorded yield values above the mean value, and six hybrids recorded yield values below the mean at the experiment level (Table 2).

Table 2. Statistical values resulting from the comparative analysis of corn hybrids

Corn hybrid	Given mean	Difference	Statistical parameters			
			95% conf. interval	t	p (same mean)	Significance
F8021	5994.47	1189.90	(815.43 1564.3)	-6.8158	8.38E-06	***
F8022	4476.24	-328.37	(-46.052 702.8)	1.8810	0.0809	ns
F8023	5556.68	752.07	(377.64 1126.5)	-4.3080	0.0007	***
F8024	4441.40	-363.21	(-11.212 737.64)	2.0806	0.0563	ns
F8025	5321.82	517.21	(142.78 891.63)	-2.9627	0.0103	*
F8026	5032.14	227.53	(-146.9 601.95)	-1.3033	0.2135	ns
F8027	4167.51	-637.10	(262.68 1011.5)	3.6495	0.0026	oo
F8028	4954.47	149.86	(-224.57 524.28)	-0.8584	0.4051	ns
F8029	4986.21	181.60	(-192.83 556.02)	-1.0402	0.3159	ns
F8030	5325.20	520.59	(146.16 895.01)	-2.9820	0.0099	**
F8031	3765.40	-1039.20	(664.79 1413.6)	5.9528	3.53E-05	ooo
F8032	4772.83	-31.78	(-342.64 406.21)	0.1821	0.8582	ns
F8033	4930.32	125.71	(-248.72 500.13)	-0.7201	0.4833	ns
F8034	4956.70	152.09	(-222.34 526.51)	-0.8712	0.3983	ns
F8035	3387.80	-1416.80	(1042.4 1791.2)	8.1158	1.16E-06	ooo

In the case of hybrids with values above the mean, in four hybrids the differences from the mean presented statistical certainty, respectively hybrids F8021 and F8023 at the p<0.001 level (***), hybrid F8030 at the p<0.05 level (**), and hybrid F8025 at the p<0.05 level (*).

In the case of negative differences, statistical certainty was recorded in the case of hybrids F8031 and F8035 at the p<0.001 (ooo), in the case of hybrid F8027 at the p<0.01 (oo).

In the case of the other hybrids, the differences from the mean at the experimental level (positive or negative) did not present statistical certainty.

The positive yield increase (ΔY) was between ΔY = 125.71 kg ha^{-1} (hybrid F8033) and ΔY = 1189.86 kg ha^{-1} (hybrid F8021). The negative yield increase was between ΔY = -328.37 kg ha^{-1} (hybrid F8022) and ΔY = -1416.81 kg ha^{-1} (hybrid F8035).

Genetic advantage for yield, under the study conditions, was shown by the hybrids F8021, F8023 (***), F8030 (**) and F8025 (*). The graphic representation of the yield differences for the studied hybrids, in relation to the mean value of the experiment, is presented in Figure 3.

Descriptive statistical analysis facilitated the determination of threshold values for the delimitation of quartiles.

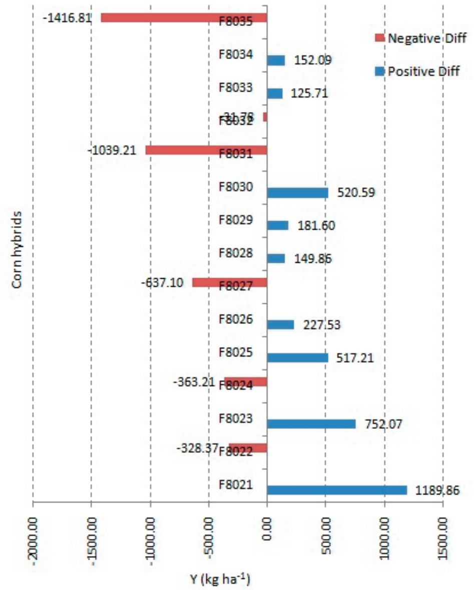


Figure 3. Distribution of yield differences compared to the mean value for the studied corn hybrids

The lower quartile included values lower than 4441.40, the middle quartile included values between the two thresholds (4441.40 > Mean Quartile < 5321.82), and the upper quartile included values higher than 5321.82.
Based on the yield values generated under the experimental conditions, the corn hybrids were classified into quartiles according to the scheme in Figure 4.

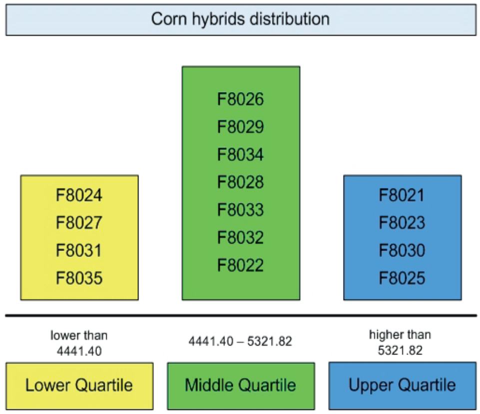


Figure 4. Distribution of corn hybrids by quartiles, in relation to yield

Four hybrids were classified in the lower quartile, F8024, F8027, F8031 and F8035. Seven hybrids were classified in the middle quartile, respectively F8026, F8029, F8034, F8028, F8033, F8032, and F8022. Four hybrids were classified in the upper quartile, respectively F8021, F8023, F8030, and F8025.
Cluster analysis grouped corn hybrids based on yield (Coph. corr. = 0.825) (Figure 5). Two distinct clusters resulted, with SDI values in Table 3.
One cluster (C1) included two hybrids with lower yield values (F8031 and F8035).

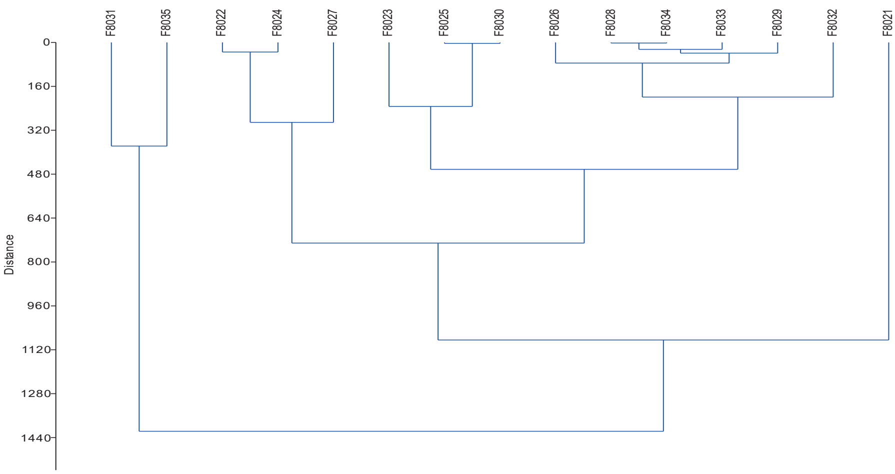


Figure 5. Cluster dendrogram of corn hybrids grouping based on Euclidean distances, in relation to yield values

Table 3. SDI values that describe the level of similarity between corn hybrids based on yield

	F8021	F8022	F8023	F8024	F8025	F8026	F8027	F8028	F8029	F8030	F8031	F8032	F8033	F8034	F8035
F8021		1518.20	437.79	1553.10	672.65	962.33	1827.00	1040.00	1008.30	669.27	2229.10	1221.60	1064.20	1037.80	2606.70
F8022	1518.20		1080.40	34.84	845.58	555.90	308.73	478.23	509.97	848.96	710.84	296.59	454.08	480.46	1088.40
F8023	437.79	1080.40		1115.30	234.86	524.54	1389.20	602.21	570.47	231.48	1791.30	783.85	626.36	599.98	2168.90
F8024	1553.10	34.84	1115.30		880.42	590.74	273.89	513.07	544.81	883.80	676.00	331.43	488.92	515.30	1053.60
F8025	672.65	845.58	234.86	880.42		289.68	1154.30	367.35	335.61	3.38	1556.40	548.99	391.50	365.12	1934.00
F8026	962.33	555.90	524.54	590.74	289.68		864.63	77.67	45.93	293.06	1266.70	259.31	101.82	75.44	1644.30
F8027	1827.00	308.73	1389.20	273.89	1154.30	864.63		786.96	818.70	1157.70	402.11	605.32	762.81	789.19	779.71
F8028	1040.00	478.23	602.21	513.07	367.35	77.67	786.96		31.74	370.73	1189.10	181.64	24.15	2.23	1566.70
F8029	1008.30	509.97	570.47	544.81	335.61	45.93	818.70	31.74		338.99	1220.80	213.38	55.89	29.51	1598.40
F8030	669.27	848.96	231.48	883.80	3.38	293.06	1157.70	370.73	338.99		1559.80	552.37	394.88	368.50	1937.40
F8031	2229.10	710.84	1791.30	676.00	1556.40	1266.70	402.11	1189.10	1220.80	1559.80		1007.40	1164.90	1191.30	377.60
F8032	1221.60	296.59	783.85	331.43	548.99	259.31	605.32	181.64	213.38	552.37	1007.40		157.49	183.87	1385.00
F8033	1064.20	454.08	626.36	488.92	391.50	101.82	762.81	24.15	55.89	394.88	1164.90	157.49		26.38	1542.50
F8034	1037.80	480.46	599.98	515.30	365.12	75.44	789.19	2.23	29.51	368.50	1191.30	183.87	26.38		1568.90
F8035	2606.70	1088.40	2168.90	1053.60	1934.00	1644.30	779.71	1566.70	1598.40	1937.40	377.60	1385.00	1542.50	1568.90	

In cluster C2, the other hybrids were grouped into different subclusters based on similarity. The hybrid F8021 with the highest yield had an independent position. The other hybrids were grouped into three subclusters.

Based on the yield values, a ranking of the corn hybrids was made. The result is the hierarchy in Figure 6, which shows the order of the hybrids, and the interevent distance values.

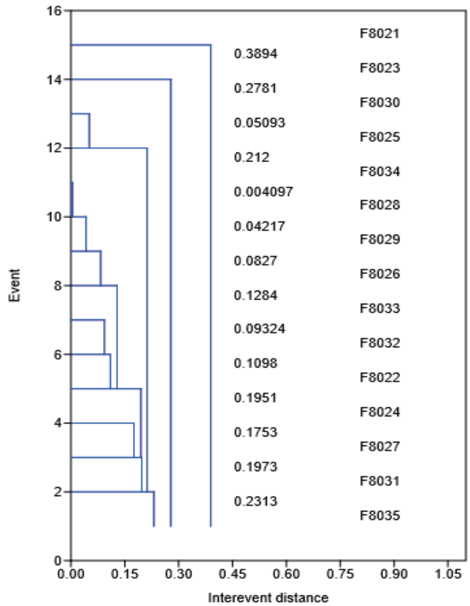


Figure 6. Ranking scaling dendrogram of corn hybrids based on yield values

Hybrids positioning in scattergram format is presented in Figure 7, with the confidence interval.

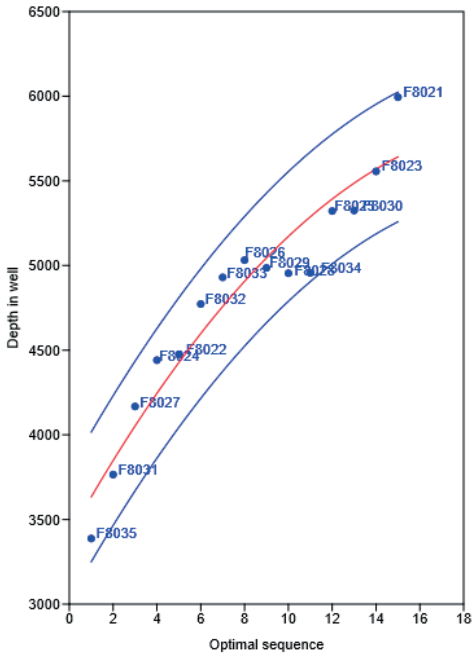


Figure 7. Distribution of corn hybrids in scattergram format, with confidence interval (95%)

Evaluating the degree of adaptation and productivity of corn hybrids through "multi-zonal" comparative testing in field conditions and technology is important, necessary and promoted by various studies and research (Lingua et al., 2023; Bougma et al., 2024).

The variation in yield of the tested corn hybrids was recorded in relation to the potential of the genotype, but also to environmental conditions (Bhat et al., 2024; Bougma et al., 2024).

Alam et al. (2022) identified maize genotypes that expressed differential genetic potential in relation to certain climate and soil conditions, and the authors formulated in the selection of the tested genotypes "hybrids with potential centered on the geographical region". Similar results have been reported in other studies, regarding the evaluation of morphological, phenotypic parameters, productivity and yield elements in corn, which confirms the high importance of these approaches (Perkins et al., 2024; Tashikalma and Giroh, 2024).

In the context of the present study, uniform cultivation conditions facilitated the expression of the genetic potential for yield of the 15 tested hybrids. Through adequate analysis of the results, four hybrids positioned in the upper quartiles, with high yield values, were identified. The yield increase generated by these hybrids ranged between $\Delta Y = 517.21$ kg ha^{-1} (F8025), and $\Delta Y = 1189.86$ kg ha^{-1} (F8021).

The comparative analysis of the hybrids differentiated the hybrids with a genetic advantage with statistical certainty compared to the mean of the experiment. The cluster analysis and the ranking analysis clearly detected the hybrids based on similarity and ranked the hybrids based on yield performance.

CONCLUSIONS

Corn hybrids created within NIARD Fundulea, and comparatively tested in the pedoclimatic conditions specific to the Western Plain of Romania, expressed differentiated genetic yield potential.

Nine hybrids showed positive differences compared to the experimental mean. Of these hybrids, four hybrids (F8021, F8023, F8030 and F8025) were placed in the upper quartiles, with yield values higher than 5321.82 kg ha^{-1}, which was the threshold of this quartile. The F8021 and F8023 hybrids showed differences at the $p<0.001$ level compared to the experimental mean, the F8030 hybrid showed differences at the $p<0.01$ level, and the F8025 hybrid showed differences at the $p<0.05$ level.

Cluster analysis based on hybrid similarity, and ranking of hybrids through ranking analysis based on yield performance, facilitated the analysis and selection of hybrids with a high level of confidence for the breeding program, as well as for crop recommendations.

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INFLUENCE OF SOWING DENSITY ON GROWTH PROCESSES OF AMARANTHUS PLANTS IN THE CONDITIONS OF SOUTHERN UKRAINE

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Abstract

Recently, the population of Ukraine has been paying more attention to a balanced diet and preferring products that contain the necessary complex of proteins, fats, carbohydrates, vitamins and micro- and macro elements. To meet such needs, manufacturers are constantly replenishing the range of products by adding non-traditional types of food raw materials to the recipes. One of these is a fairly new agricultural crop for our country - Amaranth. The extremely wide possibilities of using Amaranth are gradually increasing the demand for its production. This encourages agro-producers to improve cultivation technology, to obtain stable high yields of grain of this crop. One of the ways to increase the yield of agricultural crops is to regulate the sowing density. This measure allows plants to develop well and accumulate above-ground mass for the formation of a subsequent harvest. The article presents the results of a study of the Amaranth plants growth and development peculiarities depending on the post-emergence plant density. The highest values of amaranth plant height were recorded at the highest post-emergence plant density (180·10^3 plants/ha). In the phase of full grain ripeness, amaranth plants of the Kharkivs`kyi 1 variety were 164.0 cm high, plants of the Liera variety were 170 cm high. The greatest stem thickness was at a post-emergence plant density of 90 10$^3$ plants/ha. The stem thickness of plants of the Kharkivs`kyi 1 variety was 3.0 cm, the stem thickness of plants of the Liera variety was 3.2 cm. The greatest values of (Leaf mass ratio) LMR were at a post-emergence plant density of 150 10^3 plants/ha. For the Kharkivs`kyi 1 variety, this indicator was 0.197 g/g, and for the Liera variety it was 0.232 g/g. To ensure the best conditions for plant growth and development, the optimal post-emergence plant density was 150 10^3 plants/ha. The obtained results will help agricultural producers to optimize the sowing parameters when growing Amaranth.

Key words: *Amaranth, plant growth and development, phenological observations, post-emergence plant density, sowing parameters.*

INTRODUCTION

Human health depends on many factors, including nutrition. Recently, food manufacturers and farmers have been paying considerable attention to the development of new recipes of food products, enriching them with unconventional types of food raw materials. Amaranth is one of such types of non-traditional plant raw materials for Ukraine (Sots et al., 2024; Yaniuk et al., 2022).

Amaranth is a valuable fodder, grain, technical, food, medicinal and vegetable crop (Figure 1).

The green mass, the yield of which reaches 100 t/ha, is used in animal husbandry in fresh form, for making silage with other crops, and for obtaining protein-vitamin flour and compound feed. The protein of the green mass of amaranth has high nutritional value (Adhikary et al., 2020; Hoptsii et al., 2018; Joshi et al., 2018).

Of particular value is Amaranth oil, which contains up to 18% squalene and almost 76% unsaturated fatty acids (Levchuk et al., 2015; Malik et al., 2023).

Amaranthus is an annual, monoecious plant from the *Amaranthaceae* family (Hoptsii et al., 2018; Weerasekara et al., 2020; Joshi et al., 2018). Amaranth is a fairly tall, branched, well-leafed plant.

The stem of Amaranth is 1 to 3 meters high, straight and has an unevenly rounded, grooved shape, bright red or green color. Plants acquire branching in a sparse stand (Hoptsii et al., 2018; Joshi et al., 2018).

The leaves of the plant are arranged alternately, whole, elongated at the base into a petiole. They are oval, rhombic, ovate, lanceolate in shape. The color varies from shades of purple, orange, red and green, depending on the species (Hoptsii et al., 2018; Weerasekara et al., 2020). The inflorescence is a complex panicle 23-57 cm long, which has a green, golden or red color of varying intensity. Amaranth flowers are typically bisexual and actinomorphic, small, consist of five petals with five stamens, collected in a panicle (Joshi et al., 2018; Weerasekara et al., 2020).

Amaranth root is taproot, thickened near the root neck, branched in the arable layer. At the same time, the taproot makes up about 50% of the total mass of the root system, 18-20% are first-order roots, 30-32% are second-order roots (Hoptsii et al., 2018; Weerasekara et al., 2020).

Amaranth seeds have a rounded lenticular shape, a smooth surface and are small in size (depending on the variety from 0.3 to 2.5 mm) (Hoptsii et al., 2018). The mass of 1000 seeds ranges from 0.6 to 1.2 g depending on the varietal characteristics and growing conditions (Hoptsii et al., 2018).

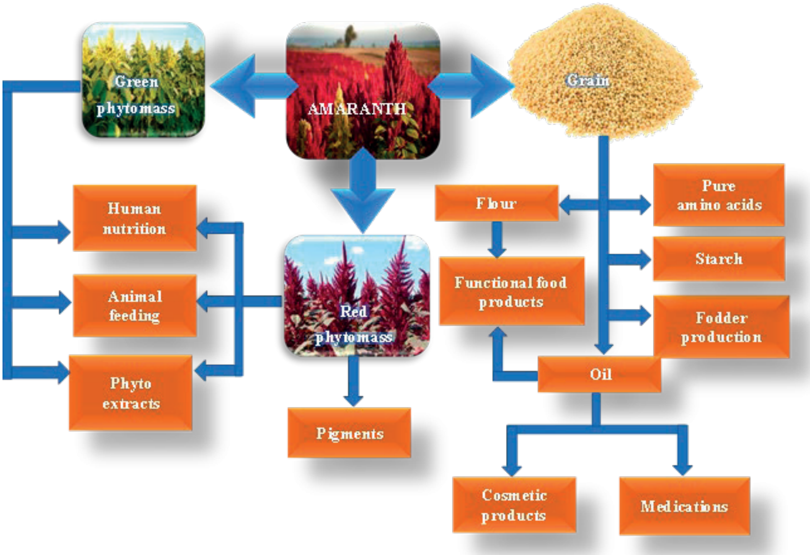

Figure 1. Modern use of amaranth phytomass and graine. Source: Authors' concept of the experiments

Amaranth is a thermophilic plant. The optimum temperature for photosynthesis is around 40°C, which is 10-15°C higher than most traditional crops (Hoptsii et al., 2018; Weerasekara et al., 2020; Joshi et al., 2018).

Amaranth is a plant of tropical origin with C_4 photosynthetic pathway (Hoptsii et al., 2018). This plant has a high ability to withstand environmental stress, which makes it a crop suitable for growing in extremely dry conditions caused by climate change (Weerasekara et al., 2020).

Amaranth grows on various types of soil in forest, forest-steppe and steppe zones, except for very acidic and saline soils, and soils with close groundwater (Mambetova et al., 2022; Dudka, 2019).

The uniqueness of this plant is evidenced by the fact that, unlike other crops, amaranth consumes the least amount of water to form 1 g of dry matter (Silva et al., 2019). According to research, it was found that amaranth consumes of 260 g water to form 1 g of dry matter while millet requires 300 g, corn requires 370 g, barley requires 520 g, wheat requires 550 g, sunflower requires 600 g, rye requires 630 g, clover requires 640 g, bean requires 700 g, alfalfa requires 840 g. (Silva et al., 2019). This makes amaranth promising for cultivation in a zone of insufficient and unstable moisture, which includes the Southern Steppe of Ukraine.

Despite the presence of a significant number of scientific studies related to the study of the biochemical composition of various parts of amaranth plants, directions and features of breeding work to develop new varieties, possibilities of use as food and medicinal raw materials (Weerasekara et al., 2020; Idowu-Agida et al., 2020), substantiation of post-harvest processing and storage regimes of amaranth grain (Stankevych et al., 2021; Valentiuk et al., 2020), there are some contradictions regarding agrotechnical techniques aimed at increasing amaranth productivity (Dudka, 2019; Pelech et al., 2021; Tyrus et al., 2023). This is especially true for the optimization of the sowing parameters, which involves the choice of sowing dates and methods, sowing rates, seeding depth, and sowing s density (Casini et al., 2020; Gomes et al., 2023; Tyrus, 2023).

Therefore, a scientific field experiment was conducted in the Southend Steppe of Ukraine in order to determine the most effective planting density of grain amaranth adapted to specific soil and climatic conditions.

MATERIALS AND METHODS

In 2022, a two-factor field experiment was established on the field of LLC "Aisberg", located within the boundaries of the Velykymykhaylivka settlement community of the Rozdlininsky district of the Odesa region which is located in the conditions of the Danubian province of the Southern Steppe subzone of Ukraine.

2 varieties of amaranth of domestic selection were selected for research (Figure 2).

Figure 2. General view of amaranth plants in the flowering phase:
a - amaranth plants of the Kharkivs`kyi 1 variety;
b - amaranth plants of the Liera variety

Varieties are included in the Register of Plant Varieties Suitable for Distribution in Ukraine and have different purposes. The Kharkivs`kyi 1 variety has a universal purpose. The Liera variety has a grain purpose. Both varieties belong to the species *A. hypochondriacus*.

Experimental scheme:

Factor A – different varieties of amaranth:

a_1 – Kharkivs`kyi 1 (control);

a_2 – Liera.

Factor B – post-emergence plant density:

b_1 – 90 $\cdot 10^3$ plants/ha;

b_2 – 120 $\cdot 10^3$ plants/ha (control);

b_3 – 150 $\cdot 10^3$ plants/ha;

b_4 – 180 $\cdot 10^3$ plants/ha.

The experimental variants are placed in 3 replications using the split-plot method.

To establish the calendar sowing dates based on the climatic indicators of the zone and the biological characteristics of the crop, the meteorological method was used. The measuring and weighing method were used to determine the biometric parameters of plant growth and development.

Leaf mass ratio (LMR) is the ratio of leaf dry mass to the dry mass of the entire plant (g/g)

The mean values and standard errors were used to analyze the data. The regression statistics and analysis of variance (ANOVA) within Microsoft Excel 2010 were used to provide mathematical analyses.

All data were compared using Least Significant Difference (LSD) test at 5% probability level.

Immediately after harvesting the pre-crop (winter wheat), the soil was disked to a depth of 10-12 cm in two tracks. The dry post-harvest period did not require additional tillage before autumn plowing due to the absence of weeds. At the end of October, a moldboard plowing was carried out to a depth of 23-25 cm.

With the acquisition of physical ripeness of the soil in the spring, harrowing was carried out. After 2 weeks, with the appearance of early spring and perennial weeds, the first cultivation was carried out with harrowing to a depth of 10-12 cm. With the appearance of a new wave of weeds in the "white thread" phase, the field was harrowed and immediately before sowing, pre-sowing cultivation was carried out to a depth of 4-5 cm with followed by compaction.

Amaranth was sown to a depth of 2-3 cm in a wide-row method (row spacing 60 cm) with a seeding rate of 1 kg per hectare and followed compaction. Before and after the emergence of seedlings, harrowing was carried out.

The research program did not provide for the use of crop protection products from harmful organisms and the application of mineral fertilizers in connection with the production of organic amaranth.

Before the beginning of amaranth branching, manual weeding was carried out with the simultaneous formation of the crop density according to the experimental scheme.

After the formation of the planned crop density of amaranth in the experiment, inter-row cultivation was carried out.

Amaranth was harvested in a continuous manner in the phase of full ripeness of amaranth grain.

RESULTS AND DISCUSSIONS

The monitoring of weather conditions over the years of research indicates their certain diversity and impact on the formation of the amaranth grain yield in the experiment. It can certainly be stated that the 2021-2022 and 2022-2023 agricultural years were close to the average long-term indicators and were generally favorable for amaranth plants. The 2023-2024 agricultural year was extremely unfavorable for most agricultural crops, including amaranth, since higher temperature values and an extreme lack of precipitation were observed during the growing season, which in most cases were torrential in nature (Figure 3).

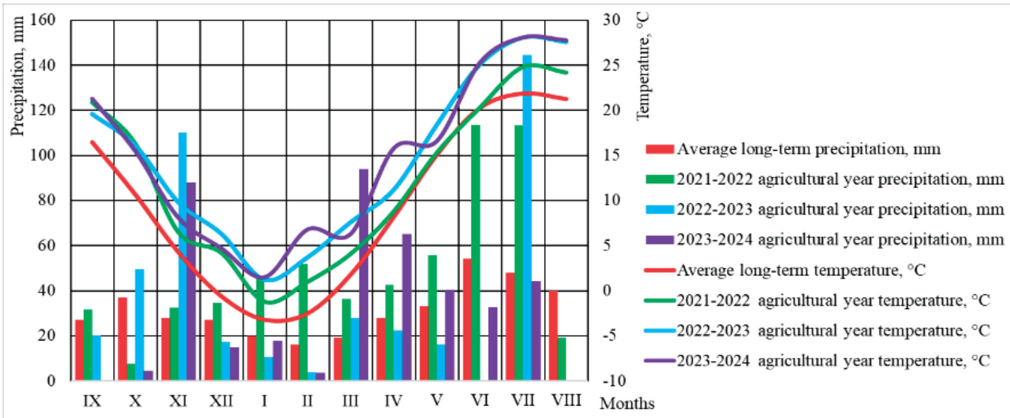

Figure 3. Monitoring of weather conditions over the research years

During 3 years of research, observations of the dynamics of the height of amaranth plants showed that the Liera amaranth variety, due to its genetic characteristics, was noticeably taller than the Kharkivs`kyi 1 variety (Table 1).

In the phase of full ripeness, at a post-emergence plant density of $120 \cdot 10^3$ plants/ha, amaranth plants in the control variant reached a height of 152 cm, while plants of the Liera variety at the same post-emergence plant density had an average height of 158 cm, which is 6 cm higher than the control indicators.

Plants of the Liera variety in the variant with $90 \cdot 10^3$ plants/ha were per 8 cm higher, in the variant with $150 \cdot 10^3$ plants/ha were per 3 cm higher and in the variant with $180 \cdot 10^3$ plants/ha were per 6 cm higher compared to similar indicators of variants with the Kharkivs`kyi 1 variety. However, it is also worth noting that the thickening of amaranth crops to some extent affects the height of the plants.

The highest height indicators were recorded at the highest post-emergence plant density of $180 \cdot 10^3$ plants/ha. For the Kharkivs`kyi 1 variety, the height of plants in the panicle formation phase was 86.0 cm, in the flowering phase it was 152.0 cm, and in the phase of full grain ripeness it was 164.0 cm. For the control variant with a post-emergence plant density of $120 \cdot 10^3$ plants/ha, these indicators were lower and were 80.0 cm, 147.0 cm and 152.0 cm, respectively, which were per 6.0, 5.0 and 12.0 cm less. At the same time, the lowest plant height was recorded at the lowest planting density of $90 \cdot 10^3$ plants/ha.

Table 1. Change in the height of amaranth plants during the growing season depending on the planting density

average for 2022-2024Variant of the experiment		Height of amaranth plants, cm					
Factor A, amaranth variety	Factor B, post-emergence plant density, plants/ha	Phase of panicle formation	Flowering phase	Grain full ripeness phase	Phase of panicle formation	Flowering phase	Grain full ripeness phase
					Average by factor B		
Kharkivs\`kyi 1	$90 \cdot 10^3$	75	142	145	76	143	149
	$120 \cdot 10^3$ (control)	80	147	152	84	149	155
	$150 \cdot 10^3$	83	150	161	87	156	163
	$180 \cdot 10^3$	86	152	164	90	159	167
Average by factor A		81	148	156			
Liera	$90 \cdot 10^3$	77	144	153			
	$120 \cdot 10^3$	87	150	158			
	$150 \cdot 10^3$	90	162	164			
	$180 \cdot 10^3$	93	166	170			
Average by factor A		87	156	161			
*LSD_{05}, cm		A=1.989 B=1.216	A=3.951 B=0.783	A=0.762 B=1.111			

*LSD - Least Significant Difference

With an increase in the post-emergence plant density from 90 to $180 \cdot 10^3$ plants/ha, amaranth plants increased in height according to the development phases. For the Kharkivs\`kyi 1 variety, for the period from the panicle formation phase to the flowering phase, the increase in plant height for the post-emergence plant density variants of $90 \cdot 10^3$; $120 \cdot 10^3$; $150 \cdot 10^3$ and $180 \cdot 10^3$ plants/ha was 67 cm, 67 cm, 67 cm and 66 cm, respectively. For the period from the flowering phase to the phase of full grain ripeness, these indicators were 3 cm, 5 cm, 11 cm and 12 cm, respectively. At the same time, for the Liera variety, for the period from the panicle formation phase to the flowering phase, the increase in plant height for the post-emergence plant density variants of $90 \cdot 10^3$; $120 \cdot 10^3$; $150 \cdot 10^3$ and $180 \cdot 10^3$ plants/ha was 67 cm, 63 cm, 72 cm and 73 cm, respectively. For the period from the flowering phase to the phase of full grain ripeness, these indicators were respectively at the level of 9 cm, 8 cm, 2 cm and 4 cm.

That is, for the Kharkivs\`kyi 1 variety, regardless of the planting density, the increase in plant height was stable (66-67 cm) until the flowering phase, and from the flowering phase to full maturity phase this indicator increased slightly with thickening of the crops. For the Liera variety with thickening of the crops, this indicator increased from the panicle formation phase to the flowering phase, and from the flowering phase to the full maturity phase, on the contrary, it decreased.

During the growing season, the thickness of the stems of amaranth plants increases. However, the post-emergence plant density also has a certain effect on the thickness of the stems of amaranth plants (Table 2).

The greatest thickness of the stems of amaranth plants of both varieties was observed at a post-emergence plant density of $90 \cdot 10^3$ plants/ha. For the Kharkivs\`kyi 1 variety, the thickness of the plant stems varied according to the development phases within the range from 2.5 to 3.0 cm. For the Liera variety, this indicator varied from 2.8 to 3.2 cm depending on the post-emergence plant density.

The smallest stem thickness was recorded at a post-emergence plant density of $180 \cdot 10^3$ plants/ha. For the Kharkivs\`kyi 1 variety, it ranged from 2.0 (panicle formation phase) to 2.3 cm (full ripeness phase), and for the Liera variety, these indicators ranged from 2.1 to 2.7 cm.

Other options for post-emergence plant density for both varieties, which were studied in the experiment by the indicator of stem thickness, had an intermediate value. This can be explained by the fact that when the crops are thickened, the plants spend a significant amount of energy on increasing height, competing with each other for the receipt of light energy and were unable to form a thick and strong stem.

Table 2. Dynamics of changes in the stem thickness of amaranth plants during the growing season depending on the planting density, average for 2022-2024

Variant of the experiment		Stem thickness of amaranth plants, cm					
Factor A, amaranth variety	Factor B, post-emergence plant density, plants/ha	Phase of panicle formation	Flowering phase	Grain full ripeness phase	Phase of panicle formation	Flowering phase	Grain full ripeness phase
					Average by factor B		
Kharkivs\`kyi 1	$90 \cdot 10^3$	2.5	2.7	3.0	2.7	2.9	3.1
	$120 \cdot 10^3$ (control)	2.4	2.7	2.8	2.5	2.7	2.9
	$150 \cdot 10^3$	2.3	2.5	2.5	2.2	2.3	2.7
	$180 \cdot 10^3$	2.0	2.1	2.3	2.1	2.2	2.5
Average by factor A		2.3	2.5	2.7			
Liera	$90 \cdot 10^3$	2.8	3.0	3.2			
	$120 \cdot 10^3$	2.5	2.6	3.0			
	$150 \cdot 10^3$	2.1	2.5	2.8			
	$180 \cdot 10^3$	2.1	2.3	2.7			
Average by factor A		2.4	2.6	2.9			
*LSD_{05}, cm		A=0.065 B=0.129	A=0.744 B=0.549	A=0.065 B=0.116			

*LSD - Least Significant Difference

From literary sources it is known that the rate of leaf formation, the total area of the photosynthetic surface and its productivity have a significant impact on yield, since 95% of the dry matter of the crop is formed from organic compounds.

The process of leaf formation in both varieties of amaranth occurs before the flowering phase, which accounts for the largest number of leaves per plant for all variants of post-emergence plant density in the experiment (Table 3). At the same time, plants of the Liera variety were characterized by greater leaf mass ratio (LMR) and at the peak of leaf formation this indicator was 0.267-0.281 (g/g) depending on the post-emergence plant density, while in the Kharkivs\`kyi 1 variety it was only 0.232-0.253 (g/g).

Table 3. Change in leaf mass ratio of amaranth plants during the growing season depending on crop density, average for 2022-2024

Variant of the experiment		Leaf mass ratio, g/g					
Factor A, amaranth variety	Factor B, post-emergence plant density, plants/ha	Phase of panicle formation	Flowering phase	Grain formation phase	Phase of panicle formation	Flowering phase	Grain formation phase
					Average by factor B		
Kharkivs\`kyi 1	$90 \cdot 10^3$	0.155	0.246	0.190	0.155	0.260	0.208
	$120 \cdot 10^3$ (control)	0.163	0.249	0.190	0.163	0.263	0.211
	$150 \cdot 10^3$	0.169	0.253	0.197	0.169	0.267	0.215
	$180 \cdot 10^3$	0.141	0.232	0.183	0.134	0.249	0.201
Average by factor A		0.157	0.245	0.190			
Liera	$90 \cdot 10^3$	0.155	0.274	0.225			
	$120 \cdot 10^3$	0.162	0.277	0.232			
	$150 \cdot 10^3$	0.169	0.281	0.232			
	$180 \cdot 10^3$	0.127	0.267	0.218			
Average by factor A		0.153	0.275	0.227			
*LSD_{05}, cm		A=0.00234 B=0.00113	A=0.00065 B=0.00093	A=0.00300 B=0.00105			

*LSD - Least Significant Difference

In addition, as the conducted studies have shown, with an increase in the amaranth post-emergence plant density from $90 \cdot 10^3$ to $150 \cdot 10^3$ plants/ha, the LMR during the growing season initially increases, but at the highest post-emergence plant density ($180 \cdot 10^3$ plants/ha) it begins to decrease. This indicates that to achieve maximum LMR, the optimal planting density was $150 \cdot 10^3$ plants/ha for both of varieties.

As the analysis of variance result, the share of the factors influence on the amaranth plants growth processes was determined (Figure 4). It was established that the greatest influence (71.4%) on the growth processes of amaranth plants is exerted by factor B (post-emergence plant density).

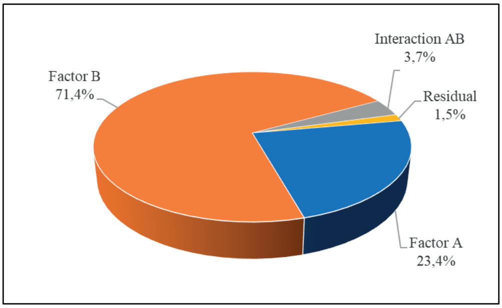

Figure 4. The share of influence of factors on the growth processes of amaranth plants

It was established that there is a weak correlation between the height of amaranth plants and Leaf mass ratio (Figure 5).

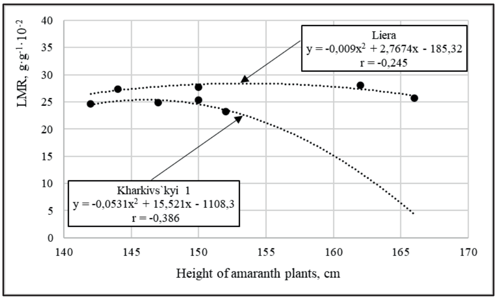

Figure 5. Correlation-regression models of the dependence of the LMR and height of amaranth plants in the flowering phase

The correlation coefficient between the height of amaranth plants and their leafiness for the Kharkivs`kyi 1 variety was r = -0.386, and for the Liera variety it was r = -0.245. Analysis of correlation-regression models shows that with an increase of the planting density to $150 \cdot 10^3$ plants/ha, the plants height increased with a simultaneous LMR increase. With further thickening of crops to $180 \cdot 10^3$ plants/ha, amaranth plants begin to compete with each other for the main factors of plant life, including the factor of light energy, which leads to an increase in plant height and simultaneous drying of the leaf's lower tiers.

CONCLUSIONS

Over the years of research, observations of the dynamics of amaranth plant height showed that the Liera amaranth variety was noticeably taller than the Kharkivs`kyi 1 variety. Thus, in the Kharkivs`kyi 1 variety in the phase of full ripeness, at a post-emergence plant density of $120 \cdot 10^3$ plants/ha (control), amaranth plants reached a height of 152 cm, while plants of the Liera variety at the same post-emergence plant density had an average height of 158 cm, which was per 6 cm higher.

The highest indicators of amaranth plant height were recorded at the highest post-emergence plant density ($180 \cdot 10^3$ plants/ha). Thus, for the Kharkivs`kyi 1 variety, the plant height in the panicle formation phase was 86.0 cm, in the flowering phase it was 152.0 cm, and in the phase of full grain ripeness it was 164.0 cm, while for the Liera variety, in the same phases the plants were higher by 7 cm 14 cm and 6 cm, respectively. At the same time, the lowest plant height for both varieties were recorded at the lowest ($90 \cdot 10^3$ plants/ha) post-emergence plant density of amaranth plants.

The greatest stem thickness was observed at a post-emergence plant density of $90 \cdot 10^3$ plants/ha and for the Kharkivs`kyi 1 variety it varied from 2.5 to 3.0 cm according to the development phases, and for the Liera variety this indicator ranged from 2.8 to 3.2 cm. The smallest stem thickness was recorded for both varieties at post-emergence plant density of $180 \cdot 10^3$ plants/ha and for the Kharkivs`kyi 1 variety it was in the range from 2.0 to 2.3 cm, and for the Liera variety it was in the range from 2.1 to 2.7 cm according to the development phases.

The greatest values of LMR were at a seeding density of $150 \cdot 10^3$ plants/ha. For the Kharkivs`kyi 1 variety, this indicator was 0.197 g/g, and for the Liera variety it was 0.232 g/g. To ensure the best conditions for plant growth and development, the optimal seeding density is $150 \cdot 10^3$ plants/ha.

The obtained results contribute to the optimization of the amaranth sowing complex in the Southern Steppe of Ukraine, which allows obtaining high permanent yields of this crop.

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THE BEHAVIOR OF ROMANIAN WINTER WHEAT BREEDING LINES AND VARIETIES IN TRANSYLVANIAN PLAIN CONDITIONS

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Abstract

Genotype selection is a key requirement for high yields in wheat crop management; higher yields can only be realized with good genetic potential. The performance of different varieties is also influenced by the environment, and it is important to understand that no genotype is perfect in all growing areas. To assess the behavior of 24 winter wheat genotypes regarding their germination energy, germination capacity, thousand kernel weight, grain yield, grain protein content and test weight, a study was conducted over two consecutive growing seasons at ARDS Turda, under two different amount of nitrogen fertilizer applied, and it has been investigated whether there is any connection between each of these traits. The experimental factors and their interactions had a different influence on the studied traits. The growing season and the nitrogen fertilization had a highly significant effect on yield, protein content and test weight. The genotype had a highly significant effect on all the studied traits. Thousand kernel weight in comparison with the other traits studied was strongly influenced by the genotype and less by the growing season and nitrogen fertilization.

Key words: *germination, protein content, thousand kernel weight, winter wheat, yield.*

INTRODUCTION

Winter wheat is a vital crop that feeds billions of people worldwide and contributes to about 21% of global food production (Cao et al., 2024). According to Romanian National Institute of Statistics cereals were also the most important crop in the European Union in 2023, wheat represented 47.2% of the total area cultivated with cereals, with barley coming in second at 20.4% and maize in third, at 16.5% of the total. The importance of winter wheat in Romania’s agriculture is given by the considerable percentage of 2023's total cereal production that went to wheat (46.3%), followed by maize (42.1%) and barley (9.6%). In 2023, Romania produced 9624.1 thousand tons of wheat, which was more than the 8684.2 thousand tons produced in 2022 (Brodeală et al., 2024; insse.ro).

The fundamental process by which plant species develop from a single seed into a plant is known as seed germination. It determines the effective use of water and fertilizer resources and affects crop quality as well as yield. (Xue et al., 2021). In agriculture the use of healthy, viable, and high-quality seeds is essential for maintaining a crop's ideal plant density. Indicators of seed vitality (germination energy and germination capacity) play a direct role in determining an optimal plant number per hectare, which is one of the three main components of yield (Mrđa et al., 2011). Germination comprises all the physiological and biochemical changes that take place in the seed during the transitions from dormant to active life. Germination evaluation in the laboratory indicates the embryo's capacity to develop into a normal plant when the field conditions are suitable. For this there are two factors needed to determine seed germination: germination energy and germination capacity. Germination energy (%) represents the speed at which the seeds placed under germination conditions initiates the germination process. Germination capacity (%) it is the ability of seeds to germinate in a limited number of days set for each species (winter wheat 8 days). The germination percentage (%) of the seed is a particularly important analysis for the seeds intended to be sown, given that the germination value is necessary for the Seed Rate calculation (Duda et al., 2003).

It is well known that nitrogen is an essential component for winter wheat to grow properly, it is one of the key elements to increase the yield and quality of winter wheat (Hamani et al., 2024). It is a crucial component of all proteins and amino acids, as well as hormones, enzymes, chlorophyll, and nucleic acids. In addition to having a significant impact on the protein content, a reasonable application of nitrogen fertilizer can promote the growth and development of winter wheat roots, stems, leaves, and other vegetative organs. Excessive application of chemical fertilizers on farmland will cause serious soil and groundwater pollution. The increase in the use of chemical fertilizers and the pollution caused by excessive use of chemical fertilizers to the soil and water sources has attracted more and more attention (Pacifico et al., 2024). Therefore, the application of fertilizers must be aimed at increasing crop yield, improving crop nutrient utilization efficiency, and reducing the impact on soil and environment (Kubar et al., 2021). Chemical fertilizers must be applied wisely in accordance with the crop growth and development requirements; they cannot be utilized excessively. Increases in nitrogen rates will result in higher crop yields when fertilization level fall within a certain range (Ju et al., 2009). Reasonable application of nitrogen fertilizer can promote the yield and quality of winter wheat. A suitable quantity of nitrogen can help winter wheat roots, stems, and leaves grow and development; increase the green area of plants; increase photosynthesis and nutrient accumulation; promote tillering; and support in the growth and development of reproductive organs (Kubar et al., 2021).

Environmental conditions have a considerable effect on protein content and yield, with both being managed through N application rates and timing (Burton et al., 2024). Wheat yield is also strongly influenced by the agronomic methods, and different wheat varieties used (Chen W. et al., 2024).

Protein is the main component of wheat grains, and its content is closely related to wheat quality (Shewry & Hey, 2015). Protein is the most essential component of the wheat grain and influences its nutritional value as well as its baking attributes (Muntean et al., 2014). Frequently the protein content is use to determine the baking quality, although protein composition is genetically determined, their proportion is strongly influenced by agronomic conditions (Peigné et al., 2014). Protein synthesis in wheat grains is regulated by the nitrogenous substances stored in the reproductive organs before flowering, as well as by nitrogen absorption and redistribution capacity after flowering. Additionally, the nitrogen compounds stored in the stem sheath and leaves before flowering are transported to the grains after flowering (Cao et al., 2024). Aside from protein content, test weight (TW) and thousand kernel weight (TKW) serve as fundamental quality attributes for baking and milling. These grain attributes are crucial indicators of the quality of wheat (Ingver et al., 2024). Wheat genotypes seeds with test weight values above 80 kg hl^{-1} show very good grain filling capacities and indicates their high quality (Tabără et al., 2008).

MATERIALS AND METHODS

This study was conducted to assess the behaviour of 24 winter wheat genotypes regarding germination energy (GE), germination (G), grain yield (GY), thousand kernel weight (TKW) grain protein content (GPC) and test weight (TW) over two consecutive growing seasons (2022-2023 and 2023-2024) under two different amount of nitrogen fertilizer applied, and it has been investigated whether there is any connection between each of these traits.

The genotypes chosen for this experiment were developed at NARDI Fundulea (12), ARDS Lovrin (1), ARDS Turda (10), and an old Russian genotype with very good quality that was mainly used as a quality control.

Field experiments were carried out at the Agricultural Research and Development Station Turda (46°35’ N; 23°47’E) which is located in the Transylvanian Plain, Romania and the experimental design consists in randomized blocks in six replications. The experiment was established on a typical clay Chernozem soil, typical for the forest steppe encountered over half of the Transylvanian Plain. The agrochemical indexes for this soil type had the following average values: the soil reaction is neutral (pH 6.81-6.84) and the humus content is 3.36-3.73% in the arable layer. The supply soil is good in nitrogen (0.177-0.205%) and rich in potassium content (220-320 ppm), and poor in mobile phosphorus (11-35 ppm).

The climate of the area is continental with 4 distinct seasons. Monthly meteorological variables were recorded from a weather station placed on site. Normal long-period average climatic data, were compared with the two-year trial data. The monthly air temperature and rainfall throughout the two growing seasons and long term are presented in Figure 1. Temperatures during the two years of the experiment were higher than the 65 years average, especially in October 2023, February, April, June and July 2024. The temperature was lower during 2022-2023 growing season, in comparison with 2023-2024 growing seasons, except for January 2023. From all two seasons 2023-2024 was the warmest. Regarding the amount of rainfall, the two experimental years were very different, 2022-2023 vegetation period was mostly rainy, the rainiest month was June, the total amount of rainfall during this period was 457.5 mm. On the other hand, 2023-2024 was mostly dry, the total amount of rainfall during this growing season was 317.6 mm.

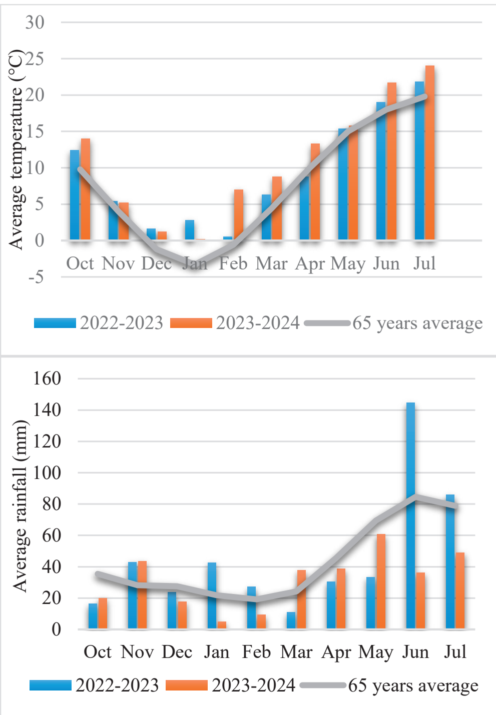

Figure 1. Weather conditions during winter wheat growing season (Oct 1st to July 31st) in Turda, Romania

The 24 winter wheat genotypes including both cultivars and breeding lines were tested over two consecutive growing seasons (2022-2023 and 2023-2024). Winter wheat was sown after pea in a three years crop rotation maize – pea - winter wheat.

The fertilizer amount was 50 kg ha^{-1} active substance of nitrogen applied in autumn before sowing (F1) in all six replications, and another 50 kg ha^{-1} was applied during vegetation at boot stage (F2) in the first three replications.

Winter wheat was sown with the experimental machine, at 2-4 cm depth, at density of 550 grains m^{-2} during the second decade of October. The plot surface area was 6.25 m^{-2}. Herbicides were always applied to the whole experiment during initial wheat growth and were chosen according to the dominant weed species. Glyphosate was applied before crop seeding, because of a very high weed pressure. Fungicide-dressed seeds were used to control seedborne diseases and insecticide were also used according to integrated crop protection principles. The plots were harvested mechanically at maturity independently for each plot, and grain yield GY (kg ha^{-1}) was expressed on a 14% grain moisture basis.

The quantity of seed harvested from each plot was weighed and the production per hectare was calculated for each experimental plot. Sample was taken from the seed harvested and using the Inframatic 9500 Near Infrared grain analyser. In the laboratory TW and TKW was determined.

In order to determine GE and G was carried out in Petri dishes at an ambient temperature of 20-22°C, by placing uniformly 100 seeds in 3 replications for 8 days. On the 4th day GE was determined. On the 8th day G was determined. Both GE and G were determined by counting (Duda et al., 2003).

Analysis of variance (ANOVA) was used to estimate the effects of genotype, year (climatic conditions) and nitrogen fertilization, and their interactions on grain yield and other tested traits. The results were also statistically treated using MS Excel 2012 tools. Correlation was assessed using the Pearson correlation coefficient.

RESULTS AND DISCUSSIONS

The results of ANOVA showed that the experimental factors and their interactions had a different influence on the studied traits (Table 1). The growing season had a highly significant effect on GE, also significantly influenced GY, GPC and TW. Nitrogen fertilization had a highly significant effect on GY, GPC and TW. The genotype had a highly significant effect on all the studied traits. G and TKW in comparison with the other traits studied were strongly influenced by the genotype and less by the growing season and nitrogen fertilization.

As shown in Figure 2 the statistical analysis according to the Duncan test classification, indicates that the 24 genotypes from this study differ from each other regarding GE, and does not demonstrate a significant difference between the genotypes studied regarding G, all the genotypes registered very good G, over 98%, mostly because the experiment's seeds came from the 2022 and 2023 yield. Voinic and Dacic had the best G from all the genotypes, on the other hand Ursita, Pitar and FDL Consecvent had the lowest G from this study. Bezostaia, Andrada and Dacic had the best GE from all the genotypes. Genotypes with good GE were Semnal, Andrada, Cezara, Luminiţa, T 28-19. Genotypes with low GE but good G were Glosa, FLD Miranda, FDL Abund, FDL Columna, FDL Evident, Codru, T 75-16. The only genotypes with low GE were Ursita, FDL Consecvent and FDL Emisar. It is reasonable to assume that a low GE does not always indicate a low G.

Table 1. Analysis of variance (ANOVA) of the effects of growing season (GS), nitrogen fertilization (F) and genotype (G), and their interaction for all studied traits

Experimental factors	DF	GE	G	GY	TKW	GPC	TW
Growing season (GS)	1	424.360***	2.619ns	160.471**	20.436*	102.382**	151.579**
Nitrogen fertilization (F)	1	248.669**	5.255ns	296.737***	21.865*	2033.491***	877.669***
Genotype (G)	23	6.494**	3.294**	75.336***	59.429***	54.692***	99.452***
GSxF	1	165.024**	0.455ns	23.424*	0.090ns	272.744***	34.368*
GSxG	23	1.312ns	3.936**	16.023***	4.457**	10.971**	4.959**
FxG	23	3.661ns	3.192ns	2.482ns	3.516ns	4.127ns	4.071ns
GSxFxG	23	3.254**	3.443**	2.257**	1.761*	1.599ns	3.555**

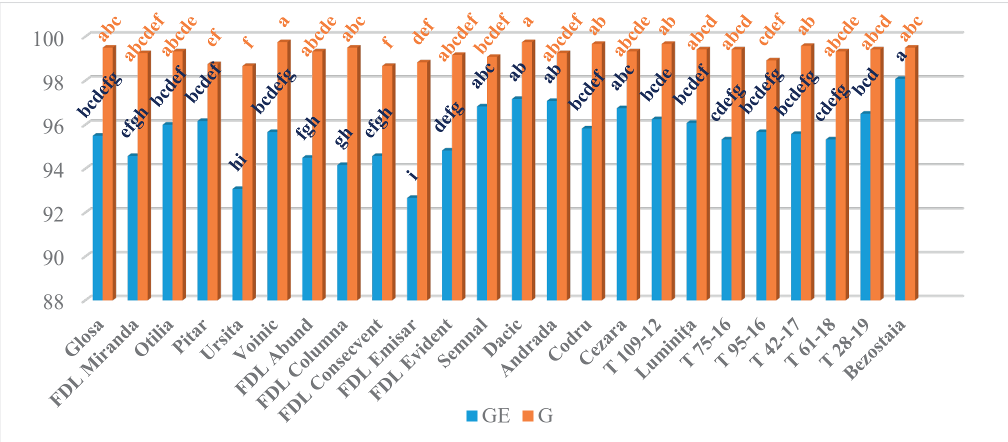

Figure 2. Genotype germination energy (GE) and germination (G) classification according to Duncan test

Between all the traits from this study Pearson correlation was calculated (Table 2), it turns out there's no correlations between GE, G or TKW and the other traits studied. TKW is the one yield component associated with grain quality, higher TKW indicates better germination of wheat grain and better milling quality (Jevtic et al., 2024), however in our study, TKW is positive correlated but not significant with GE (r= 0.156) or G (r= 0.292. There is a high correlation between GPC and TW (r = 0.583**), and a strongly negative correlation between GY and GPC (r -0.569**). This negative correlation has been documented in wheat breeding: higher grain yield is also linked to lower grain protein content. This negative correlation between grain yield and grain protein concentration is often with r values ranging from − 0.13 to − 0.60 (Donaire et al., 2023; Hoang et al., 2024).

Table 2. Pearson Correlation between all studied traits

	GE	G	TKW	GPC	TW	GY
GE	1					
G	0.323	1				
TKW	0.156	0.292	1			
GRP	-0.207	-0.099	-0.396	1		
TW	-0.056	0.050	-0.301	0.583**	1	
GY	-0.335	-0.119	0.113	-0.569**	-0.303	1

We investigated how the 24 genotypes behaved each year in comparison with the genotypes average (Ct.) for each fertilization rate used regarding TKW, GY (Table 3), GPC and TW (Table 4).

According to what other authors have observed, in our experiment N treatment had a significant and positive effect on yield. As total N applied increased, average yields across all years also increased (Burton et al., 2024). In F2 where we applied another 50 kg ha^{-1} nitrogen at boot stage, in comparison with F1 the yield increased in both experimental years. Similar to the effect on crop yield, applying N fertilizer before the beginning of grain filling period had a positive effect on TKW as well. The highest TKW mean was in 2024 F2 (46.75 g) followed by 2024 F1(45.68 g), 2023 F2 (45.58 g) and the lowest TKW were in 2023 F1 (44.60g). Mean GY obtained in 2024 F2 was the highest (9797 kg ha^{-1}), followed by 2024 F1 (9154 kg ha^{-1}), 2023 F2 (8583 kg ha^{-1}), and the lowest in 2023 F1 (7438 kg ha^{-1}) (Table 3). As we can see the environmental conditions and N fertilization had a strong influence on both TKW and GY, in our experiment as well.

Growing season and wheat genotypes significantly affected TKW, this yield component was significantly different between the genotypes, though controlled by the genetic features of a specific genotype TKW perform differently in different conditions during the process of yield formation (Hoang et al., 2024; Jevtic et al., 2024; Chen et al., 2024). The genotypes' response to N fertilization regarding TKW were very different. From of all the genotypes studied Luminiţa and T 75-16 are the only ones who recorded highly significant TKW values in both years in all the N fertilization rates. The varieties and breeding lines developed at ARDS Turda are characterized by a greater TKW, while the genotypes created at NARDI Fundulea are generally characterized by a medium TKW. The lowest values of TKW had Otilia, Voinic, FDL Emisar and FDL Evident in both years in all the N fertilization rates (Table 3).

Genotype it is a significant factor in determining yield, as well the environmental conditions have a considerable effect on yield (Burton et al., 2024; Hoang et al., 2024). The yield of the 24 genotypes in our two-year trial varies from one year to another despite using the same methodology (Lobell et al., 2012; Semenov & Shewry, 2011). From all two seasons 2023-2024 was the warmest, high temperatures and the lack of precipitations at the end of the winter wheat vegetation period determined the speed up of the heading and ripening stages of wheat plants, GY in 2024 was significantly higher, compared to 2023 when April and May were dry and it rained a lot in June and July.

It stands out the genotypes that had a highly significant GY in F1, where no N were applied during vegetation, like FDL Columna, FDL Evident, T 95-16, T 42-17, T 28-19 in 2023, FDL Abund, FDL Consecvent, FDL Emisar, Dacic, Codru, T 109-12, T 75-16, T 95-16, T 42-17, T 61-18 in 2024. This indicates that genes, more than the environment, influence the genotypes production, as is known that wheat productivity is constrained by genetic, agronomic, and climate factors (Vafa et al., 2024; Leonte, 2011)

Certain genotypes, such as Glosa, FDL Miranda, Pitar, Voinic, and FDL Columna, had significantly decreased GY, but this was not because they were less productive; rather, it was because they are less adapted to the experiment's environment, these genotypes being developed at NARDI Fundulea, are more adapted for the southern region of Romania.

The N fertilizer effect on yield is obvious, as other authors observe, but fertilizer application during important crop growth stages could improve yield (Jarecki, 2024; Hamani et al., 2024; Sun et al., 2022). N is the most important crop yield limiting-factor (Pacifico et al., 2024). The highest yield was obtained by Ursita (10592 kg ha $^{-1}$), followed closely by FDL Emisar (10549 kg ha $^{-1}$), and Dacic (10322 kg ha $^{-1}$) in 2024 in F2. The breeding lines created at ARDS Turda like T 75-16 recorded yields over 10000 kg ha $^{-1}$ in 2024 both in F2 and F1 and T 109-12, T 95-16, T 42-17, T 61-18 recorded yield over 10000 kg ha $^{-1}$only in F2. In 2023 in F2 T 75-16 had the highest yield (9293 kg ha $^{-1}$), followed by Cezara (9111 kg ha $^{-1}$), FDL Evident (9094 kg ha $^{-1}$) and Semnal (9005 kg ha $^{-1}$) (Table 3).

Table 3. The influence of the experimental factors on TKW and Grain Yield

Experimental factors	TKW g				Yield kg ha^{-1}			
	2023		2024		2023		2024	
Genotype	F1	F2	F1	F2	F1	F2	F1	F2
Glosa	45.70	48.40**	46.75	46.90	7319	7908^{000}	8094^{000}	8864^{000}
FDL Miranda	45.00	44.75	45.25	45.75	7192	8138^{0}	8717^{0}	9230^{00}
Otilia	41.40^{00}	42.60^{00}	42.50^{00}	43.50^{00}	7560	8546	9295	9975
Pitar	42.95	44.60	46.45	47.65	7590	8603	8661^{0}	9524
Ursita	39.75^{000}	44.30	43.60^{0}	45.70	7251	8506	9321	10592***
Voinic	41.45^{00}	41.70^{000}	41.85^{000}	40.95^{000}	7424	8223	8580^{00}	9549
FDL Abund	42.00^{00}	44.55	45.85	47.40	7242	8635	9601*	10275*
FDL Columna	45.40	48.35**	46.65	49.00*	7851*	8606	9053	9326^{0}
FDL Consecvent	42.25^{0}	44.95	43.25^{0}	45.85	7350	8735	9567*	9922
FDL Emisar	41.95^{00}	40.45^{000}	42.50^{00}	42.50^{000}	7029^{0}	8701	9689**	10549***
FDL Evident	41.25^{000}	42.10^{000}	42.40^{00}	42.75^{000}	8097**	9094*	9080	9761
Semnal	43.40	45.75	43.55^{0}	45.05	7425	9005*	8977	9977
Dacic	47.55**	44.75	47.35	49.95**	7397	8494	9625*	10322**
Andrada	48.55***	49.40***	49.25***	48.00	7248	8479	8957	9773
Codru	49.25***	49.05***	49.00**	49.55**	7479	9111**	9553*	10140
Cezara	42.20^{0}	38.90^{000}	45.20	44.90	7470	7895^{000}	9443	10141
T 109-12	49.40***	50.60***	48.45**	48.40	7620	8549	9806**	10364**
Luminița	49.60***	49.75***	49.05***	49.45**	7119	8849	9269	9958
T 75-16	47.40**	49.45***	49.25***	51.30***	7738	9293***	10162***	10347**
T 95-16	47.55**	50.15***	47.30	50.10**	8186***	9311***	9571*	10104
T 42-17	43.35	42.45^{00}	43.70^{0}	44.80^{0}	7947*	9046*	9912***	10186
T 61-18	42.65^{0}	44.25	44.25	47.65	7284	8710	9874***	10458**
T 28-19	45.55	48.20**	48.40**	49.60**	7979**	8678	8896	9361^{0}
Bezostaia	44.95	44.45	44.45	46.15	5710^{000}	6879^{000}	5985^{000}	6419^{000}
Mean (Ct.)	44.60	45.58	45.68	46.75	7438	8583	9154	9797
	p 5% 1.95; p 1% 2.58; p 0.1% 3.33				p 5% 393.62; p 1% 519.50; p 0.1% 668.96			

GPC is strongly influenced by variety, N fertilization, and year. The environmental conditions like the amount of rainfall during the growing season can play a significant role in determining GPC (Burton et al., 2024). Because there were more precipitations during the 2022-2023 growing season than during the 2023-2024 growing season, in our experiment, the GPC was higher in 2023 than in 2024. Also, the temperatures during winter wheat vegetation period, especially during the grain filling period, strongly influenced GPC. In our two experimental years as we can see in Table 4, during 2023-2024 growing season the temperatures were higher in comparison with 2022-2023 growing season, the values of GPC were lower (mean GPC in 2024 was 10.69%, and in 2023 was 11.94% both in F2). This could be connected to the impact of high temperatures on reducing the duration of dry matter accumulation, shortening the grain-filling period, and finally reducing GPC (Hoang et al., 2024). Recent evidence has indicated that an increase in temperature has led to a serious deterioration in wheat grain quality, when the temperature exceeded 32ºC, the winter wheat experienced heat stress, leading to a decrease in the grain protein content (Cao et al., 2024).

The nitrogen fertilization effect on GPC and on TW is evident (Table 4), Nitrogen is necessary for the growth of all crops, but especially winter wheat requires N for storing proteins in the grains (Pacifico et al., 2024). With the reduction in the nitrogen fertilizer application rate, the GPC decreased, as other authors have noticed (Cao et al., 2024) in both years in F1 GPC values were lower than in F2. Therefore, these genotypes necessarily require nitrogen fertilization during vegetation, even in amounts greater than 50 kg ha^{-1} as used in this experiment, in order to obtain a good percentage of GPC, supplementary application of Nitrogen at heading or anthesis, is generally recognized as beneficial for increasing grain protein content (Hamani et al., 2024). In 2023, the best GPC had FDL Columna (12.90), followed by FDL Emisar, Bezostaia (12.80), and Pitar (12.70); on the other hand, in 2024, Bezostaia had the best value of GPC (12.17), followed by FDL Columna (11.93), Glosa (11.67), Pitar, and Voinic (both with 11.30).

Table 4. The influence of the experimental factors on Grain protein content and Test weight

	Protein Content, %				Test weight, kg hl^{-1}			
	2023		2024		2023		2024	
Genotype	F1	F2	F1	F2	F1	F2	F1	F2
Glosa	9.30	12.50**	10.20***	11.67***	79.60**	81.20*	77.37	78.40
FDL Miranda	8.50^{000}	11.30^{00}	9.13	10.47	76.40^{000}	79.20^{000}	75.33^{000}	76.47^{000}
Otilia	9.30	12.43*	9.73	11.27**	79.50**	81.60***	78.13***	79.10**
Pitar	9.20	12.70***	9.90*	11.30**	78.10	80.40	76.73	77.80
Ursita	9.40	12.10	9.70	11.07	80.20***	81.60***	78.50***	79.73***
Voinic	9.47	12.50**	10.07**	11.30**	80.40***	82.00***	78.27***	79.73***
FDL Abund	10.10***	12.33	9.73	10.70	78.80	80.20	76.97	77.63
FDL Columna	9.73*	12.90***	10.23***	11.93***	79.80***	80.40	76.40	78.00
FDL Consecvent	10.00***	12.43*	9.37	10.80	78.20	80.40	76.57	77.77
FDL Emisar	10.40***	12.80***	9.37	10.17^{0}	78.50	79.60^{0}	76.57	77.83
FDL Evident	8.80^{0}	11.97	9.50	10.70	79.70***	80.00	77.07	77.43
Semnal	9.03	12.07	9.33	10.63	77.60^{0}	80.80	76.43	77.50
Dacic	8.60^{00}	10.67^{000}	8.73^{000}	9.90^{000}	75.00^{000}	76.80^{000}	74.13^{000}	75.70^{000}
Andrada	9.50	11.83	8.57^{000}	9.90^{000}	78.30	80.80	77.57*	79.10**
Codru	9.00	11.20^{000}	8.57^{000}	9.80^{000}	76.90^{000}	79.60^{0}	75.50^{000}	77.17^{0}
Cezara	8.90	11.20^{000}	9.13	10.00^{000}	80.20***	80.80	76.73	78.80*
T 109-12	9.00	11.70	9.33	10.13^{00}	80.60***	81.60***	77.63**	78.97**
Luminiţa	9.43	11.60	8.77^{00}	10.03^{00}	78.60	80.80	77.37	79.00**
T 75-16	8.60^{00}	11.30^{00}	9.30	10.43	78.60	80.80	77.27	78.80*
T 95-16	8.20^{000}	10.90^{000}	8.63^{000}	10.03^{00}	75.80^{000}	77.60^{000}	73.30^{000}	74.80^{000}
T 42-17	9.03	11.50^{0}	9.47	10.17^{0}	78.90	80.40	76.47	77.47
T 61-18	9.37	11.70	9.40	10.57	76.60^{000}	80.50	76.57	78.07
T 28-19	9.03	12.20	9.90*	11.37***	77.10^{000}	80.20	76.77	77.80
Bezostaia	10.13***	12.80***	10.47***	12.17***	79.90***	81.30**	77.97***	79.27***
Mean (Ct.)	9.25	11.94	9.44	10.69	78.47	80.36	76.73	78.00
	p 5% 0.40; p 1% 0.53; p 0.1% 0.68				p 5% 0.68; p 1% 0.90; p 0.1% 1.16			

Aside from protein content, TW acts as an essential attribute of quality for baking and milling. Because of the high correlation between GPC and TW (Table 2), TW values were higher in 2023 when GPC increased, and in 2024, TW values were lower when GPC decreased. Similar to the effect of Nitrogen fertilization on GPC, TW values were higher in F2 in comparison with F1. In 2023 best TW values had Voinic (82.0), Otilia, Ursita, T 109-12 (81.60), Bezostaia (81.30) and Glosa (81.20). In 2024 Ursita and Voinic had the best TW value (79.73), followed by Bezostaia (79.27), Otilia, Andrada (79.10) and Luminiţa (79.0) (Table 4).

CONCLUSIONS

The 24 genotypes from this study differ from each other regarding GE, and does not demonstrate a significant difference between the genotypes studied regarding G, all the genotypes registered very good G. It is reasonable to assume that a low GE does not always indicate a low G.

Growing season and nitrogen fertilization highly influenced GY and GPC. As total Nitrogen applied increased, average yields across both years also increased.

Genotype it is a significant factor in determining yield. The yield of the 24 genotypes in our two-year trial varies from one year to another despite using the same methodology.

A negative correlation has been documented in wheat breeding: higher GY is also linked to lower GPC; in 2023, when the yield was low, the GPC was high, and in 2024, when we obtained the highest yields, the GPC was low.

Growing season and wheat genotypes significantly affected TKW, this yield component was significantly different between the genotypes, though controlled by the genetic features of a specific genotype TKW perform differently in different conditions during the process of yield formation.

The nitrogen fertilization effect on GPC and on TW is evident. With the reduction in the nitrogen fertilizer application rate, the GPC decreased, in both years in F1 GPC values were lower than in F2. Therefore, the genotypes from this study necessarily require nitrogen fertilization during vegetation, even in amounts greater than 50 kg ha^{-1} as used in this experiment, in order to obtain a good percentage of GPC and a high TW value.

The genetic potential when it comes to grain yield of the new varieties and breeding lines is evident in comparison with the Bezostaia variety. Thus, showing the genetic progress of the new varieties and breeding lines. However, in terms of grain quality, some varieties like FDL Columna, FDL Emisar, Glosa have a GPC that are comparable to those recorded by Bezostaia.

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EXPLORING SPRING BARLEY GENOTYPE X ENVIRONMENT INTERACTION IN THE SOUTH-EAST REGION OF ROMANIA

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Abstract

The main objective of this study was to evaluate genotype-by-environment interactions in a spring barley germplasm panel with different geographic provenance and to identify useful germplasm that can be exploited in the barley breeding program. Spring barley genotypes usually show wide variation under climatic conditions in the south-east of Romania, which negatively affects agronomical traits. During the 2021-2023 period, at National Agricultural Research and Development Institute (NARDI) Fundulea, a spring barley panel was tested under three different environments, and data of heading (DH), data of flowering (DF), plant height (PLH), yield (Y), one thousand kernels weight (TKW), protein (P) and starch (S) content were determined. The significant genotype × environment interaction on the traits showed different responses of the genotypes across the testing environments, offering the possibility of identifying some genotypes of interest for future crosses and describing the genetic resources for stakeholders.

Key words: *spring barley, germplasm, traits, environment.*

INTRODUCTION

Barley is an ideal model for understanding the response to climate change (Dawson et al., 2015).

Typically, spring barley varieties serve as a high-quality source for malting and distillation, which is the main reason why they are intensively studied (Schreiber et al., 2024).

The most well-known aspect is that spring barley varieties have different behaviour and yield under various growing conditions due to genotype-environment-technology interactions (Leistrumaitė and Razbadauskienė, 2008).

Under Romanian growing conditions, spring barley usually reaches maturity in 90-100 days (Vătămanu, 2013) compared with winter barley, which has a different period of vegetation based on geographical location (winter barley reached maturity between 250 and 280 days in Banat and the Danube Plain; between 270 and 280 days in Moldova and the Transylvanian Plain). The growing zone of spring barley is divided into three areas: very favourable, favourable, and less favourable. The degree of favourability for the spring barley growing is determined first of all by the temperatures and precipitation falling in the period March-July, and secondly by the soil characteristics. The very favourable areas include the Bârsa Land, the Sf. Gheorghe and Târgu Secuiesc depressions, the Olt, Someş and Mureş depressions, and the Suceava Plateau.

The favourable area includes the Crişuri valleys, parts of the Someşului Plateau, the Siret Valley, and some pre-Carpathian hilly lands of Moldova. Less favourable for spring barley are the areas with a pronounced continental climate in Moldova and Muntenia, the lands with acidic soils, hardly permeable to water, in the area of the Piedmont Hills in the west of the country (Vătămanu, 2013), as well as the light, sandy soils with a reduced water retention capacity.

According to this ecological map of the growing-favourability zone, spring barley should be cultivated only in Transylvania, Banat, and Northern Moldova. Only in the climatic conditions of these regions (humid and cool) can a barley grain meet the requirements of malt and breweries (Vătămanu, 2018). Spring barley genotypes usually show wide variation in agronomic traits, especially low yield levels under climatic conditions in the South-Eastern part of Romania, and these conditions negatively affect some grain quality traits (one thousand kernel weight, protein and starch content).

This study aims to explore spring barley genotype x environment interaction in the South East region of Romania, at the National Agricultural Research and Development Institute (NARDI) Fundulea, and identify the best cultivar for future introduction as parents in the breeding programme based on the traits which contribute to agronomic performance (heading data, flowering data, plant height, yield, and protein and starch content).

MATERIALS AND METHODS

During the period 2021-2023, under three different climatic environments, a spring barley panel comprising 48 varieties (Table 1), with various provenances and part of the European project AGENT, was tested at NARDI Fundulea in South-Eastern Romania.

The panel was evaluated under three distinct environmental conditions, with differing rainfall and temperatures each year during the growing season (March to July).

The traits measured on tested genotypes and observed in two replications, had included: heading (DH), expressed in days from sowing; flowering (FD), in days from heading to flowering; plant height (PLH), in centimetres (measured from the soil to the tip of the spike without awns); yield (Y), reported in kg/ha; one thousand kernels weight (TKW), in grams; and protein (P) and starch content (S), expressed in percentage.

The first three phonological traits (DH, FD, and PLH) were assessed in the experimental field at three stages: BBCH 60 (heading data), BBCH 65 (flowering data), and BBCH 70 (plant height) (https://www.julius-kuehn.de/en/jki-publication-series/bbch-scale/).

Yield was calculated in kilograms per hectare (kg/ha) after harvest, and the seed quality parameter, one thousand kernels weight (TKW), was determined by averaging two samples of 500 seeds each, counted with the Contador instrument and weighed on an electronic balance to two decimal places.

Table 1. Tested spring barley panel genotypes and sample geographical provenance

No.	Variety	Seeds provenance
1	Accordine	Czech Republic
2	AF Cesar	Czech Republic
3	Aligator	Czech Republic
4	Bojos	Czech Republic
5	Francin	Czech Republic
6	Spitfire	Czech Republic
7	Avalon	Germany
8	Barke	Germany
9	Bowman	Germany
10	Ditta	Germany
11	Golden Promise	Germany
12	Optic	Germany
13	Quench	Germany
14	Solist	Germany
15	Steptoe	Germany
16	Zeisig	Germany
17	Conchita	Hungary
18	Malz	Hungary
19	GK-Toma	Hungary
20	GK-Habzo	Hungary
21	Concerto	Hungary
22	Ma’anit- 6 row	Israel
23	Noga-2 rows	Israel
24	Alastro	Italy
25	LG Aragona	Italy
26	Pariglia	Italy
27	Chifaa	Morroco
28	Compass	Morroco
29	Rihane-03	Morroco
30	Taffa	Morroco
31	V Morales	Morroco
32	Applaus	Netherland
33	KWS Irina	Netherland
34	Avatar	Poland
35	Radek	Poland
36	Oberek	Poland
37	Suweren	Poland
38	Fandaga	Romania
39	Daciana	Romania
40	Kangoo	Romania
41	Romanita	Romania
42	Overture	Romania
43	Tunika	Romania
44	IS Maltea	Slovakia
45	IS Maltigo	Slovakia
46	IS Perlina	Slovakia
47	Karmel	Slovakia
48	PS-1/PS Krupko	Slovakia

Additionally, the protein and starch contents were determined using the INFRATECH 1241 (NIR instrument) using a 500g seed sample per replication.

Climatic data were collected and provided by NARDI Fundulea meteorological station

(minimum, maximum, and mean temperatures and daily rainfall were registered).
These parameters varied during the tested period (2021-2023), and the data are very suggestive compared with the 60-year average for monthly average temperature and rainfall (Tables 2 and 3).

Table 2. Monthly average temperatures during the 2021-2023 period and the 60-year average

Year/Month	2021	2022	2023	60 years average
January	1.6	2.1	4.9	-2.4
February	3.2	4.7	3.3	-0.4
March	5.1	4.4	8.2	4.9
April	9.7	12.1	10.8	11.3
May	17.2	17.9	16.9	17.0
June	21.1	22.6	22.3	20.8
July	25.3	25.0	26.1	22.7

Comparing the 60-year average temperature with the monthly average for each year, it can be observed that the level was higher in June and July, ranging from 0.3°C to 1.8°C (Table 2).
Rainfall was unevenly distributed, with a very large amount recorded only in June 2021 (135 mm), compared to the multi-annual average (Table 3).

Table 3. Monthly average rainfall during the 2021-2023 period and the 60-year average

Year/Month	2021	2022	2023	60 years average
January	77.0	4.8	64.2	35.1
February	16.2	5.4	5.8	32.0
March	59.0	12.3	10.0	37.4
April	31.0	47.6	77.2	45.1
May	57.6	30.1	32.4	62.5
June	135.0	59.6	40.2	74.9
July	21.2	29.2	43.8	71.1

The collected experimental results were analysed statistically using a combined ANOVA to evaluate the significance of year (Y), varieties (V), and their interaction (Y x V). Pearson's correlation coefficients were calculated to examine the relationships among the measured traits with the OPSTAT online software (www.opstat.pythonanywhere.com).
The main aim of this study was to evaluate the genotype-by-environment interaction in a spring barley germplasm panel with diverse geographic origins (or provenance) and to identify valuable germplasm that can be utilised in the barley breeding programme.

RESULTS AND DISCUSSIONS

Analyses of variance revealed the influence of year (Y) and the interaction between year and varieties (Y x V) on all the studied traits. In contrast, varieties significantly influenced heading data (HD) and plant height (PLH). The variety as a source of variation did not affect the flowering data (FD) (Table 4).

Table 4. Heading data, flowering data, and plant height combined analysis of variance for pooled data (LSD for analysed factors and probability significance)

Source of variation	DF	HD	FD	PLH
Year	2	0.00000	0.00000	0.00002
Varieties	47	0.03070	0.07341	0.03730
Year X Varieties	94	0.00000	0.00000	0.00434
LSD (Year)		0.37	0.28	0.50
LSD (Varieties)		9.48	4.88	1.92
LSD (Year X Varieties)		2.61	2.61	2.61

Table 5. Yield, TKW, starch, and protein content combined analysis of variance for pooled data (LSD for analysed factors and probability significance)

Source of variation	DF	Y	TKW	P	S
Year	2	0.00077	0.00001	0.00000	0.00000
Varieties	47	0.00000	0.00001	0.06457	0.00001
Year X Varieties	94	0.00000	0.00006	0.00000	0.00000
LSD (Year)		1.06	0.31	0.83	0.55
LSD (Varieties)		2.37	2.15	3.55	2.50
LSD (Year X Varieties)		2.61	2.61	2.61	2.61

The quantitative and qualitative traits (yield, TKW, and starch content) were also affected by the year, variety, and the interaction between year and variety (Table 5). The variety alone did not influence the protein content data.
Regarding the heading data (HD), as the first phenological trait registered (Table 6), this ranged from 62.5 days (Rihane-03 variety) to 75.7 days (Kangoo variety).
The 13-day difference between the minimum and maximum value showed significant diversity. The flowering data (FD) varied from 69.8 days (Chifaa and Rihane-03 varieties from Morocco) to 79.2 days (Kangoo variety released in Poland).

Table 6. Phenological data observed during the 2021-2023 period (heading data, flowering data, plant height)

No.	Variety	HD (days)	FD (days)	PLH (cm)
1	**Accordine**	66.5	73.7	**77.0**
2	AF Cesar	71.2	74.2	71.8
3	Alastro	67.2	73.8	70.7
4	Aligator	66.7	71.7	71.4
5	Applaus	66.5	71.3	72.9
6	Avalon	67.2	72.2	71.9
7	Avatar	67.7	72.8	73.2
8	Barke	68.7	74.8	**77.1**
9	Fandaga	65.8	72.5	72.2
10	Bojos	67.2	72.3	73.7
11	**Bowman**	**63.7**	71.5	75.7
12	**Chifaa**	64.2	**69.8**	72.4
13	Compass	67.8	73.5	72.9
14	Conchita	67.7	72.7	70.9
15	Daciana	68.0	75.2	74.4
16	Ditta	68.8	75.7	72.2
17	Francin	68.5	74.3	76.2
18	Golden Promise	67.8	73.7	76.5
19	IS Maltea	69.8	73.5	72.7
20	IS Maltigo	68.7	76.0	75.8
21	IS Perlina	69.2	76.0	74.1
22	Concerto	67.8	75.7	75.7
23	Karmel	66.5	72.8	74.4
24	KWS Irina	66.7	73.5	76.9
25	LG Aragona	66.0	75.2	67.8
26	Ma'anit- 6 row	67.8	70.2	**66.6**
27	Malz	69.5	73.0	72.6
28	Noga-2 rows	70.2	76.0	74.5
29	Radek	70.0	74.8	74.4
30	Optic	69.3	76.2	76.1
31	Pariglia	68.7	73.7	74.0
32	**Kangoo**	**75.7**	**79.2**	68.0
33	PS-1/PS Krupko	68.2	74.3	69.9
34	Quench	69.5	75.5	73.0
35	Oberek	68.5	73.8	72.5
36	**Rihane-03**	62.5	**69.8**	72.3
37	Romanita	68.2	75.5	76.5
38	Suweren	67.5	73.0	71.9
39	GK-TOMA	69.0	74.8	73.1
40	Solist	68.0	75.7	75.8
41	Spitfire	65.8	73.8	71.8
42	Steptoe	66.0	72.3	75.4
43	GK-HABZO	64.8	70.3	70.0
44	Taffa	62.7	67.5	**66.4**
45	Overture	70.2	75.0	74.4
46	V Morales	66.5	71.7	72.4
47	Tunika	68.3	71.8	73.7
48	Zeisig	68.8	73.2	71.6
Mean		**67.7**	**73.5**	**73.1**
Min.		**62.5**	**67.5**	**66.4**
Max.		**75.7**	**79.2**	**77.1**

Table 7. Quantitative and qualitative data obtained during the 2021-2023 period (yield, TKW, protein, and starch content)

Variety	Yield (kg/ha)	TKW (g)	P (%)	S (%)
Accordine	**5494.0**	**45.8**	14.2	**62.4**
AF Cesar	4104.7	**41.4**	17.0	60.7
Alastro	4371.2	**40.2**	15.4	**59.2**
Aligator	**6183.2**	**42.8**	14.8	61.4
Applaus	**5008.7**	**37.5**	15.1	61.2
Avalon	4844.2	**43.8**	15.5	60.9
Avatar	4698.2	**43.2**	15.7	60.9
Barke	**5173.8**	**43.9**	15.1	61.0
Fandaga	**5322.2**	**41.4**	14.9	61.3
Bojos	**5351.3**	**42.8**	15.4	61.4
Bowman	**5430.3**	**46.1**	14.4	60.5
Chifaa	**3321.0**	**39.4**	17.1	60.2
Compass	**5417.2**	**48.2**	14.5	61.5
Conchita	4374.7	**45.0**	15.1	61.5
Daciana	3962.0	**39.7**	15.9	61.2
Ditta	4826.3	**43.0**	15.4	61.4
Francin	4795.3	**44.7**	14.3	60.6
Golden Promise	**5461.7**	**40.0**	15.3	61.3
IS Maltea	4941.8	**41.0**	14.6	61.8
IS Maltigo	4730.3	**45.7**	14.7	62.2
IS Perlina	**5246.7**	**42.7**	15.2	61.4
Concerto	**5842.5**	**43.5**	14.1	60.6
Karmel	**5313.7**	**43.0**	15.5	60.9
KWS Irina	**5073.3**	**44.4**	15.1	61.4
LG Aragona	4714.5	**42.6**	14.5	**59.7**
Ma'anit- 6 row	**3295.7**	**40.0**	**17.6**	**58.6**
Malz	**5366.5**	**43.5**	15.1	61.6
Noga-2 rows	4566.8	**44.4**	14.6	61.4
Radek	**5093.5**	**42.3**	14.5	61.2
Optic	4572.8	**44.6**	14.5	60.8
Pariglia	4075.5	**49.8**	16.1	61.0
Kangoo	**2300.5**	**41.9**	**17.3**	**59.5**
PS-1/PS Krupko	**5752.7**	**43.2**	15.0	60.8
Quench	**4873.5**	**48.6**	14.6	60.6
Oberek	**5779.3**	**42.4**	15.0	61.6
Rihane-03	**2951.2**	**41.9**	**20.0**	**60.6**
Romanita	**4698.8**	**43.3**	14.5	61.3
Suweren	**5759.0**	**40.5**	14.6	62.0
GK-TOMA	**5379.7**	**46.9**	13.9	61.5
Solist	**5282.5**	**43.8**	14.2	61.6
Spitfire	**5170.0**	**45.7**	14.3	61.0
Steptoe	**4440.2**	**46.1**	**13.7**	61.0
GK-HABZO	**4454.8**	**44.5**	16.7	**59.9**
Taffa	**3431.8**	**38.8**	15.7	60.0
Overture	**4931.5**	**43.6**	14.6	61.7
V Morales	**5225.0**	**46.6**	15.0	61.4
Tunika	**5544.2**	**45.0**	15.2	61.4
Zeisig	**5042.5**	**44.8**	14.8	61.6
Mean	**4833.1**	**43.4**	**15.2**	**61.0**
Min.	**2300.5**	**37.5**	**13.7**	**58.6**
Max.	**6183.2**	**49.8**	**20.0**	**62.4**

On average, plant height (PLH) ranged from 66.4-66.6 cm (Taffa from Morocco and Ma'anit-6 row from Israel) to 77.1 cm (Barke variety from Germany).
The lowest level of yield as a three-year average (Table 7) was 2300 kg/ha (Kangoo variety), and the maximum was 6183 kg/ha (Aligator variety). This high amplitude suggests promoting future crosses with genotypes that have a high yield under different growing seasons (Aligator, Oberek, Bowman, Compass, Golden Promise, PS Krupko, and Tunika varieties) and a TKW value over 42 g.
The spring barley variety Applaus from the Netherlands registered the smallest value of TKW (37.5 g), and the spring barley variety Pariglia from Italy registered the highest value (49.8 g).

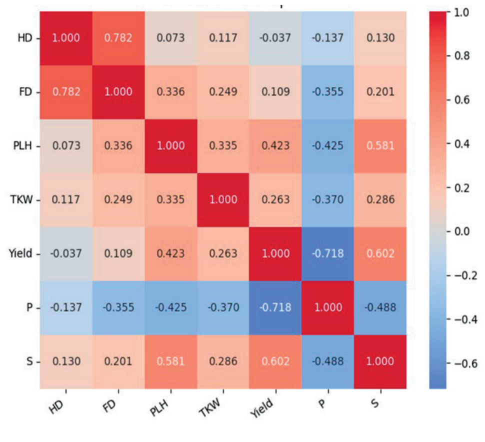

Figure 1. Spring barley traits correlation heatmap

According to the Pearson correlation heatmap (Figure 1), the variable Yield had the strongest negative correlation (-0.718) with P and showed significant correlations with the variables S and PLH.
The variable HD was significantly correlated with FD, with the highest positive correlation value (0.782). In contrast, it has been observed that HD exhibited non-significant correlations with PLH, TKW, Yield, P, and S variables.
The variable PLH showed the highest positive correlation (0.581) with S and significant correlations with S, P, Yield, FD, and TKW. It has been observed that variable PLH had non-significant correlations with HD variables. The variable TKW showed the strongest negative correlation (-0.370) with P and significant correlations with P, PLH, and S.
The variable Yield had the highest negative correlation (-0.718) with P and significant correlations with variables P, S, and PLH. The variable P showed significant negative correlations with the variables Yield, S, PLH, TKW, and FD. The variable S had the highest positive correlation (0.602) with Yield and significant correlations with variables Y, PLH, P, and TKW (all results are interpreted at the 5% level of significance).

CONCLUSIONS

This study indicated significant effects of year, variety, and V × Y interaction on all the studied agronomic traits, except flowering data and protein content.
The significant effect of the interaction between variety and environment interaction (V x E) on the studied traits revealed different responses of the genotypes across the three testing environments, providing the opportunity to identify some germplasm of interest for future crosses in the breeding programme, especially for yield and TKW, and also to have more agronomical descriptors for these valuable barley genetic resources.
Bowman variety was on average the most precocious (63.7 days) with a reasonable grain yield (5430 kg/ha) besides Accordine, Barke, Compas, Concerto, Oberek, Suweren and Tunika. The highest yield was registered by the Aligator variety (6183 kg/ha), which had a TKW over 42 g and a starch content of 60% (this variety combines three important traits, yield as a criterion for farmers, and two criteria met for the malt and beer industry).
The negative correlation between yield and protein content was maintained, as in the case of winter barley varieties (only Steptoe two-row barley variety registered the lowest average protein content).
One of the most important aspects is that several 19 varieties have cumulatively achieved two of the most important indices: yield of over 5000 kg/ha and a thousand-kernel weight of over 42 g, indicating high yield and large grains under south-east climatic conditions.
Five varieties achieved low yield (under 3500 kg/ha) and a low thousand-kernel weight (under

42 g), and several four varieties recorded yields of over 5000 kg/ha but with a thousand-kernel weight under 42 g.

A good choice for improvement must also be based on the knowledge of another important aspect, namely the type of growth, because the Kangoo variety was the latest in terms of heading date (an 8 days difference compared to the average and almost 13 days difference compared to the most precocious variety), which suggests performing an additional test to determine precisely the growth habit (winter, facultative or spring).

All varieties exceeded the protein content, except the Steptoe variety, which suggests testing it with different nitrogen doses to study its absorption from the soil.

Only 5 varieties recorded a starch content below 60%, which shows that 89.6% of the total maintained their leaves green for a longer time, resulting in good translocation of assimilates to the grains.

The country of origin can explain differences between varieties, as well as differences due to the different parents used in the breeding programmes in which the spring barley variety was tested and released.

The selection of varieties for future crosses, promising in terms of heading data, plant height, yield, thousand-kernel weight, protein and starch content, must be based on their ability to realise their full potential across different environments.

However, the spring barley varieties V Morales released outside Europe (in Morocco), or other spring barley varieties namely Steptoe released in the UK in 1960, Golden Promise and Bowman released in 1973 and 1984, respectively, in the USA, have recorded good agronomic performances, which leads to the deepest research into the background of old and new varieties to discover useful resources in a barley breeding program.

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CHLOROPHYLL CONTENT IN MAIZE HYBRIDS

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Abstract

One of the most important factors determining productivity is the photosynthesis process. The work aimed at to find the chlorophyll content in leaves of maize hybrids from two vegetation groups. Maize hybrids FAO 500-599 (Kneja 560, Kneja 564, Kneja 570A, Kneja 572 and KWS Camillo), and FAO 600-699 (Kneja 648, Kneja 649, Kneja 650A, Kneja 650 and Kneja 683) were tested in field conditions and no irrigation. Chlorophyll 'a' and chlorophyll 'b' were determined in green leaf samples 60 DAS. The total chlorophyll a+b and chlorophyll 'a' to chlorophyll 'b' ratio was calculated. Generally, the values for chlorophyll concentration were found higher for the maize hybrids FAO 600-699 group. The exceeding compared to FAO 500-599 group was for chlorophyll 'a' by 12.53%, for chlorophyll 'b' by 9.38% and for total chlorophyll by 11.47%, respectively. The chlorophyll 'a' to chlorophyll 'b' ratio was relatively similar for the hybrids from two groups. It was concluded the highest total chlorophyll concentration in maize hybrids was found in Kneja 648 (5.39 mg/g) and Kneja 560 (4.49 mg/g).

Key words*: chlorophyll 'a', chlorophyll 'b', maize, total chlorophyll.*

INTRODUCTION

Maize, often called the "Queen of the Field" is the most common fodder crop in Bulgaria and is grown for grain and silage. Maize for silage has a high energy nutritional value and is most preferred in intensive animal husbandry (Ljubičić et al., 2023; Karnatam et al., 2023).
One of the most important factors determining productivity is the photosynthesis process. Chlorophyll a and chlorophyll b are essential components of chlorophyll and thus photosynthesis. The main chlorophyll is chlorophyll a, which ensures a higher efficiency of the process of converting carbon dioxide and water into organic compounds. Both are the main components of chlorophyll and affect the capacity and speed of its photosynthetic activities. Although both are active components, chlorophyll a has significant potential for light binding, energy acquisition and sugar production, especially in photosystem I and photosystem II (Sarieva et al., 2010).
As reported by Yokoya et al. (2007) and Zhao et al. (2016), these photosynthetic pigments are responsible for collecting and transmitting absorbed light to photosynthetic reaction centres, and their concentration is linked to the effectiveness of photosynthesis. In addition, according to Zhao et al. (2016), increased content of these pigments may be one of the factors increasing photosynthetic activity.
The content of photosynthetic pigments is also one of the indicators of the reaction of plants to changes in the environment and the degree of adaptation to new environmental conditions.
The present work aimed at to determine the plastid pigments content in leaves of maize hybrids from two vegetation maturity groups (mid-late, FAO 500-599 and late, FAO 600-699, respectively).

MATERIALS AND METHODS

The experiment was conducted in the field of the Maize Research Institute - Knezha, Bulgaria under non-irrigated conditions with standard cultivation technology of growing of maize. Ten hybrids of maize from two vegetation maturity groups (mid-late, FAO 500-599 and late, FAO 600-699) were tested as follows: i) FAO 500-599: Kneja 560, Kneja 564, Kneja 570A, Kneja 572 and KXC 1395, and ii) FAO 600-699: Kneja 648, Kneja 649, Kneja 650A, Kneja 650 and Kneja 683. The sowing density was 75000 plants/ha.
For analysis to determine the content of plastid pigments, the ear leaf of the hybrids at the time of flowering of the tassel was used for each test variant. The plastid pigments content was

determined in green leaf samples 60 DAS, using the protocol of Bozova et al. (1993). Chlorophyll a, chlorophyll b, chlorophyll a+b, carotenoids, total plastid pigments content [chlorophyll (a+b) + carotenoids], (mg/g FW) was found. The chlorophyll 'a' to chlorophyll 'b' ratio was calculated.

Minimum and maximum values, coefficient of variation (CV%), standard deviation (SD) were determined. Pearson's correlation coefficient (r) was used to determine the strength and direction of the linear relationship between indicators.

RESULTS AND DISCUSSIONS

For the hybrids in the FAO 500-599 group, chlorophyll content ranged between 2.16 and 2.88 mg/g FW for chlorophyll a and between 1.12 and 1.61 mg/g FW for chlorophyll b, respectively (Table 1). The highest chlorophyll a, chlorophyll b and total contents were found for plants of hybrid Kneja 560 (2.88 mg/g FW, 1.61 mg/g FW and 4.49 mg/g FW, respectively). Relating the values to the group average, the chlorophyll content for hybrid Kneja 560 was 9.17% higher for chlorophyll a, 15.66% higher for chlorophyll b and 11.41% higher for chlorophyll a+b. Our results related to chlorophyll a are in agreement with those of Łącka et al. (2021) and for chlorpphyll a and chorophyll b with Vulchinkova et al. (2018).

On average, the highest coefficient of variation was found for carotenoid content (CV% 24.42) for hybrids in the FAO 500-599 group.

Table 1. Plastid pigment content (mg/g FW) in maize hybrids of FAO 500-599 maturity group

Hybrids	Chl a	Chl b	Chl a+b	Carotenoids	Total
Kn 560	2.88	1.61	4.49	0.347	4.837
Kn 564	2.79	1.43	4.22	0.266	4.486
Kn 570A	2.71	1.43	4.14	0.250	4.390
Kn 572	2.16	1.12	3.28	0.204	3.484
KXC1395	2.65	1.37	4.02	0.192	4.212
Max	2.88	1.61	4.49	0.347	4.837
Min	2.16	1.12	3.28	0.192	3.484
Average	2.64	1.39	4.03	0.250	4.282
SD	0.281	0.177	0.453	0.061	0.501
CV%	10.65	12.69	11.25	24.42	11.69

For hybrids in the FAO 600-699 group, chlorophyll content ranged between 2.55 and 3.54 mg/g FW for chlorophyll a and between 1.24 and 1.85 mg/g FW for chlorophyll b, respectively (Table 2). The highest chlorophyll a, chlorophyll b and total contents were found for plants of hybrid Kneja 648 (3.54 mg/g FW, 1.85 mg/g FW and 5.39 mg/g FW, respectively). Relating the values to the group average, the chlorophyll content for hybrid Kneja 648 was 17.37% higher for chlorophyll a, 20.4% higher for chlorophyll b and 18.41% higher for chlorophyll a+b. Higher values compared to the group average were also found for plants of hybrid Kneja 650A, by 8.42% for chlorophyll a, by 0.7% for chlorophyll b and by 9.18% for chlorophyll a+b, respectively.

For hybrids in the FAO 600-699 group, chlorophyll content ranged between 2.55 and 3.54 mg/g FW for chlorophyll a and between 1.24 and 1.85 mg/g FW for chlorophyll b, respectively (Table 2). The highest chlorophyll a, chlorophyll b and total contents were found for plants of hybrid Kneja 648 (3.54 mg/g FW, 1.85 mg/g FW and 5.39 mg/g FW, respectively). Relating the values to the group average, the chlorophyll content for hybrid Kneja 648 was 17.37% higher for chlorophyll a, 20.4% higher for chlorophyll b and 18.41% higher for chlorophyll a+b. Higher values compared to the group average were also found for plants of hybrid Kneja 650A, by 8.42% for chlorophyll a, by 0.7% for chlorophyll b and by 9.18% for chlorophyll a+b, respectively.

On average, for the hybrids of the FAO 600-699 group, the highest coefficient of variation was found for carotenoid content (CV% 21.18).

Table 2. Plastid pigment content (mg/g FW) in maize hybrids of FAO 600-699 maturity group

Hybrids	Chl a	Chl b	Chl a+b	Carotenoids	Total
Kn 648	3.54	1.85	5.39	0.312	5.702
Kn 649	2.55	1.24	3.79	0.189	3.979
Kn 650A	3.27	1.7	4.97	0.342	5.312
Kn 650	2.99	1.52	4.51	0.271	4.781
Kn 683A	2.73	1.37	4.1	0.259	4.359
Max	3.54	1.85	5.39	0.342	5.702
Min	2.55	1.24	3.79	0.189	3.979
average	3.02	1.54	4.55	0.270	4.830
SD	0.400	0.245	0.645	0.058	0.697
CV%	13.25	15.98	14.17	21.18	14.43

In general, the plastid pigment content was found higher in the hybrids of the FAO 600-699 maturity group as follows: by 12.53% for chlorophyll a, by 9.38% for chlorophyll b, by 11.47% for chlorophyll a+b, by 8.30% for carotenoids, and by 11.29% for total plastid pigments content (chlorophylls+carotenoids).

Figure 1 presents data on the percentage of plastid pigments content in the tested hybrids. It can be seen that chlorophylls occupied a part of 92.83 to 95.44%, while that of carotenoids ranged from 4.56 to 7.17%. The highest coefficient of variation was found for carotenoids content (CV% 21.92). Their percentage as a proportion of total plastid pigments content was higher for hybrids of FAO 500-599 maturity group.

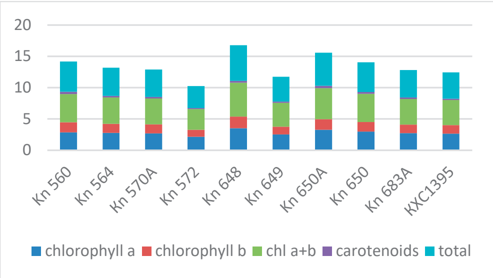

Figure 1. Plastid pigments content (mg/g FW) in maize hybrids of two maturity groups (FAO 500-599 and FAO 600-699), (CV% 13.50 for chlorophyll a, CV% 14.71 for chlorophyll b, CV% 13.82 for chlorophyll a+b, CV% 21.92 for carotenoids and CV% 14.05 for total content)

Szulc and Bocianowski (2011) found that "stay-green" hybrids are genetically more stable than classical hybrids. "Stay-green" maize hybrids compared to conventional hybrids are characterized not only by higher chlorophyll content, but also by significantly better ability to form higher yields of both grain and silage (Szulc et al., 2021). Chlorophyll content is a relatively more stable trait compared to carotenoid content (CV% 21.18 and 21.92 vs. CV% 11.25 and 14.17, respectively). Ghimire et al. (2015) demonstrated that chlorophyll content was positively correlated with grain yield.

Figure 2 shows the chlorophyll a to chlorophyll b ratio. The chlorophyll a to chlorophyll b ratio was relatively similar for the hybrids from two maturity groups (1.900 for FAO 500-599 against 1.971 for FAO 600-699), CV% (2.931 for FAO 600-699 against 3.430 for FAO 500-599). This indicator is considered genetically determined, which explains the low variation, SD=0.07 and CV% 3.57, respectively.

Some relationships between the plastid pigments content were calculated. Pearson's correlation coefficient (r) was used to determine the strength and direction of the linear relationship between the parameters (Table 3 and Table 4).

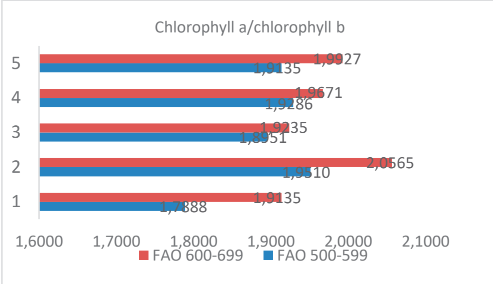

Figure 2. Chlorophyll a to chlorophyll b ratio in maize hybrids of two maturity groups (FAO 500-599, CV% 3.324; FAO 600-699, CV% 2.931)

The following correlations were found for the hybrids in the FAO 500-599 maturity group (Table 3): a significant positive correlation between chlorophyll a and carotenoids (r=0.683), a strong positive correlation between chlorophyll b and carotenoids (r=0.818), and between chlorophyll a+b and carotenoids (r=0.742). A very strong positive correlation was found between chlorophyll a and chlorophyll b (r=0.962), between chlorophyll a and chlorophyll a+b (r=0.994), and between chlorophyll b and chlorophyll a+b (r=0.986).

A moderate negative correlation was found between chlorophyll a and chlorophyll a/b (r= -0.447). A significant negative correlation was found between chlorophyll a+b and chlorophyll a/b (r= -0.539), as well as between chlorophyll b and chlorophyll a/b (r= -0.673) and a strong negative correlation between chlorophyll a/b and carotenoids (r= - 0.828).

Table 3. Correlations between plastid pigments content in maize hybrids of FAO 500-599 maturity group

	Chl a	Chl b	Chl a+b	Chlo a/b
Chl a	0.962	0.994	-0.447	0.683
Chl b		0.986	-0.673	0.818
Chl a+b			-0.539	0.742
Chlo a/b				-0.828

In the studies of Łącka et al. (2021) the next correlations were observed between contents of: chlorophyll a and carotenoids (r=0.778), contents of chlorophyll b and carotenoids (r=0.807), contents of chlorophyll a+b and carotenoids (r=0.785), chlorophylls a and b (r=0.990), chlorophylls a and a+b (r=1.000). The significant negative correlations were observed between contents of: chlorophylls a and a/b (r=-0.678), chlorophylls b and a/b (r=-

0.767), chlorophylls a+b and a/b (r=-0.698) as well as contents of chlorophyll a/b and carotenoids (r=-0.711).

The following correlations were found for the hybrids of FAO 600-699 maturity group (Table 4): strong positive correlation between chlorophyll a and carotenoids (r=0.873), between chlorophyll b and carotenoids (r=0.890), and between chlorophyll a+b and carotenoids (r=0.880). A very strong positive correlation close to the functional correlation was found between chlorophyll a and chlorophyll b (r=0.999), between chlorophyll a and chlorophyll a+b (r=0.999), and between chlorophyll b and chlorophyll a+b (r=0.999).

Table 4. Correlations between plastid pigments content in maize hybrids of FAO 600-699 maturity group

	Chl a	Chl b	Chl a+b	Chlo a/b
Chl a	0.999	0.999	-0.954	0.873
Chl b		0.999	-0.964	0.890
Chl a+b			-0.958	0.880
Chlo a/b				-0.967

A very strong negative correlation was found between chlorophyll a and chlorophyll a/b (r= -0.954), between chlorophyll b and chlorophyll a/b (r= -0.964) and between chlorophyll a+b and chlorophyll a/b (r= -0.958).

CONCLUSIONS

When determining the content of plastid pigments (chlorophylls and carotenoids) in the leaves of maize hybrids of two vegetation maturity groups (mid-late, FAO 500-599 and late, FAO 600-699), it was found that the highest chlorophyll a, chlorophyll b and total contents for plants of hybrid Kneja 560 (2.88 mg/g FW, 1.61 mg/g FW and 4.49 mg/g FW, respectively). For the hybrids of the FAO 600-699 group, the highest chlorophyll a, chlorophyll b and total contents were found for plants of hybrid Kneja 648 (3.54 mg/g FW, 1.85 mg/g FW and 5.39 mg/g FW, respectively). Higher than the group average values were also found for plants of hybrid Kneja 650A, respectively by 8.42% for chlorophyll a, by 10.7% for chlorophyll b and by 9.18% for chlorophyll a+b.

In general, the plastid pigment content was higher in the hybrids of the FAO 600-699 maturity group as follows: by 12.53% for chlorophyll a, by 9.38% for chlorophyll b, by 11.47% for chlorophyll a+b, by 8.30% for carotenoids and by 11.29% for total plastid pigment content (chlorophylls+carotenoids). The percentage contribution of carotenoids as a proportion of total plastid pigment content was higher for hybrids in the FAO 500-599 group, reaching 7.17% versus 6.44% for hybrids in the FAO 600-999 group.

For the hybrids of FAO 500-599 maturity group a very strong positive correlation was found between chlorophyll a and chlorophyll b (r=0.962), between chlorophyll a and chlorophyll a+b (r=0.994), and between chlorophyll b and chlorophyll a+b (r=0.986) and a strong negative correlation between chlorophyll a/b and carotenoids (r= - 0.828).

For the hybrids of maturity group FAO 600-699, a very strong positive correlation close to the functional correlation was found between chlorophyll a and chlorophyll b (r=0.999), between chlorophyll a and chlorophyll a+b (r=0.999), and between chlorophyll b and chlorophyll a+b (r=0.999). A very strong negative correlation was found between chlorophyll a and chlorophyll a/b (r=-0.954), between chlorophyll b and chlorophyll a/b (r=-0.964) and between chlorophyll a+b and chlorophyll a/b (r=-0.958).

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THE ROLE OF METHYLOBACTERIUM SYMBIOTICUM IN AGRICULTURE

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Abstract

The current scientific work presents a review of the role of Methylobacterium symbioticum not only as a tool for increasing agricultural yields but also as a strategic ally in transitioning to a greener and more sustainable agricultural model. Methylobacterium symbioticum is a symbiotic bacterium known for its ability to utilize single-carbon compounds, especially methanol, as a source of carbon and energy. In the context of modern agriculture, the use of beneficial microorganisms has become an essential component for enhancing sustainability and productivity. Among these, Methylobacterium symbioticum stands out as a symbiotic bacterium with significant potential for improving plant health and their adaptation to environmental stress. This bacterium has the capability to colonize plants and form symbiotic relationships, thereby contributing to increased nutrient use efficiency, stimulating growth and development, and enhancing plant tolerance to adverse conditions. It stimulates the physiological processes of the host plant by synthesizing phytohormones, such as auxins, and by producing enzymes that contribute to increased stress resistance.

Key words*: methylobacterium symbioticum, biofertilizer, phytohormones, biostimulant.*

INTRODUCTION

The use of beneficial bacteria for agriculture is a growing area of interest in sustainable crop production (Campobenedetto et al., 2020). With pressures rising for more production with less environmental impact, the agricultural sector is turning to biological solutions that can augment or even replace traditional chemical inputs (Kloepper & Schroth, 1978; Chițu et al., 2024; Sitnicki et al., 2024). Bacteria associated with plants and those found in soil are some of those that have demonstrated significant potential in plant growth promotion, nutrient enhancement, and soil structure and fertility improvement (Saharan & Nehra, 2011; Pandey et al., 2012; Hurek et al., 2002).

Moreover, the use of bacterial inoculants aligns with current efforts to reduce dependency on synthetic fertilizers and plant protection products, whose long-term use has been associated with soil degradation, biodiversity loss, and groundwater contamination. By enhancing natural soil microbial communities and promoting nutrient cycling, beneficial bacteria offer a promising alternative that supports both crop productivity and environmental resilience. Recent advances in microbiome research, genomics, and bioformulation technologies have further expanded the possibilities for practical applications of bacteria in agriculture. However, successful implementation depends on factors such as strain selection, soil type, crop species, and environmental conditions. As such, continued interdisciplinary research is needed to optimize the use of microbial inoculants under field conditions and to integrate them effectively into modern farming systems (Amaresan et al., 2020; Butta et al., 2023). This article reviews the current understanding of the role of beneficial bacteria in agriculture, focusing on *Methylobacterium symbioticum*.

Bacteria from the *Methylobacterium* genus (Patt et al., 1976) are classified under the class *Alphaproteobacteria* and are Gram-negative bacteria characterized by a pink pigmentation due to carotenoid synthesis (Van et al., 2003), primarily xanthophylls. These bacteria have a bacillus shape, are strictly aerobic, and can grow by utilizing single-carbon (C1) compounds such as methanol or methylamine (Toyama et al., 1998; Konovalova et al., 2007). *Methylobacterium* spp. can occupy various habitats, including soil, water, leaf surfaces, root nodules, seeds, and air (Tani et al., 2012a; Wellner et al., 2012), having been detected in more than 70 plant species (Omer et al., 2004).

In the context of modern agriculture, the use of beneficial microorganisms has become an essential component in increasing sustainability and productivity. Among these, *Methylobacterium symbioticum* is a symbiotic bacterial species with significant potential for improving plant health and enhancing adaptation to environmental stresses. This bacterium has the ability to colonize plants and form symbiotic relationships that contribute to increased nutrient use efficiency, stimulation of growth, and enhanced plant tolerance to adverse conditions (Szparaga et al., 2019).

While traditional fertilizers and plant protection products provide immediate effects, they often come with long-term ecological and economic risks. In contrast, *Methylobacterium symbioticum* and other symbiotic bacteria contribute to the development of a sustainable agricultural model by providing ecological solutions that support plants naturally, without negative effects on ecosystems. *Methylobacterium symbioticum* has been studied and reported to have the ability to produce phytohormones and other bioactive compounds that not only stimulate plant growth but also enhance resistance to pathogens and abiotic stresses such as drought or nutrient-poor soils (Green & Ardley, 2018; Zhang et al., 2024).

This paper examines the role of *Methylobacterium symbioticum* in agriculture, focusing on the mechanisms through which this bacterium supports plants and its potential agronomic applications. Additionally, the ecological and economic benefits of using this bacterium in sustainable agriculture will be explored, along with the challenges and perspectives for its integration into large-scale agricultural practices. Thus, *Methylobacterium symbioticum* stands out not only as a tool for increasing agricultural yields but also as a strategic ally in the transition toward a greener and more sustainable agricultural model.

MATERIALS AND METHODS

The working methodology involved the selection of currently known information on the fixed theme from academic databases such as Google Scholar, PubMed and Web of Science, aiming to integrate microbiological, physiological and agronomic approaches to comprehensively evaluate *Methylobacterium symbioticum* as a plant growth promoting bacterium (PGPB) on the one hand and as a support for the development of sustainable agricultural practices, which reduce dependence on synthetic fertilizers, while improving crop productivity and soil health, on the other hand.

RESULTS AND DISCUSSIONS

The species *Methylobacterium symbioticum*, from the *Methylobacterium* genus, has garnered considerable attention in recent years due to its potential applications in promoting plant growth and increasing productivity. Studies have demonstrated that this bacterium forms symbiotic associations with numerous plant species, leading to improved physiological and biochemical performance, which is reflected in higher crop yields. The mechanisms through which *Methylobacterium symbioticum* exerts its beneficial effects include nitrogen fixation, phytohormone synthesis, and enhanced nutrient absorption processes that are essential for sustainable agriculture.

One of the major advantages of *Methylobacterium symbioticum* is its ability to fix atmospheric nitrogen, a vital nutrient for plant growth. Research has shown that species from the *Methylobacterium* genus, including *M. symbioticum*, can significantly increase nitrogen availability in the rhizosphere, thereby reducing the need for synthetic nitrogen-based fertilizers. For example, the role of leaf-resident *Methylobacterium* species in nitrogen fixation, a process that favors biomass accumulation and increased seed production in *Jatropha curcas*, has been highlighted (Madhaiyan et al., 2007). These findings are also supported by other studies that have observed that, under nitrogen stress conditions, plants can intensify their symbiotic relationship with *Methylobacterium symbioticum*, leading to improved photosynthetic activity and better overall plant health (Torres et al., 2024).

Beyond nitrogen fixation, *Methylobacterium symbioticum* is known for producing phytohormones such as auxins and cytokinins, which play a crucial role in regulating plant growth and development. The production of these hormones has been correlated with enhanced root system development and increased nutrient uptake efficiency. Other studies have reported that foliar application of *Methylobacterium symbioticum* led to higher root length density (RLD) and an increase in root area density (RAD) in common wheat, indicating a positive impact on root architecture and function (Valente et al., 2024). Similarly, it has been observed that the beneficial effects of *Methylobacterium* spp. on plant growth are linked to their ability to produce growth regulators, supporting their role in promoting plant development through hormonal regulation (Senthilkumar et al., 2021).

The ability of *Methylobacterium symbioticum* to colonize plant tissues is another essential factor in manifesting its beneficial effects. Colonization efficiency significantly influences the extent of growth promotion observed in plants. Some studies suggest that efficient colonization of plant tissues by *Methylobacterium* species is crucial for establishing a symbiotic relationship that benefits both the plant and the bacterium (Yim et al., 2012). Additionally, the mutualistic symbiosis between *Methylobacterium* species and various plant species has been investigated, suggesting that this interaction is facilitated by methanol emission from plants, which serves as a carbon source for the bacteria (Tani et al., 2012b). This mutualistic relationship not only stimulates plant growth but also contributes to ecosystem health.

Moreover, the application of *Methylobacterium symbioticum* as a microbial inoculant has demonstrated its potential to reduce agricultural production costs. Studies have highlighted the potential for using lower amounts of *Methylobacterium*-based inoculants once substantial colonization has been achieved, thereby reducing expenses associated with traditional fertilizer use (Zhang et al., 2024). This characteristic is particularly relevant in the context of sustainable agriculture, where integrating biological inoculants can contribute to the development of more environmentally friendly farming practices.

The advantages of *Methylobacterium symbioticum* are not limited to a specific plant species but can be extended to various agricultural crops. Research has shown that *Methylobacterium* can enhance the growth and yield of economically valuable crops such as strawberries and maize. The effectiveness of *Methylobacterium symbioticum* as a biological inoculant for these crops has been particularly evident under nitrogen stress conditions, where the symbiotic relationship proved highly beneficial (Torres et al., 2024). Furthermore, the diversity of *Methylobacterium* species in the phylloplane of different crops has been documented, indicating a broad potential for promoting plant growth across various agricultural systems (Cheng et al., 2022).

Additionally, the role of *Methylobacterium symbioticum* in phytoremediation has become a promising research direction. Studies have highlighted the potential of *Methylobacterium*-enriched *Crotalaria pumila* seeds in improving phytoremediation efforts for soils contaminated with heavy metals (Sánchez-López et al., 2018). This application not only supports plant growth but also contributes to soil health and environmental sustainability by facilitating the restoration of contaminated lands.

Research indicates that *Methylobacterium symbioticum* can effectively reduce the need for synthetic nitrogen-based fertilizers without compromising crop productivity. Studies have demonstrated that its application in maize and strawberry crops has led to comparable or even superior results in terms of growth and yield compared to conventional fertilization methods (Torres et al., 2024). This aspect is particularly relevant in the context of sustainable agriculture, where the ecological impact of chemical fertilizers is an increasing concern. As a biofertilizer, *Methylobacterium symbioticum* not only stimulates plant growth but also contributes to soil health by enhancing microbial diversity and activity (Valente et al., 2024; Torres et al., 2024; Rodrigues et al., 2024).

Mechanisms of Action of *Methylobacterium symbioticum*

The mechanisms through which *Methylobacterium symbioticum* exerts its beneficial effects are complex and multidimensional. One of the fundamental mechanisms is the production of phytohormones such as auxins and cytokinins, which are essential for plant growth and development.

These hormones regulate processes such as root elongation, nutrient uptake, and stress tolerance, thereby improving overall plant health and productivity (Senthilkumar et al., 2021; Bogas et al., 2016; Grossi et al., 2024). Additionally, *Methylobacterium* species are known for their ability to produce indole-3-acetic acid (IAA), a type of auxin essential for root architecture and function, which significantly enhances plants' ability to absorb water and nutrients from the soil (Grossi et al., 2024; Kwak et al., 2014).

Moreover, the ability of *Methylobacterium symbioticum* to fix atmospheric nitrogen represents a significant advantage, especially in nitrogen-deficient soils. This characteristic reduces dependence on chemical nitrogen-based fertilizers, which are often associated with negative environmental effects such as water eutrophication and soil degradation (Torres et al., 2024; Rodrigues et al., 2024).

Integrating *Methylobacterium symbioticum* into agricultural practices can thus contribute to the development of more sustainable farming systems that maintain high productivity levels while minimizing ecological impact. In addition to its role in nitrogen fixation and phytohormone production, *Methylobacterium symbioticum* improves plant resistance to abiotic stresses such as drought and salinity. By modulating metabolic pathways associated with stress responses and stimulating root growth, this bacterium helps plants withstand unfavorable environmental conditions, ultimately leading to stabilized yields under fluctuating climatic conditions (Palberg et al., 2022; Choudhury et al., 2023). Its ability to mitigate stress effects is particularly important in the context of global climate change, which is placing increasing pressure on agricultural systems.

The application of *Methylobacterium symbioticum* is not limited to a single type of crop; it has been successfully tested on various species, including rice, olive, and potato. For example, studies have demonstrated that inoculating olive trees with *Methylobacterium symbioticum* resulted in improved growth and increased nitrogen fixation efficiency, highlighting its versatility as a biofertilizer (Rodrigues et al., 2024; Grossi et al., 2020). Similarly, in potato cultivation, *Methylobacterium* has been reported to enhance phosphorus and nitrogen absorption, further reinforcing its role as a valuable agricultural agent (Grossi et al., 2020).

Furthermore, integrating *Methylobacterium symbioticum* into agricultural practices aligns with agroecological principles, which emphasize the importance of biodiversity and ecological balance in farming systems. By promoting beneficial microbial communities in the soil and rhizosphere, *Methylobacterium symbioticum* contributes to the overall health of agroecosystems, fostering sustainable agricultural practices capable of addressing the challenges of modern farming (Valente et al., 2024; Christian et al., 2021).

Methylobacterium symbioticum offers multiple benefits for plant growth and productivity through mechanisms such as nitrogen fixation, phytohormone production, and optimized nutrient absorption. Its ability to establish symbiotic relationships with various crops positions it as a valuable tool for sustainable agriculture, potentially reducing dependence on chemical fertilizers while enhancing crop yields. Ongoing research into the diverse applications of *Methylobacterium symbioticum* underscores its importance in modern agricultural practices and environmental management.

Recent studies and practical applications have confirmed that *Methylobacterium symbioticum* can bring significant benefits to essential agricultural crops, contributing to increased yield, improved quality, and enhanced plant tolerance to stress. As research progresses, *Methylobacterium symbioticum* is on its way to becoming a fundamental tool in sustainable agricultural practices, providing farmers with a method to boost productivity in an environmentally friendly and economically viable manner.

In recent years, the application of foliar biostimulant products has gained universal acceptance as a promising practice due to their ease of application and cost-effectiveness, particularly when combined with other agronomic practices such as herbicide, fungicide, and fertilizer application. Numerous studies have investigated the efficacy of these products, which often contain growth-promoting substances such as amino acids, humic acids, or beneficial microorganisms, including plant growth-promoting bacteria (PGPB) (Glick et al., 1998; Dal et al., 2017).

These studies have explored the possibility of reducing chemical fertilization while maintaining production and quality standards. However, the *Methylobacterium symbioticum* strain, isolated by Pascual et al. in 2020, has not yet been extensively studied, and the effects of an inoculant based on this bacterium on common wheat remain untested.

Consistent with previously reported results for crops such as maize and strawberry (Torres et al., 2024; Pascual et al., 2020), this study confirmed the beneficial effects on vegetative parameters (SPAD and NDVI) following inoculation with *Methylobacterium symbioticum*. The inoculant extended the crop's stay-green period, prolonging photosynthetic activity throughout the growth cycle. In biomass crops such as maize, this aspect represents an agronomic advantage, as it allows the plant to continue the photosynthesis process for a longer period, thereby increasing CO_2 assimilation and total biomass accumulation at harvest.

In the case of common wheat, as expected, SPAD values before harvest were significantly lower in plots fertilized with a reduced nitrogen dose (75%N) compared to control plots. However, the "75%N + bact" treatment mitigated this difference, demonstrating the bacterium's ability to delay chlorophyll degradation in the final stages of the growth cycle. Additionally, although not statistically significant, a trend was observed in which plots treated with the microbial biostimulant exhibited higher SPAD values than untreated ones, particularly at the reduced nitrogen dose. This suggests that the bacteria express their effects more effectively under nitrogen deficiency conditions. It is presumed that plants in a nitrogen-deficient nutritional state are more favorable for establishing and maintaining an endophytic relationship with these beneficial bacteria. Similar *Biofertilizer* results were obtained in an *in vitro* study on maize (Torres et al., 2024), where bacterial application at a reduced nitrogen dose (50%N) resulted in higher SPAD values compared to the fully fertilized control, further confirming a more efficient association between plants and bacteria under nutritional stress conditions.

NDVI analysis demonstrated that, at the end of the growth cycle, values were similar across all treatments but were higher in the "75%N + bact" treatment compared to "75%N" alone.

Previous studies have shown that various strains from the *Methylobacterium* genus possess ACC deaminase activity (Yim et al., 2014). Based on this hypothesis, preliminary *in vitro* tests confirmed that the tested strain exhibits this enzymatic activity, allowing it to delay senescence by breaking down the ethylene precursor ACC, thereby prolonging the stay-green period of leaves. This enzyme degrades ACC, reducing ethylene synthesis and enhancing plant stress tolerance (Madhaiyan et al., 2007).

Although the bacterial treatment did not have significant effects on morphological traits (plant height, upper internode length, LAI, and aerial biomass), notable effects were recorded on photosynthetic parameters. The "75%N + bact" treatment increased stomatal conductance and transpiration rate compared to the "100%N" and "75%N" plots without inoculation. Additionally, improved CO_2 assimilation rates and enhanced PSII efficiency were observed, confirming that plants establish associations with microorganisms to compensate for nutritional deficits.

Previous studies on other plant growth-promoting bacteria (PGPB) have highlighted similar benefits. For example, *Azospirillum brasilense* improves the growth of various crops by fixing atmospheric nitrogen and synthesizing growth-promoting substances (Bhattacharyya & Jha, 2012). Similarly, *Pseudomonas fluorescens* has demonstrated its ability to increase plant tolerance to saline stress by producing phytohormones and ACC deaminase, thereby reducing ethylene levels and improving plant development (Egamberdieva & Lugtenberg, 2014).

Regarding yield, the bacterial treatment did not significantly impact wheat productivity. However, other studies have reported yield increases in crops such as maize, grapevine, rice, and strawberry, particularly under reduced nitrogen doses (Torres et al., 2024; Pascual et al., 2020). These results suggest that improved crop performance is associated with the microorganisms' ability to assimilate ammonium through nitrogen fixation, reducing the plant's energy requirements for mineral nitrogen conversion.

Regarding wheat quality, the microbial biostimulant did not significantly increase protein content but influenced protein quality by increasing the glutenin-to-gliadin ratio and the proportion of high-molecular-weight (HMW) subunits relative to low-molecular-weight (LMW) subunits. This modification led to an increase in dough tenacity and stability.

From an economic perspective, applying the inoculant does not incur additional costs for agricultural equipment, as it can be integrated with post-emergence weed control treatments or fungicides, thereby saving time and fuel. Although potential interactions between the bacterium and applied plant protection products need further investigation, economic viability is largely determined by fertilizer prices, which have risen due to raw material shortages. Beyond economic considerations, the ecological benefits of reducing chemical fertilizer applications such as decreased nitrogen leaching after heavy rainfall represent another agronomic advantage.

CONCLUSIONS

A rapid transition to a sustainable and productive agricultural system is necessary to maintain soil fertility and reduce soil biodiversity loss.

Microbial biostimulants represent an innovative solution for achieving safe and long-term agricultural production with high nutritional value (Castiglione et al., 2021). Given the continuous growth of the global population, the reduction of arable land, and the depletion of crops' genetic potential, the implementation of innovative agricultural technologies is essential. Agronomic solutions with low environmental impact, aimed at improving plant resilience to challenging soil conditions, are becoming indispensable for meeting the high demand for nutrient-rich food (Szparaga et al., 2019; Mannino et al., 2021).

Plant biostimulants (PBs) are a new generation of products available on the market that can contribute to achieving sustainability goals in agriculture. According to EU Regulation 2019/1009, they are defined as "products that stimulate plant nutrition processes, independently of their nutrient content, with the sole purpose of improving one or more of the following characteristics of the plant or its rhizosphere: nutrient use efficiency, tolerance to abiotic stress, quality characteristics, and the availability of limited nutrients in the soil or rhizosphere" (Campobenedetto et al., 2020; Campobenedetto et al., 2021).

PBs can consist of substances, mixtures, or microorganisms and are thus classified into microbial and non-microbial biostimulants (European Commission). A microbial plant biostimulant consists of a microorganism or a consortium of microorganisms included in CMC-7 (Component Material Categories, number 7), which comprises four distinct genera: *Azotobacter* spp., mycorrhizal fungi, *Rhizobium* spp., and *Azospirillum* spp.

However, although the existence of regulations and limitations can be beneficial for ensuring food safety and product quality, the strictness and exclusivity of the positive list may significantly affect the potential benefits of these innovative products. Economically, the application of the inoculant does not entail additional spending on farm machinery, as it can be mixed with post-emergence weed control treatments or fungicides, thereby conserving time and fuel. Although the possible interaction of the bacteria with sprayed plant protection products must be the subject of further research, economic viability is decided primarily by the cost of fertilizers, which has increased because of the shortage of raw materials. Therefore, it would be appropriate to consider reducing the negative list and expanding the positive list with new microorganisms, provided that scientific evidence demonstrates and supports their safety for both the environment and consumers.

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AGROCLIMATIC ASSESSMENT OF THE PROSPECTS FOR GROWING WINTER PEAS IN THE ODESA REGION

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Abstract

Peas are a key leguminous crop in Ukraine due to their valuable properties, which are beneficial for both agriculture and ecology. Pea grain is widely used in the food industry, serves as an important component of animal feed, and acts as a natural nitrogen fixer, improving soil fertility. These characteristics ensure its important role in enhancing the productivity of agricultural systems and supporting agroecological stability. Climate change, particularly warming and uneven rainfall distribution, presents challenges for growing traditional crops, including spring peas. As a result, the development of winter pea varieties becomes a promising solution. These varieties better utilize moisture during the late autumn and early spring periods, provide more stable yields, and protect the soil from erosion. This is especially important for regions at risk of soil degradation, such as the southern regions of Ukraine. The article presents the results of numerical calculations of the productivity of winter peas under the agrometeorological conditions that prevailed in 2023, compared to the agroclimatic conditions expected for 2031-2050. The obtained results confirm the potential of growing winter pea varieties as a strategy for adapting Ukrainian agriculture to climate change, as it increases yield, preserves soil, and ensures the stability of agroecosystems.

Key words*: winter peas, productivity, yield, climate change, scenario, modelling, moisture availability of crops.*

INTRODUCTION

Pea (*Pisum sativum*) holds a leading position among leguminous crops in Ukraine due to its unique properties, which make it highly valuable for both agricultural production and agroecology. Compared to other legumes, peas exhibit high grain yield potential, excellent quality characteristics, and a short growing season. Pea grain serves as an essential component of human nutrition and a crucial element in livestock feed. Moreover, peas are an exceptionally effective precursor crop for many agricultural species, as they act as a natural nitrogen fixer. As a result, over 100 kg/ha of bound nitrogen remains in the soil after pea cultivation, reducing the need for mineral fertilizers, minimizing humus mineralization, and significantly enhancing soil fertility (Kyryllov & Zhyhaylo, 2024; Solomonov et al., 2022; Boincean et al., 2014; Vann et al., 2019).

Modern challenges, particularly climate change, have a significant impact on crop productivity. Global warming is accompanied by uneven precipitation distribution throughout the year, contributing to an increase in drought occurrences. These climatic changes negatively affect the yield of many crops, including spring peas, making the implementation of alternative cultivation approaches increasingly relevant (Vozhehova et al., 2023a, 2023b; Zhyhaylo et al., 2024a; Stepanenko et al., 2018).

In the past decade, there has been a growing trend toward the adoption of winter pea varieties, which offer several key advantages over spring peas (Zhyhaylo et al., 2024b; Kaminskyy et al., 2013; Sukhova, 2014). These varieties ensure more stable yields of both green biomass and grain due to their efficient utilization of moisture resources and moderate temperatures during the late autumn and early spring periods. Additionally, winter peas contribute to soil protection against wind and water erosion, which is particularly relevant for regions with a high risk of soil degradation. Investigating the agroclimatic conditions for winter pea cultivation may facilitate the expansion of its cultivation area in the southern regions of Ukraine, where climatic conditions are favorable for this crop.

MATERIALS AND METHODS

A study was conducted on the formation of productivity and yield of winter peas using the WINTERPEAS-24 mathematical model, which simulates the water-thermal regime and productivity of the crop. This model is a modified version of the fundamental mathematical model for crop yield formation proposed by A. M. Polovyi (Polovyi, 2007).

The natural-physical system "soil-plant-atmosphere" in the model is represented by three main components. The first component, the input, includes information about environmental characteristics and initial model parameters. The second component describes the internal structure of the system, which is characterized by equations governing radiation, heat, and water balance, mineral nutrition, and biomass accumulation in the plant cover. The third component, the output, represents the system's performance and provides quantitative indicators of the dynamics of dry biomass, leaf area, total biomass accumulation, and the economically valuable yield of winter pea grain.

The key conceptual principles of the model are as follows: plant growth and development are determined by genotype and environmental factors; biomass accumulation is simulated based on the distribution of photosynthesis products and absorbed mineral nutrients, considering the assimilate requirements of different plant organs for growth; and radiation, heat, and water regimes within the "soil-plant-atmosphere" system are modeled.

The model has a modular structure consisting of five blocks: input data, radiation and water-thermal regimes, photosynthesis, respiration, and growth and assimilate distribution (Figure 1).

The verification of the WINTERPEAS-24 model was performed by comparing the simulated productivity and yield indicators with actual field data from 2023.

To assess the prospects for winter pea cultivation in the coming decades, we used the Representative Concentration Pathway (RCP) climate change scenario - specifically, RCP8.5, which represents a high greenhouse gas emissions trajectory.

Numerical calculations of crop productivity and yield were conducted for two climate periods: 1986-2005, which serves as the baseline (Adamenko, 2011), and 2031-2050 under the RCP8.5 scenario.

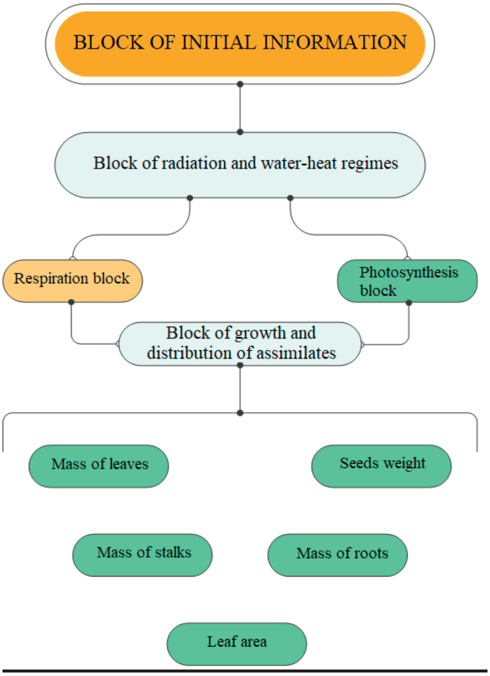
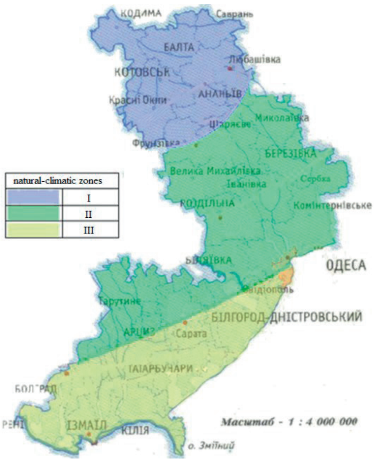


Figure 1. Block diagram of the WINTERPEAS-24 mathematical model for the water-thermal regime and productivity of winter peas

The calculations were carried out for two natural-climatic zones of the Odesa region: the Forest-Steppe and the Steppe zone, with further subdivisions into the Northern and Southern Steppe subzones (Figure 2).

RESULTS AND DISCUSSIONS

In 2023, the resumption of winter pea vegetation was observed in all natural-climatic zones of the Odesa region at the beginning of the first decade of March (Table 1). Pea maturation occurred in the Forest-Steppe and Northern Steppe in the middle of the first decade of June, while in the Southern Steppe, it was observed nine days earlier (May 29 vs. June 6).



The Forest-Steppe zone - I, the Northern Steppe subzone - II, the Southern Steppe - III.

Figure 2 - Natural-climatic zones (subzones) of the Odesa region

The duration of the spring-summer growing season in the Southern Steppe subzone was 90 days, which was 6-8 days shorter than in the Forest-Steppe zone and the Northern Steppe subzone.

Compared to the average long-term dates of the baseline period, the resumption of vegetation in 2023 began almost ten days earlier across the entire study area. However, the timing of pea maturation remained nearly unchanged from the long-term averages, resulting in an extended growing season by 7-12 days.

The average air temperature increases from north to south. In the Forest-Steppe zone, it matched the baseline value, whereas in the Steppe zone, temperatures were lower by 0.9°C and 1.0°C in the Northern and Southern Steppe subzones, respectively. Precipitation levels in all natural-climatic zones of the region exceeded the norm, reaching 168%, 174%, and 148%, respectively.

In 2023, soil moisture availability for crops in the Forest-Steppe zone was 91% of the baseline due to a moisture deficit of 153%, amounting to 0.73 relative units compared to the baseline value of 0.80 (Figure 3). In the Northern Steppe, moisture availability was higher at 0.75 relative units compared to 0.70 in the baseline period. In contrast, the Southern Steppe had the lowest moisture availability, at 0.59 relative units, which was only 89% of the norm. Thus, the best soil moisture conditions for pea crops were observed in the Northern Steppe subzone.

On average, in the region, the resumption of winter pea vegetation typically occurs at the end of the first or the beginning of the second decade of March (Table 1).

The seeds of this crop reach maturity in the first decade of June. The earliest maturation dates are observed in the Southern Steppe subzone, while in the Forest-Steppe zone, this process occurs the latest.

According to the RCP8.5 climate change scenario, vegetation resumption in the Forest-Steppe zone is expected to shift five days later compared to the baseline period. In contrast, in the Steppe zone, resumption is projected to occur 6-9 days earlier.

During the baseline climatic period, the average air temperature from vegetation resumption to maturation gradually increased from the northern to the southern part of the region. However, under the climate change scenario, this difference is expected to be nearly eliminated, with the average temperature in the north almost matching that of the south.

Table 1. Comparative assessment of the agrometeorological conditions of 2023 and the agroclimatic conditions of the multi-year periods of 1986-2005 and 2031-2050 during the spring-summer growing season of winter peas

Natural-climatic zones (subzones)	Period, years	Agrometeorological and agroclimatic characteristics							
		Date		duration of the vegetation period, days	average air temperature for period ($T_{cp.}$),°C	amount of precipitation for period (R), mm	total evaporation for the period (E), mm	evaporation for period, (E_0), mm	moisture deficit (D), mm
		resumption of vegetation	maturation						
Forest-Steppe zone	[1]1986-2005	13.03	09.06	89	10.9	117	181	226	45
	2023	02.03	06.06	96	10.8	197	186	255	69
	[2]2031-2050	18.03	13.06	88	11.0	161	174	188	14
Northern Steppe subzone	[1]1986-2005	11.03	04.06	86	11.8	101	157	226	69
	2023	01.03	06.06	98	10.9	176	144	192	48
	[2]2031-2050	05.03	08.06	100	10.5	133	158	203	45
Southern Steppe	[1]1986-2005	10.03	01.06	83	12.3	100	145	219	74
	2023	01.03	29.05	90	11.3	148	147	251	104
	[2]2031-2050	01.03	02.06	94	11.4	133	162	208	46

Note: 1 - Average multi-year data in the baseline climatic period; 2 - Climatic period under the scenario RCP8.5

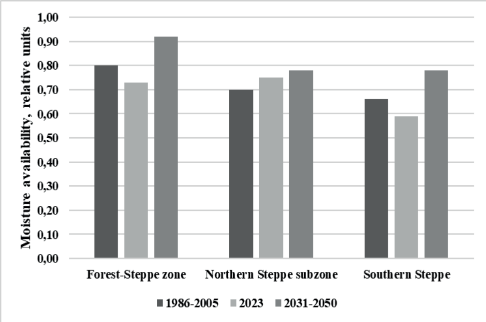


Figure 3. Soil Moisture Availability for Winter Pea Crops on Average During the Period from Vegetation Resumption to Maturation in Odesa Region

During the spring-summer growing season of the baseline period, precipitation levels decrease from north to south. However, under the RCP8.5 scenario, precipitation is expected to increase compared to the baseline across all zones and subzones, although the northern part of the region will continue to receive more rainfall than the south.

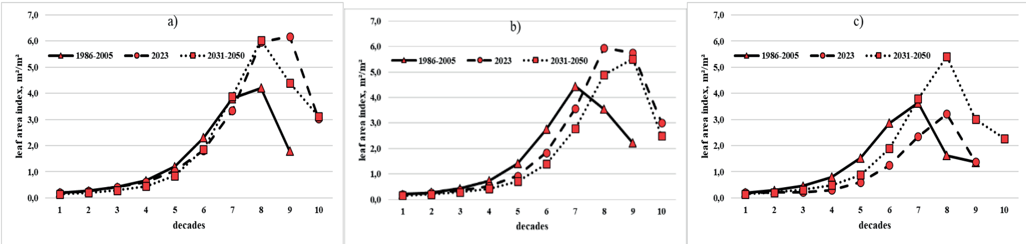
An analysis of soil moisture availability for winter pea crops showed that during the 1986-2005 period, this indicator in the Forest-Steppe zone (Figure 3) was 0.80 relative units, indicating favorable moisture conditions. In the Steppe region, particularly in both subzones, moisture availability was lower - 0.70 and 0.66 relative units, respectively - suggesting satisfactory conditions for plant growth. According to forecasts for the 2031-2050 period, moisture availability is expected to improve significantly compared to the baseline period. In the Forest-Steppe zone of Odesa region, it is projected to reach an excellent level of 0.92 relative units compared to 0.80, while in both Steppe subzones, moisture availability is expected to be good, reaching 0.78 relative units.

The agrometeorological conditions of 2023 contributed to high productivity of winter pea crops in the Forest-Steppe and Northern Steppe (Table 2). Modeling results showed that in the Forest-Steppe zone, the leaf area index reached 6.18 m^2/m^2 by the ninth decade of vegetation, which is 1.5 times higher than the long-term average (Figure 4a). The photosynthetic potential of plants during this period was 151% of the baseline value. Additionally, a more intense accumulation of dry biomass was observed. The maximum increase in dry biomass reached 378 g/m^2, while the total biomass at maturity was 960 g/m^2, significantly exceeding the long-term average of 604 g/m^2.

Table 2. Comparative assessment of the productivity indicators of winter pea crops in 2023, the baseline climatic period, and under the scenario RCP8.5.

Characteristics		Natural-climatic zones (subzones)								
		Forest-Steppe zone			Steppe					
					Northern Steppe subzone			Southern Steppe		
		Period, years								
		1986-2005	2023	2031-2050	1986-2005	2023	2031-2050	1986-2005	2023	2031-2050
Maximum values	leaf area index, m^2/m^2	4.2	6.1	6.0	4.4	6.0	5.5	3.6	3.2	5.4
	biomass accumulation, g/m^2	239	378	441	273	331	358	204	224	363
	total biomass, g/m^2	604	960	1243	731	919	852	664	446	1070
Photosynthetic potential (PP), m^2/m^2 for period		151	229	196	151	218	189	117	96	169

The agrometeorological conditions in the Northern Steppe this year also contributed to the high productivity of winter pea crops. The maximum leaf area index by the eighth decade of vegetation reached 6.0 m^2/m^2 (Figure 4b), which is nearly 1.5 times higher than the long-term average. As a result, the photosynthetic potential for the growing season amounted to 144% of the long-term average. The total biomass at the time of pod maturation was 30% higher than the long-term average, reaching 919 g/m^2. In the Southern Steppe, agrometeorological conditions were less favorable compared to the long-term data. The maximum leaf area index by the eighth decade of vegetation reached 3.2 m^2/m^2 (Figure 4c), which is lower than the average value of 3.6 m²/m². Due to a precipitation deficit in the early decades of vegetation, the photosynthetic potential of leaves decreased to 96 m²/m², amounting to 82% of the norm.



a) Forest-Steppe b) Northern Steppe c) Southern Steppe

Figure 4. Dynamics of winter pea leaf area from spring vegetation renewal - maturity. Odesa region

Maximum biomass accumulation by the eighth decade of the growing season exceeded the baseline (224 g/m² vs. 204 g/m²), but the total biomass at the end of the season was 50% lower than the long-term average, amounting to 446 g/m² compared to 664 g/m².

Under the implementation of the RCP8.5 climate scenario, agro-climatic conditions in the period 2031-2050 in both natural-climatic zones of the Odesa region are expected to positively influence the productivity of winter peas. The relative leaf area will increase: in the Forest-Steppe zone, it will reach 6.0 m²/m², in the Northern Steppe subzone - 5.5 m²/m², and in the Southern Steppe subzone - 5.4 m²/m². The photosynthetic potential, compared to the baseline, will increase by 130% in the Forest-Steppe zone, by 126% in the Northern Steppe subzone, and by 144% in the Southern Steppe subzone.

The maximum biomass accumulation during the budding stage is expected to exceed the baseline by 185%, 131%, and 178%, respectively. The total biomass estimates indicate an increase of 639 g/m² in the Forest-Steppe zone, and 121 g/m² and 406 g/m² in the Steppe subzones compared to the baseline period.

Grain yield of winter peas under baseline agro-climatic conditions is 3.4 t/ha in the Forest-Steppe and Northern Steppe zones, while in the Southern Steppe zone, it is 3.0 t/ha (Figure 5). Under the agro-meteorological conditions of 2023, pea yield in the Forest-Steppe and Northern Steppe subzones reached 176-177% of the baseline, while in the Southern Steppe, it matched the baseline level. Under the RCP8.5 scenario, with improved agro-climatic conditions, yield in the Forest-Steppe zone is projected to double, while in the Steppe zone, it is expected to increase 1.5 times.

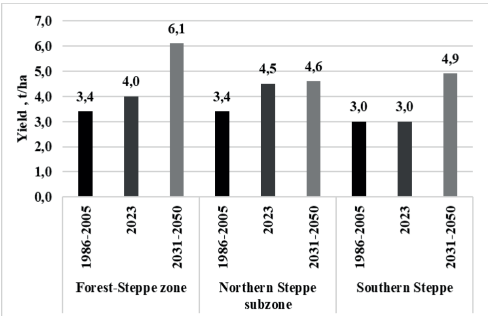


Figure 5. Winter pea yield in Odesa region

CONCLUSIONS

A series of numerical calculations were conducted using the WINTERPEAS-24 model. Agroclimatic indicators and productivity parameters of winter pea cultivation were assessed across the natural-climatic zones of the Odesa region, including the Forest-Steppe, Northern Steppe, and Southern Steppe.

It was established that, during the baseline climatic period (1986-2005), winter pea vegetation typically resumed in the region between the end of the first (March 10) and the beginning of the second decade of March (March 13), with pod maturation occurring in the first decade of June. The earliest maturation was observed in the southern steppe subzone

(June 1), while the latest occurred in the forest-steppe zone (June 9).

Compared to the climatic norms of the baseline period, the resumption of vegetation in 2023 occurred nearly a decade earlier (March 1-2) across the region, while pod maturation was observed three days earlier than the average historical date. The duration of the vegetation period from regrowth to maturation was extended by 7-8 days.

Under the RCP8.5 scenario for the period 2031-2050, vegetation regrowth in the forest-steppe zone is projected to begin one pentad later (March 18) than in the current climatic period, whereas in the steppe zone, it is expected to commence 6-9 days earlier (March 5 and March 1, respectively). Maturation in the Forest-Steppe and Northern Steppe zones is anticipated to occur four days earlier, while in the Southern Steppe, it will remain nearly unchanged.

The agroclimatic conditions of the baseline climatic period in the Forest-Steppe ensure favorable moisture availability for crops (0.80 relative units), while in both subzones of the Steppe, moisture availability is assessed as satisfactory (0.70 and 0.66 relative units, respectively). The temperature regime, on average, increases from north to south (10.9°C, 11.8°C, and 12.3°C, respectively).

In 2023, the temperature regime in the Forest-Steppe was nearly identical to the baseline, whereas in both Steppe subzones, the mean air temperature was 0.9°C and 1.0°C lower than the long-term average. Moisture conditions were most favorable for winter pea cultivation in the Northern Steppe (0.75 relative units), less favorable in the Forest-Steppe (0.73 relative units), and least favorable in the Southern Steppe, which experienced drought conditions (0.59 relative units).

According to projections, the temperature regime in the Forest-Steppe zone will remain nearly unchanged (11.0°C), while in the Northern and Southern Steppe zones, it will be lower (10.5°C and 11.4°C, respectively). Moisture availability is expected to improve (0.92, 0.78, and 0.78 relative units, respectively).

The optimal temperature regime and favorable to satisfactory moisture conditions during the actual climatic period have supported winter pea yields of 3.4 t/ha in the Forest-Steppe and Northern Steppe and 3.0 t/ha in the Southern Steppe.

Agrometeorological conditions in 2023 contributed to a high winter pea yield of 4.0 t/ha in the Forest-Steppe and 4.5 t/ha in the Northern Steppe of Odesa, while the yield in the Southern Steppe remained at the long-term average level of 3.0 t/ha.

Future agroclimatic conditions are projected to further enhance winter pea productivity, with yields reaching 6.1 t/ha in the Forest-Steppe and 4.6-4.9 t/ha in both Steppe subzones.

The findings of this study will contribute to improving the efficiency of winter pea cultivation and optimizing agronomic practices, which are crucial for the development of Ukraine’s agricultural sector under changing climatic conditions.

Winter pea cultivation should be considered as an adaptive strategy for mitigating the impacts of climate change on agricultural production.

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THE EVALUATION OF THE QUALITY INDICES OF PHYTOMASS FROM ENERGY CROPS AND AGRICULTURAL RESIDUES

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Abstract

The objective of this research was to evaluate the quality indices of the solid dry phytomass from energy crops Miscanthus giganteus 'Titan', Silphium perfoliatum 'Vital', Sorghum bicolor, var. saccharatum 'SAŞM1' and agricultural residues – stems of Brassica napus oleifera and Pisum sativum collected in the experimental plot of the NBGI Chişinău. It has been found that elemental composition the collected dry phytomass was 41.36-50.00% carbon, 4.32-6.14% hydrogen, 0.22-1.37% nitrogen, 0.03-0.10% sulphur, 2.18-5.66% ash and gross calorific value varied from 18.2 to 19.6 MJ/kg phytomass. The solid dry phytomass contained 361-520 g/kg cellulose, 191-320 g/kg hemicellulose, 83-122 g/kg acid detergent fibre and the estimated theoretical ethanol yield averaged 410-592 L/t organic dry matter. The studied energy crops were characterized by optimal quality indices of phytomass and can serve as feedstock for the production of pellets and cellulosic bioethanol. The agricultural residues have higher content of ash, nitrogen, sulphur and lower concentration of structural carbohydrates and energy value, which make them suitable to be used as a part of a diverse mix with biomass from woody species.

Key words: *agricultural residues, energy crops, Brassica napus oleifera, Miscanthus giganteus, quality indices of phytomas, Pisum sativum, Silphium perfoliatum, Sorghum bicolor.*

INTRODUCTION

Nowadays, energy has become a common topic for reporters and analysts around the world. The demand for fossil fuel, such as petroleum, coal and natural gas, increases worldwide, and a huge amount of fuel is used as an energy source. Because of such a high demand, the price of fuel keeps increasing, while the resources of fossil fuels are depleting. Besides, burning fossil fuels has several adverse effects, such as releasing greenhouse gas and increasing pollution, which, in turn has a harmful impact on human health. As a result, the depletion of fossil energy resources and the desire to decrease greenhouse gas emissions are two major issues that have driven the research for a secure and sustainable energy from a renewable source.

Agriculture is one of the largest sectors, which produces high amounts of biomass that can be an important input for the bioeconomy. Traditionally, some crop residues have been used as animal fodder, roof thatching, composting, soil mulching, matchstick and paper production. Lignocellulosic biomass from crop residues and energy crops can serve as a sustainable source of biodiesel, bioethanol, biogas, biohydrogen and solid fuel production, in order to mitigate the fossil fuel shortage and climate change issues.

The Republic of Moldova import 95 % fossil energy resources. Therefore, the issue of renewable energy sources is still relevant. According to the Energy Strategy of the Republic of Moldova, the energy from renewable sources should be increased to 20 % by the year 2030 and ¾ of this amount will make from biomass. To determine crops that are the most suitable for energy production, its agro-biological peculiarities, biochemical composition and thermo- physical properties, environmental impact and production economy must be investigated thoroughly. As a result of the research conducted in the "Alexandru Ciubotaru" National Botanical Garden (Institute) the collection of energy plants were founded, new cultivars of energy crops were created, registered in the Catalogue of Plant Varieties and patented by the State Agency on Intellectual Property of the Republic of

Moldova. These cultivars can be placed to use of marginal, polluted, eroded, salinized lands (Țîței & Roșca, 2021; Țîței, 2023).

The main objective of this research was to evaluate the quality indices of the solid dry phytomass from energy crops *Miscanthus giganteus*, *Silphium perfoliatum*, *Sorghum bicolor*, var. *scacharatum* and agricultural residues- stems of *Brassica napus oleifera* and *Pisum sativum.*

MATERIALS AND METHODS

The local cultivars *'Titan'* of *Miscanthus giganteus, 'Vital'* of *Silphium perfoliatum 'Vital', 'SAȘM 1'* of *Sorghum bicolor, var. saccharatum 'SAȘM1'* and agricultural residues, namely, stems of *Brassica napus oleifera* and *Pisum sativum* collected in the experimental plot of the "Alexandru Ciubotaru" National Botanical Garden (Institute) of MSU, Chişinău, located at latitude 46°58′25.7″N and longitude 28°52′57.8″E, served as subjects of the research. The harvested phytomass was chopped into small pieces using a stationary forage chopping unit. The chopped phytomass was then crushed in a beater mill, equipped with a sieve with mesh diameter of 6 mm. To perform the analyses, the milled phytomass samples were dried in an oven at 85°C and then milled (<1 mm) and homogenized. After that, the total carbon, hydrogen, nitrogen and sulphur amounts were determined by dry combustion in a Vario Macro CHNS analyser; pelleting equipment was used to perform biomass densification; the ash content and energy value of dry biomass and pellets were determined according to standard protocols at the Technical University of Moldova.

To determine the cell wall components in the dry mass of tested species, the amounts of neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were assessed using the near infrared spectroscopy (NIRS) technique PERTEN DA 7200 at the Research-Development Institute for Grassland Brasov, Romania. The amount of cellulose was calculated as ADF minus ADL and hemicelluloses – NDF minus ADF. The Theoretical Ethanol Potential (TEP) was calculated according to the equations of Goff et al. (2010) based on conversion of cellulose and hemicellulose into hexose (H) and pentose (P) sugars:

$$H = [\%Cel + (\%HC \times 0.07)] \times 172.82$$

$$P = [\%HC \times 0.93] \times 176.87$$

$$TEP = [H + P] \times 4.17$$

RESULTS AND DISCUSSIONS

The use of phytomass as solid fuel for energy supply requires characterizing elemental chemical components. The main constituents of dry biomass are carbon, oxygen and hydrogen. The energy released during the combustion process is positively correlated with the carbon and hydrogen contents as a function of the energy value of these elements. In contrast, high oxygen and nitrogen values decrease the calorific value. The higher hydrogen content determines and leads to a higher net caloric value. Nitrogen, sulphur and chlorine concentrations are some of the main causes of air pollution from biomass combustion. A higher percentage of these elements generally results in a higher level of air contaminants being released.

Table 1. The elemental composition of the dry phytomass from the studied species, %

Indices	*Miscanthus giganteus*	*Silphium perfoliatum*	*Sorghum bicolor*	*Brassica napus*	*Pisum sativum*
Carbon	50.00	46.28	49.35	45.60	41.37
Nitrogen	0.47	0.22	0.41	0.92	1.37
Hydrogen	5.86	6.14	5.60	5.14	4.32
Sulphur	0.03	0.03	0.06	0.07	0.10
Oxygen	43.60	47.33	44.58	48.30	52.84

The average elemental composition of the studied species for energy biomass is presented in Table 1. We found that the phytomass from *Miscanthus giganteus* and *Sorghum bicolor* is characterized by a very high concentration of carbon and very low concentration of nitrogen and sulphur, as compared with *Brassica napus* and *Pisum sativum* phytomass. The phytomass from *Silphium perfoliatum* is characterized by lower levels of nitrogen and higher - of hydrogen, as compared with other investigated species.

Different results regarding the elemental composition of the dry biomass from the studied species are given in the specialized literature. Karaosmanoglu et al. (1999) mentioned that rapeseed straw and stalks consisted of 45.17% carbon, 5.15% hydrogen, 0.75% nitrogen, 42.92% oxygen, 0.14% sulphur. Greenhalf et al. (2012) reported that rapeseed straw contained 48.35% carbon, 5.80% hydrogen, 1.15% nitrogen and 44.70% oxygen, but wheat straw – 47.24% carbon, 6.00% hydrogen, 0.66% nitrogen and 46.09% oxygen. Huang (2014) revealed that *Miscanthus giganteus* contained 44.21% carbon, 6.21% hydrogen, 0.56% nitrogen, 0.45% chlorine. Moon et al. (2014) found that *Miscanthus giganteus* had 44.00% carbon, 5.8% hydrogen and 0.023-0.038% sulphur. Stolarski et al. (2014) reported that *Silphium perfoliatum* harvested in March contained 47.40% carbon, 5.70% hydrogen and 0.36% sulphur, while *Miscanthus giganteus* – 49.80% carbon, 5.70% hydrogen and 0.026% sulphur. Šiaudinis et al. (2015) mentioned that *Silphium perfoliatum* contained 45.44% carbon, 5.28% hydrogen, 0.68% nitrogen, 38.57% oxygen, 0.07% sulphur. Ivanova et al. (2017) mentioned that pure sweet sorghum contained 343.1% carbon, 5.27% hydrogen, 0.61% nitrogen, 0.04% sulphur, 0.09% chlorine. Mohammadi et al. (2017) mentioned that *Miscanthus giganteus* pellets had 4.78% 49.45% carbon, 6.24% hydrogen. Dahunsi et al. (2019) found that *Sorghum bicolor* stalks had 41.24% carbon and 2.33% nitrogen. Babich et al. (2021) revealed that *Miscanthus giganteus* contained 47.1-49.7% carbon, 5.38-5.92% hydrogen, and 41.4-44.6% oxygen. Dok et al. (2021) reported that the pellets obtained from *Sorghum* stems had 43.98-54.19% carbon, 5.42-5.90% hydrogen, 0.45-0.76% nitrogen, 39.65-49.52% oxygen. Pegoretti et al. (2021) revealed that *Miscanthus giganteus* had 43.7% carbon, 6.21% hydrogen, 0.31% nitrogen, 0.1% sulphur. Szufa et al. (2021) mentioned that *Miscanthus giganteus* biomass contained 48.5% carbon, 6.20% hydrogen, 0.27% nitrogen, 42.56% oxygen, 0.05% sulphur, 0.015% chlorine. Szyszlak-Bargłowicz et al. (2021) mentioned that *Miscanthus giganteus* contained 48.45% carbon, 6.09% hydrogen, 0.24% nitrogen and 0.04% sulphur. Güleç et al. (2022) revealed that *Miscanthus* biomass had 0.10% nitrogen, 47.09% carbon, 0.10% sulphur, 6.30% hydrogen, but pea plant waste – 0.90% nitrogen, 44.06% carbon, 0.39% sulphur, 4.73% hydrogen, respectively. Šuric et al. (2023) found that the elemental composition of the investigated *Miscanthu*s biomass was 0.08-0.15% nitrogen, 51.6-52.6% carbon, 0.1-0.17% sulphur, 5.4-6.12% hydrogen. Țîței (2023) reported that biomass from *Miscanthus giganteus* contained 46.34% carbon, 5.95% hydrogen, 0.33% nitrogen, 0.05% sulphur, while from *Silphium perfoliatum*- 45.07% carbon, 5.96% hydrogen, 0.21% nitrogen, 0.03% sulphur. Angelova & Koleva (2024) remarked that the *Silphium perfoliatum* biomass had 0.846% nitrogen, 40.5% carbon, 0.052% sulphur, 5.7% hydrogen, 46.03% oxygen, 0.074% chlorine. Mohammadi et al. (2024) mentioned that the key elemental components of *Miscanthus* pellets were 45.47 % carbon, 5.62% hydrogen and 48.91% oxygen.

Table 2. The ash content and the energy value of phytomass and pellets from the studied species

Indices	*Miscanthus giganteus*	*Silphium perfoliatum*	*Sorghum bicolor*	*Brassica napus*	*Pisum sativum*
Ash content of phytomass, % DM	2.18	4.24	3.64	5.85	5.66
Gross calorific value of phytomass, MJ/kg DM	19.60	18.54	18.45	18.60	18.20
Net calorific value of phytomass, MJ/kg DM	18.33	17.35	17.22	17.40	17.20
Net calorific value of pellets, MJ/kg DM	16.20	15.11	15.26	15.70	15.20

While handling, transporting, storing and using biomass as fuel in its original form considerable difficulties are to be faced. For this reason, the densification of biomass, in the form of pellets and briquettes, is usually preferred and aimed at reducing the volume of biomass, which subsequently leads to lower transportation costs, easier usage and increased quantity of energy per unit of volume.

Pellet fuels are also more consistent in their structure, and therefore more suitable for the automated fuel system in the corporate and individual boilers. The ash content and the energy value of phytomass and pellets from the studied species are illustrated in Table 2. Ash content is one of the main factors of biomass quality, since higher amounts of ash decrease the quality of fuels, especially solid ones. The *Miscanthus giganteus* phytomass is characterized by excellent ash content (2.18%). The *Sorghum bicolor* phytomass had optimal ash concentration, while the *Silphium perfoliatum* phytomass has higher ash content, but lower than agricultural residues – stems of *Brassica napus oleifera* and *Pisum sativum.* The gross calorific value is higher in *Miscanthus giganteus* biomass (19.60 MJ/kg) and lower in *Pisum sativum* stems (18.20 MJ/kg). The level of net calorific value of dry phytomass from *Silphium perfoliatum*, *Sorghum bicolor, Brassica napus oleifera* and *Pisum sativum* does not differ considerably (17.20-17.40 MJ/kg). The pellets made from *Miscanthus giganteus* phytomass are characterized by net calorific value of 16.20 MJ/kg. The net calorific value of the pellets made from *Brassica napus oleifera* reached 15.70 MJ/kg, which was higher than in pellets from *Silphium perfoliatum*, *Sorghum bicolor* and *Pisum sativum* (15.11-17.26 MJ/kg).

Some authors mentioned various findings about the physical and mechanical properties of phytomass and pellets from the studied species. Karaosmanoglu et al. (1999) mentioned that rapeseed straw and stalks had 12.64% moisture content, 5.87% ash, 75.55% volatile matter, 18.58% fixed carbon and 141.17 kg/m^3 bulk density. Zabaniotou et al. (2008) revealed that rapeseed residues contained 3.95% ash, 71.01% volatile matter, 23.04% fixed carbon, 16.8 MJ/kg gross calorific value and 16.37 MJ/kg net calorific value. Greenhalf et al. (2012) determined that rapeseed straw had 6.58% ash, 76.9% volatile matter, 11.88% fixed carbon, 18.94 MJ/kg gross calorific value, but wheat straw 4.89% ash, 79.92% volatile matter, 15.18% fixed carbon and 18.69 MJ/kg gross calorific value, respectively. Maroušek (2013) reported that rapeseed straw pellets had 15.4 MJ/kg calorific value and 944 kg/m^3 specific density. Huang (2014) revealed that *Miscanthus giganteus* contained 1.70% ash, 74.28% volatile matter, 17.7 MJ/kg gross calorific value, 16.09 MJ/kg net calorific value. Melgarejo et al. (2014) found that residual biomass from *Pisum sativum* contained 11.65 % moisture content, 83.61 % volatile matter, 12.28% fixed carbon, 4.11% ash, and 11040 kcal/kg calorific power. Moon et al. (2014) reported that the pellets from *Miscanthus giganteus* had 2.2% ashes, 4.025 kcal/kg gross calorific value. Stolarski et al. (2014) reported that harvested in March *Silphium perfoliatum* had 3.04% ash, 18.70 MJ/kg gross calorific value and 13.35 MJ/kg net calorific value, but *Miscanthus giganteus* 2.06 % ash, 19.12 MJ/kg gross calorific value and 11.12 MJ/kg net calorific value. Heuzé et al. (2015) reported that pea straw contained 824-924 g/kg dry matter 8.1-12.12.1% ash, and 18.1 MJ/kg gross calorific value. Jasinskas et al. (2016) reported that pellets from *Silphium perfoliatum* had 11.6% humidity, 9.96%ash, 16.82 MJ/kg calorific value and density 1072.3 kg/m^3. Ivanova et al. (2017) mentioned that sweet sorghum biomass contained 3.9% ash, 70.8% volatile matter, 18.9MJ/kg gross calorific value and 17.7MJ/kg net calorific value. Ferreira et al. (2017) reported that *Sorghum* pellet properties were: 3% ash, 14.45% fixed carbon, 4525.0 kcal/kg gross calorific value, 3605.31 kcal/kg net calorific value and 735.1 kg/m^3 bulk density. Gageanu et al. (2018) reported that rapeseed stalk pellets had 10.54% moisture content, 3780.21 kcal/kg energy values, but pellets from wheat straws 8.16% moisture content, 3965.56 kcal/kg energy value, respectively. Muntean et al. (2018) determined that biomass from *Miscanthus giganteus* contained 2.51% ash and 19.3 MJ/kg gross calorific value, while the biomass from *Sorghum almum* – 3.71% ash and 18.6 MJ/kg gross calorific value. Babich et al. (2021) mentioned that *Miscanthus giganteus* had 2.7% ash, 73.6-73.9% volatile matter, 19.3–19.8% coke residue and specific heat of combustion ranged from 17 to 20 MJ/kg. Bury et al. (2021) found that the heat of combustion of *Silphium perfoliatum* varied from 14.59 MJ/kg in the first year to 17.68 MJ/kg in the third year. Szyszlak-Bargłowicz et al. (2021) mentioned that the *Miscanthus giganteus* biomass had 7.20% water, 2.36% ash, 73.61% volatile matter, 16.40% fixed carbon, 17.578 MJ/kg gross calorific value, 16.303 MJ/kg net calorific value. Dok et al. (2021) determined that the pellets obtained from the *Sorghum* stems were characterized by 4226-4412 kcal/kg gross calorific value and 512.3-705.5 kg/m^3 bulk density. Pegoretti et al. (2021) revealed that *Miscanthus giganteus* had 2.67% ash, 19.0MJ/kg gross calorific value, 17.76 MJ/kg net calorific value. Güleç et al. (2022) remarked that *Miscanthus* biomass had 9.60% ash, 79.00 % volatile matter, 11.40% fixed carbon, 18.07 MJ/kg gross calorific value, but pea plant waste – 5.80% ash, 78.00% volatile matter, 15.90% fixed carbon, 17.35 MJ/kg gross calorific value. Mill (2022) mentioned that the harvested *Miscanthus* biomass contained 15.0% water, 3.7% ash and 17.5 MJ/kg net calorific value. Witaszek et al. (2022) reported that *Silphium perfoliatum* had 15.58 MJ/kg heat of combustion measured in the calorimetric test and 14.08 MJ/kg calorific value. Šuric et al. (2023) determined that *Miscanthus* plants harvested in the spring period contained 84.64-85.15 % dry matter with 1.49-1.55 % ash, 11.68 - 12.16 % coke, 10.14 - 10.68 % fixed carbon, 82.44 -83.42 % volatile matter, 17.83-18.7 MJ/kg gross calorific value and 16.49-17.53 MJ/kg net calorific value. Țîței (2023) reported that biomass from *Miscanthus giganteus* had 1.75% ash and 19.5 MJ/kg gross calorific value, while from *Silphium perfoliatum*- 3.83% ash and 18.65 MJ/kg gross calorific value. Angelova & Koleva (2024) mentioned that the *Silphium perfoliatum* biomass contained 9.1% ash, 77.34 % volatile matter, 16.40% fixed carbon and 16.56 MJ/kg net calorific value. Mohammadi et al. (2024) reported that *Miscanthus* pellets had 8.16% moisture content, 5.13% ash, 18.39 MJ/kg gross calorific value and 1030 kg/m^3 specific density.

Table 3. The biochemical composition and the theoretical ethanol potential of phytomass from the studied species

Indices	*Miscanthus giganteus*	*Silphium perfoliatum*	*Sorghum bicolor*	*Brassica napus*	*Pisum sativum*
Acid detergent fibre, g/kg DM	617	632	537	484	446
Neutral detergent fibre, g/kg DM	937	880	779	675	649
Acid detergent lignin, g/kg DM	122	110	92	83	85
Cellulose, g/kg DM	495	522	445	401	361
Hemicellulose, g/kg DM	320	248	242	191	203
Theoretical ethanol potential, L/t ODM	592.37	558.80	498.90	492.62	409.64
- from hexose sugars, L/t ODM	372.87	388.69	332.90	298.62	270.40
- from pentose sugars, L/t ODM	219.50	170.11	166.00	131.00	139.24

Bioethanol production from lignocellulosic biomasses has been proved a promising alternative energy source, and its advantages include not only the possibility to compensate for the fast depleting petroleum resources, but also the low cost, the great potential availability and the possibility to reduce toxic emissions in the transportation sector. The bioethanol produced from lignocellulosic biomasses is currently promoted as an alternative transportation fuel, because of its antiknock properties, which help increasing octane ratings and improve fuel efficiency. The bioethanol yields are influenced by tissue composition, ratios of cellulose, hemicellulose and lignin. By analysing the cell wall composition of dry matter substrates (Table 3), we found that the highest average concentration of structural carbohydrates was generally observed in energy crops substrates from *Miscanthus giganteus* and *Silphium perfoliatum* as compared with agricultural residues – stems of *Brassica napus oleifera* and *Pisum sativum*. The *Sorghum bicolor* stalk biomass substrate has lower levels of cellulose, hemicellulose and lignin than energy crops substrates, which are due probable to the higher concentration of soluble carbohydrates, but they are still higher than in agricultural residues substrates. The theoretical ethanol yield from fermentable sugars averaged 592.37 L/t in *Miscanthus giganteus* substrate, 558.80 L/t in *Silphium perfoliatum* substrate, 498.90 L/t in *Sorghum bicolor* substrate, as compared with 492.62 L/t in *Brassica napus oleifera* substrate and 409.64 L/t in *Pisum sativum* substrate.

Several literature sources describe the composition of cell walls in studied plant species and the calculated ethanol yields. Greenhalf et al. (2012) determined that rapeseed straw had 37.55% cellulose, 31.37% hemicellulose, 21.30% lignin, 3.76% soluble, 6.02% ash. Stefaniak et al. (2012) studied the biomass composition of 152 sorghum samples and found that sorghum biomass types contained 6.3% ash, 3.3% protein, 9.0% sucrose, 13.7% lignin, 16.4% xylans, 29.1% glucans, 5.6% starch and the calculated ethanol yields reached 452 L/t; forage sorghum types – 8.4% ash, 4.5% protein, 1.1% sucrose, 13.0% lignin, 16.2% xylans, 37.2% glucans, 1.8% starch and the calculated ethanol yields were 456 L/t; sorghum-Sudan grass types – 8.8% ash, 3.7% protein, 2.4% sucrose, 13.5% lignin, 17.2% xylans, 33.2% glucans, 1.1% starch and calculated ethanol yields was 452 L/t; sweet sorghum types – 5.7% ash, 3.3% protein, 9.8 % sucrose, 13.0% lignin, 15.4% xylans, 29.9% glucans, 7.3% starch and 533 L/t. Melgarejo et al. (2014) found that residual biomass from *Pisum sativum* contained 26% cellulose, 20.5% hemicellulose and 3.92% lignin and soybean hulls 46-51% cellulose, 16–18% hemicellulose and 1.4–2% lignin. Heuzé et al. (2015) reported that pea straw contained 43.9-61.5 % NDF, 27.6-42.5% ADF, 4.5-9.8 % lignin. Lee & Kuan (2015) remarked that the contents of cellulose in dried biomass of *Miscanthus* × *giganteus* was 41.1% and theoretical ethanol yields were 0.211-0.233 g/g raw biomass if only cellulose is taken into account. Ferreira et al. (2017) found that *Sorghum bicolor* biomass residuals contained 29.05% lignin, 52.8% holocellulose and 15.6% extractives. Xue et al. (2017) remarked that *Miscanthus* straw contains about 41-45% cellulose, 20.6-33.0% hemicellulose, and 19.0-23.4% lignin. Scagline-Mellor et al. (2018) reported that *Miscanthus giganteus* biomass composition and theoretical ethanol yield was 4.54% ash, 87.78% aNDF, 5.46% ADL and 465L/T. Viel et al. (2018) remarked that the chemical characterization of agro-resources of rapeseed straw had the following indices: 53.06% cellulose, 18.13% hemicellulose, 9.63 % lignin, 17.68% soluble and 0.79% ash. Allison et al. (2019) revealed that *Miscanthus giganteus* contained 86.69-89.28% NDF, 11.08-12.06% ADL, 45.80-48.11% cellulose, 27.22-31.78% hemicellulose; *Miscanthus sacchariflorus* 84.38-87.178% NDF, 9.85-10.46% ADL, 41.17-44.18% cellulose, 31.67-33.35% hemicellulose and *Miscanthus sinensis* 84.02-85.77% NDF, 8.97-9.17% ADL, 42.08-43.27% cellulose, 32.98-33.98% hemicellulose. Almeida et al. (2019) reported that sorghum biomass had 68.39-73.06% NDF, 40.61-46.84% ADF, 4.79-7.77% ADL. 35.81-39.07% cellulose and 25.34-28.91% hemicellulose. Alaei et al. (2022) remarked that the chemical composition of green pea residues was 971.7g/kg dry matter, 9.66% crude protein, 8.49% ash, 47.33% ADF, 62.66% NDF, 44.9% cellulose, 20.4% hemicellulose, and 13.7% lignin. Hajj Obeid et al. (2022) mentioned that the chemical composition of the rapeseed straws was 51.40-55.20% cellulose, 9.30-15.00% hemicellulose, 8.40-10.90 % lignin, 20.90-29.90% soluble and 0.40-0.90% inorganic materials. Mill (2022) reported that *Miscanthus* biomass contained 43.06–52.20% cellulose, 24.83–33.98% hemicellulose 9.27–12.58% lignin, 2.16–3.47% ash. Çelik et al. (2023) revealed that sweet sorghum biomass had 30.72-40.27% cellulose and 18.34-24.90% hemicellulose. Țîței (2023) found that *Miscanthus giganteus* had 50.8% cellulose, 30.5% hemicellulose and theoretical ethanol yield was 591 L/t. Witaszek et al. (2022) reported that *Silphium perfoliatum biomass* had 30.96% cellulose, 22.6% hemicellulose and 21.62% lignin. Tóth Š. (2023) reported that the ligno-cellulose quality of *Silphium perfoliatum* green phytomass was: 31.32-48.94% ADL, 34.94–54.69% NDF, 7.21-12.54% ADL, 24.11-37.30% cellulose, 24.11 – 37.30%, 2.33–5.75% hemicellulose.

CONCLUSIONS

The local cultivars of energy crops *'Titan'* of *Miscanthus giganteus, 'Vital'* of *Silphium perfoliatum* and *'SAȘM 1'* of *Sorghum bicolor var. saccharatum* were characterized by optimal quality indices of phytomass and can serve as feedstock for the production of pellets and cellulosic bioethanol, as renewable energy sources.

The agricultural residues – stems of *Brassica napus oleifera* and *Pisum sativum* have higher content of ash, nitrogen, sulphur and lower concentration of structural carbohydrates, which make them suitable to be used as a part of a diverse mix with biomass from woody species for energy production.

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A CASE STUDY ON GRAIN LEGUMES GENETIC RESOURCES AVAILABLE FOR USE IN BREEDING FOR SUSTAINABLE AGRICULTURE - A REVIEW

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Abstract

Plant genetic resources are essential for sustainable agriculture and for securing the global food supply. Sustainable agriculture and global food security depend on the availability of well-described plant genetic resources. The work presents challenges related to the availability of grain legumes genetic resources for use in breeding for sustainable agriculture, germplasm held in gene banks, and approaches to enhance the use and access to conserved resources. A better understanding of the stability and potential of investigated traits can be achieved by considering data from different experiments, thereby improving the prospects of using genetic resources in novel breeding programs. Consequently, genetic resource collections can become more utilized through enhanced cooperation and sharing not only of seeds, but also the accumulated knowledge gained over many years of resource regeneration and/or research. Legumes are a vital group of crops that include beans, lentils, chickpeas, lupins, and many others, contributing significantly to global food security, nutrition, and sustainable agriculture. The genetic resources of legumes are essential for breeding programs aimed at improving yield, disease resistance, and adaptability to changing climates. However, these genetic resources face several safeguarding challenges.

Key words: *sustainable agriculture, germplasm, crops, genetic variation.*

INTRODUCTION

Sustainability is imperative, particularly within agri-food systems, where it becomes even more critical amid experienced global challenges, as climate changes, conflicts, and pandemics. Agrobiodiversity conservation, sustainable agricultural practices, and food security demand feasible solutions that are both economically competitive and environmentally beneficial. Enhancing sustainability is essential, particularly for agri-food chains, given the current global challenges related to climate changes, food security and need to preserve the quality of environment. The grain legumes species, featured by unique biological and nutritional characteristics, play a pivotal role in addressing critical societal issues related to agri-food, including agrobiodiversity conservation, sustainable farming practices, food security, and human health.

Capturing the full diversity of grain legumes and ensuring availability and access to well-described, well-managed genetic resource collections is of paramount importance for advancing grain legume crops. This is essential for achieving a competitive level of agronomic performance and sustainability.

Legumes stand out as a sustainable, cost-effective, water-efficient, and low-carbon footprint crop, making them a vital component of environmentally conscious agriculture. Globally, grain legumes represent approximately 15% of the 7.4 million accessions preserved in gene banks (Tripathi, et al. 2020). However, same authors stated that a significant challenge is that over half of this germplasm lacks essential characterization and evaluation data, which severely limits its use in legume improvement programs. The comprehensive characterization of gene bank accessions is crucial for unlocking their full potential.

Recognizing the importance of accessible and well-documented grain legumes genetic resources, the establishment of a robust framework for selection and creation of practical, harmonized research plans become an imperative. These efforts aim to promote the utilization of plant genetic resources, thereby securing long-term economic, environmental, climatic, and socio-economic advantages.

Grain legumes become components of sustainable agriculture, driven by emerging research opportunities in key areas. A major challenge in this endeavor is securing adequate expertise and funding to ensure the network's sustainability and continued impact.

A critical task involves strategically selecting legumes, species and cultivars that can be effectively integrated into diverse cropping systems. This selection process must carefully balance specific challenges as economic yield, environmental and agronomic benefits, ensuring both profitability and ecological resilience.

It is crucial to pinpoint the traits valued by end-users (breeders, farmers, technicians), necessitating improved documentation of legume germplasm diversity in *ex situ* collections. This process involves several steps which may include characterizing genetic material through DNA markers, evaluating responses to specific biotic and abiotic stresses, and assessing organoleptic qualities as well as health-related attributes or anti nutritional profile of legumes

Most of the current strategies are focussed on modernizing and refining pre-breeding and breeding techniques for legumes by exploring genetic resources - such as crop wild relatives, landraces, minor crops, cultivars, and breeding lines - to maximize benefits for human nutrition, environmental sustainability, and end-user needs within divers agri food systems. These approaches will facilitate the development of advanced methods for evaluating and breeding legume cultigens, ultimately leading to a more diverse and efficient pipeline of improved cultivars. Additionally, addressing abiotic stress limitations, particularly water deficit, salinity, and thermal shocks, requires extensive investigation to develop resilient legume varieties.

Amid rising demand for plant-based products like proteins and oils, coupled with increasing economic and environmental pressures on agroecosystems, grain legumes are set to play a pivotal role in shaping the future of sustainable cropping systems. Their ability to enhance soil health, reduce input dependency, and provide nutritious yields positions them as indispensable allies in addressing global agricultural challenges.

This work aims to strengthen a strategic approach that leverages genetic variation of grain legumes to enhance the access and the share of plant genetic resources, in a context of actively engaging European farmers, advisory services, research institutes, breeding companies, and food producers across diverse socioeconomic contexts.

BACKGROUND AND CASE STUDY DOCUMENTATION

A search for studies that link legumes genetic resources and their role for food and resilient agriculture was undertaken by screening case studies, reviews, projects, initiatives and open access data bases from the ScienceDirect, Scopus and Google Scholar databases. Peer-reviewed literature was complemented, where appropriate, with relevant project reports and databases. Sector-specific searches were undertaken to ensure adequate coverage legumes genetic resources, as their diversity to meet breeding objectives like nutritional quality, yield, and tolerance to a diverse source of biotic and abiotic stresses, role, and the impact of *ex situ*, *in situ* and *on farm* conservation. The search results, consisting in 56 sources were reviewed to determine which threats were overrepresented and which might represent research gaps. The conclusions and recommendations of this thematic study are built upon the reviews papers, which include background literature on the various sectors and topics addressed. Case studies, projects, databases were included in the analysis met the following criteria: include a description of legumes diversity, address one or more ways in which biodiversity contributes to coping with or managing environmental stresses and change in production systems. Based on this search specific recommendations to facilitate the use and the share of plant genetic resources are provided.

THE CONTEXT OF PLANT GENETIC RESOURCES CONSERVATION

The tasks of preserving and characterizing genetic resources are requiring expertise, labour, equipment, and significant funding. A systematic approach is mandatory for effective plant genetic resource management. This will maximize the value and use capacity, adhering to high-quality standards. According to Genetic Resources Strategy for Europe, 2021, this systematic approach includes implementing agreed-upon ontologies and following FAIR principles (Findable, Accessible, Interoperable, Reusable) for open data sharing, documentation, and archiving. Moreover, conservation of genetic resources requires interventions to ensure the ecological or management processes necessary for the preservation of populations *in situ* (including on-farm), and to support the collection and management of population samples in dedicated *ex situ* facilities. The success of screening programs in crop breeding, focused on identifying valuable traits, hinges on the availability of a broad and genetically diverse pool of plant resources sourced globally. Following the screening process, only a few breeding lines are ultimately chosen to develop the final, commercially viable variety (Ebert et al., 2023).

Based on our experience and also stated generally, traditional plant variety innovation in agriculture relies heavily on centralized efforts by seed companies, scientists, and experts, who develop new varieties using the vast genetic diversity stored in germplasm banks. However, this model limits plant genetic resource utilization to a small group of contributors, resulting in innovative varieties reaching farmers only after a lengthy process (10–20 years). This slow, narrow approach minimally contributes to agricultural diversification and hinders the adoption of minor crops, especially when rapid transitions are needed.

Participatory and decentralized innovation strategies, rooted in localized plant genetic resources conservation (essential for supporting plant breeding programs), offer a promising alternative to promote agricultural diversification and climate-economic adaptation. To maximize their effectiveness, these strategies should integrate cutting-edge technologies, including data analytics, genomics, metabolomics, high-throughput phenotyping, digital tools. Additionally, leveraging satellite-based weather data and developing crop-specific climatic predictions can enhance resilience to environmental variability, as developed and implemented by pulsesincrease.eu.

Another critical factor is the development of new food products, based on genetic resources conserved and explored in breeding programs. According to FAO reports, most food products derive from a limited number of species, varieties, and market classes, constrained by the scale of industrial production. To overcome this bottleneck, diversifying food types requires not only farmer and stakeholder involvement but also broader societal engagement. This collective effort can drive cultural and economic innovation in agricultural and food production systems.

Due to the rising demand for plant-based products, such as proteins and oils, coupled with increasing economic and environmental pressures on agro-ecosystems, grain legumes play a significant role in future cropping systems. Improving the nutritional quality of food, especially plant-based proteins, is essential for enhancing human health. Achieving this dual goal of quantity and quality is further complicated by environmental shifts, including unpredictable rainfall patterns, extreme weather events like floods and storms, and rising temperatures, which can lead to soil erosion, pest infestations, and reduced crop yields. Additionally, transitions to low-input agricultural systems, driven by evolving farming conditions and regulatory policies on land and energy use, may exacerbate food production challenges.

Legumes are essential in tackling these challenges, as they provide food, fodder, energy, and nutritional security, while simultaneously benefiting the environment through their nitrogen-fixing capabilities and promotion of agrobiodiversity in cereal-centric systems. (Bellucci et al., 2021). Enhancing the European production of grain legumes crops is amongst the first steps in the protein transition. The most cultivated grain legumes species in Europe are faba bean (*Vicia faba* L.), pea (*Pisum sativum* L.) and soybean (*Glycine max* (L.) Merr.) (Eurostat, 2023, Sepngang et al., 2020). Expanding legume cultivation would substantially strengthen regional protein supply independence. Leveraging diverse genetic resources in legume breeding and incorporating new cultivars into both conventional and organic farming systems offers significant potential to develop climate-resilient production systems for current and future needs.

STRATEGIES TO CONSERVE THE GRAIN LEGUMES DIVERSITY

Despite the abundance of grain legumes genetic resources, their application in breeding remains underdeveloped, yielding suboptimal results. Key barriers include insufficient metadata (such as essential passport data and user-relevant descriptors), heterogeneity among accessions, and non-standardized data formats. These challenges not only hinder the effective use of genetic resources but also impede efforts to secure funding for their conservation (Bellucci et al., 2021). The main strategies and initiatives to conserve plant genetic resources and information related their taxonomy are summarised in Figure 1.

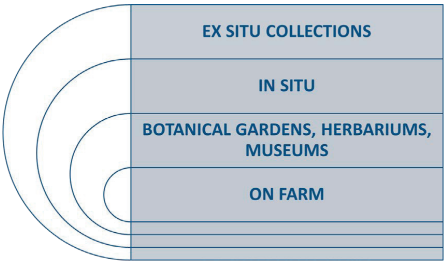

Figure 1. Ways to conserve grain legumes genetic resources

Ex Situ Germplasm Collections - gene banks host valuable plant genetic resources that require multiplication, regeneration, and thorough evaluation to promote their use. Additionally, there is a wealth of advanced materials and data from various projects and activities that could be integrated and broadly shared with stakeholders throughout the European agri-food chain.

▪Chickpea and lentil are two crops present in worldwide collections, according to the querying of the most important online platforms, Genesys (Virtual Gene bank of Plant Genetic Resources for Food Agriculture, https://www.genesys-pgr.org) and EURISCO (European Search Catalogue for Plant Genetic Resources, https://eurisco.ipk-gatersleben.de/apex/eurisco). Gene banks across the world conserve about 97,400 accessions of chickpea germplasm. (Brezeanu et al., 2024).

Based on GENESYS (https://www.genesys-pgr.org) data cited by pulsesincrease.eu the largest collections of chickpeas are maintained at International Centre for Agricultural Research in the Dry Areas (ICARDA) and International Crops Research Institute for the Semi-Arid Tropics. (ICRISAT), centres of Consultative Group for International Agricultural Research (CGIAR) with unique accessions estimated at more than 15,000 and 20,000, respectively. California's UC Davis houses the world's most extensive collection of wild materials and their derived introgression lines.

▪ Current lentil cultivars suffer from a limited genetic base, making them vulnerable to diverse biotic and abiotic stresses. The solution to these challenges lies in the genetic diversity preserved within gene banks. This germplasm diversity is of paramount importance, as it enables the identification of valuable genes that can serve as essential resources for lentil breeding programs. There are currently over 58,000 lentil accessions held in various gene banks worldwide. Genesys displays information for about 70% of these (https://www.genesys-pgr.org/c/lentil).

ICARDA, with 12,463 accessions, is the centre with the largest lentil collection (Figure 2).

ICRISAT and ICARDA continue to provide global leadership in the conservation of chickpea and lentil germplasm. Over time, both collections have expanded substantially, and today, the two institutes collectively safeguard 33.9% of chickpea and 43.5% of lentil accessions in their facilities (Piergiovanni, 2022).

▪Common bean genetic resources are secured in some focal points, based on GENESYS data the most important *P. vulgaris* collections are maintained at Centro Internacional de Agricultura Tropical (CIAT) and USDA-ARS, at Washington State University (USA), with more than 24,000 and 18,500 accessions respectively. In Europe large collections are conserved at Leibniz Institute of Plant Genetics and Crop Plant Research-IPK (~8,500 accessions) as it is shown in Figure 2.

▪Lupinus has its largest number of accessions held in two gene banks: the Australian Grains Gene bank and IPK possess majority of *L. albus*

(800 accessions) and *L. mutabilis* (ca. 700 accessions), respectively. Other important lupin resources are maintained at Plant Breeding and Acclimatization Institute (IHAR-PIB, Poland, ~300 accessions) and IGR-PAN (~300 accessions), according to pulsesincrease.eu.

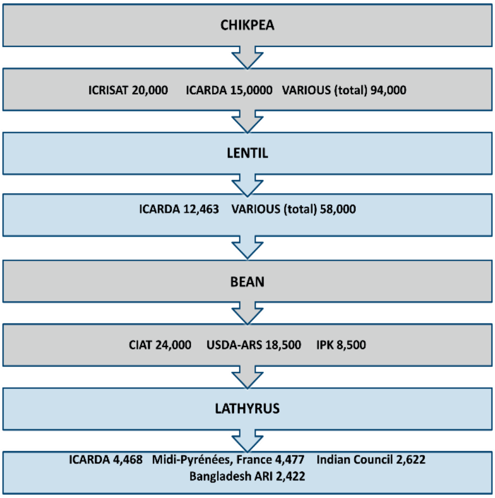

Figure 2. Representative centers for legumes genetic resources conservation and the number of conserved accessions

The largest collections of *Lathyrus* are held by the Conservatoire botanique national Midi-Pyrénées, France (4,477), ICARDA, Lebanon (4,468), the Indian Council of Agricultural Research National Bureau of Plant Genetic Resources, India (2,622), and the Bangladesh Agricultural Research Institute, Bangladesh (2,422) (Singh et al., 2024). The *Lathyrus* (Figure 2) conservation strategy highlight the importance of enhancing documentation systems, ensuring safe multiplication and duplication, and adopting international standards to manage existing collections (Global Crop Diversity Trust Strategy, 2007).

Botanical Gardens, Herbariums and Museums were established to respond to the growing interest for taxonomy, and no provision has been made for biodiversity conservation and although the Convention on Biological Diversity (CBD) mentions the opportunity of capacity building, if countries will take the necessary steps. Dixon (2007) mentioned botanic gardens as “mandated with conserving the world’s flora” but in fact there is no such mandate and the task of making and maintaining the necessary *ex situ* collections of wild species has largely fallen to botanic gardens. Research conducted at botanical gardens has highlighted the importance of addressing the risks associated with hybridization in *ex situ* conservation efforts for threatened plant species. Studies have demonstrated that spontaneous hybridization within these facilities can compromise the genetic integrity of preserved collections, potentially leading to contamination of open-pollinated seeds or seedlings (Ye et al., 2006; Zhang et al., 2010).

In Situ Conservation of Crop Wild Relatives of targeted species is even more acute than that for *ex situ* conservation as discussed by Heywood & Dulloo (2006). Conducting multi-crop gene pool analyses, potentially focusing on legume species found in each of the Vavilov Centres, should be a globally important priority. Once *in situ* locations are identified, they should be leveraged to enhance global food security, maintaining as it possible the original selection pressures exerted. This necessitates understanding plant (and human) demography, the diversity of bean mixtures, the variety of selection pressures, and allowing sufficient time for continued evolution (Smýkal et al., 2014).

Planty populations of wild bean relatives can thrive in protected areas across different regions, mainly the Americas. The challenging issue remains the absence of published records, assessing which species and populations are currently conserved and were. Similarly, the absence of published inventories makes monitoring the success or failure of *in situ* conservation equally difficult (Debouck, 2014).

On-farm conservation of landraces is feasible if sustainable economic mechanisms are applied, and farmers are financial supported for the conservation of higher diversity. This was previously suggested (Zeven 1996) for the conservation of landraces in western Europe – he raised the attention on the fact that farmers will only have an interest to grow landraces when and if they are paid to do so. Some exceptions can work in case of specialty markets asking for specific landraces as in central Italy (Negri and Tosti 2002), in any case, markets may change.

If for example, beans are used by the food processing industry, it is possible that interest for specific colours, colour patterns and size will continue to fall, causing little incentive for farmers to keep all that diversity on farm (Kaplan and Kaplan 1992).

CHALLENGES RELATED TO THE AVAILABILITY OF GRAIN LEGUMES GENETIC RESOURCES FOR USE IN BREEDING WORKS

The role and the importance of grain legumes in sustainable agriculture can be substantially elevated by harnessing genetic diversity across breeding programs, with a focus on enhancing yield, resilience, nutritional quality and antinutritional profile.

A key task in advancing sustainable agriculture is selection of legume species and cultivars that can seamlessly integrate into diverse cropping systems, according to the current climatic pressure. A critical challenge lies in striking a balance between maximizing yield - a primary driver of economic returns (highly demanded by farmers) and enhancing environmental and agronomic benefits (recommended by different practices, programs and strategies). Securing legume genetic resources requires a comprehensive strategy that includes robust conservation measures, supportive policies, capacity development, and the promotion of sustainable farming practices. Among the most pressing threats (Figure 3) related to the availability and utilization of genetic resources are:

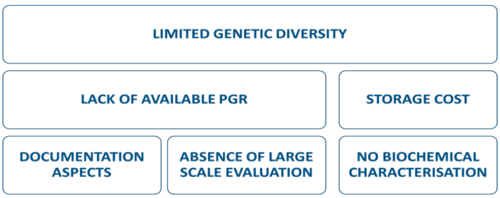

Figure 3. Threats related to the availability of grain legumes genetic resources for use in breeding works

Lack of material available for the distribution - in general, the total amount of seeds available in gene banks and research units is limited and this is challenging in terms of development of collections for research topics in frame of joint research projects. Moreover, this in a major limitation that affects the access of farmers to materials to be tested in their fields.

The narrow genetic diversity of modern legume varieties presents a substantial obstacle to their further enhancement, a critical need for addressing present and future agricultural challenges. For instance, the most widely cultivated chickpea varieties are especially susceptible to water stress and climate change impacts (Muehlbauer, 2017). To utilize available germplasm more efficiently, it is essential to broaden the genetic diversity of commercial varieties and maximize genetic gains during the breeding process (Jha, 2020). Core collections serve as an invaluable starting point for germplasm users, as they streamline the process by eliminating the time and resource-intensive task of screening large germplasm segments to identify parents with desirable traits.

The absence of large-scale germplasm evaluation is another significant limitation. Efforts to identify useful traits are often confined to a national framework, focusing on a limited set of bean germplasm from a single geographic origin. Evaluation should be done at the crop (biological) level, not at the national level. Vavilov, implemented in the 1920s an evaluation network of 400 experimental stations with 20,000 staff (Hawkes, 1990, Reznick & Vavilov 1997) in one country. The model was replicated by CIAT that followed that approach for a period of twenty years, starting at the middle of 70s. (CIAT 1973), with many useful traits disclosed (Hidalgo and Beebe 1997) and a significant impact in breeding (Voysest, 2000) unmatched to date.

The costs of storage, distribution and multiplication of materials, and costs for acquisitions of new samples (Halewood, 2020). Ensuring proper storage conditions and seeds availability to researchers, breeders, farmers and so on, represents great efforts to setting up core collections or mini core collections. These core collections consist of a minimized set of accessions that maximally represent the genetic diversity of the entire collection. This approach significantly reduces the volume of material requiring distribution and multiplication. In recent years, ICRISAT and ICARDA have developed several chickpea and lentil core collections, incorporating diverse traits such as pest resistance and morphological characteristics. However, this process remains ongoing due to the continuous acquisition of new materials and the rapid advancements in knowledge from omics techniques.

Lack of characterisation of biochemical traits – It is estimated a large percentage, up to 70% of conserved germplasm is well characterised. This is done based on distinct morpho-agronomic traits including resistance to biotic and abiotic stresses using crop-specific descriptor sets (Upadhyaya et al., 2011). Despite this, just a small percentage of collections have been characterized for biochemical traits. As model can serve a comprehensive study on common bean (*Phaseolus vulgaris*), chickpea (*Cicer arietinum*), lentil (*Lens culinaris*), white lupin (*Lupinus albus*) and pearl lupin (*Lupinus mutabilis*), at the tissue level. 3,400 metabolites covering major nutritional and anti-nutritional compounds were detected and quantified. This structure includes 224 derivatized metabolites, 2,283 specialized metabolites and 923 lipids. This kind of data ensure a foundation to advance metabolomics-assisted crop breeding and enable metabolite-based genome-wide association studies, thereby unravelling the genetic and biochemical underpinnings of metabolism in legume species (Bulut et al., 2023).

Documentation aspects at accession level might be challenging in many cases related to wide data availability on the internet. This is a crucial aspect in terms of management of collection. Documentation units have been set up in nearly every gene bank globally. These units collectively grapple with the critical task of ensuring that information about gene bank accessions is consistently maintained and effectively transferred from one generation to the next. (Weise et al., 2020)

APPROACHES TO FACILITATE THE ACCESS TO THE GRAIN LEGUMES GENETIC RESOURCES FOR A HIGH STANDING USE IN BREEDING

Facilitating proper access to grain legumes genetic resources is crucial for advancing breeding programs, as it enables researchers and breeders to harness diverse traits for improved yield, resilience, and nutritional quality. By streamlining access, breeders can more efficiently develop varieties that address global challenges such as climate change, food security, and sustainable agriculture, ultimately benefiting farmers and consumers alike.

The following tools contribute to a successful management of data and genetic diversity evaluation and utilization. The final goal is to ensure the access to unlock the stored collection and to make them useful for food and agriculture, ensuring environmental benefits and worldwide human nutrition. The useful tools and approaches for facilitating the access and use are listed in Figure 4 and described in this section. The list is non-hierarchical, and its application should be tailored to individual contexts.

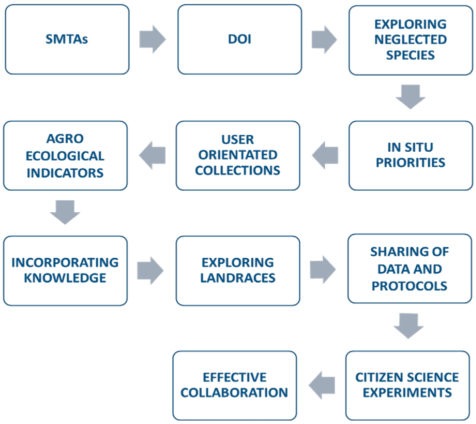

Figure 4. Approaches for a successful management of data and genetic resources

Standard Material Transfer Agreement (SMTA) It is a tool developed to ensure the implementation of principles of the Treaty "Treaty on Plant Genetic Resources for Food and Agriculture" (ITPGRFA, "Treaty"). Its standardized format facilitates access to and fair use of plant genetic resources for food and agriculture (PGRFA) for conservation, development and training to promote sustainable agricultural development. The PGRFA allows the use or free of charge redistribution for research and breeding purposes. Farmers are invited to exploit this tool in their farm management.

Transparent exchanges will enhance accessibility and make information readily available, supported by mechanisms such as subscription systems or seed sales taxes. The primary benefit of this system lies in eliminating legal uncertainties and minimizing transaction costs for conservers, curators, and users of genetic resources, thereby enabling plant breeders to fulfil their mission more effectively.

Identity of collection - assignment of Digital Object Identifiers to avoid the difficulty of collaboration on conservation, research and breeding. Without a common standard for identification, it is challenging to locate information associated with the material. Millions of accessions are currently preserved in germplasm collections and breeding pools, yet numerous duplicates often contain valuable data that is frequently lost during transfers between custodians. Compounding this issue, diverse user groups, including plant breeders, data curators, researchers, and extensionists, often employ distinct methods to assign identifiers based on their specific requirements. This lack of standardization has historically impeded the global exchange of Plant Genetic Resources for Food and Agriculture (PGRFA) data, a longstanding barrier to their effective conservation and sustainable use. By adopting the Digital Object Identifier (DOI) standards endorsed by the Governing Body of the International Treaty, users can ensure unique and permanent identification and documentation of their plant material, significantly enhancing data interoperability across various systems. (Alercia, et al., 2018).

Explore the effects and benefits promote access to neglected grain legumes featured by resilience suitable for introduction in breeding activities - development of open and distributed seed system involving farmers and other main actors to support innovation for agricultural diversification. These species possess an extensive adaptation to poor soils and resilience to biotic and abiotic stresses, having a great potential to improve the livelihoods of smallholder farmers globally (Chandra et al., 2020; Kebede et al., 2020). Given climate change, legumes crops integrated into agroecosystems for diversification can break the cycle of pests and diseases and contribute to balancing the deficit in plant protein production worldwide (Chandra et al, 2020; Sita et al., 2020).

Establishing in situ conservation priorities, having greater practical value and being cost efficient to establish multi-gene pool conservation targets irrespective of individual gene pool results (Smýkal et al., 2014). Development of GAP analysis according to (Maxted et al., 2008) which involves steps as: identify priority taxa, identify genetic (or ecogeographic as a proxy for genetic) diversity and complementary hotspots using distribution and environmental data, match current *in situ* and *ex situ* conservation actions with the identified genetic (or ecogeographic) diversity and complementary hotspots to identify 'gaps' formulate revised *in situ* and *ex situ* conservation actions derived from identification of the gaps.

The GAP analysis is a valuable method for diversity conservation, explored and applied by Maxted et al. (2008), to illustrate its potential utility in both *in situ* and *ex situ* genetic diversity conservation approaches. The authors outlined four key stages: (i) defining the focal species and geographic region; (ii) evaluating native biodiversity alongside potential threats; (iii) reviewing the effectiveness of existing complementary *in situ* and *ex situ* conservation measures; and (iv) refining the conservation approach by examining disparities between the natural diversity and the components of that diversity currently safeguarded through *in situ* and *ex situ* strategies.

This methodology has been previously explored for several legumes CWR groups, as vetch (Maxted, 1995), lentils (Ferguson et al., 1998), Asiatic *Vigna* (Tomooka et al., 2009), African *Vigna* species (Maxted et al., 2004), perennial *Medicago* (Bennett et al., 2006), garden pea, faba bean and cowpea as important food crop gene pools (Maxted and Kell, 2009), *Phaseolus* species (Ramırez-Villegas et al., 2010), *Lathyrus* species (Shehadeh et al., 2013).

Developing and mapping crop-specific agro-ecological indicators across current and future climate scenarios to enhance the understanding of the benefits legumes offer for improving climatic resilience and adaptive capacity. This approach provides valuable insights across a wide range of spatial and temporal conditions, supporting more informed agricultural strategies.

Development of specific user-oriented collections at gene bank level – this can include populations and genetically purified lines, complemented by novel information services, able to guide users based on their specific interest (van Treuren and van Hintum, 2014).

Incorporating knowledge of related organisms, such as pathogens and pests, into the utilization of new germplasm in breeding programs would significantly enhance the development of improved crop varieties. These varieties would be better equipped to withstand climate change challenges and contribute to more sustainable agricultural practices. By leveraging this integrated approach, breeding efforts can address both biotic stresses and environmental changes, fostering resilience and sustainability in agriculture (Erbert, 2023).

The development and sharing of guidelines, protocols and data (Piergiovanni, 2022) The characterization, maintenance, and utilization of food legume genetic resources are critical for breeding new varieties with enhanced adaptability, improved quality traits, and higher nutritional value. Recent advancements in genomics and metabolomics provide unprecedented opportunities to explore heritable diversity, identify the genetic basis of phenotypic traits, and develop genomic prediction tools. These technologies also deepen the understanding of Genotype × Environment interactions, enabling more precise breeding strategies (Rocchetti et al., 2022).

In this context, creating well-described collections based on pure single-seed-descent (SSD) lines, where phenotypic traits are directly linked to specific genotypes, offers a powerful approach to efficiently manage genetic resources and generate unique, accessible information for users. This concept, termed "Intelligent Collections" (ICs), has been proposed for chickpea, as well as for common bean (Cortinovis et al., 2021), lentil (Guerra-García et al., 2021), and lupin (Kroc et al., 2021). ICs are developed through standardized protocols, with phenotypic characterization integrated during the multiplication and seed-increase stages, ensuring a robust foundation for future breeding and research efforts.

Advanced tools such as genomics, climate-based crop suitability modelling, plant phenotyping, soil biodiversity analyses, metabolomics, and nutritional quality assessment are being increasingly explored to enhance agricultural research and conservation efforts. Additionally, breakthroughs in sequencing technologies have significantly reduced production costs, enabling the efficient analysis of large volumes of germplasm. This progress has opened new possibilities for screening gene bank collections more effectively, particularly in identifying DNA sequence variations. (van Treuren and van Hintum, 2014).

Designing a multi-location variety testing system and evaluation networks – one successful example is EVA networks. All activities are designed to deepen understanding of valuable traits in publicly available crop germplasm, with the aim of integrating these traits into public and private breeding programs. Evaluation data from diverse environments across Europe are meant to identify adapted accessions for developing climate-resilient crops. The primary goal is to unite diverse stakeholders of Plant Genetic Resources for Food and Agriculture (PGRFA) to collectively enhance understanding of the genetic diversity preserved in gene banks and to make this material accessible for breeding and research purposes. This European Initiative holds strategic importance for Europe, offering a platform to promote the sustainable use of PGRFA, support the adaptation of European agriculture to climate change, and contribute to achieving relevant Sustainable Development Goals (SDGs). Through collaborative projects involving public and private sector partners, as well as participatory plant breeding initiatives, EVA is generating standardized evaluation data, both phenotypic and genotypic, for seven legumes crop groups (EVA legumes, 2024).

Exploring the landraces - the local germplasm, oftentimes associated with traditional sustainable farming systems, incorporates diverse and dynamic gene pools associated to adaptive traits that allow subsistence, in a changing climate. Initiatives such as (ExploDiv, 2022) are focused on exploring the potential of locally adapted and genetically diverse resources. Previous studies on landraces showed their potential to address ongoing and emerging challenges in real farms under biotic and abiotic stressful conditions. Despite their variable phenology and typically modest yields, local ecotypes often boast high nutritional value. Landraces are essential in plant breeding, possessing traits for improved nutrient efficiency and resilience to abiotic stresses such as drought, salinity, and heat. Systematic evaluation of these resources can uncover genetic diversity, aiding in the discovery of alleles that enhance both yield and stress tolerance. This strategy holds promise to increase the productivity and resilience of staple crops in environmentally vulnerable regions. (Dwivedi et al, 2016).

Citizen science experiments for creating participatory and decentralized innovation framework. The goal is to implement a decentralized approach for conserving food legume genetic resources. citizens are actively engaged in evaluation and conservation efforts, as well as in seed sharing and exchange initiatives. The gain is to disseminate knowledge related to legume biodiversity and to empower citizens to participate directly in these activities (pulsesincrease.eu).

Effective collaboration between research institutions, farmers, and international organizations is crucial to tackle these challenges and secure the enduring availability of legume genetic diversity for the benefit of future generations. As the most important directions to consolidate the availability and the access for valuable use of legumes genetic resources, some synergic and imperative activities are in progress at different levels, in national and international initiatives:

- enhancement of farm diversity and practices by preserving local biological resources and improving access to new resources and knowledge, focusing on legume traits that enhance adaptability to variable and changing climates;
- conserving the wide legume diversity, including neglected species, landraces, traditional and modern breeds, and varieties;
- preservation of the complexity and heterogeneity of farms to ensure continuous ecosystem services, support recovery from disturbances, and provide habitats for associated biodiversity;
- embrace participatory and transdisciplinary approaches in research and conservation, using a social-ecological systems framework to develop and promote locally relevant strategies that strengthen resilience, informed by local knowledge;
- strengthen research efforts, including: (i) investigating how legume resources enhance the resilience of production systems, particularly in recovering from multiple stresses; (ii) studying resilience strategies that integrate diverse legume components across scales; and (iii) conducting long-term studies to evaluate the contribution of legume genetic resources to resilience over medium and long periods;
- expand institutional and policy support to encourage the broader adoption of diversity-rich practices that enhance resilience;
- mobilizing experts to collaboratively plan for the more efficient and effective conservation and the use of legumes diversity;
- allocation of funding to generate data and to support regional strategies for *ex situ* and *in situ* conservation and utilization of crop diversity.

CONCLUSIONS

Sustainability is imperative, particularly within agri-food systems. Agrobiodiversity conservation demands feasible solutions that are both economically competitive and environmentally beneficial. Facilitated access to Plant Genetic Resources for Food and Agriculture is essential for promoting sustainable crop production and enhancing food and nutrition security. Access and utilization of genetic resources and well documented data must be streamlined. Making related information available under a Standard Material Transfer Agreement (SMTA), and combining it with additional measures, would eliminate legal ambiguities and reduce transaction costs for conservers, curators, and users of genetic resources. This, in turn, would support plant breeders and farmers in effectively fulfilling their mission.

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Passiflora incarnata L. - CULTIVATION IN OPEN FIELD

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Abstract

The article presents the synthesis of the results of the investigations conducted over several years, regarding the acclimatization and cultivation of the species Passiflora incarnata L. in open field, under the conditions of the Republic of Moldova, the plant being native to subtropical and tropical areas. P. incarnata is commonly used as an herbal medicine, possessing sedative, spasmolytic, hypertensive, cardiotonic properties etc. The dry raw material productivity from field cultivation reached 3.6 t/ha from 2 cuts. About 2.7 t/ha pharmaceutical raw material and edible, delicious, fragrant fruits can be harvested at a single cut. After several years of testing, a high-performance form of the aforementioned species, with stable characteristics in terms of raw material, fruit and seed productivity, has been selected and after the DUS and VCU testing, we came to the conclusion that it can be proposed as a new cultivar, with high value for medicinal plant growers and pharmaceutical companies.

Key words: *species, sedative, production, raw material.*

INTRODUCTION

The species is native to South America, but can also be found quite often in Asia and Australia (Smruthi et al., 2021). The genus *Passiflora* is very large and includes over 550 species (Fonesca et al., 2020) The study of introduction, multiplication and use of the species *Passiflora incarnata* L. was initiated, in our region and in the neighboring ones
(Krasovsky et al., 2022) due to the high interest in this species for the taste of the fruits, the medicinal and decorative properties (Boboc & Cantor, 2017) possessed by this species (Cerqueira Silva, 2014).

The use of all products derived from *Passiflora incarnata* plants was confirmed by multiple publications (Deepika et al., 2024), from various research centers in different countries.

Thus, extracts from the aerial part of the plant, that is, the leaves, stems, fruits and even the roots are used to treat various conditions such as helminth infestations, gastric tumors, to relieve stress (Costa et al., 2005), (Cerqueira-Silva, 2014), (Amata et al., 2009). Besides, it possesses antibacterial (Tassew & Chou, 2022), antimicrobial properties (Badalova & Atay, 2022), and in China it was discovered that preparations from *Passiflora incarnata* L. have beneficial soothing effects in cases of heart and liver disorders (Hou et al., 2021).

In addition to being delicious and flavored, the fruits of this species also contain vitamins A, C and D (Dhawan, 2004), being an important source of alkaloids, flavonoids and carotenoids (Khan & Nabavi, 2019), providing numerous health benefits (Zeraik et al.2010).

The seeds of *Passiflora incarnata* L. are a source of essential fatty acids such as linoleic (55-60%), oleic (18-20%), palmitic (10%–14%), used in the food industry and in the cosmetic industry as an ingredient in soaps, shampoos and moisturizers (Faleiro et al., 2014).

Extensive studies on the biochemical compounds of the seeds have demonstrated that they contain a large amount of sterol – β-sitosterol, which has anti-hypercholesterolemic and anti-inflammatory effects (Al-Fartosy, 2011). The aerial part of the plant is often used as a sedative remedy (Krenn, 2002).

In addition to all their medicinal properties, the seeds of *Passiflora incarnata* constitute the main propagation material of the genus *Passiflora*
(Angelini & Tavarini, 2021). Extracts from the flowers of *Passiflora incarnata* can be used to improve the duration and the quality of sleep (Ngan et al., 2019).

Butanol extracts from the leaves of *Passiflora incarnata* are effective in the prevention of neurodegenerative and Parkinson's disease (Ingale & Kasture, 2022) as well as neurotic dysfunctions (Gad et al., 2022).
All products obtained over time from *Passiflora incarnata* L. have been validated as beneficial for human health and wellbeing (Singh & Nagpal, 2022). Recent research has confirmed the calming and anti-stress effect of *Passiflora incarnata* L. preparations not only on the nervous system of humans, but also on some animals. Thus, a beneficial tranquilizing effect of *Passiflora incarnata* L. preparations was observed in suckling piglets during the post-weaning period, which was very stressful for the animals, but which proceeded normally, with even a weight gain in the animals being recorded during the respective period (Pastorelli & Luzi, 2022). It was also observed that the addition of a mixture of *Passiflora incarnata* grass to the feed significantly influenced the behavior of minks under stress, which improved animal welfare and optimized production parameters (Wlazło & Żebracka, 2022).
In accordance with current publications (Pacheco, 2021), obtaining high quality pharmaceutical raw material, seeds and planting material requires research on the productivity of the raw material and the quality indices of the species under the growing conditions of the Republic of Moldova, by applying single- and double-harvest cultivation techniques, in different years.

MATERIALS AND METHODS

The study was conducted on a separate sector, in open ground with irrigation possibilities, on the territory of the Institute of Genetics, Physiology and Plant Protection of Moldova State University. The data obtained are results of the harvesting of raw material in the first and second years of life of *Passiflora incarnata* L. plants. The yield of fresh and dried pharmaceutical raw material from the first- and second-year plantations was determined. Simultaneously with determining the production of raw material, phenological phases, morphometric indices such as plant mass, axial shoot length, number of lateral shoots, proportion (yield) of leaves, fruits, stems in the total mass of raw material and seed production were also determined.
The plants were harvested at the full budding stage - beginning of flowering – for plant raw material and then – at full fruit development and ripening.
After the first cut, to obtain the plant raw material, mineral nitrogen fertilizers (NO_3) were applied to the "stubble", then the area was irrigated and the space between the rows, which initially constituted 0.7m, was loosened.
The experiment included the following options: obtaining two harvests per season – the first one – at the flowering stage and the second one – at the initial stage of fruit development, in the first and second year of vegetation, and obtaining production at full fruit maturity in the first and second year of vegetation.
Seedlings obtained from seeds of the selected biotype of *Passiflora incarnata* L, improved and acclimatized to the environmental conditions of R. Moldova, were used to establish the plantations. The seedlings were grown under controlled greenhouse conditions, in boxes, at temperatures above 25 °C and high humidity, obtained by covering the boxes in the early stages with "Agril" film with a density of 19 g/cm, which helped maintaining humidity and temperatures at a constant level, thus favoring faster and more even seed germination. Sowing to obtain seedlings was carried out, depending on the year, at the end of March or the beginning of April. The seedbed was made up of a mixture of forest soil, peat and sand in a ratio of 2:1:1. During the seedling production period, the soil in the nursery was irrigated sufficiently to keep it slightly moist, which facilitated the emergence of the seedlings within the optimal time frame of 48-62 days. After the seedlings emerged, they started growing rapidly and the protective film was removed to provide more light and to lower the temperature, which helped hardening the seedlings.
After reaching 10-15 cm in height, with 5-7 leaves on the stems, the seedlings were transplanted into open ground. At the end of May, the seedlings were transplanted manually in abundantly irrigated soil, incorporating the root system and the lower part up to the first leaf into the soil at a depth of 18-20 cm, at a density of 60.000-70.000 units/ha.

In the 2nd year of life, *Passiflora incarnata* L. plants started growing from the rhizome buds, occupying the space between the rows and the one adjacent to the plantation. During our research, another propagation method was applied to the *Passiflora incarnata* L. species – sowing the seeds directly in open ground, and covering the seedlings with Agril film with a density of 21 g/cm.

RESULTS AND DISCUSSIONS

The seedlings planted in open ground formed 25-30 cm long shoots in 35-40 days (Figure 1), and in the first half of July, developed flower buds, which bloomed in 6-8 days and, by the end of July, produced fruits, which reached the stage of full ripening in late September or early October.

Figure1. *Passiflora incarnata* L. seedlings

The second-year *Passiflora incarnata* L. plants started growing 15-20 days earlier than the seedlings were planted in the first year, that is – in early May.

Figure 2. *Passiflora incarnata* L. plants in full bloom

At the beginning of June, the plants formed 8-9 leaves, and after 15 days – flower buds, which bloomed (Figure 2.) at the beginning of July. In the middle of the July, the early stage of fruit development was recorded and at the end of September – the full ripening stage.
At the first harvest, the plants were in the full flowering - beginning of fruit development stage (Figure 3.), the shoots were leafy (leaf ratio 52.3-59.8%), the presence of fruits was 2.6-5.8%. The regeneration of plants after the first cut lasted 68 days.

Figure 3. *Passiflora incarnata* L. fruits

The plant mass obtained from the second harvest (Table 1), performed at the beginning of fruit development stage, consisted of leafy shoots (leaf ratio 52.9-64.0%).

Table 1. The structure of the *Passiflora incarnata* L. raw material obtained in the first year of vegetation

Variant	Technology	Development stage	The share of organs in raw material, %		
			leaves	stems	fruits
V_1	with 2 cuts: I (86 days)	flowering 50%	59.8	37.4	2.6
	II (62 days)	fruit development	64.0	30.9	5.1
	with 1 cut (150 days)	fruit ripening	47.1	24.4	28.6
V_2	with 2 cuts: I (99 days)	flowering 70%	52.9	41.7	5.4
	II (68 days)	fruit development	52.3	41.9	5.8
	with 1 cut (178 days)	fruit ripening	44.5	28.7	26.8

*V_1 – transplanted seedlings; V_2 – seeds sown in the field, under Agril film

The raw material in the double-cut technology consisted of 65-80 cm long shoots, weighing 85-89 g in V_1, the most vigorous being the plants from V_2 with a weight of 114-169 g, with 5-7 lateral shoots, 10-14 floral buds and 0.9-2.0 fruits with a diameter of 10-15 mm at the beginning stage of their development (Table 2). The raw material in the double-cut technology was of very high quality, without the presence of any mature fruits, which are not admissible for obtaining high quality pharmaceutical raw material.

Table 2. Morphometric and numerical parameters of the plants

Variant	Technology	Parameters of the axial shoot				
		length cm	shoots, pcs.	leaves, pcs.	buds, pcs.	fruits, pcs.
Vi	with 2 cuts: I	65.1	5.5	24.1	10.4	0.9
	II	50.7	6.8	20.1	1.2	0.3
	with 1 cut	89.0	10.4	30.8	0.5	4.3
V_2	with 2 cuts: I	79.9	6.7	21.0	13.2	2.0
	II	85.7	4.2	24.8	1.6	1.4
	with 1 cut	116.8	11.2	31.6	0.7	6.8

*V_1 – transplanted seedlings; V_2 – seeds sown in the field, under Agril film

When applying the single-cut technology, shoots from both variants harvested during the fruit maturation stage were more vigorous, with a weight of 223.0-285.5 g, and fruits with mature seeds with a weight of 63.8-76.5 g (average from 25 plants, Table 3). The quality of the raw material in the single-cut crop was average, with a high content of stems lignified in the basal part and devoid of leaves, requiring manual work to remove the fully ripe fruits, which were still valuable as a source of seeds.

Table 3. The structure of the production of *Passiflora incarnata* L. plants in the 1st year of vegetation

Variant	Technology	Weight, g			
		plant	leaves	buds	fruits
V_1	with 2 cuts: I	85.9	51.4	32.2	2.3
	III	82.8	53.0	25.2	4.3
	with 1 cut	223.0	105.0	54.2	63.8
V_2	with II cuts: I	169.5	89.6	70.8	9.1
	II	113.9	59.5	47.8	6.6
	with 2 cuts	285.5	127.0	82.0	76.5

*V_1 – transplanted seedlings; V_2 – seeds sown in the field, under Agril film

The harvest obtained by sowing in the field was 15 t/ha of raw material consisting of 12 t/ha of herba and 3 t/ha of fruits. The useful production (fresh mass without fruits) obtained by direct sowing in the field in the single-cut technology was twice as high as in the variant obtained by transplanting seedlings. The raw material production in the single-cut variant, obtained by transplanting seedlings, was 7.76 t/ha, of which 6.24 t/ha was herba and 1.52 mature fruits (Table 4).

The two-cut (harvest) technology proved to be more efficient due to the higher productivity of fresh herba – of 14.75 t/ha and its high quality.

Tabelul 4. The productivity of the species *Passiflora incarnata* L.

Variant	Technology	The productivity of:			
		fresh herba t/ha	pharmaceutical herba, t/ha	fruits t/ha	seeds, kg/ha
V_1	with 2 cuts: I	3.10	0.99	-	
	II	4.66	1.63	-	
	total	7.76	2.62	-	
	with 1 cut	6.16	2.76	1.52	103
V_2	with 2 cuts: I	5.51	1.87	-	
	II	9.24	3.33	-	
	total	14.75	5.20	-	
	with 1 cut	12.38	4.56	2.83	185

*V_1 – transplanted seedlings; V_2 – seeds sown in the field, under Agril film

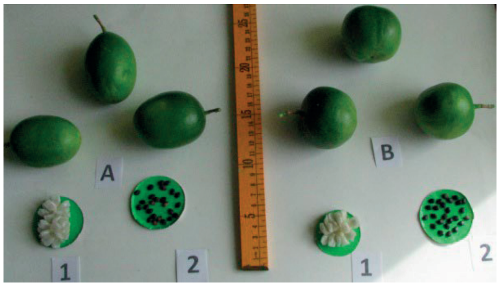

Figure 4. Fruits with seeds of *Passiflora incarnata* L.

When ripe, the fruits of *Passiflora incarnata* L. are yellow-green, buy oblong (Figure 4. A, ori ovoid forms – B), soft to the touch, slightly wrinkled, the lower skin is thinner. Inside the fruit, there is a white protective layer, containing a round, also white sac enclosing the seeds. The seeds (Figure 4. A-2 and B-2) are surrounded by a yellowish gelatinous pulp (Figure 4. A-1and B-1) with a very pleasant aroma and taste, somewhat similar to melon, with a slight flavor. The fruits are on average 58.1 mm long and 50.3 mm wide, with an average weight of 42.2 g (Table 5). The average multiannual germination capacity of the seeds is 66%.

Table 5. Morphometric indices of *Passiflora incarnata* L. fruits

Parameters	Values
Fruit length, mm	58.1
Fruit diameter, mm	50.3
Fruit weight, g	42.2
Number of seeds in a fruit, pcs	106.4
Ratio of fruits with mature seeds	63.4
Weight of 1000 seeds, g	35.2

*average of 25 fruits in 5 repetitions

CONCLUSIONS

The research has shown that *Passiflora incarnata* L. plants adapted to the climatic conditions of the Republic of Moldova can be successfully cultivated and propagated by sowing in open ground, being able to bear fruit and form mature seeds in the first year of vegetation. In early spring, the young seedlings can be successfully protected from low temperatures by covering them with Agril film.

The raw material obtained by two cuts (harvests) per season is characterized by very high quality, leaf content of 52-64% and lack of fully developed fruits and lignified stems, which meets the quality standards for pharmaceutical herba.

According to productivity indices, the variant with plants obtained by sowing directly in open filed had a higher raw material production, in the single-cut option, of 12.38 t/ha, or 201% as compared with the variant obtained by transplanting seedlings – 6.16 t/ha. When applying the technology of sowing directly in the open field followed by two cuts, yields of 14.75 t/ha were obtained, as compared with 7.76 t/ha obtained from the variant with transplanted seedlings, or almost twice as much. The production of pharmaceutical herba was also higher in the variant obtained by direct sowing in the field, with 4.56 in the single-cut option and 5.2 t/ha in the double-cut option, as compared with 2.76 and 2.62 t/ha, the positive difference being 63% and 86%, respectively. For the production of high-quality raw material, it is preferable to use the double-cut technology.

The determined morphological parameters demonstrated that the fruits of *Passiflora incarnata* L. grown in open ground, under the meteorological conditions of our area, correspond to the species description in published sources, with a seed number of 106.4 units per fruit and the weight of 1000 seeds of 35.2 g.

To obtain edible and viable fruits and high-quality seeds, it is necessary to use the technology with a single harvest per season, carried out at the end of October.

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THE ASSESSMENT OF HEAVY METAL BIOACCUMULATION IN PEPPER PLANTS (*Capsicum annuum*) CULTIVATED IN GREEN-HOUSE CONDITIONS, USING CONTAMINATED SOILS FROM THE INDUSTRIAL AREA OF COPȘA MICĂ

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Abstract

The study investigated the effects of cadmium, lead, zinc, and copper contamination on the bioaccumulation of heavy metals in pepper plants (Capsicum annuum L.). The experiment was conducted using soil materials contaminated with heavy metals, collected from 24 individual households in the Copșa Mică area, selected to ensure a large range of soil reactions and total heavy metal content. To evaluate the mobility and bioaccessibility of the metals, pepper seedlings (Capsicum annuum L.) were cultivated in green-house conditions. The mobility of metals were assessed by using two extraction methods:extraction with solution NH_4NO_3 (1M) for the easily exchangeable forms and DTPA-$CaCl_2$-TEA, for the bioavailable forms. Experimental data indicated a significant correlation between the cadmium and lead content in soil, in their bioavailable forms, and their content in edible parts of pepper plants. Cadmium demonstrated higher mobility and bioaccumulation compared to lead. The results showed that pepper plants have a relatively low capacity to bioaccumulate zinc and copper but can accumulate cadmium and lead under highly soil contamination conditions.

Key words: *bioaccumulation, heavy metals, soil contamination pepper.*

INTRODUCTION

The contamination of soils with heavy metals is a global issue, particularly in industrialized regions, having a major impact on environmental quality and food safety (Kabata-Pendias, 2011; Alloway, 2013;). Heavy metals such as lead (Pb), cadmium (Cd), and zinc (Zn) are extremely toxic even in low concentrations and can severely affect human health when ingested through food grown in contaminated soils. Nagajyoti et al., 2010, argues that metals are not biodegradable and tend to accumulate in the soil, entering the food chain and eventually reaching human and animal consumers.

Mousavi et al. (2013) and Sabău (2019), presented some causes of anthropogenic soil pollution, including the processing and exploitation of non-ferrous metals, waste and residues, which play an important role in soil pollution. Exhaust gases from road traffic and improper waste disposal practices are also another source of soil pollution, as well as the treatment of soil with fertilizers and pesticides, sludge from wastewater treatment, irrigation with contaminated water, and the burning of fossil fuels, all contributing to soil pollution.

The industrial region of Copșa Mică in Romania is one of the most polluted in Europe, affected by the contamination of soil and groundwater with heavy metals as a result of decades of intense industrial activity. For several decades, Copșa Mică has been a major center of non-ferrous metal processing and the main source of pollution being emissions from industrial platform. This has led to the degradation of surrounding soils and significant accumulation of heavy metals, especially lead and cadmium, in the soil and vegetation.

Yang et al. (2009), Hu et al. (2013) and Jolly et al. (2013), claim that vegetables are edible crops and an essential part of the human diet. They are rich in nutrients necessary for the human body and are an important source of carbohydrates, vitamins, minerals, and fiber. At the same time, they argue that heavy metals can be easily absorbed by the roots of vegetables and can accumulate at high levels in the edible parts of the vegetables, even when heavy metal contents in the soil are at low levels.

According to Ali et al.(2013) and Singh et al. (2015), different plant species have the ability to absorb and accumulate heavy metals through complex transport and cellular retention mechanisms. These processes, are well documented in metal-tolerant species, often used in phytoremediation, such as Indian mustard *(Brassica juncea)* and sunflower *(Helianthus annuus)(Rizwan et al., 2016)*. For example, a study conducted at the National Institute for Laser, Plasma, and Radiation Physics showed that heavy metals such as Pb and Cd are extremely toxic and can cause mutations or cancer, even in small quantities (Achim et al., 2016).

Several researchers (Goyer, 1993; Klaassen et al., 1999; Navas-Acien et al., 2007; Hartley, 2008; Ekong et al., 2016;) considered that heavy metals can persistently accumulate in the body throughout life. They state that chronic exposure to heavy metals, especially cadmium (Cd), can affect the immune system, damage the lungs and liver, negatively impact the development of the nervous system in children, and induce hypertension and cardiovascular diseases.

Capsicum annuum L., known as bell pepper, is an important horticultural crop and is widely consumed globally due to its nutritional value and high content of vitamins and antioxidants. Pepper plants, like other vegetable species, are capable of absorbing heavy metals from the soil and transfer them to different parts of the plant, including the fruit, which can pose a health risk to consumers when grown in contaminated soils (López-Millán et al., 2009).

Bell pepper is one of the plants sensitive to heavy metals, and bioaccumulation in its tissues can vary significantly depending on the type of metal and its concentration in the soil (Maleki et al., 2008; Nagajyoti et al., 2010). Thus, the health risks associated with the consumption of peppers from contaminated areas are significant, as exposure to heavy metals can lead to chronic conditions, including neurological disorders, renal and hepatic dysfunction, as well as carcinogenic effects (Tchounwou et al., 2012).

Given the toxic impact of heavy metals and the lack of effective soil remediation measures in highly contaminated areas, studies on the accumulation capacity of these metals by different crops are essential to assess the risk to food safety (Sharma et al., 2005).

The objective of this study is to evaluate the levels of accumulation of heavy metals (Cu, Cd, Pb, Zn) in bell pepper plants grown in protected spaces, using contaminated soil materials. The study also aims to estimate the concentrations of these metals in edible parts of bell pepper plants (fruits), with the goal of providing essential data on the risks of consumption and supporting the need for soil decontamination measures in the region.

MATERIALS AND METHODS

For this study, 24 soil samples were collected from various rural households located in the industrial area of Copșa Mică. The purpose of this selection was to evaluate the diversity of soil conditions and their impact on the bioaccumulation of heavy metals in vegetables.

The soil samples were analyzed in the Physico-Chemical Analysis Laboratory of the National Institute for Research and Development in Soil Science, Agrochemistry, and Environmental Protection (ICPA Bucharest), to assess the mobility and bioaccessibility of heavy metals (Cu, Zn, Cd, Pb) in the soil using two extractants: NH_4NO_3 (1M), at a soil:solution ratio (w/v) of 1:2 for extracting exchangeable forms, and DTPA-$CaCl_2$-TEA for extracting the potentially available forms of metals.

Each soil material was placed in 3L pots, in which sweet pepper plants (*Capsicum annuum* L.) were cultivated to evaluate the accumulation of heavy metals. The experiment was conducted under greenhouse conditions to control environmental factors. The planting material was obtained from a local producer and planted in May 2023.

The sweet pepper plants were grown in greenhouses (Figure 1) for 10 weeks under controlled humidity and temperature conditions. The development of the sweet pepper plants grown in the 24 soil types, with different levels of heavy metal contamination, is presented in Figure 2.

Figure 1. Experimental setup organized in greenhouses for estimating the bioaccumulation of heavy metals in bell peppers (Vegetation House, ICPA, 2023)

Figure 2. Development of bell pepper plants in the experiment organized in vegetation pots for estimating the bioaccumulation of heavy metals (Vegetation House, ICPA, 2023)

At the end of the experimental period, the edible part (fruit) of the sweet pepper plants, harvested from the pots, was washed, chopped, treated with 10 ml of HNO_3, and then mineralized in the Ethos Easy microwave oven. To determine the concentrations of heavy metals extractable with NH_4NO_3 and DTPA-$CaCl_2$-TEA from the soil and to measure the concentrations of heavy metals in the edible parts of the sweet pepper plants (fruits), both the soil and plant samples extracts were analyzed by atomic absorption spectrometry (AAS) using the iCE 3000 equipment.

For the bioaccumulation analysis in the sweet pepper fruits, both data on the total heavy metal content in the soil and data on their potentially mobile forms were used. The evaluation of correlations between the metal concentrations in the soil and those in the sweet pepper fruits was carried out using power regression charts, considering both total and bioaccessible forms.

RESULTS AND DISCUSSIONS

In the study, correlations between the heavy metal contents (cadmium, lead, zinc, and copper) in the soil and their accumulation in bell pepper plants (*Capsicum annuum* L.) were investigated using logarithmic regression curves of the power type. The main goal was to estimate the bioaccumulation of these metals in bell peppers based on their different forms in the soil (total forms and potentially available forms), considering that the mobility of metals in soil and the plants' ability to translocate them into the fruits can vary considerably.

In Figure 3, logarithmic plots for the power-type regression curves are presented, estimating the stochastic dependence between cadmium content in soil (total forms and potentially available forms) and cadmium content in bell peppers (fruits) cultivated on contaminated soils. The transfer of cadmium from soil to plant is facilitated by the presence of this element in potentially available forms for plants. Numerous conventional extraction methods, universally accepted, are used to estimate the cadmium content present in various forms in the soil. In our study, two extractants, recognized for their ability to extract cadmium in the exchangeable form (extraction with NH_4NO_3) and the potentially available form (extraction with DTPA-$CaCl_2$-TEA buffered solution), were used.

Given the relatively high mobility of cadmium in soil, both the total cadmium content in the soil and the data on cadmium contents in the two potentially mobile forms in the soil were used to estimate the bioaccumulation of this metal in bell pepper fruit. The values of the parameters used varied as follows:

- Cd_{total}: 0.24 mg/kg and 19.27 mg/kg;
- Cd_{DTPA}: 0.20 mg/kg and 8.83 mg/kg;

- Cd_{NH4NO3}: 0.01 mg/kg and 0.15 mg/kg;
- Cd_{plant}: 0.029 mg/kg and 0.106 mg/kg.

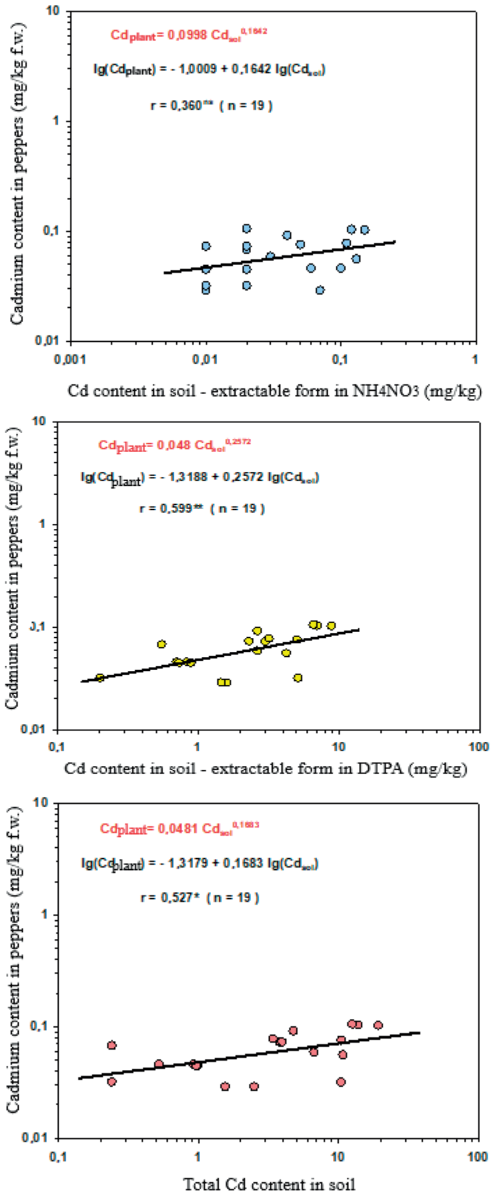

Figure 3. Logarithmic diagrams for power regression curves estimating the stochastic dependence between cadmium concentrations in soil and cadmium content in peppers

The strongest correlation was obtained between the cadmium content extractable in DTPA-$CaCl_2$-TEA buffered solution and the cadmium content in bell peppers (fruits). The form of cadmium extractable in this buffered solution is considered by some authors to be the most accessible for plants, which explains the strong dependence obtained in the greenhouse experiment.

Furthermore, the bioaccumulation of this element in bell peppers can also be estimated based on the total cadmium content in the soil, using power regression curves. The linear correlation coefficient obtained for this stochastic dependence was significantly different from zero, indicating a close correlation between the two studied variables. Using the values for cadmium extractable in NH_4NO_3, considered to be the easily exchangeable form, did not yield satisfactory results in estimating cadmium bioaccumulation in bell peppers edible parts (Figure 3).

According to studies, different plant species have the ability to absorb and accumulate heavy metals through complex transport and cellular retention mechanisms. However, bell peppers are a plant sensitive to heavy metals, and bioaccumulation in tissues can vary depending on the type of metal and its concentration in the soil. For example, a study conducted at the National Institute for Laser, Plasma, and Radiation Physics showed that heavy metals such as Pb and Cd are extremely toxic and can cause mutations or cancer, even in small amounts (Achim et al., 2016). Singh et al. (2012), showed that different vegetable crops grown on soils contaminated with heavy metals demonstrated significant differences in terms of accumulation, absorption, and distribution of metals. The cultivated species also showed remarkable differences in heavy metal concentrations in different parts of the plants. For example, root vegetables like radishes and carrots accumulated smaller amounts of heavy metals, except for zinc, while leafy vegetables such as spinach and mustard accumulated larger amounts of both essential and non-essential metals, except for cadmium and nickel. Potatoes and onions accumulated more cadmium and nickel, while cauliflower and cabbage showed greater accumulation of lead and nickel. In terms of absorption, cauliflower and cabbage absorbed more zinc, lead, and nickel, and mustard absorbed more zinc and cadmium. They concluded that leafy vegetables appear unsafe for soils contaminated with heavy metals, while fruit-type vegetables,

such as peas, are more suitable for soils polluted with cadmium, but not for those with nickel and lead.
Literature data indicate bell pepper as having a low rate of translocation of heavy metals to the fruit (Angelova et al., 2009; Morikawa, 2017). Morikawa (2017), analyzing results from a study conducted in 52 individual gardens located in a contaminated area, found that the cadmium content in bell pepper fruits varied between 0.02-0.09 mg/kg fresh weight, with differences depending on the analysed variety. In our experiment, it is notable that for all experimental variants, the cadmium contents in bell peppers (fruits) exceeded the limit value (0.020 mg/kg fresh weight) set for this element by Commission Regulation (EU) 1323/2021 of August 10, 2021.
In Figure 4, logarithmic plots for the power-type regression curves are shown, estimating the stochastic dependence between lead content in the soil (total forms and potentially accessible forms) and lead content in bell peppers (fruits). Lead mobility in the soil is much lower compared to that of cadmium. This also explains the low lead contents in the plant as well as in the potentially accessible forms in the soil.
The values of the parameters used varied as follows:

- Pb_{total}: 19 mg/kg and 668 mg/kg;
- Pb_{DTPA}: 5.6 mg/kg and 134 mg/kg;
- $Pb_{NH_4NO_3}$: 0.02 mg/kg and 0.12 mg/kg;
- Pb_{plant}: 0.010 mg/kg and 0.080 mg/kg.

Based on the correlations obtained in this study, it can be concluded that the bioaccumulation of lead in bell peppers (fruits) can be satisfactorily described using both the total lead content in the soil and the bioavailable lead content (extractable with DTPA-$CaCl_2$-TEA solution).
The linear correlation coefficients obtained for the stochastic dependencies between the lead content in the plant and the total Pb and bioavailable lead contents were significantly different from zero, indicating a strong correlation between the considered parameters.
As with cadmium, the lead extractable in NH_4NO_3 solution did not correlate with the lead content in the plant, excluding this parameter from being used to estimate lead bioaccumulation in bell peppers.

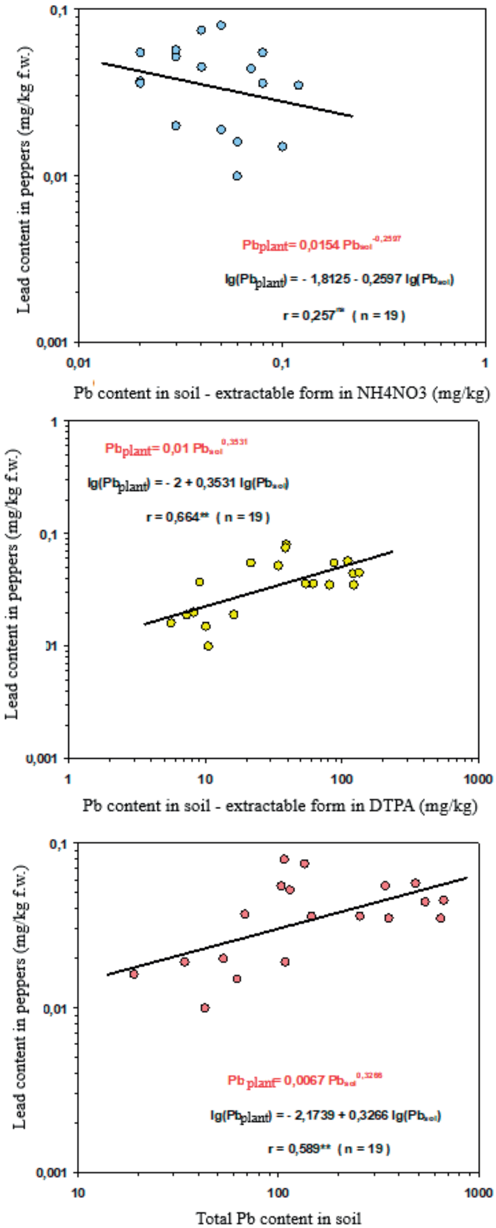

Figure 4. Logarithmic diagrams for power regression curves estimating the stochastic dependence between lead concentrations in soil and lead content in peppers

Regarding the lead content in the plant, it was observed that in none of the experimental variants considered, the maximum level set by Commission Regulation (EU) 1317/2021 of August 9, 2021, was exceeded (Figure 4).

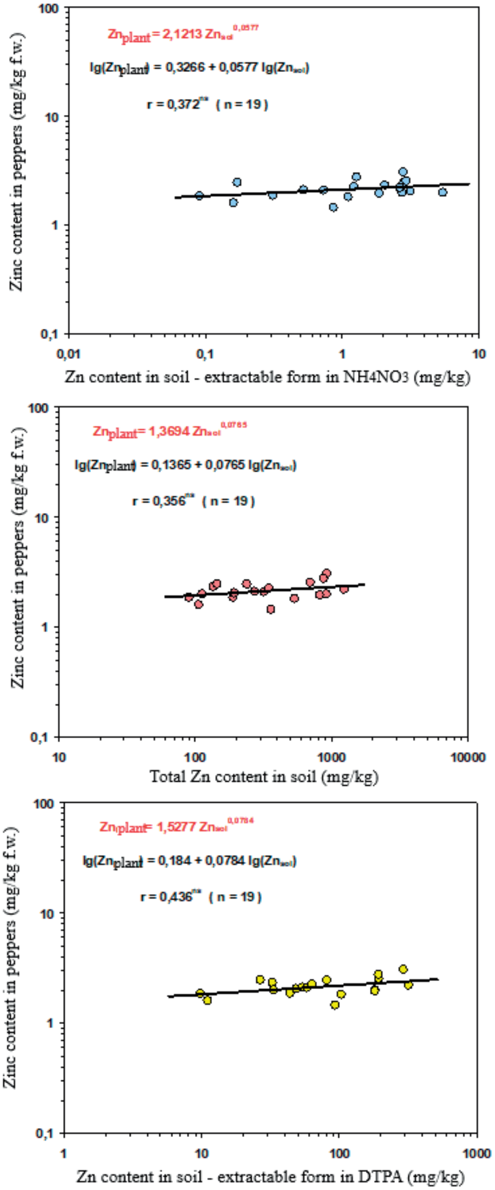

Figure 5. Logarithmic diagrams for power regression curves estimating the stochastic dependence between zinc concentrations in soil and zinc content in peppers

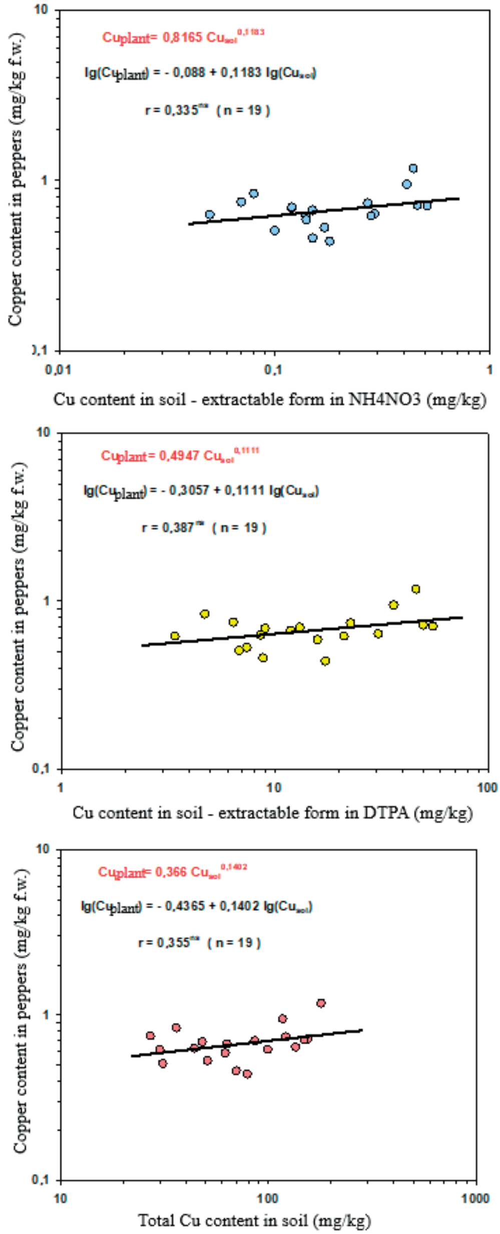

Figure 6. Logarithmic diagrams for power regression curves estimating the stochastic dependence between copper concentrations in soil and copper content in peppers

The reduced potential for lead accumulation observed in bell peppers is also confirmed by the data obtained in this experiment. Thus, bell pepper plants grown on a soil material with a total lead content of 668 mg/kg accumulated reduced quantities of lead in the fruit (0.080 mg/kg fresh weight).

The data collected from the bell pepper experiment, organized in experimental pots using soil materials with different contamination degrees, were also used to parameterize the bioaccumulation models for zinc in bell peppers (fruits). Due to the reduced ability of this vegetable species to accumulate heavy metals, as well as the role of zinc as a micronutrient, the correlations established between the considered parameters had no significantly linear correlation coefficients (Figure 5).

It is noteworthy that peppers, although grown in soils with excessive zinc content (1,323 mg/kg), accumulated very small amounts in the fruit (3.08 mg/kg dry weight). Moreover, although the zinc concentrations in the soil had a wide range of variation, the zinc concentrations determined in peppers (fruit) varied within a very narrow range. The values of the parameters used varied as follows:

- Zn_{total}: 90 mg/kg and 1,232 mg/kg;
- Zn_{DTPA}: 9.7 mg/kg and 318 mg/kg;
- $Zn_{NH_4NO_3}$: 0.09 mg/kg and 5.43 mg/kg;
- Zn_{plant}: 1.46 mg/kg and 3.08 mg/kg.

The logarithmic diagrams for power regression curves estimating the stochastic dependence between copper concentrations in soil and copper content in peppers (fruit) are shown in Figure 6. Since copper is not a major contaminant of the soil materials used for experimentation, and it also plays an essential role in plant development, no strong correlations were established between the parameters considered to describe the accumulation of this element in peppers. Furthermore, the range of variation of the parameters considered was relatively narrow. Thus, the values of the parameters used varied as follows:

- Cu_{total}: 27 mg/kg and 179 mg/kg;
- Cu_{DTPA}: 3.4 mg/kg and 55 mg/kg;
- $Cu_{NH_4NO_3}$: 0.05 mg/kg and 0.51 mg/kg;
- Cu_{plant}: 0.44 mg/kg and 1.18 mg/kg.

For none of the correlations tested, significant linear correlation coefficients different from zero were obtained. Therefore, the data collected during this study do not allow the development of stochastic models for estimating copper bioaccumulation in peppers. Moreover, the obtained stochastic dependencies confirm the ability of these vegetables to reduce the transfer of metals into the fruit.

CONCLUSIONS

The study demonstrated that peppers have a reduced capacity to accumulate heavy metals, being capable of accumulating cadmium and lead in the fruits, but with a low potential for accumulating zinc and copper. The power logarithmic regression models showed significant correlations between the bioaccumulation of cadmium and lead and their bioavailable contents in the soil, especially in the forms extractable with the DTPA-$CaCl_2$-TEA buffered solution. These results are valuable for evaluating the risks of vegetable contamination, particularly in the case of soils contaminated with heavy metals, and for developing monitoring and protection strategies for crops.

The experimental results obtained in our study are supported by literature data (Angelova et al., 2009; Morikawa, 2017 and Lidikova et al., 2021), which mentioned peppers as having a low rate of heavy metal translocation into the fruit, making this vegetable a relatively safe option in terms of heavy metal accumulation in conditions of soil contamination.

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THE INTERESTING CASE OF PHOSPHORUS IN FOREST SOILS: A BIBLIOMETRIC REVIEW

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Abstract

Phosphorus is an essential element in soils. By using VOSviewer and Web of Science tools, we conducted a bibliometric analysis considering titles, and abstracts to identify the types of publications, the scientific fields they belong to, the years, the authors distributed by country, the publishing journals, and the keywords used. The results revealed the existence of 613 publications, of which 595 are articles in the priority fields of Environmental Sciences, Ecology, Forestry, and Soil Science, with an exponential increase in the number of articles published on this topic, especially in the last 10 years. A total of 201 authors were inventoried, with the top two countries being China and the USA. Journals where articles on this topic were published belong to the fields of soil science and forestry, but also include general journals; most articles were published in Forest Ecology and Management, Science of the Total Environment, and Forests. The most frequently used keywords were nitrogen, phosphorus, carbon, soil, and diversity, with an increased emphasis in the last 5 years on the implications of phosphorus in forest soils.

***Key words**: publications, keywords, topic, journals, phosphorus.*

INTRODUCTION

Phosphorus (P) is a key element in soil organic matter and originates from the weathering of minerals in parent rock material within natural terrestrial ecosystems. It is often the second most limiting nutrient for terrestrial primary production after nitrogen (Lajtha & Jarrell, 1999). P is vital for ecosystem productivity and agricultural output, but its misuse can undermine agricultural sustainability and lead to serious environmental issues (George et al., 2016). Globally, the phosphorus resource is quite limited, garnering attention as a nonrenewable resource (Cordell et al., 2009).

In forest ecosystems, phosphorus is typically a limiting nutrient, with its soil levels dependent on its presence in plant litter (Lemanowicz, 2018). Understanding the mechanisms of phosphorus availability is crucial for predicting forest productivity in a changing environment. Phosphorus distribution in forest soils is highly variable, both between and within different soil types, regarding content, speciation, availability, and sources (Bol et al., 2016). Human activities are expected to impact phosphorus cycling in temperate forests (Pistocchi et al., 2022).

A significant portion of these articles are from the fields of medicine (Williams et al., 2019; Jiang et al., 2021; Feng et al., 2023), economics (Khan et al., 2022; Jajic et al., 2022; Mi'raj et al., 2024; Rabbani et al., 2024), and environmental sciences (Wu & Wang, 2018; Oh & Lee, 2020; Burki et al., 2021; Putra et al., 2024).

In contrast, bibliometric review articles specifically addressing forest soils are scarce. Existing reviews focus on topics such as global soil water content (Zhang et al., 2022; Singh et al., 2023), soil erosion (Yu et al., 2024), forest litter decomposition (Liu et al., 2024), and soil metagenomics (Vieira et al., 2021).

The objective of this article is to conduct a bibliometric review of publications related to phosphorus in forest soils. This analysis examines the distribution of the main types of publications, the primary scientific fields they pertain to, the yearly distribution of articles, the authors and their countries of origin, the journals in which the articles were published, and the main keywords used.

MATERIALS AND METHODS

The information was sourced from the online version of the SCI-Expanded database on Web of Science. This multidisciplinary database is managed by the Institute for Scientific Information (ISI) in Philadelphia, PA, USA. As Based on the Journal Citation Reports (JCR), the SCI-Expanded database, as of 2015, included 11,149 key journals, featuring citation references across 237 scientific fields and spanning 82 countries. Abstracts have been included in each SCI publication since 1991.
For over four decades, the Institute for Scientific Information (ISI), now part of Thomson Reuters, provided the exclusive bibliographic databases that enabled bibliometricians to develop large-scale bibliometric indicators. These citation indexes, now part of the Web of Science (WoS), have served as the primary sources for bibliometric data.
For this bibliometric study, the online SCI-Expanded database was searched using the keywords "phosphorous in forest soils" to gather a bibliography of all relevant research papers from 1984 to the present.
The data were processed using the facilities offered by the Web of Science Core Collection as well as the Vosviewer program version 1.6.20 Excel and Geocharts.

RESULTS AND DISCUSSIONS

A total of 613 publications were identified and analyzed. Of these, 595 are articles, 19 are proceeding papers, 7 are review articles, and 2 are book chapters (Figure 1).

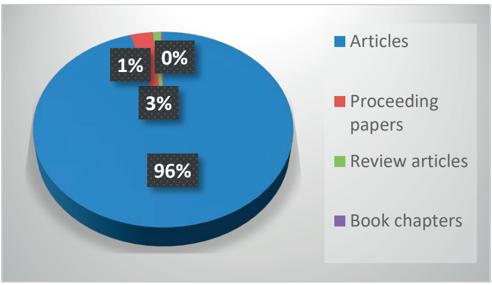

Figure 1. Distribution of the main types of publications used in the bibliometric analysis

Regarding the scientific fields to which the published articles belong, the most representative are Environmental Sciences (172), Ecology (131), Forestry (111), and Soil Science (102) (Figure 2).

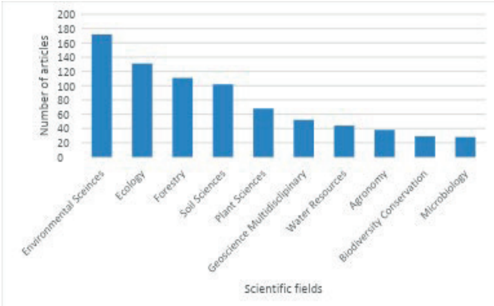

Figure 2. Distribution of the main 10 scientific fields of publications used in the bibliometric analysis

Concerning the years in which the articles were published, there is an exponential increase in the number of articles on this topic, especially in the last 10 years (Figure 3).
The first article on this topic was published in a renowned scientific journal in 1984.

Figure 3. Distribution of articles published per year

A total of 201 authors have published articles on this topic, with the most articles written by Jorge Mataix-Solera (8), Xavier Ubeda (6), and Manuel Esteban Lucas-Borja (6), all of whom are Spanish.
The published articles have authors from almost all countries (except some from Africa or Asia) and continents (Figure 4).

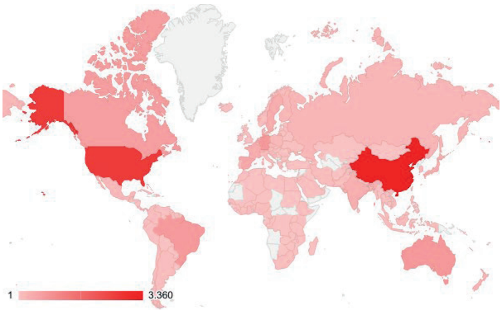

Figure 4. Geography of the use of phosphorus in soil in published articles. 1-3000 the number of articles

The countries with the highest representation are: China = 164; USA = 134; Spain = 49. In total, there are authors from 88 countries, with significant contributions from Germany, Brazil, India, Italy, Sweden, France, England, and many others in addition to the top three countries mentioned above. The node size and thickness of the connecting lines are proportional to the number of documents assigned to each country. The connections represent the collaboration network among research institutions (Figure 5).

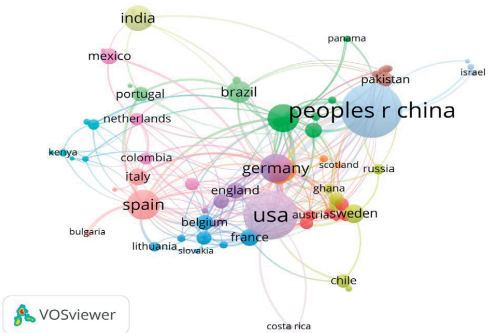

Figure 5. Countries with authors of articles on phosphorus in soil

Similar to the distribution of authors by country is the representation of their respective institutions. The most numerous are from China: Chinese Academy of Sciences = 54; University of Chinese Academy of Sciences = 20; Chinese Academy of Forestry = 17.

Articles on this topic are published in 207 journals, with the most articles appearing in Forest Ecology and Management (33), Science of the Total Environment (28), and Forests (16). Based on total link strength, the most important journals are Science of the Total Environment, Catena, Ecological Engineering, and Geoderma (Table 1).

Table 1. The most representative journals where articles about the phosphorus in soil have been published

Crt. No.	Journal	Documents	Citations	Total link strength
1	Science of the Total Environment	28	622	23
2	Catena	14	293	14
3	Ecological Engineering	8	245	9
4	Geoderma	8	291	9
5	Forests	16	114	8
6	Land Degradation & Development	7	195	6
7	Forest Ecology and Management	33	717	5
8	Applied Soil Ecology	7	243	4
9	Journal of Environmental Management	7	109	4
10	Journal of Forestry Research	12	96	4
11	Plant and Soil	15	358	4
12	Pedologia	5	271	3
13	Plos One	11	252	3
14	Soil Biology and Biochemistry	14	806	3

In terms of clusters, the journals are grouped into three clusters: one comprising journals in Forestry and Ecosystems (Forest Ecology and Management, Forestry, Frontiers in Microbiology, Ecosystems), another in Environmental Sciences (Science of the Total Environment, Ecological Engineering, Ecological Indicators), and another in applied sciences (Applied Soil Ecology, Land Degradation & Development, Geoderma, Journal of Environmental Management) (Figure 6).

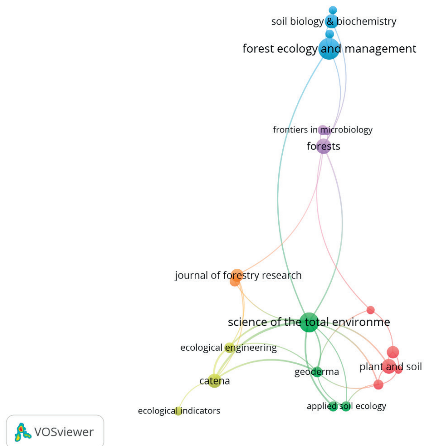

Figure 6. Main journals where articles on phosphorus in soil have been published

There are 102 publishers, with the most prominent being Elsevier (194 articles), Springer Nature (125 articles), and Wiley (60 articles).
The most frequently used keywords are nitrogen, phosphorus, carbon, soil, and diversity (Figure 7 and Table 2).

Table 2. The most commonly used keywords in articles about the phosphorus in soil

Crt. No.	Keyword	Occurrences	Total link strength
1	nitrogen	14	51
2	carbon	10	43
3	phosphorus	10	38
4	traits	3	18
5	climate	3	17
6	quality	3	17
7	soil	6	17
8	ecological stoichiometry	3	16
9	N-P stoichiometry	3	16
10	storage	3	16
11	organic matter	4	15
12	diversity	5	12
13	plant	3	12
14	water	3	10
15	biomass	3	9
16	vegetation recovery	3	7
17	fire severity	3	6
18	growth	3	6

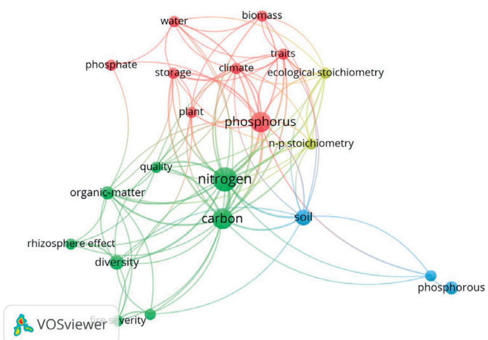

Figure 7. Authors' keywords regarding phosphorus in soil

We can observe two main clusters: one includes keywords such as nitrogen, carbon, quality, organic matter, rhizosphere effect, diversity, and fire severity; the other includes keywords such as climate, plant, storage, water, biomass, and traits. Additionally, there is a smaller cluster represented by the keywords N-P stoichiometry and ecological stoichiometry.

Regarding the distribution of keywords over the years, it is observed that in the last 5 years, there has been an increasing emphasis on the implications of phosphorus in forest soils, with keywords such as accumulation, strategy, root functional traits, and ecosystem services being used (Figure 8).

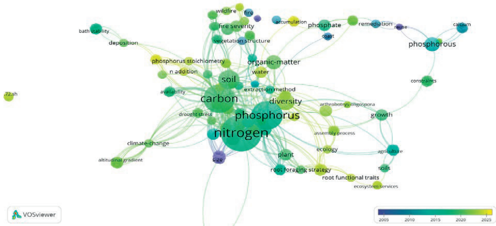

Figure 8. Distribution of keywords concerning phosphorus in soil over the years

The subject areas considered in this review are diverse but often interconnected and mutually inclusive. For instance, the majority of articles fall under the category of Environmental Science, which broadly encompasses the categories ranked 2nd and 7th (Ecology and Biodiversity). Naturally, the field of Soil Science is also prominently featured (ranked 4th in this case), along with other areas closely related to the studied subject (Plant Science, indicating an inclination of the authors to study not only the effects of phosphorus in soil but also its influence on vegetation) and Forestry (where soil phosphorus plays a significant role). Other scientific fields are also well-represented, including Geosciences, Microbiology, and Agronomy.
The sustained increase in the number of published articles over the past 10 years concerning phosphorus in forest soils mirrors the trend observed in nearly all topics analyzed through bibliometric review methods (Hsieh et al., 2004; Chiu & Ho, 2005; Ho, 2007; Buffardi & Ruberti, 2023). This trend is attributed to the growing number of published articles in the last decade and the increasing interest of researchers in the analyzed subject.
The largest number of authors comes from the USA and China. These two countries also top the list in other bibliometric review topics: severe acute respiratory syndrome (Chiu et al., 2004), adsorption technology in environmental science (Chiu & Ho, 2005), and land subsidence in coastal and alluvial plains (Buffardi &

Ruberti, 2023). The large number of authors from the USA and China can be attributed not only to the size of these countries and the number of researchers but also to specific issues related to soil phosphorus in these regions.

Global soil phosphorus levels are lowest in tropical and subtropical regions (Taylor, 1964; Zhang et al., 2005), which includes parts of China. Additionally, total phosphorus in soils decreases with weathering (Walker & Syers, 1976), a phenomenon common in China (Duan et al., 2002; Shao et al., 2015). These factors lead to limitations in net primary production (Pan et al., 2011) and decreases in biomass and diversity (Vitousek et al., 2010), which are sensitive issues for Chinese researchers.

In the USA, the dominant research aspects include phosphorus in wetlands (Bruland et al., 2006; Odhiambo et al., 2018) and the influence of harvesting on soil properties (Lockaby et al., 1997; Rapp et al., 2021).

Among the authors, besides those from China and the USA, Spanish researchers are notable. In Spain, wildfires are a common phenomenon, leading researchers to publish numerous articles related to this issue and phosphorus in forest soils (Braga et al., 2024; Peña-Molina et al., 2024). The country also has numerous degraded soils in semi-arid regions, an aspect correlated by authors with soil phosphorus (Fernandez et al., 2007; Mataix-Solera et al., 2011; Cerdà et al., 2021).

Journals where articles on this topic have been published primarily belong to the fields indicated in the title: soil science (Pedologia, Plant and Soil, Soil Biology and Biochemistry, Catena) and forestry (Forests, Forest Ecology and Management, Journal of Forestry Research). However, the most well-represented journal is a general environmental journal (Science of the Total Environment), along with others in the same category (Ecological Engineering, Land Degradation & Development). Thus, besides strictly specialized journals, the subject of phosphorus in forest soils is also addressed in general journals.

Keywords are essential in research outputs, providing insights into the content of each publication (Ashraf et al., 2022), highlighting the most relevant topics, and indicating main research trends (Medina-Mijangos & Seguí-Amórtegui, 2020).

The appearance of nitrogen and carbon alongside phosphorus in the predominant keywords demonstrates the interconnectivity of these three chemical elements (Hume et al., 2018; Smith et al., 2000) and their importance in soil studies (Fatemi et al., 2016; Brödlin et al., 2019).

Nitrogen appears even more frequently than phosphorus due to its significant role in plant nutrition and development (Rennenberg & Dannenmann, 2015; Cánovas et al., 2019). The concentration of carbon in forest soils is also a highly topical subject (Dincă, et al., 2012; Grüneberg et al., 2014; Dincă et al., 2015; Zhao et al., 2019).

The grouping of keywords into two clusters occurs in the first case according to organic matter and its representatives (organic matter, nitrogen, carbon) and their influence on ecosystems (rhizosphere effect, diversity, and fire severity), and in the second case according to environmental factors (climate, water, biomass, and traits). The smallest cluster is represented by stoichiometry, a topic studied in relation to its connection with soil microorganisms (Li et al., 2014; Zederer et al., 2017; Maaroufi et al., 2020; Cui et al., 2022) or with organic matter (Cui et al., 2022; Stahr et al., 2018; Gan et al., 2020; Spohn & Stendahl, 2022).

CONCLUSIONS

We can conclude that phosphorus in forest soils is indeed an interesting topic due to the numerous scientific aspects it raises and the diversity of studies conducted. The role and importance of phosphorus in forest soils are highlighted by the large number of research efforts undertaken. Since 1984, and especially in the last 10 years, numerous articles (as well as proceeding papers and book chapters) have been published in specialty journals in the fields of Environmental Sciences, Ecology, Forestry, and Soil Science. A total of 207 journals where articles have been published were identified, with the most representative being Forest Ecology and Management, Science of the Total Environment, and Forests, by authors from 88 countries, the most significant being China, the USA, and Spain. The most frequently used keywords were nitrogen, phosphorus, carbon, soil, and diversity. Journals can be grouped into two clusters, and keywords into three clusters, based on importance and the connections between different components. This topic has been an intensely studied scientific aspect, both independently and in correlation with other soil chemical elements, particularly nitrogen and carbon.

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DYNAMICS OF THE MOTOR MECHANISM OF INTERNAL COMBUSTION ENGINES

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Abstract

Engines that equip self-propelled tractors and combines are diesel engines. These engines have a construction adapted to working over a wide range of speeds and powers. This ensures the operation of all agricultural machines. However, the areas of economic use are more restricted, which is why agricultural aggregates should be properly made up and exploited to fit into them. The tractor engine is used at maximum speed regime when it has maximum power requests or when operating machines is also necessary from the independent power source. The studies in this work were carried out on a D-110 Diesel engine, an engine that equips the U-650M tractor. Studies refer to the dynamics of the motor mechanism. The parts of the engine mechanism studied were: the cylinder, the piston, the piston bolt, the connecting rod, the crankshaft, and the steering wheel. In each of these parts, the size and weight were measured. For the calculation of the operating indices of the D-110 engine, measurements were made on a stand to try and roll internal combustion engines.

Key words*: diesel engine, agricultural machines, tractor, cylinder, bolt, piston.*

INTRODUCTION

The cycle of an internal combustion engine can be divided into five phases:
-Intake or filling of the cylinder with motor fluid;
-Compression of the motor fluid intake in the engine cylinder;
-Ignition and burning of fuel;
-Gas relaxation;
-Evacuation of burnt gases from the engine cylinder (Copcea Anişoara Claudia Duma, et al, 2023; Anișoara Duma Copcea et al., 2024; Mihuț et al., 2024).

It follows from the above that the engine with compression fluid has a chemical energy that is partially or completely hot during its evolution.

In engines with compression ignition, mechanical energy is obtained by the engine fluid action on the pistons that have an alternative translation movement (Mateoc-Sîrb, et al., 2024). The translation movement of the pistons is transformed into a rotation movement of the engine shaft through connecting rod-crank mechanisms. In the four-stroke cycle, the succession of the processes is performed in four piston races, i.e., in two rotations of the crankshaft. (Relly et al., 2017). The periodic resumption of the motor cycle, which ensures the continuous operation of the engine, requires the emptying of the motor fluid cylinders that transmitted the mechanical work of the pistons (relaxed burn products), followed by the filling of the cylinders with a fresh motor fluid (Gunston, 1999). The diesel cycle has the advantage of high thermal efficiency due to the possibility of using high values of the compression ratio (Ɛ = 12-24) (Voleac, 2011).

The usual representation of the motor cycle is a diagram illustrating the variations of the gas pressure in the cylinder during the cycle. When gas pressure (p) is represented according to the volume (V) they occupy in the cylinder, the diagram is called the p-V diagram. The p-V diagram of the four-stroke actual engine cycle includes its phases (Mihuț et al., 2022; Mihut et al., 2023; Boca et al., 2019). The value and evolution of the gas pressure in the cylinder depends on the position of the piston, the moments of opening the intake holes (i.h.) and evacuation (e.h.), on their closing moments (b.e., b.a), of the injection (i), as well as of the nature of the process in which the cylinder gases are used: aspiration, compression, burning and relaxation, evacuation (Petrescu et al., 2009; Petrescu, 2011).

The main processes that take place in combustion engines in four stages, depending on the p-V diagram of the engine cycle are presented in Figure 1.

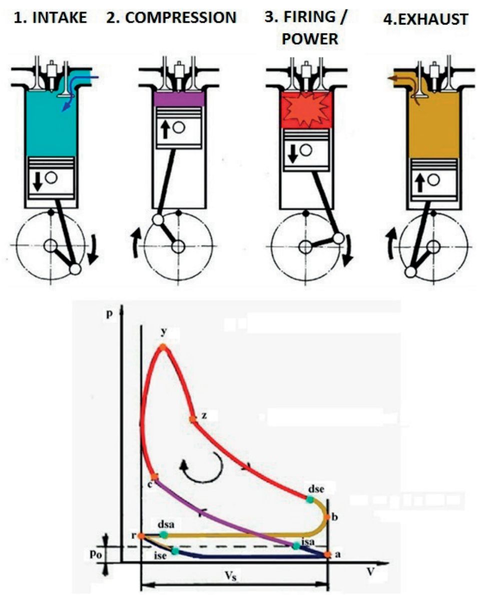

Figure 1. The main processes that take place in combustion engines in four stages, depending on the p-V diagram of the engine cycle

Time 1 – Air intake. After opening the intake hole with a certain advance compared to the IDP (inner dead point), the filling of the cylinder is possible under the action of the depression created by moving the piston to the ODP (outer dead point). The average speed of air entry through the intake hole is 70-90 m/s. During the movement of the piston to the ODP, in the so-called main phase of the intake, most of the air is aspired. The intake hole closes late compared to ODP for a complete air intake in the cylinder (Daicu Anatolie et al., 2015).

Time 2 – Compression. The piston moves from ODP to IDP. The intake and evacuation holes are closed. The air in the cylinder is compressed. The pressure and air temperature increase. The volume is shrinking. With a certain advance before the IDP, the fuel is injected into the cylinder. The very fine fuel drops sprayed in the cylinder by the injector are lit in contact with the compressed and heated air. The burning takes several thousands of seconds. The process of burning is carried out at constant volume and pressure.

Time 3 – Relaxation. The gases resulting from the burning of the fuel presses strongly on the piston forcing it to move from IDP to ODP. Relaxation is active time or engine time. The beginning of the relaxation is considered from the moment of reaching the maximum gas pressure; the end, in the opening of the exhaust hole (e.h.) (Levente et al., 2015).

Time 4 – Evacuation. Removal of the burnt gases from the engine cylinder begins with the opening of the exhaust hole and continues until its closing. The piston moves from ODP to IDP. The exhaust hole closes late compared to the IDP for a complete emptying of the burnt gases. During a motor cycle, the crankshaft performs two rotations (720^0 RAC) and the camshaft, one rotation (Novorojdin, 2011).

For a complete intake and evacuation, it is necessary for the intake and evacuation holes to open in advance and close late from the dead points (Agape et al., 2018).

Each four-stroke internal combustion engine has a certain diagram with distribution phases, with certain values of advance and delay angles (Hăbăşescu et al., 2005).

MATERIALS AND METHODS

The motor mechanism achieves the transformation of thermal energy into mechanical energy, having the role of transforming the translation movement of the cylinder into a rotational movement of the crankshaft.

The piston of an internal combustion engine plays a special role due to the complex requests to which it is subjected. The power of the engine is limited by the resistance of the piston to thermal and mechanical demands.

The piston is a mechanical organ, in alternative translation motion, which, together with the parts that accompany it (segments and bolt), perform the following functions:

Achieving the volume variation inside the cylinder;

Ensuring the evolution of the motor fluid in the cylinder (gas intake and evacuation);

Guiding the rod movement by transmitting the gas pressure forces at the same time;
Contributing to the evacuation of the heat resulting during burning;
Ensuring the sealing of the cylinder, thus preventing gas leaks and excess oil penetration.
The pistons are made of metal materials: aluminium, steel, and, in some cases, cast iron. Because these materials have a thermal coefficient of expansion, the piston dimensions are not fixed but variable, depending on the temperature.
In order for the piston to move into the cylinder, between the piston and the cylinder there should be a clearance. This clearance is higher when the engine is cold and drops as the temperature increases. The thermal expansion of the piston is not uniform: it is higher in the area of the piston head due to the larger amount of material. The distribution of temperature in the body of the piston is not uniform, being larger in the head area and smaller in the lower part, in the area of the mantle. For this reason, the shape of the piston should be slightly conical, so that the expansion becomes cylindrical.
Due to the force of piston pressing on the bolts and thermal dilation, the piston mantle is deformed, taking the form of an ellipse, with the large axis arranged after the axis of the bolt. If the large axis of the ellipse is greater than the bore of the cylinder, the danger of fluctuating/locking the piston in the cylinder occurs. For this reason, the piston mantle is not continuous: it is cut in the areas below the axis of the bolt.
The diameter clearance of the piston induces a tilting effect around the bolt during the engine operation. This occurs with a shock: the cylinder starts vibrating and there are characteristic noises called “piston beat”. In addition to the unpleasant acoustic effect, the piston beat intensifies the wear effect of the piston and segments. To diminish this tilting effect without changing the diameter clearance, the axis of the piston is shifted in relation to the cylinder axis.
A diesel engine is noted by the fact that the gas pressure in the cylinder has much higher values than in the case of a petrol engine. In petrol engines, the maximum pressure reaches around 60-90 bars while, in diesel engines, it reaches up to 130-160 bar. This requires diesel engines to use mechanical parts that have a much higher resistance.
It should be noted that a diesel engine piston is more robust compared to the petrol engine piston, has a taller mantle, and contains the combustion chamber in the piston head. The disadvantage is that higher robustness means greater mass. This is one of the reasons why the maximum speed of a diesel engine is lower, generally with 2,000-2,500 rpm than that of a petrol engine. All the moving parts of a diesel engine have a larger mass and, by default, larger inert. The maximum speeds at the level of a petrol engine are not possible in a diesel engine because it induces very large shocks and vibrations that can lead to partial or total engine damage.
Part of the heat resulting from the burning is evacuated through the piston. Most of the heat is evacuated through the segment-port region (70%); at the level of the mantle, around 25% are evacuated; and the rest is transmitted to the bolt, the crankcase gases, and the oil. The maximum temperature level depends on the operating regime of the engine.
Temperature can rise in two ways:
- By increasing the load, due to the greater amount of fuel inserted into the cylinder;
- By increasing speed, due to the greater number of cycles performed per time unit.
The connecting rod is the connection piece between the piston bolt and the crankshaft. It transmits the gas pressure force (during gas relaxation) from the piston to the crankshaft. The connecting rod includes the small head of the connecting rod, provided with a brass bushing in which the piston bolt, the connecting rod, the large head of the connecting rod and the connecting rod bearing, provided with semi-bearings, connecting the connecting rod of the crankshaft.
The connecting rod should withstand the intense mechanical requests produced by the gas pressure force and the inertia of the moving parts. The piston is fixed to the connecting rod through a bolt, which is fixed in the connecting rod and can rotate in the piston places. There is no direct contact between the foot of the connecting rod and the bolt: between them, there is a softer metal (bronze) that has the role of reducing friction.

Two screws are used to attach the connecting rod lid. Newer rods do not provide for bolts: they are fixed in the lid. On the lid and on the connecting rod, there are pins and holes that allow the lids to be installed only in one position. In an engine, the rod lids are not interchangeable: a lid will always be mounted at the same connecting rod.
Connecting rods are made of high resistance allied steels; in some cases, titanium-based connecting rods are used.
The piston bolt makes the articulated bond between the piston and the connecting rod. It has a cylindrical shape with an inner hole along the entire length. The mechanical resistance of the bolt should be high because it is subject to compression, shear, and bending requests. Because of the heavy operating conditions and of the need for wear resistance, the bolt is made of alloy steel.
To have an increased resistance to wear, the bolt is subjected to thermal hardening treatments (through high frequency currents). In thermal engines for cars, the bolt is floating, and there is clearance between the bolt, the shoulders of the piston, and the foot of the connecting rod. Due to this clearance, during the operation of the engine, an oil film is created between the moving parts that amortizes the shocks and reduces the friction.
To limit the axial displacement of the bolt in the piston places, the bolt is fixed with the help of metal rings located in special piston cans. Limiting the clearance is necessary because excessive clearance can lead to mechanical stress that could result in piston deformation.
The bearings are mounted in the head of the connecting rod, between the connecting rod and the crankshaft. On each connecting rod, there are two bearings, one in the lid and the other on the connecting rod. A bearing consists of a thin metal layer covered by an antifriction layer that comes in contact with the crankshaft bearing.
Between the bearing and the crankshaft, clearance is provided to allow the formation of a hydrodynamic oil layer to reduce the friction. To observe this clearance, the tightening of the connecting rod covers will always be done in the couple specified by the engine manufacturer.
Each bearing has a fixing spur that has the role of correctly positioning the bearing and, at the same time, of ensuring the mounting of the bearing only on the lid or only on the connecting rod. Like the rod lids, the bearings are not interchangeable: they are mounted all the time on the same piece.
The crankshaft is the most requested piece of the engine because, through the piston and the rod, it takes over the forces due to the pressure in the cylinder. The crankshaft is the piece that takes over the forces in the connecting rod, adds the mechanical things produced in the cylinders, and transmits the resulting energy to the wheels through the transmission. Also, the crankshaft involves some motor auxiliary systems (distribution, oil pump, water pump, compressor, alternator, etc.). The crankshaft is mounted in the engine block through the level bearings.
The items that make up a crankshaft for the engine are:
Level bearings (making the crankshaft rest on the motor block, in its places);
Handlers (on which the connectors are caught);
Arms (achieving the connection between the levels bearings and handlers;
Heads (coving areas).
Inside, the crankshaft has channels for the circulation of the lubrication oil that correspond to the feeding holes of the levels and handlers; most crankshafts have only one channel along them.
The crankshaft has a number of level bearings equal to the number of cylinders plus one. The level bearings are placed on the same geometric axis, and their width is different. The number of handlers is equal to that of the cylinders. The handler together with the two arms of the handler forms the crank (elbows). The diameter of the handler is smaller than that of the level bearing. The clearing of the bearings is done according to their number, thus ensuring a uniform engine operation and a balancing of the crankshaft.
The crankshaft is balanced with the help of the counterweights placed in the extension of the crankshaft arms (opposite them) and of the correct collapse of the crank. The balancing checking is done on special machines, and the share of the crankshaft, by partial releases (drilling or milling of counterweights).
Because it works under special disadvantageous conditions, the crankshaft has a complicated construction influenced also by the type of engine it equips. In general, the elements of the

crankshaft are oversized to achieve the rigidity necessary to limit the deformations.

Of all the engine organs, the crankshaft is subjected to the highest requests for the gas pressure force and the inertia forces of the masses with translation and rotation movement. Under the action of these forces in the elements of the crankshaft, important requests for stretching, compression, bending, and twisting occur, requests that have a shock character due to the mounting clearances, the high speed of pressure during the burning period, and the change of the forces application. The forces being variable produces dangerous demands of fatigue especially in the areas of voltage concentrators, such as the crossings from the arms and holes for lubrication oil. Apart from these, the crankshaft is stressed by additional stresses produced by the bending and twisting vibrations. The twisting vibrations of the crankshaft also produce disturbances in the functioning of the distribution mechanism, in the ignition distributor; in vehicle engines, they propagate in the mechanical transmission. All these requests cause the crankshaft deformation, which results in premature wear or even in breaking the crankshaft.

Given the difficult operating conditions, the construction and material of the crankshaft should meet the following important requirements: high mechanical stiffness and resistance, high wear resistance of the bearing surfaces, high fatigue resistance, manufacturing accuracy and dimensions, static and dynamic balancing, and avoiding resonance for both twisting and bending vibrations.

The construction and dimensions of the crankshaft depend on the type of engine, the number and disposition of the cylinders, the order of work, the balancing of the engine, etc.

Crankshafts are executed in two main variants: non-removable and removable, the first solution being the most used.

The front end of the crankshaft is constructively realized, taking into account that it serves mainly to operate the distribution, fan, cooling pump (except for air-cooled engines), and current generator (alternator or dynamo). It is also envisaged to place the oil sealing element, for example sealing ring and, in some engines, the location of the twisting shock absorber; the amplitudes of the twisting vibrations reach maximum values at the front end of the crankshaft, which justify the assembly of the shock absorber, if necessary, at that end.

Crankshafts are currently built of steel. The wide dimensioning demanded by the assurance of rigidity sometimes allows the use of quality carbon steel, being encountered in the manufacture of the connecting rod. For the most requested crankshafts, generally MAC, allied steels with Cr, Ni, Mo and possibly V, with breaking resistance up to 1,450 N/mm² are required.

The semi-finished product is elaborated by hot deformation – free moulding or forging. The first process is applied to small- and medium-sized crankshafts, whose final mass does not exceed 250 kg, using successively closed moulds (more heating). It presents the advantage of ensuring the continuity of the fibres of the material, as the semi-finished product has the shape of the crankshaft. This form is not made through free forging (large crankshafts), so that the elbows are obtained by removing the material between the arms by cutting, which causes the fibre to be interrupted and increases the cost of manufacture; newer technologies are based on the individual forging of each elbow, using a device that allows the eccentric movement of the respective handler bearing and then the rejection of the arms; continuous fibre is, thus, respected, and the homogeneity of the material and the resistance to fatigue are improved.

After elaboration, the semi-finished product is subjected to a normalization treatment to ease the processing. Large crankshafts forged with continuous fibre usually require axial recovery, and normalization is performed in a vertical position. Before finishing, the qualities of the crankshaft are improved by a chemical or thermochemical treatment. To increase wear resistance, the bearings are superficial. On the large scale, for this purpose, it applies to chill by induction or flame, on a depth of 3-5 mm, achieving a minimum hardness of 50 HRC.

Of great importance is the structure obtained by the treatment of return, which follows the hardening. For the hardening of the bearings, the cementation or fuelling is sometimes practiced, cheaper than hardening. In Cr - Mo or Cr - Mo - V steel crankshafts, bearing nitriding is very efficient, which increases not only hardness, but

also fatigue resistance; the treatment is more expensive.
Special results were obtained by constructing the crankshafts of some traction engines made of special micro-alloy steels. In the case of steel 49 Mn VS 3, the semi-finished product is uniformly cooled with air, without the need for normalization or correction of the geometric shape, and has a break resistance of 800-900 N/mm². From this material, for example, the crankshaft of a car engine with six cylinders in line is built; the crankshaft has non-removable counterweights at all arms and a mass of 35.5 kg. Optimizing its geometric form, the processing additions were reduced by lowering the semi-finished products by 10% and 35% of the volume of cutting operations.
Newer procedures for manufacturing the crankshaft are performed by casting.
The general characteristics of the crankshaft have some elements of superiority compared to those of the crankshafts performed from hot deformed semi-finished products: the mechanical characteristics can be located above the level of the forged piece, the resistance to creep is slightly higher, and hot behaviour is better. The technological difficulties, the less homogeneous structure, the more pronounced susceptibility of the dispersion of the physico-mechanical properties from one casting to another and the lower behaviour to fatigue have delayed the casting of crankshafts. Such obstacles were removed due to the progress made in the field of casting. As a result, the casting of crankshafts introduces important advantages, in relation to their forging. Thus, the consumption of metal is reduced by 30-70%, as the casting offers high accuracy of execution, greatly diminishing the processing additions (by 40-80%) because the amount of chips decreases 2.5-3 times and the number of operations with 20-25%. The mass of the finished crankshaft is 10-15% smaller, because the holes can be easily achieved along the bearings, which, in addition, ensures the uniform solidification and the increase of the rigidity.
The steering wheel is mounted on the engine crankshaft and has the role of accumulating a moment of inertia in the useful phase of the motor cycle (relaxation) that it gives in the energy consuming phases (evacuation, intake, and compression), thus realizing the uniform movement of rotation of the engine crankshaft. It is a massive piece of steel or cast iron on which the gear (the steering wheel) is mounted for training the crankshaft with the electromotor when starting the engine.

RESULTS AND DISCUSSIONS

The study of the dynamics of the motor mechanism aims to determine the forces and moments acting on the parts of the mechanism. This study is important for carrying out resistance calculations.
The forces that act in the motor mechanism are: inertia forces, pressure forces, gravitational forces, and reactions.
For the measuring of forces and moments, the dynamic model in Figure 2 is used.

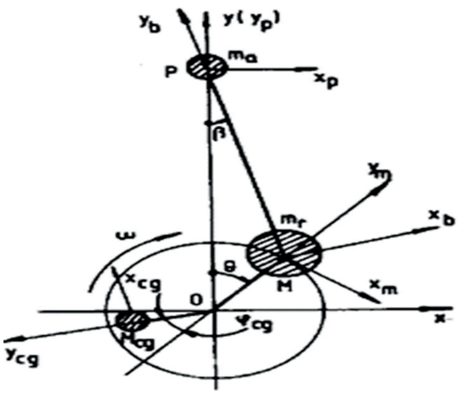

Figure 2. The dynamic model of the motor mechanism

m_a – masses in motion of translation; m_r – masses in rotational motion; m_{cg} – mass of counterweights
The values of the concentrated masses (of the subassemblies of the D-110 engine) are: $m_a = m_p + m_{ba}$,
where:
m_p – piston mass ($m_p = 0.86$ kg);
m_{ba} – connecting rod mass relative to piston in translational motion ($m_{ba} = 0.34$ kg)
Inertial forces are: $F_a = -m_a \cdot a_p$.
The pressure force of the gases is determined with the relationship: $F_p = \frac{\pi \cdot D^2}{4} \cdot p$
The force applied by the piston in the joint is: $F = F_a + F_p$.
The normal reaction is: $N = F \cdot tg\beta$.

The forces that act in the motor mechanism for a complete rotation of the crankshaft were calculated from 20 to 20 degrees. The results are centralized in Table 1.
Based on the data in Table 1, the forces diagram in Figure 3 was drawn.

Table 1. The forces acting on the motor mechanism

α (ºRAC)	F_p [daN]	F_a [daN]	F [daN]	N [daN]
0	295	- 79	216	0
20	389	- 72	317	27
40	192	- 51	141	23
60	96	-24	72	16
80	57	39	96	15
100	39	26	65	17
120	30	40	70	15
140	25	46	71	12
160	18	48	66	6
180	51	48	99	0
200	53	47	100	- 4
220	61	46	107	- 8
240	78	40	118	- 10
260	190	26	216	- 9
280	173	38	211	- 6
300	54	- 24	3	- 2
320	98	- 51	47	- 3
340	170	- 72	98	- 1
360	295	- 79	216	0

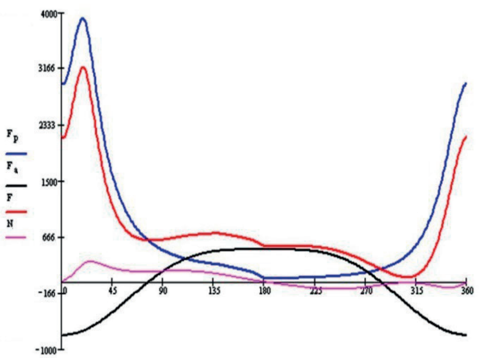

Figure 3. Diagram of forces acting on the motor mechanism

Analysing the diagram in Figure 3, it is found that the inertia forces have maximum values at the end of the race, i.e., when the piston is found in the dead points. This is due to the fact that the direction of travel of the piston changes.

CHECKING THE PISTON BOLT

The piston bolt is requested for shear in two ring cross sections. The sizes of the piston bolt are:
-Length: 88 mm;
-Outer diameter: 40 mm;
-Inner diameter: 23 mm.

Knowing the shape and dimensions of the transversal section, the permissible resistance of the material from which the bolt is made and the maximum size of the cutting force, the shear check is done with the relationship:

$$\tau_{ef} = \frac{T_{\max}}{A_{ef}} \leq \tau_a$$

where:

τ_{ef} – the actual tangential voltage occurring in the cross-section;

$T_{\max}$ – maximum cutting force ($T_{\max} = 400\ daN$);

A_{ef} – actual area required for shearing ($A_{ef} = 2 \cdot 0{,}785 \left(D_1^2 - d_2^2\right) = 16{,}81\ cm^2$);

τ_a – permissible shear resistance of the material from which the piston bolt is made $\tau_a = 500\ bari$

By replacement, it is obtained:

$$\tau_{ef} = \frac{T_{\max}}{A_{ef}} = \frac{400}{16{,}81} = 23{,}80\ bari < \tau_a.$$

In conclusion, the piston bolt withstands the shear request.

CHECKING THE CONNECTING ROD

The connecting rod mechanism is requested at combustion. It is considered that the main request is bending and that the task is distributed triangularly (Figure 4).

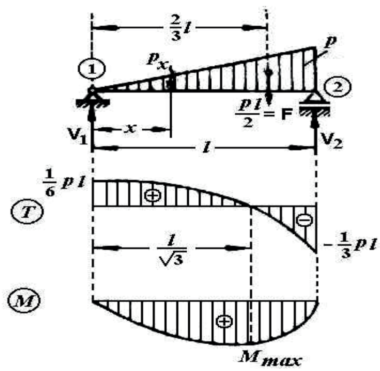

Figure 4. The connecting rod mechanism is requested at combustion

Because the load is distributed triangularly, its value differs from one cross-section to another of the connecting rod. For these reasons, the connecting rod is made in the form of an equally resistant beam to another beam.

The total task that requires the connecting rod is: $F = p \cdot l / 2$.

The total load is considered to be applied in the centre of gravity of the triangle and the reactions in the supports (connecting rod bearings) are determined: $V_1 = \frac{1}{3}F = \frac{pl}{6}$ $\quad V_2 = \frac{2}{3}F = \frac{pl}{3}$

In any section *x*, the distributed load has the intensity: $p_x = \frac{x}{l} \cdot p$

Taking into account that the derivative of the shear force with respect to the variable is equal to the distributed force taken with a changed sign ($\frac{dT}{dx} = -p$) and that the derivative of the bending moment with respect to the variable is equal to the shear force in the analysed section ($\frac{dM}{dx} = T$), cutting forces and bending moments can be determined by integrating these relationships.

$$T_x = -\int p_x dx = -\int \frac{x}{l} p dx = -\frac{px^2}{2l} + C_1$$

The constant C1 is determined by applying the relation in origin (*x* = 0), where $T = V_1 = \frac{pl}{6}$, hence: $T_x = \frac{pl}{6} - \frac{px^2}{2l}$.

It is observed that the cutting force varies parabolically from $\frac{pl}{6}$ (for $x = 0$) to $-\frac{pl}{3}$ (for $x = l$) and is cancelled at $\frac{pl}{6} - \frac{px^2}{2l} = 0$ for $x^2 = \frac{l^2}{3}$ and $x = \frac{l}{\sqrt{3}}$, respectively.

The expression of the bending moment in a section will be:

$$M_x = \int_0^x T_x dx = \int_0^x \left(\frac{pl}{6} - \frac{px^2}{2l} \right) dx = \frac{p\,l\,x}{6} - \frac{px^3}{6l} + C_2$$

.

The constant C_2 is determined for $x = 0$, where $M = 0$, hence $C_2 = 0$ and $M_x = \frac{plx}{6}\left(1 - \frac{x^2}{l^2}\right)$.

From the previous relation it follows that the bending moment varies according to a cubic parabola whose maximum is $x = \frac{l}{\sqrt{3}}$, hence:

$$M_{max} = \frac{pl^2}{9\sqrt{3}}.$$

From the relation $F = p \cdot l / 2$, it results $p = 2 \cdot F / l.$

Taking into account that the maximum force of inertia is $F_i = F = 400\ daN$ and that the length of the connecting rod, between the small head and the large head, is $l = 0{,}26\ m$, the distributed load will be:

$$p = 2 \cdot F / l = \frac{2 \cdot 400}{0{,}26} = 3077\ daN / m.$$

Therefore, the maximum moment will be:

$$M_{max} = \frac{pl^2}{9\sqrt{3}} = \frac{3077 \cdot \sqrt{3} \cdot 0{,}26^2}{27} = 13{,}346\ daNm = 1335\ daN \cdot cm$$

The dangerous net section of the connecting rod is at the small head, having the shape of an I_{26} profile. The cross-sectional strength modulus for profile I_{26} is: $W_z = 442\ cm^3$.

The actual voltage that occurs in the dangerous section, according to Navier's relation, has the value: $\sigma_{ef} = \frac{M_{max}}{W_z} = \frac{1335\ daN \cdot cm}{442\ cm^3} = 3.02\ bari$.

The connecting rod is made of cast iron alloyed with nodular graphite. The permissible strength for the connecting rod material is: $\sigma_a = 200 \div 400\ bari$, value much higher than the actual voltage in the dangerous net section. Therefore, the connecting rod is checked from the point of view of the resistance calculation.

CHECKING THE CRANKSHAFT

The crankshaft of the D-110 engine is made by alloy steel forging and treated in the outer layer in high frequency currents. It has five level bearings with a diameter of 85 mm and 4 bearings with a diameter of 75 mm. The length of the crankshaft between the first and last level bearing is 600 mm, and the handler range of 63 mm (r = S/2 – half of the piston race).

The main request of the crankshaft is the twist. The calculation is made for one of the handlers (smaller circular section).

The twisting checking relationship is: $\tau_{ef} = \frac{M_t}{W_p} \le \tau_a$, where:

τ_{ef} – effective tangential tension occurring in the cross-section of the spindle;

τ_a – permissible tensile strength of the material of which the crankshaft is made ($\tau_a = 300 \div 1000\ bari$);

M_t – maximum torque moment ($M_t = 29{,}5\ daN \cdot m$);

W_P – polar resistance modulus of the cross-section, given by the relationship: $W_P = \frac{\pi \cdot D^3}{16} = \frac{3{,}14 \cdot 7{,}5^3}{16} = 82\ cm^3$

The effective tension in the cross-section of the lever spindle will be: $\tau_{ef} = \frac{M_t}{W_p} = \frac{2950\ daN \cdot cm}{82\ cm^3} = 36\ bari < \tau_a$.

So, the crankshaft is checked at the twisting request.

CHECKING THE FLYWHEEL

The steering wheel is a heavy wheel, travelled on the crankshaft, having a high time of inertia in relation to the axis of rotation (*Figure 5*).

The steering wheel does not allow sudden variations in the angular speed, fixing the variation of the angular speed between the desired limits. The steering wheel works as a battery, storing energy when the movement accelerates, energy that then returns when the movement slows down. The disadvantage of the flywheel is that it makes it difficult to start or stop the car and reduce its maximum speed.

The D-10 engine steering wheel has the following features:

Outer diameter: D = 400 mm;

Generator (thickness): g = 80 mm;

Mass (without clutch): 36 kg.

Material of the flywheel: ash cast iron FC 25;

Material density: $\rho = 7{,}8\ grame / cm^3$.

The flywheel must ensure the quiet operation of the engine between two speed limits:

Minimum speed: $n_1 = 1000\ rot / min$ ($\omega_1 = 105\ rad / s$);

Maximum speed: $n_2 = 2600\ rot / min$ ($\omega_1 = 271\ rad / s$).

For this, the moment of inertia of the flywheel J_Δ should be as large as possible.

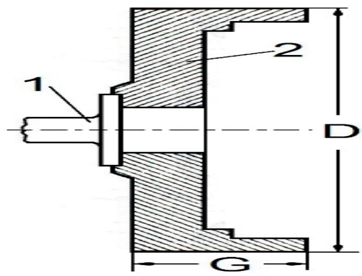

Figure 5. 1 - crankshaft; 2 - flywheel; D - flywheel diameter; G - Generator (thickness)

Moment of inertia relative to the axis of rotation J_Δ is determined by the relationship: $J_\Delta = \frac{1}{2} \cdot M \cdot R^2 \left[kg \cdot m^2\right]$, where:

M – flywheel mass [kg];

R – flywheel radius [m].

Angular velocity variation $\omega_2 - \omega_1$ during engine operation must not exceed n_r part of the average angular velocity: $\omega_m = \frac{\omega_1 + \omega_2}{2} = \frac{105 + 271}{2} = 188\ rad/\sec$

.Therefore, $\omega_2 - \omega_1 = \frac{\omega_m}{n_r} = \frac{\omega_1 + \omega_2}{2 \cdot n_r}$, hence: $n_r = \frac{\omega_1 + \omega_2}{2 \cdot (\omega_2 - \omega_1)} = \frac{105 + 271}{2 \cdot (271 - 105)} = 1{,}13$.

n_r It is called the regularity coefficient of the motor and the higher it is, the closer the motor has to the uniform one.

Applying the kinetic energy variation theorem, one can write: $E_{C_2} - E_{C_1} = \Delta L$, or $\frac{1}{2} J_\Delta \cdot \omega_2^2 - \frac{1}{2} J_\Delta \cdot \omega_1^2 = \Delta L$, hence $J_\Delta = 2 \cdot \frac{\Delta L}{\omega_2^2 - \omega_1^2} = \frac{n_r \cdot \Delta L}{\omega_m^2}$.

Taking into account that $\Delta L = P \cdot t$, where $P = 48000\ W$ and $t = 1\ s$, hence: $\Delta L = P \cdot t = 48000\ Jouli$, the axial moment of inertia will be:

$$J_{\Delta} = \frac{n_r \cdot \Delta L}{\omega_m^2} = \frac{1{,}13 \cdot 48000}{188^2} = 1{,}54\ kg \cdot m^2$$

Knowing the moment of inertia, the mass of the flywheel and its radius can determine the generator (thickness) of the flywheel.

The relationship $J_{\Delta} = \frac{1}{2} \cdot M \cdot R^2$ results in

$$M = \frac{2 \cdot J_{\Delta}}{R^2} = \frac{2 \cdot 1{,}54}{0{,}2^2} = 77\ kg$$

Knowing the density of the material, the volume of the flywheel (cylinder) V and its generator G are determined:

$$V = \frac{M}{\rho} = \frac{77000\ grame}{7{,}8} = 9872\ cm^3 = 0{,}785 \cdot D^2 \cdot G$$

$$G = \frac{V}{0{,}785 \cdot D^2} = \frac{9872}{0{,}785 \cdot 40^2} = 7{,}87\ cm,$$

which corresponds to the measurements made (G = 80 mm).

Therefore, the steering wheel is correctly sized and ensures the operation of the engine at constant speed.

CONCLUSIONS

The present paper presents a study on diesel engines that equip self-propelled tractors and machines. Using a vast bibliography, we analysed the evolution and performance of internal combustion engines and the dynamics of the motor mechanism.

Following the study conducted, the following conclusions resulted:

-Internal combustion engines that equip agricultural tractors are large mass engines that perform mechanical energy due to inertial mass properties;

-Diesel engines on tractors are “high engines” that perform the large cylinder race (they have a higher engine block than large bore engines) and therefore the crankshaft levers are larger, resulting in high torque;

-Using supercharged diesel engines with electronic injection in tractors reduces fuel consumption and polluting emissions, and improves their yield;

-Periodic technical checks and current internal combustion engines maintenance lead to reduction of costs and increase their reliability.

Resistance calculations on the subassemblies of the motor mechanism show that they resist the dynamic requests to which they are subjected during operation.

The steering wheel of the motor mechanism is correctly sized to keep the engine in constant speed for all operating regimes.

Regarding the energy base of the agricultural aggregates, the tendency to use tractors equipped with heavy power engines is noted. Under these conditions, their rational use in energy and technological aspect is ensured by the formation of combined aggregates, capable of performing several works, so that the duration of the work is reduced and savings regarding cost prices are made.

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FIELD PEA AND ITS IMPORTANCE FOR A SUSTAINABLE AGRICULTURE AND BETTER FOOD SYSTEMS

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Abstract

The cultivation of peas (Pisum sativum) presents a promising opportunity for promoting sustainable agriculture and ensuring food security in the face of contemporary challenges. This leguminous plant (Fabaceae family), plays a crucial role in the process of nitrogen fixation in the soil, significantly enhancing its fertility while simultaneously reducing dependence on synthetic fertilizers. Within a global context characterized by food insecurity, driven by climate change and market fluctuations, there is an urgent need to promote less conventional crops that possess significant agronomic potential. This article aims to consolidate the numerous advantages offered by pea cultivation, including its origins, relevance, and adaptability to various agro-climatic conditions. Moreover, it will highlight the high nutritional value of peas, alongside their potential to address increasingly urgent global challenges, such as the need to produce sustainable and healthy food for a constantly growing population. In this regard, peas are not only emerging as an important agricultural crop but also as a key pillar in the sustainable food strategy.

Key words: *pea, sustainable, agriculture, food security.*

INTRODUCTION

One of the main goal of scientists is to find optimal methods to maintain high productivity of crops under climate changes condition as well as developing crops with enhanced nutritional value, such as legumes. In support of this objective comes genetic engineering, which is revolutionising agriculture, increasing resilience and improving crop adaptation (Bonciu et al., 2021; De Souza and Bonciu, 2022).
The plant-based protein market has been steadily growing globally. Globally, the plant-based protein market will continue to increase (Thavarajah et al., 2023).
Pisum sativum, commonly known as pea, is an important leguminous crop grown globally for its nutritional and ecological benefits. One of the primary reasons for the increasing interest in *Pisum sativum* is its remarkable nutritional profile. Peas are an excellent source of plant-based proteins, making them an essential food for vegetarians and vegans (Shanthakumar et al., 2022; Thavarajah et al., 2023).
Rich in essential amino acids, they provide a high-quality protein alternative to animal-based products. In addition to protein, peas are packed with dietary fiber, which promotes digestive health, lowers cholesterol levels, and aids in maintaining a healthy weight. The high content of vitamins, particularly vitamin C, and minerals such as iron, potassium, and magnesium, further contribute to their importance in human diets (Dahl et al., 2012). However, the benefits of pea extend far beyond its nutritional value.
As a leguminous crop, peas have the unique ability to fix atmospheric nitrogen in the soil through a symbiotic relationship with soil bacteria (Jakobsen, 1985). This process not only enhances soil fertility but also reduces the need for synthetic fertilizers, which are widely recognized for their negative environmental impact.
The widespread use of chemical fertilizers in conventional farming has led to numerous environmental issues, including soil degradation, water pollution, and the release of greenhouse gases (Finez et al., 2023). By incorporating nitrogen-fixing crops like peas into crop rotations, farmers can reduce their reliance on chemical inputs, improving both the sustainability and resilience of their agricultural

systems. Furthermore, pea can serve as a climate-resilient crop.

With the increasing unpredictability of weather patterns and the growing threat of climate change, farmers are seeking crops that are more adaptable to extreme conditions. Peas, which are hardy and drought-tolerant once established, offer potential for cultivation in regions facing water scarcity or fluctuating climatic conditions (Zhang et al., 2016). Their relatively short growing season also makes them an attractive option for farmers in areas with shorter growing periods, allowing them to diversify their crop portfolios and reduce risk. With the environmental and ethical concerns surrounding animal agriculture becoming more prominent, peas and other legumes are gaining recognition as key components of a sustainable food future. As demand for plant-based protein sources increases, peas are poised to become an even more integral part of global food systems.

In this context, the purpose of this paper was to present the high nutritional value of peas, alongside its potential to address increasingly urgent global challenges.

MATERIALS AND METHODS

The methodology includes a comprehensive analysis of recent literature published on pea, focusing on its agricultural, nutritional, and environmental contributions. Studies published in the last five years were prioritized to ensure the inclusion of the latest developments in the field.

The review covers various aspects of pea, including its cultivation practices, genetic improvements, ecological benefits, and uses in human nutrition, livestock feed, and food processing. Additionally, the environmental impact of pea cultivation, such as its role in nitrogen fixation and its potential in crop rotation systems, was explored.

RESULTS AND DISCUSSIONS

Nutritional Benefits of pea

Pisum sativum is a nutrient-dense crop that provides high-quality protein, fiber, and micronutrients essential for human health (Table 1).

Table 1. The nutritional value of peas

Component	Range/Content	Reference
Carbohydrates	59.32–69.59% of dry weight	Arif et al., 2020
Starch	39.44%–46.23%	Raghunathan et al., 2017
Dietary Fiber (Total)	23.23%–30.72% of seed weight	Kan et al., 2018
Soluble Fiber (SDF)	3.91%–8.01%	Kan et al., 2018
Insoluble Fiber (IDF)	19.32%–23.1%	Kan et al., 2018
Protein	20–25% of dry weight	Shanthakumar et al., 2022
Lipid	3.06%–7.3%	Kan et al., 2018
Ash Content	~3.07%	Arif et al., 2020
Pea Protein Composition	55%–65% globulin	Lu et al., 2020
Lysine in Pea Protein	Rich in lysine	Ge et al., 2020
Fatty Acids in Lipids	42.01%–60.68% polyunsaturated fatty acids	Ciurescu et al., 2018
Main Fatty Acids	Palmitic acid (12.39%–19.24%), Linoleic acid (34.56%–47.74%), Linolenic acid (7.37%–12.55%)	Ciurescu et al., 2018
Minerals	Nitrogen (28.49–54.78 g/kg), Phosphorus (1.648–4.04 g/kg), Potassium (13.13–50.41 g/kg)	Nadeem et al., 2021
Selenium	28.6 µg/100 g	Liu et al., 2019
Tocopherols (Vitamins)	48.44–57.00 µg/g of total tocopherols	Padhi et al., 2017

Recent studies have shown that peas are particularly rich in essential amino acids like lysine, which is often limiting in other plant-based proteins (Smith et al., 2023).

A growing body of evidence suggests that *Pisum sativum* can be used as a substitute for animal proteins in various plant-based food products, including meat analogues, dairy alternatives, and protein-rich snacks (Vastolo et al., 2025). The protein content of dried peas typically ranges from 20 to 25%, with an amino acid profile comparable to that of soybeans (Michaud et al., 2020). Furthermore, peas are an excellent source of fibers, particularly soluble fibers, which contribute to gut health and have been linked to reduced risks of cardiovascular disease (Johnson et al., 2021).

Pisum sativum is an excellent source of high-quality protein, containing all nine essential amino acids required for human health. The protein content in peas typically ranges between 20% and 25% of dry weight, with significant levels of lysine, an amino acid often limited in many other plant-based proteins (Michaud et al., 2020). Recent studies emphasize the potential of pea protein as a sustainable alternative to animal-based proteins, making it particularly valuable in the context of growing global demand for plant-based products (Smith et al., 2023). In addition to protein, peas are rich in dietary fibers, with levels ranging from 15% to 20% of dry weight. This high fibers content includes both soluble and insoluble fibers, which promote digestive health, regulate blood sugar levels, and contribute to lower cholesterol levels, thereby reducing the risk of cardiovascular diseases (Johnson et al., 2021).

Soluble fibers, particularly, plays a significant role in managing cholesterol by binding to bile acids and helping eliminate them from the body (Smith et al., 2022). Peas are also a valuable source of essential micronutrients. They are rich in vitamins such as folate (vitamin B9), which is important for cell division and proper neural development, particularly during pregnancy. In addition, peas contain a high concentration of vitamin C, an antioxidant that supports the immune system, promotes skin health, and aids in iron absorption (Michaud et al., 2021). Moreover, peas provide essential minerals such as iron, magnesium, potassium, and phosphorus, which contribute to overall health by supporting metabolic functions, bone health, and muscle function.

Recent studies have highlighted the antioxidant properties of peas, which can help neutralize free radicals in the body and reduce oxidative stress, a major factor in chronic diseases such as cancer, diabetes, and cardiovascular conditions (Johnson et al., 2021). Moreover, pea protein has been shown to have a positive effect on satiety, making it an ideal ingredient in weight management and health-conscious diets (Jones et al., 2023).

With a relatively low glycemic index (GI) compared to many other carbohydrate-rich foods, peas are beneficial for individuals with diabetes or those seeking to manage blood sugar levels. This makes peas a versatile addition to a balanced diet, especially for people following plant-based diets or those with dietary restrictions (Baldwin et al., 2021).

The digestibility of pea protein has also been shown to be high, with studies indicating that pea protein is easily absorbed by the human body, similar to animal proteins like whey (Michaud et al., 2021). This is especially important for athletes or individuals with higher protein needs, as well as for individuals who experience digestive issues with other protein sources.

Carbohydrates constitute one of the primary chemical components of pea seeds, making up 59.32–69.59% of their dry weight (Arif et al., 2020). Starch content ranges from 39.44% to 46.23% (Raghunathan et al., 2017), higher than that of faba beans (38.4-41.8%) (Abdel-Aal et al., 2019). Peas are also rich in dietary fibers, with 23.23%-30.72% of pea seeds containing 3.91%-8.01% soluble fibers and 19.32%-23.1% insoluble fibers (Kan et al., 2018). Protein content in pea seeds is about 20-25% of the dry weight (Shanthakumar et al., 2022), similar to adzuki beans (23.51%) and kidney beans (23.44%-24.90%) (Ge et al., 2021).

Lipid content ranges from 3.06% to 7.3%, similar to cowpea (4.22%-7.17%) (Kan et al., 2018), while ash content is about 3.07% (Arif et al., 2020). Nutrients in pea seeds are influenced by cultivar, environment, and planting year (Wang et al., 2010), highlighting the need for further studies on the systematic comparison of chemical composition across different cultivars for precise use in the food industry.

Pea seeds are rich in dietary fiber, comprising 23.23%-30.72% of the seed, including 3.91%-8.01% soluble dietary fiber (SDF) and 19.32%-23.1% insoluble dietary fiber (IDF) (Kan et al., 2018). Pea seed SDF content is comparable to that of broad beans, kidney beans, and cowpeas (Kan et al., 2018). Ultrafine grinding technology can increase SDF content in pea seeds from 1.26% to 4.97% (Wang et al., 2021).

Dietary fiber helps lower cholesterol and glycemic indexes, suggesting that peas may aid in the prevention of diabetes and hypercholesterolemia. Pea SDF consists mainly of galacturonic acid, arabinose, galactose, glucose, mannose, rhamnose, xylose, and fucose (Wu et al., 2019), with galacturonic acid being the predominant sugar. IDF in pea seeds contains glucose, arabinose, galacturonic acid, xylose, galactose, mannose, and rhamnose, suggesting it is made of cellulose, xylans, and arabinans. Pea dietary fiber and polysaccharides exhibit significant antioxidant and hypoglycemic effects (Mayengbam et al., 2019). However, further research is needed to better understand their chemical structures.

Pea protein is categorized into globulin, albumin, prolamin, and glutenin, with globulin accounting for 55%-65% of total protein in field peas (Lu et al., 2020). Pea proteins are rich in lysine, complementing the amino acid profile of cereal-based diets and offer health benefits such as antioxidant, anti-diabetic, and anti-hypertensive effects, as well as supporting gut health. Also, pea proteins are used in various food applications, such as encapsulating bioactive compounds and in alternative meat products (Ge et al., 2020).

Allergenic proteins in peas, including Pis s_1 and Pis s_2, are associated with allergic reactions in sensitive individuals (Popp et al., 2020). The amino acid composition of pea proteins is well-balanced but is limited by methionine and cysteine (Ge et al., 2021), with aromatic amino acids and lysine being limiting for different age groups (Han et al., 2020). Pea proteins share similar physicochemical characteristics with soybean proteins, including pH-dependent solubility and isoelectric points (Zhao et al., 2020).

High-pressure processing and heat treatment reduce pea protein solubility (Hall et al., 2021), and pea proteins have better foaming stability and water adsorption capacity than rice and wheat proteins (Zhao et al., 2020). Pea proteins may serve as a potential substitute for soybean proteins in meat and sausage products (Zhao et al., 2020). However, further studies are needed for more accurate and conclusive data due to sample size and genotype limitations.

Pea seeds are low in lipids, making peas a low-fat food. The lipids in peas primarily consist of polyunsaturated fatty acids, accounting for 42.01%-60.68% of total fatty acids, with lower levels of unsaturated fatty acids (Ciurescu et al., 2018). The main fatty acids in peas include palmitic acid (12.39%-19.24%), linoleic acid (34.56%-47.74%), and linolenic acid (7.37%-12.55%) (Ciurescu et al., 2018). The bioavailability of these unsaturated fatty acids during digestion remains uncertain and requires further investigation.

Peas are a source of several minerals, such as nitrogen, potassium, phosphorus, manganese, copper, and zinc, with their content varying across genotypes (Nadeem et al., 2021). The primary minerals in peas include nitrogen (28.49-54.78 g/kg), phosphorus (1.648-4.04 g/kg), and potassium (13.13-50.41 g/kg) (Nadeem et al., 2021). Minor amounts of selenium are also found in peas (28.6 μg/100 g) (Liu et al., 2019), higher than in mung beans. The bioavailability of these minerals in peas remains unclear and requires more research. Peas also contain vitamins like α-tocopherol and γ-tocopherol, with total tocopherols ranging from 48.44 to 57.00μg/g, higher than lentils and kidney beans but lower than chickpeas (Padhi et al., 2017).

Ecological and Environmental Benefits

As a leguminous crop, pea offers significant ecological and environmental benefits, positioning it as a key player in sustainable agricultural practices.

One of the most remarkable advantages of peas is their ability to fix nitrogen, a process that significantly enhances soil fertility and reduces the need for synthetic fertilizers. Peas, like other legumes, form a symbiotic relationship with rhizobial bacteria in the soil, which allows them to convert atmospheric nitrogen into a bioavailable form that plants can use for growth (López et al., 2020). This natural nitrogen fixation can reduce the reliance on synthetic fertilizers, whose production and use contribute significantly to environmental pollution, greenhouse gas emissions, and soil degradation.

Studies have demonstrated that the inclusion of peas in crop rotation systems can lead to a significant reduction in the use of nitrogen fertilizers by up to 40%, which in turn decreases the overall environmental footprint of agriculture. This is particularly important in areas with intensive agricultural practices, where fertilizer overuse leads to eutrophication of water bodies, contributing to algae blooms, aquatic ecosystem imbalances, and contamination of groundwater sources (Baldwin et al., 2021).

By replacing synthetic nitrogen inputs, pea cultivation helps mitigate these adverse environmental effects and promotes a more sustainable approach to farming. Additionally, pea's nitrogen-fixing ability enhances soil health by improving its organic matter content and microbial diversity.

Research has shown that peas can increase soil microbial activity, leading to improved soil structure and water retention capacity. This is particularly valuable in regions affected by desertification and soil erosion, where maintaining soil health is critical to ensuring long-term agricultural productivity (Smith et al., 2022). Peas also help reduce the risk of soil compaction due to their deep root system, which can penetrate compacted soils and improve aeration; further enhancing soil fertility. The reduced need for chemical inputs not only protects pollinators and other beneficial organisms but also helps preserve the surrounding ecosystems from chemical contamination. In this regard, pea is an excellent choice for organic farming systems, where the use of synthetic chemicals is limited or avoided entirely. Furthermore, peas have demonstrated excellent resilience to drought, making them an ideal crop for regions facing water scarcity due to climate change. In fact, the main factors that impact any agronomic crops yield and quality are represented by the management of biotic and abiotic constrainers: the weather conditions during the growing season of plants, crop rotation, the previous crop, the soil tillage system, cropping technology, nitrogen fertilization, etc. (Paraschivu et al., 2024; Partal et Paraschivu, 2020; Partal et al., 2023; Săndulescu et al. 2024; Zală et al. 2023).

Peas require significantly less water compared too many other crops, such as rice or maize, and their drought-tolerant nature makes them a valuable component of dry land agriculture systems (López et al., 2020). Lastly, the versatility of pea in crop rotation systems enhances the resilience of agricultural ecosystems to pests, diseases, and environmental stress. By rotating peas with cereals or other crops, farmers can break pest cycles and reduce the overall incidence of soil-borne diseases, improving the health and sustainability of the entire farming system.

The ability to use peas in a diverse range of agricultural systems, from conventional farms to regenerative and organic practices, makes them a versatile and eco-friendly option for modern agriculture (Michaud et al., 2020).

Sustainable cultivation practices

Legume grains such as field peas and field beans can be produced on a local level, and may be reliable sources of dietary protein and energy apart from common soybean and rapeseed meals (Bachmann et al., 2020).

Pea is a quick growing, an annual herbaceous vine that requires the trellis to support growth. Is typically a cool season crop and thrives well in cool weather.

Require an average temperature range of 10-18°C during its growth period and can be cultivated on all types of soils under appropriate management conditions, but it prefers well drained soil for early crop and high yield.

A well-drained loamy soil free from excessive soluble salts with neutral pH range of 6.5 to 7.5 is suitable for successful cultivation of the crop. The field must be free from stubbles and crop residues of previous crops by ploughing through disc plough followed by 2-3 harrowing. To ensure good drainage and aeration in the field, powdery seedbeds must be avoided.

Field pea is mostly grown on residual soil moisture and can sustain drought conditions up to some extent. All soils must be analysed prior to planting so that any corrective measures may be taken before a problem becomes noticeable. Light sandy soils require more fertiliser than heavier soils. The phosphorus and potassic fertilizer should be applying as basal dose based on soil test value.

Peas are sown as early as possible in the spring, the best time being the end of February or the beginning of March, when the soil temperature reaches 4-5°C, with a tendency to increase. The last sowing period is April 1-2. For autumn crops, sowing can be done in September.

Pea is a poor competitor with weeds, especially during the first month after planting. However, perennial weeds and annual weeds that emerge early in the season are very competitive with pea.

Controlling diseases in field pea begins with crop rotation. A preferred crop rotation would have field pea planted with at least four cropping years between plantings.

Field peas should be harvested when they are fully ripe. A desiccant may be used to enhance crop drying prior to combining. Maintaining a low cutter bar height is essential to reduce pea losses.

Economic trends and potential for sustainable farming and better food systems

Global pea consumption is set to reach almost 6 million metric tons by 2026. This represents a growth of 0.4% per year since 2017. India was the top consumer in 2021 with 2.3 million metric tons. China, Ethiopia and Bangladesh followed in that order (https://www.reportlinker.com/clp/). In Europe, Spain and France dominate the cultivation of field peas (Table 2).

Table 2. Cultivated area and production of peas in Europe

Country	The cultivated area (ha)	Percentage of total (%)	Production (tons)	Percentage of total (%)
Austria	1920	1.35%	8650	1.11%
Belgium	9600	6.77%	62400	7.97%
Bulgaria	530	0.37%	1410	0.18%
Croatia	390	0.27%	3160	0.40%
Cyprus	50	0.04%	660	0.08%
Czechia	1020	0.72%	2630	0.34%
Denmark	1090	0.77%	4110	0.53%
Estonia	130	0.09%	200	0.03%
Finland	3910	2.76%	7830	1.00%
France	40150	28.33%	268200	34.30%
Germany	3820	2.69%	20310	2.59%
Greece	870	0.61%	7130	0.91%
Hungary	18490	13.04%	91350	11.69%
Italy	15170	10.68%	72530	9.27%
Lithuania	170	0.12%	190	0.02%
Luxembourg	0	0.00%	0	0.00%
Malta	0	0.00%	0	0.00%
Netherlands	6230	4.40%	37250	4.76%
Poland	8400	5.91%	49500	6.32%
Portugal	1540	1.08%	7280	0.93%
Romania	1070	0.75%	2140	0.27%
Slovakia	1330	0.94%	3560	0.46%
Slovenia	40	0.03%	130	0.02%
Spain	19700	13.87%	114530	14.64%
Sweden	6200	4.38%	17350	2.22%
EU	141820		782500	
EU Average	5672.8		31300	

The economic potential of pea has been highlighted by the increasing demand for plant-based protein sources in response to growing concerns over the environmental impact of animal agriculture.

Due to its nutritional profile and versatility, pea has become a popular ingredient in plant-based meat alternatives, dairy substitutes, and protein supplements (Jones et al., 2023). In particular, pea protein isolates are widely used in the production of plant-based burgers, sausages, and protein shakes. Moreover, pea cultivation offers economic opportunities for farmers, particularly in regions with limited access to expensive synthetic inputs.

The crop's resilience to adverse weather conditions, combined with its ability to enrich soil fertility, makes it a cost-effective option for sustainable farming. In addition, peas can be marketed as a high-value crop in both fresh and processed forms, providing income diversify-cation for farmers in rural areas (Michaud et al., 2021).

There are several ways in which farmers can be eligible for the green payment scheme, and one is by cultivating nitrogen-fixing crops. Those crops, which include peas, protect the soil from erosion and improve soil organic matter.

The global market for plant-based proteins is projected to grow significantly in the coming years. According to recent reports, the global plant-based protein market is expected to reach $17.9 billion by 2027, with a compound annual growth rate (CAGR) of 7.6% from 2020 to 2027 (Smith et al., 2022). Within this expanding market, pea has gained prominence due to its high protein content, ease of cultivation, and minimal environmental footprint.

As consumers increasingly seek plant-based alternatives for meat, dairy, and other animal products, the demand for pea protein - particularly pea protein isolates and concentrates - has risen dramatically. Pea protein has become a key ingredient in the production of plant-based meat substitutes, including burgers, sausages, and nuggets, as well as dairy alternatives such as milk, yogurt, and cheese. These products have gained significant market share due to their nutritional benefits, affordability, and versatility.

Beyond the plant-based food industry, pea has significant applications in animal feed, particularly in the growing market for sustainable and plant-based animal feeds. Also, peas are a valuable source of protein for livestock, particularly for poultry and pigs.

The increasing focus on reducing the environmental impact of animal agriculture has led to a rise in the use of plant-based ingredients, such as pea protein, in animal feed formulations. This shift is expected to continue as farmers and feed manufacturers seek to reduce their reliance on resource-intensive protein sources, such as soy and fishmeal, which contribute to deforestation, land degradation, and overfishing (Smith et al., 2022).

In addition to its role in food and feed, pea holds promise in the biofuel and bioplastics industries. Research into the use of pea biomass as a feedstock for biofuels is gaining traction, as peas are a renewable resource with relatively low environmental impact compared to traditional biofuel crops like corn and soybeans. The potential for pea-derived bioplastics, which can replace petroleum-based plastics, is also being explored. This opens up new markets for peas in the bioeconomy, where sustainability is increasingly a priority. Peas also provide an opportunity for farmers to diversify their income streams. The demand for peas in both fresh and processed forms such as frozen peas, pea flour, and pea protein products, has been steadily increasing, allowing farmers to access lucrative markets beyond traditional fresh produce sales.

As the global food system shifts towards more sustainable and plant-based products, peas offer farmers a crop that aligns with these trends, contributing to economic stability and growth in rural communities (Bonciu, 2023).

Consumption of whole peas reduces blood glucose, improves gastrointestinal health, and enhances satiety. This nutritional, functional, and sustainable benefit recommends peas as a major alternative protein source for the global food industry.

CONCLUSIONS

Pea (*Pisum sativum*) is a crop with huge potential to contribute to a more sustainable and resilient agricultural future. Its nutritional, ecological, and economic benefits position it as a key player in addressing the global challenges of food security, environmental sustainability, and climate change.

The ability of peas to fix nitrogen, reduce the reliance on synthetic fertilizers, and improve soil health makes them an essential component of sustainable farming systems. Additionally, the growing demand for plant-based proteins offers significant economic opportunities for farmers and the food industry.

To fully realize the potential of peas, continued research into its genetic improvement, cultivation practices, and market development is essential.

The European strategies provide the policy framework for supporting sustainable food systems in developing countries. In this context, growing peas can contribute to mitigate climate change and adapting to its impacts; also, peas can ensure food security, nutrition and public health.

Preserving the pea's affordability, while generating fairer economic returns in the supply chain, this sustainable food can also become one of the most affordable.

Food chain players (consumers, producers and processors) can make a real contribution to strengthening a better, more resilient and responsible food system. The adoption of pea as a staple crop in sustainable agricultural systems holds promise for a healthier, more sustainable future.

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MELLIFEROUS POTENTIAL AND BIOMASS WASTE VALORIZATION OF *Coriandrum sativum*, *Salvia hispanica*, and *Lavandula angustifolia* FOR RENEWABLE ENERGY APPLICATIONS

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Abstract

We investigated some biological characteristics of Coriandrum sativum, Salvia hispanica, and Lavandula angustifolia. It was established that the studied plant species are characterized by an extended flowering period, providing food for bees from end May to mid-August, with a honey potential ranging from 60 to 330 kg/ha. The analysis of the lignocellulose composition of the collected waste biomass (dry stalks) indicated that the dry matter contained 357-504 g/kg cellulose, 197-248 g/kg hemicellulose, and 72-135 g/kg acid detergent lignin. The estimated theoretical ethanol yield from cell wall carbohydrates ranged from 398 to 533 L/t. We found that the biochemical methane potential of the studied substrates varied from 173 to 280 L/kg ODM. The physical and mechanical properties of the dry stalk biomass showed an ash content of 3.36-4.48%, volatile matter content of 73.17-79.39%, a higher heating value (HHV) of 17.19-19.83 MJ/kg, and a lower heating value (LHV) of 15.86-18.46 MJ/kg. These characteristics indicate that the biomass can serve as a feedstock for the production of solid densified fuel (pellets) for renewable energy generation.

Key words: *biomass, biomethane potential, Coriandrum sativum, Lavandula angustifolia, melliferous potential, Salvia hispanica, solid densified fuel, pellets, theoretical ethanol yield.*

INTRODUCTION

The importance of medicinal and aromatic plants is widely acknowledged, especially given the renewed interest in phytotherapy, herbal medicine, the cosmetics industry, and perfumery. These plants have been used for thousands of years, and since the mid-20th century, interest in plant-based pharmaceuticals has grown steadily due to their greater biocompatibility with humans and animals. Being metabolically similar to endogenous compounds, they are generally better tolerated than many synthetic drugs.

The use of plants for medicinal and culinary purposes has deep roots in this region (Chisnicean et al., 2011). Today, numerous species are being researched and cultivated, both from local flora and from other parts of the world. In this context, special attention is given to species from the *Lamiaceae* and *Apiaceae* families, which serve not only medicinal and aromatic functions but also provide pollen and nectar for bees and other beneficial insects.

Lavender (*Lavandula angustifolia*) is a species in the family *Lamiaceae*, native to the Mediterranean region. It typically grows in natural habitats at lower mountain elevations. Lavender is a hardy, strongly aromatic shrub with bushy, branched stems reaching 40-50 cm in height. The plant has small, silver-grey leaves and deep violet-blue inflorescences. It is a highly valuable plant with aromatic, medicinal and ornamental uses. Lavender essential oil is especially popular and widely used in medicine, food, and cosmetics due to its antibacterial, antifungal, antioxidant, and anti-inflammatory properties. According to Gonceariuc (2014), new cultivars of *Lavandula angustifolia* developed in Moldova have reached essential oil yields of up to 245 kg/ha.

Chia (*Salvia hispanica*), also a member of the *Lamiaceae* family, is an annual herb native to Central America. It develops an extensive system of fibrous roots, forming a dense root mass under favourable conditions. The stems are erect, quadrangular, and can reach up to 200 cm in height, either simple or sparsely branched. The leaves are petiolate, with ovateelliptic blades and serrated margins; the upper (adaxial) surface is pubescent, while the lower (abaxial) surface is densely covered with whitish hairs. The inflorescence is a dense raceme composed of 6-12-flowered verticillasters. The fruits are ovoid nutlets, and the seeds are oval, measuring 1-2 mm. Seed colour varies from black and grey to white, including black-spotted forms. Today, chia is studied and cultivated in various regions around the world (Brandán et al. 2019; Rossi et al. 2020; Bhardwaj H.L. 2021; Chernov et al. 2022; Filik et al. 2022; Rodríguez et al. 2022; Rahal et al. 2023). The use of chia seeds for human consumption has been approved by the European Parliament and the European Council (European Commission, 2020).

Under the conditions of the Republic of Moldova, *Salvia hispanica* genotypes exhibit optimal growth and development, with a growing season lasting 122-126 days, ending with seed maturation. The weight of 1,000 seeds was 1.2-1.4 grams, and the potential seed yield reached 2,030 kg/ha (Chisnicean, 2017). The green biomass yield of *Salvia hispanica* varied from 6.02 to 6.56 kg/m², with a crude protein content of 87-107 g/kg, making it suitable both as forage for livestock and as feedstock for biogas production (Ababii et al., 2023).

Coriander (*Coriandrum sativum* L.), an annual herb from the *Apiaceae* family and native to the Mediterranean Basin, is an important multipurpose crop. It is an upright, branched plant that grows up to 80 cm tall. The leaves vary in shape: they are broadly lobed near the base and slender and feathery along the flowering stems. The small, white to pink flowers are arranged in compound umbels. The fruit is a globular dry schizocarp, measuring 3-5 mm in diameter. When fully mature, the seeds are light brown in colour. All parts of the plant – seeds, leaves and roots – are edible, although each has distinct flavours and culinary uses. In recent years, coriander has seen increased market demand due to its wide range of applications in industry, human and veterinary medicine, animal husbandry, and agriculture. The seeds, in particular, are valued for their content of essential oils, lipids, fatty acids, carbohydrates, amino acids, caffeic acid, chlorogenic acid, umbelliferone, scopoletin, carotenoids, vitamin C and mineral salts. The green leaves are also consumed fresh in salads, soups, and pickles. According to Yadav et al. (2001), coriander seed yields ranged from 0.34 to 1.73 t/ha, while straw yields was 0.84-3.12 t/ha. Garid et al. (2015) found seed yield was 1.236-1.925 t/ha.

Beekeeping is one of the key pillars of agricultural development in Moldova and has been practiced for thousands of years using traditional methods. Bees search for nectar and pollen not only in forests, grasslands, ruderal and marshy vegetation, but also in agrophytocenoses such as orchards, vineyards, and plantations of rapeseed, sunflower, leguminous crops, medicinal and aromatic plants.

The need to reduce dependence on fossil fuels, the growing global demand for energy, and the widespread goal of lowering atmospheric greenhouse gas emissions have all driven research into alternative fuels that utilize readily available renewable resources with minimal environmental impact. It is well known that agricultural residues from the cultivation of medicinal, spice and aromatic plants have often been burned directly in the field, while residues from the processing of raw materials were not properly stored, contributing to environmental pollution.

Currently, one way to make use of these agricultural and industrial residues is by converting them into various types of biofuels, serving as a renewable energy source.

The goal of this study was to determine the melliferous potential of *Lavandula angustifolia* (lavender), *Coriandrum sativum* (coriander), and *Salvia hispanica* (chia), as well as to evaluate the quality indices of their agricultural residues, specifically – stem biomass, for potential use as feedstock in biofuel production.

MATERIALS AND METHODS

The local cultivar *'Lavinia de Grădină'* of lavender (*Lavandula angustifolia*), the Romanian cultivar *'Omagiu'* of coriander (*Coriandrum sativum*), and the introduced ecotype of chia (*Salvia hispanica*), which grow in the experimental plot of the "Alexandru Ciubotaru" National Botanical Garden (Institute) of MSU, Chișinău, served as the subjects of the research. The cultivar *'Vital'* of cup plant (*Silphium perfoliatum*) and rapeseed (*Brassica napus* subsp. *oleifera*) were used as controls. Some biological characteristics of the studied species were investigated according to standard methodological procedures. The melliferous potential was calculated number of flowers per plants, sugar in flower and blooming duration.

Lavender stem biomass was collected in February during rejuvenation works on a 10-year-old plantation, while coriander and chia biomass were collected after seed harvesting. The harvested stem biomass was first chopped into small fragments using a stationary forage chopper. Subsequently, the chopped material was ground in a beater mill fitted with a 6 mm mesh sieve. For analysis purposes, the milled biomass was dried in an oven at 85°C, further ground to a particle size of less than 1 mm, and thoroughly homogenized. Elemental composition, including total carbon (C), hydrogen (H), nitrogen (N), and sulfur (S), was determined via dry combustion using a Vario Macro CHNS elemental analyzer (Elementar Analysensysteme GmbH, Langenselbold, Germany). Biomass densification was carried out using pelleting equipment. The ash content and energy characteristics (higher and lower heating values) of both the dry biomass and resulting pellets were evaluated following standardized protocols at the Technical University of Moldova. To analyse the structural components of the plant cell wall in the dry biomass, the contents of neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) were measured using near-infrared spectroscopy (NIRS) with a PERTEN DA 7200 instrument at the Research-Development Institute for Grassland in Brașov, Romania. Cellulose content was estimated as the difference between ADF and ADL, while hemicellulose content was calculated as the difference between NDF and ADF. The theoretical ethanol potential (TEP) was estimated based on the conversion of cellulose and hemicellulose into hexose (H) and pentose (P) sugars, following the equations developed by Goff et al. (2010). The biochemical methane potential (BMP) was calculated using the methodology proposed by Dandikas et al. (2015).

RESULTS AND DISCUSSIONS

Based on the analysis of the biological characteristics of growth and development, it was observed that lavender (*Lavandula angustifolia*) resumes vegetation in the second half of April, with the flowering period occurring from mid-May to mid-June. The flowers are visited by bees throughout the day. The estimated honey potential of lavender is approximately 230 kg/ha.

In the 'Omagiu' cultivar of coriander (*Coriandrum sativum*), seedling emergence was recorded in mid-April, followed by more vigorous vegetative growth during May. Flowering began 48-55 days after emergence, typically in June, when plants reached a height of 62-75 cm. The flowering period lasted 10-16 days. The flowers were intensely visited by bees throughout the day, with peak activity occurring in the afternoon, when nectar secretion was more abundant. The estimated honey potential for coriander is approximately 330 kg/ha.

Figure 1. *Apis melifera* visiting *Coriandrum sativum* and *Salvia hispanica* plants

In *Salvia hispanica*, seedlings emerge at the soil surface in early May, with more vigorous vegetative growth observed in June. During the flowering stage, plants reach a height of 200-210 cm. The flowering period lasts 12-18 days, and the flowers are intensively visited by bees, particularly in the first half of the day. The estimated honey potential is approximately 60 kg/ha.

The scientific literature presents various data regarding the biological characteristics and honey potential of plant species from the *Lamiaceae* and *Apiaceae* families. According to Iordache et al. (2007), the nectar production of *Coriandrum sativum* is between 0.10 and 0.15 mg per flower, with a honey potential ranging from 100 to 500 kg/ha, and under favourable conditions, up to 1500 kg/ha. For *Salvia nemorosa*, nectar production ranges from 0.3 to 1.5 mg per flower, with a honey potential of 200-400 kg/ha. In *Lavandula angustifolia*, nectar production is reported at 0.07-0.22 mg per flower, with a honey potential of 50-100 kg/ha. According to Auer (2020), Halbritter et al. (2020), and Enache et al. (2021), the flowers of *Lavandula angustifolia*, *Salvia nemorosa*, *Salvia officinalis*, *Salvia pratensis* and *Salvia verticillata* produce medium-sized, hexacolpate pollen grains. In contrast, species such as *Coriandrum sativum*, *Cichorium intybus*, *Fagopyrum esculentum*, *Foeniculum vulgare* and *Robinia pseudacacia* produce medium-sized, tricolporate pollen grains. Rodríguez et al. (2023) reported that the flowering period of *Salvia hispanica* begins 66 days after sowing, with full flowering reached at 76 days. According to Brandán et al. (2019), the flowering period of *Salvia hispanica* lasts 15 to 25 days, with each flower remaining open for 5 to 7 days. Varban et al. (2022) observed that the growing season of the studied *Salvia hispanica* accessions lasted between 150 and 170 days, with the flowering stage accounting for 13.5-18.0% of the total growing season. Dragoman et al. (2024) reported the following melliferous potentials: *Lavandula angustifolia* – 200 kg/ha, *Salvia officinalis* – 400 kg/ha, and *Salvia verticillata* – 600 kg/ha.

The use of biomass as solid fuel for energy supply requires characterizing elemental chemical components. The elemental composition of biomass is a significant asset that defines the amount of energy and evaluates the clean and efficient use of biomass materials, provides significant parameters used in the design of almost all energy conversion systems and projects, for the assessment of the complete process of any thermochemical conversion techniques. The main constituents of dry biomass are carbon (C), oxygen (O) and hydrogen (H). As carbon and hydrogen are oxidised in the combustion process, they release energy. Carbon is obviously representing foremost contributions to overall heating value. Furthermore, higher hydrogen content determines and leads to a higher net caloric value. Nitrogen (N), sulphur (S) and chlorine (Cl) concentrations are some of the main causes of air pollution from biomass combustion. A higher percentage of these elements generally results in a higher level of air contaminants being released. The energy released during the combustion process is positively correlated with the carbon and hydrogen contents as a function of the energy value of these elements. In contrast, high oxygen and nitrogen values decrease the calorific value.

Table 1. The elemental composition of the dry biomass from the studied species, %

Indices	*Coriandrum sativum*	*Salvia hispanica*	*Lavandula angustifolia*	*Brassica napus*	*Silphium perfoliatum*
Carbon	44.35	43.13	51.03	45.60	46.28
Nitrogen	0.42	1.26	0.44	0.92	0.22
Hydrogen	6.04	5.24	6.15	5.14	6.14
Sulphur	0.09	0.11	0.02	0.07	0.03
Oxygen	49.11	50.26	42.36	48.30	47.33

The elemental composition of the dry biomass from the studied species is presented in Table 1. We found that the *Lavandula angustifolia* dry biomass is characterized by a very high concentration of carbon and very low concentration of sulphur, as compared with biomass from *Salvia hispanica, Brassica napus* and *Coriandrum sativum.* The biomass from *Salvia hispanica* and *Brassica napus* had higher levels of nitrogen and lower – of hydrogen, as compared with other investigated species.

The valorization of energy biomass in the form of solid fuels, such as pellets and briquettes, is commonly preferred, as it reduces biomass volume, thereby lowering transportation costs, improving handling, and increasing the energy amount per unit of volume.

Densified solid fuels like pellets also offer structural consistency, making them particularly suitable for automated boiler systems in individual households, schools, kindergartens, and other public institutions.

The quality indices of the biomass and resulting pellets from the studied species are presented in Figures 2-7.

Ash content is a key parameter in determining the quality of solid fuels, as higher ash levels negatively affect combustion efficiency, promote clinker formation in combustion chambers, and can lead to physical damage and wear of heating systems. *Lavandula angustifolia* dry biomass exhibited an optimal ash content (3.74%). The biomass of *Salvia hispanica* and *Coriandrum sativum* had lower ash content compared to *Brassica napus* subsp. *oleifera* and *Silphium perfoliatum.*

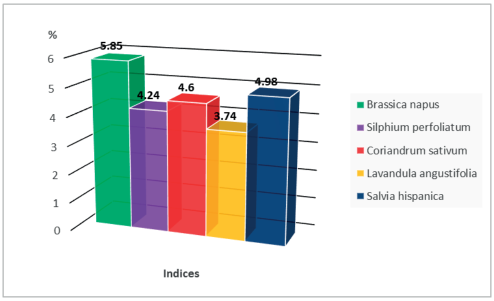

Figure 2. Ash content of biomass

The gross calorific value (higher heating value) was highest in *Lavandula angustifolia* biomass (19.84 MJ/kg), followed by *Coriandrum sativum*, which showed a significantly higher value than both *Brassica napus oleifera* and *Silphium perfoliatum*. *Salvia hispanica* biomass had a lower calorific value (17.24 MJ/kg).

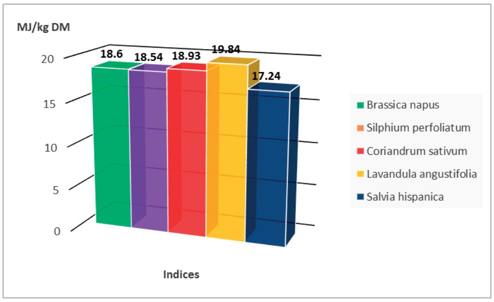

Figure 3. Gross calorific value of biomass

The net calorific value of dry biomass from the studied species varied significantly (15.88-18.46 MJ/kg). Compared to *Silphium perfoliatum* and *Brassica napus* subsp. *oleifera*, the dry biomass of *Lavandula angustifolia* exhibited a higher energy concentration, while *Salvia hispanica* and *Coriandrum sativum* showed lower values.

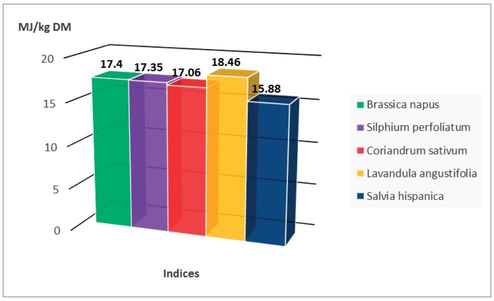

Figure 4. Net calorific value of biomass

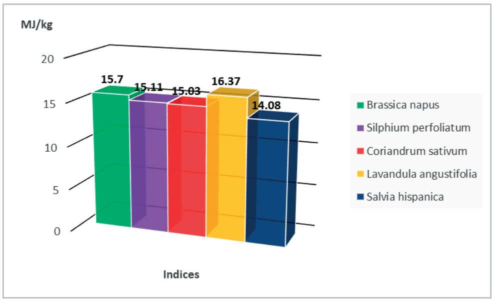

Figure 5. Net calorific value of pellets

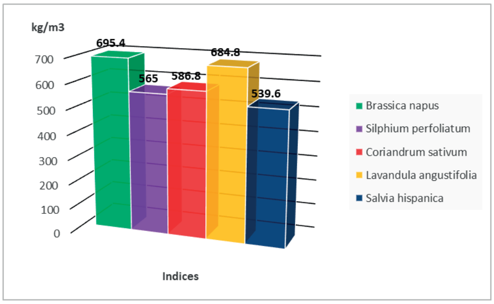

Figure 6. Bulk density of pellets

The pellets produced from *Lavandula angustifolia* biomass were characterized by a high net calorific value, as well as superior bulk density and mechanical durability. In contrast, the net calorific value of pellets made from *Salvia hispanica* and *Coriandrum sativum* was lower than that of pellets from *Brassica napus*. The net calorific value of *Coriandrum sativum* pellets did not differ significantly from that of pellets made from *Silphium perfoliatum*. However, the bulk density and mechanical durability of *Coriandrum sativum* pellets were higher than those from *Silphium perfoliatum*.

Pellets derived from *Salvia hispanica* biomass demonstrated greater mechanical durability compared to those from *Coriandrum sativum* and *Silphium perfoliatum*.

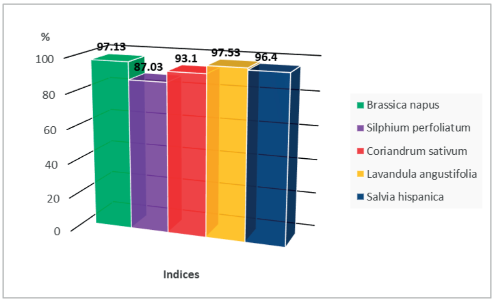

Figure 7. Mechanical durability of pellets

Differences in the quality indices of biomass and densified biofuels from these species are also reported in the literature. According to Plíštil et al. (2005), coriander biomass briquettes had a bulk density of 800-900 kg/m³, a destruction force of 30-50 N/mm, and a compaction pressure of 32-40 MPa, while rapeseed straw briquettes had a bulk density of 800-860 kg/m³, destruction force of 24-40 N/mm, and compaction pressure of 35-40 MPa. Srivastava et al. (2014) reported that coriander stalk and leaf biomass contained 3.47% ash, 78.00% volatile matter, and 18.48% fixed carbon, with a bulk density of 436 kg/m³. The resulting briquettes had a true density of 1319 kg/m³, a bulk density of 747 kg/m³, and a gross calorific value of 13.70 MJ/kg. According to Maroušek (2013), pellets from rapeseed straw had a calorific value of 15.4 MJ/kg and a specific density of 944 kg/m³. Greenhalf et al. (2012) reported that rapeseed straw contained 48.35% carbon, 5.80% hydrogen, 1.15% nitrogen, and 44.70% oxygen. Lesage-Meessenc et al. (2015) found that distilled lavender and lavandin straw contained 48.1% carbon, 5.8% hydrogen, 1.3% nitrogen, 37.8% oxygen, 0.1% sulfur, 0.2% chlorine, and 6.7% ash. Stolarski et al. (2014) reported that *Silphium perfoliatum* harvested in March contained 47.40% carbon, 5.70% hydrogen, and 0.36% sulfur. Chakyrova & Doseva (2021) mentioned that proxymate composition of lavender stalks biomass was 10.2% moisture content, 7.8% ash, 66.7% volatile matter, 15.3% fixed carbon and elemental composition 48.1 % carbon, 5.8 % hydrogen, 1.3 % nitrogen, 37.8% oxygen, 0.1% sulphur, 19.57 MJ/kg gross calorific value. Li et al. (2022) reported that *Lavandula* distilled straws had 2.93% moisture, 80.89 % volatile matter, 8.55% fixed carbon, 7.63% ash, 50.77% carbon, 6.9% hydrogen, 1.28% nitrogen, 41.05% oxygen and 21.43 MJ/kg HHV. Pulidori et al. (2023) revealed that lavender waste had 45.4% carbon, 6.77% hydrogen, 1.79% nitrogen, 45.70% oxygen, 0.112% sulphur, 6.30% ash, 19.20 MJ/kg gross calorific value; rosemary waste - 51.66% carbon, 6. 90% hydrogen, 1.19% nitrogen, 39.2% oxygen, 0.09% sulphur, 3.04% ash, 22.0 MJ/kg gross calorific value; thyme waste 42.55% carbon, 6.66% hydrogen, 1.66 % nitrogen, 46.7 % oxygen, 0.15% sulphur, 4.6% ash, 17.80 MJ/kg gross calorific value, *Artemisia vulgaris* waste contained 44.7% carbon, 6.90% hydrogen, 2.4% nitrogen, 41.8 % oxygen, 0.20% sulphur, 5.8% ash, 19.30 MJ/kg gross calorific value, *Ruta chalepensis* waste included 42.8% carbon, 6.71% hydrogen, 1.84% nitrogen, 45.0% oxygen, 0.15% sulphur, 6.7% ash, and 18.00 MJ/kg gross calorific value.

The production of biogas through anaerobic digestion (biomethanation) of phytomass is gaining increasing importance in the context of renewable energy generation. It is considered both socio-economically cost-effective and environmentally beneficial, primarily due to its potential to reduce greenhouse gas emissions. Phytomass feedstock can be used as a sole substrate for anaerobic digestion or co-digested with two or more additional substrates. Biogas generators produce not only methane for heat and electricity, but also digestate and fugate, which are considered valuable fertilizers in organic farming systems. The use of phytomass for biogas production plays a crucial role in replacing limited fossil energy sources, supporting the transition from a fossil-based to a bio-based economy.

The results concerning the quality indices of biomass substrates from the studied species and their biochemical methane potential are presented in Table 3. In the substrate derived from *Salvia hispanica*, the carbon-to-nitrogen (C/N) ratio was 32.8, the ADL content was 72 g/kg, and the hemicellulose content was 197 g/kg. The biochemical methane potential reached 280 L/kg of organic dry matter, which was significantly higher as compared to *Brassica napus* subsp. *oleifera* and *Silphium perfoliatum*. It was found that the substrates from *Lavandula angustifolia* and *Coriandrum sativum* contained higher levels of acid detergent lignin (117-135 g/kg), which contributed to a lower biochemical methane potential (173-199 L/kg ODM) as compared to the other substrates studied.

Several publications have documented the biochemical composition and biomethane production potential of substrates from the studied species. Gunaseelan (2004) reported that the biochemical methane potential of waste feedstocks from coriander leaves was 0.325 m^3/t DM, while that from coriander stems was 0.309 m^3/t DM. Fardad et al. (2012) found that a substrate from lavender waste contained 48.56% carbon, 1.44% nitrogen, with a C/N ratio of 37.72 and a methane content of 57%. Sahu et al. (2012) observed that the biogas production potential of kitchen waste and sludge in the presence of 50 mg/L coriander waste was 213 L/kg VS, compared to 340 L/kg for kitchen waste alone and 119 L/kg for sludge alone. Ababii et al. (2023) reported that fresh and ensiled biomass substrates of *Salvia hispanica*, based on their C/N ratios (29.2-36.8), acid detergent lignin content (62-72 g/kg) and hemicellulose content (166-199 g/kg), met established standards for anaerobic digestion. The biochemical methane potential of these substrates ranged from 285 to 298 L/kg ODM. According to Sibaoueih et al. (2025), coriander straw contained 898.8 g/kg DM, 6.94% ash, 4.90% CP, 43.39% CF, 73.62% NDF, 53.14% ADF and 8.52% ADL.

Table 2. Biochemical biomethane production potential of substrates from the studied species

Indices	*Coriandrum sativum*	*Salvia hispanica*	*Lavandula angustifolia*	*Brassica napus*	*Silphium perfoliatum*
Organic dry matter, g/kg	954.00	950.20	962.60	941.50	957.60
Minerals, g/kg DM	46.00	49.80	37.40	58.50	42.40
Crude protein, g/kg DM	26.25	78.75	27.50	57.50	13.75
Nitrogen, g/kg DM	4.20	12.60	4.40	9.20	2.20
Carbon, g/kg DM	443.50	431.30	510.30	456.00	522.00
Ratio carbon/nitrogen	106.00	32.80	116.00	49.57	237.20
Cellulose, g/kg DM	466.00	357.00	504.00	401.00	522.00
Hemicellulose, g/kg DM	248.00	197.00	230.00	191.00	248.00
Acid detergent lignin, g/kg DM	117.00	72.00	135.00	83.00	110.00
Biomethane potential, L/kg ODM	199.13	280.00	172.65	263.37	210.90
Biomethane potential, L/kg DM	190.00	266.63	166.19	247.96	201.96

Table 3. Cell wall composition and theoretical ethanol potential yield of the studied species

Indices	*Brassica napus*	*Silphium perfoliatum*	*Coriandrum sativum*	*Lavandula angustifolia*	*Salvia hispanica*
Acid detergent fibre, g/kg DM	484.00	632.00	583.00	639.00	429.00
Neutral detergent fibre, g/kg DM	675.00	880.00	831.00	869.00	626.00
Acid detergent lignin, g/kg DM	83.00	110.00	117.00	135.00	72.00
Cellulose, g/kg DM	401.00	522.00	466.00	504.00	357.00
Hemicellulose, g/kg DM	191.00	248.00	248.00	230.00	197.00
Theoretical ethanol potential:					
from hexose sugars, L/t ODM	298.62	388.69	348.33	374.80	267.21
from pentose sugars, L/t ODM	131.00	170.11	170.11	157.91	135.13

The bioethanol produced from lignocellulosic biomass is currently promoted as an alternative transportation fuel, because of its antiknock properties, which help increasing octane ratings and improve fuel efficiency. The bioethanol yields are influenced by tissue composition, ratios of cellulose, hemicellulose and lignin.

The composition of cell wall dry matter substrates from the studied species and their theoretical ethanol yields are presented in Table 3 and Figures 7-13. We found that the concentration of structural carbohydrates in substrates from *Lavandula angustifolia* biomass do not differ considerably as compared with control *Silphium perfoliatum* biomass. The substrate from *Coriandrum sativum* stalk biomass showed lower levels of cellulose, hemicellulose, and lignin than *Silphium perfoliatum* biomass, although these levels were still higher than those observed in *Brassica napus* subsp. *oleifera* and *Salvia hispanica* substrates. Among all species, the lowest average concentration of structural carbohydrates was generally observed in *Salvia hispanica* biomass substrates. This lower concentration, particularly of lignin compared to other energy crop substrates, is likely due to the higher content of soluble carbohydrates. The theoretical ethanol yield from hexose sugars averaged 375 L/t in *Lavandula angustifolia*, 349 L/t in *Coriandrum sativum*, 267 L/t in *Salvia hispanica*, 389 L/t in *Silphium perfoliatum* (control), and 299 L/t in *Brassica napus oleifera* substrates. Theoretical ethanol yields from pentose sugars were lower across all species, with values of 170 L/t for *Coriandrum sativum* and *Silphium perfoliatum*, 158 L/t for *Lavandula angustifolia*, 135 L/t for *Salvia hispanica*, and 131 L/t for *Brassica napus oleifera*.

Several sources in the literature describe the cell wall composition of the studied plant species and their corresponding calculated ethanol yields. Greenhalf et al. (2012) reported that rapeseed straw contained 37.55% cellulose, 31.37% hemicellulose, 21.30% lignin, 3.76% solubles, and 6.02% ash. Srivastava et al. (2014) stated that the biomass of coriander stalks and leaves contained 15.93% cellulose, 11.38% hemicellulose, and 4.51% lignin. Lesage-Meessen et al. (2015) noted that lavandin branch biomass contained 33.6% cellulose, 13.9% hemicellulose, and 25.4% lignin in winter, and 42.7% cellulose, 13.0% hemicellulose, and 23.1% lignin in summer. Tripathi et al. (2017) reported that coriander straw contained 7.43% crude protein, 73.18% NDF, 56.54% ADF, 48.0% cellulose, 8.77% lignin, and 6.64 MJ/kg metabolizable energy. Lesage-Meessen et al. (2018) found that lavandin distilled straw contained 6.85% moisture, 25.64% acid-insoluble lignin, 29.81% hemicelluloses and pectins, 0.36% rhamnose, 1.80% arabinose, 14.20% xylose, 1.06% mannose, 1.32% galactose, 14.51% glucose, 6.58% galacturonic acid, and 16.06% cellulose. In comparison, lavender distilled straw had 7.15% moisture, 24.99% acid-insoluble lignin, 27.82% hemicelluloses and pectins, 0.42% rhamnose, 1.56% arabinose, 13.14% xylose, 1.15% mannose, 1.32% galactose, 3.16% glucose, 7.05% galacturonic acid, and 17.49% cellulose. Ferdous et al. (2020) reported that chia stalk contained 23.2% lignin, 2.73% acid-soluble lignin, 60.5% holocellulose, 30.5% alpha-cellulose, 13.22% pentosane, and 2.58% ash. In contrast, lentil stalk contained 23.8% lignin, 3.57% acid-soluble lignin, 59.9% holocellulose, 23.8% alpha-cellulose, 15.20% pentosane, and 6.78% ash. Angelova et al. (2021) revealed that the proximate composition of lavender straw biomass was 38.16% cellulose, 24.48% lignin, 13.79% non-cellulosic polysaccharides, and 6.59% ash. Uitterhaegen et al. (2020, 2021) reported that coriander straw contained 8.9% moisture. In the dry matter, they determined 52.5% cellulose, 21.2% hemicelluloses, 9.8% lignin, 10.4% hot-water extractives, 4.2% minerals, 3.7% proteins and 0.8% lipids. Pulidori et al. (2023) found that the composition of aromatic plant waste was as follows: lavender waste contained 41% cellulose, 23% hemicellulose, and 16% lignin; rosemary - 32% cellulose, 25% hemicellulose and 26% lignin; thyme - 37% cellulose, 23% hemicellulose and 24% lignin; *Artemisia vulgaris* - 43% cellulose, 21% hemicellulose and 17% lignin; and *Ruta chalepensis* - 35% cellulose, 24% hemicellulose and 22% lignin. Tóth Š. (2023) reported that the lignocellulosic composition of *Silphium perfoliatum* green phytomass was as follows: 31.32-48.94% ADF, 34.94-54.69% NDF, 7.21-12.54% ADL, 24.11-37.30% cellulose and 2.33-5.75% hemicellulose.

CONCLUSIONS

Coriandrum sativum, *Salvia hispanica*, and *Lavandula angustifolia* are characterized by an extended flowering period, providing forage for bees from late May to mid-August, with honey potential ranging from 60 to 330 kg/ha.

The dry biomass of *Lavandula angustifolia* is notable for its very high carbon content, low sulphur content, optimal ash levels, and high energy value, compared to the biomass of *Salvia hispanica* and *Coriandrum sativum*. Pellets produced from *Lavandula angustifolia* biomass exhibited a high net calorific value, along with superior bulk density and mechanical durability.

The biochemical methane potential of substrate derived from *Salvia hispanica* reached 280 L/kg, significantly higher than that of the other substrates studied. The theoretical ethanol yield from hexose and pentose sugars averaged 533 L/t in *Lavandula angustifolia*, 518 L/t in *Coriandrum sativum*, and 398 L/t in *Salvia hispanica*.

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CARABID BEETLES AS ENTOMOPATHOGENIC VECTORS: A REVIEW OF THEIR ECOLOGICAL ROLE AND POTENTIAL APPLICATIONS

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Abstract

As predators of phytophagous insects and potential vectors of pathogens, ground beetles (Carabidae) are vital in agroecosystems. In this work, their interaction with entomopathogenic fungi and bacteria is presented to highlight the dual role of ground beetles as biocontrol agents and pathogen vectors. This includes understanding how disease propagation and dispersion in multitrophic complexes interact with parasitism, predation, and competition. Integrated pest management (IPM) strategies that consider the potential of ground beetles are evaluated in terms of their ability to enhance biological control methods. The current state of knowledge regarding the long-term ecological consequences and the function of these strategies as vectors of organisms, however, limits their scope. This work underscores the necessity of understanding multitrophic interactions to inform the integration of ground beetles into pest control strategies.

Key words*: Carabidae, biological control, entomopathogens, multitrophic, IPM.*

INTRODUCTION

Ground beetles (Coleoptera: Carabidae) play a fundamental role in agroecosystems, acting as biological control agents of herbivore populations, facilitating nutrient recycling, and contributing to the overall ecological stability of agricultural environments (Lundgren & McCravy, 2011; Marrec et al., 2020).
As noted by Holland (2002), these insects are among the most frequently studied entomofauna groups due to their remarkable taxonomic and ecological diversity. Additionally, their interactions with other species allow them to influence ecosystem structure and function in meaningful ways.
The factors driving ground beetle population dynamics are diverse, ranging from environmental conditions such as temperature and humidity to soil properties and food resource availability.
Cividanes (2021) highlights how vegetation structure significantly impacts their distribution, determining both how these beetles spread across landscapes and their feeding behaviors. Changes in habitat composition influence their functional diversity, leading to variations in species distribution and ecological interactions.
Because they respond quickly to environmental fluctuations, ground beetles are widely recognized as reliable indicators of agroecosystem health. A study by Makwela et al. (2023) establishes a direct correlation between beetle biodiversity and farming intensity, demonstrating that modifications in habitat structure and increased agrochemical usage have a measurable impact on these insect communities. As a result, ground beetles are valuable tools for monitoring human-induced disturbances in agricultural systems, offering crucial data on ecosystem stability and resilience.
Entomopathogens are microorganisms, primarily fungi and bacteria, that can infect and cause lethal diseases in insects, playing a crucial role in the biological control of agricultural pests. These agents are widely used in Integrated Pest Management (IPM) due to their high specificity and minimal impact on non-target organisms (Deka et al.,2021).
According to Khan & Ahmad (2019), entomopathogens represent a fundamental component of biological control strategies, offering high host specificity, low impact on non-target species, and contributing to the ecological balance within agroecosystems. These microorganisms are commonly found in

soil and other natural habitats, acting by infecting insect hosts either through the cuticle or via ingestion. Their infections often lead to epizootic outbreaks that significantly reduce pest populations.

Entomopathogenic fungi, such as *Beauveria bassiana* (Bals-Criv.) Vuill. and *Metarhizium anisopliae* (Metschn.) Sorokīn penetrate the insect cuticle, triggering a systemic and lethal infection. After the host dies, the fungi proliferate on the dead insect, releasing spores that contribute to the regulation of pest populations (Abbas et al., 2020; Ebani & Mancianti, 2021).

Entomopathogenic bacteria, such as *Bacillus thuringiensis* (Berliner, 1915) (Bt), produce specific toxins that disrupt the insect's intestinal epithelium, causing paralysis and eventual death. Unlike fungal entomopathogens, bacterial pathogens must be ingested by the host to be effective (Ebani & Mancianti, 2021).

By utilizing entomopathogens, dependence on chemical pesticides can be reduced, promoting sustainable agriculture while preserving biodiversity.

MATERIALS AND METHODS

This review aims to examine the role of carabid beetles in agroecosystems, emphasizing their dual function as both natural predators of pests and potential vectors of entomopathogens. Additionally, it explores the multitrophic interactions that shape their effectiveness in biological control. By synthesizing current research, the review assesses ecological and abiotic factors that influence carabid populations, compares their biocontrol efficiency with other methods, and identifies both the opportunities and challenges of integrating them into pest management strategies.

The study draws on peer-reviewed literature, scientific research, and other relevant sources published in English from the 1990s onward.

Through this approach, the review provides an integrated perspective on the contribution of carabid beetles to modern Integrated Pest Management (IPM) strategies, highlighting their advantages as well as the constraints they face in the context of agroecological and climatic shifts.

RESULTS AND DISCUSSION

Ecology and Diversity of Carabids

Carabids constitute one of the most diverse beetle families, comprising over 40,000 described species distributed across approximately 86 tribes (Lövei & Sunderland, 1996). Their remarkable diversity is reflected in a broad range of ecological adaptations, including active predators, phytophagous species, and opportunistic feeders. Taxonomic classification within this group is primarily based on distinct morphological features, such as antenna and leg structures, along with the presence of pygidial glands responsible for secreting defensive chemical compounds (Kotze et al., 2011). Advances in molecular and phylogenetic studies have further clarified evolutionary relationships, reinforcing the stability of the Carabinae subfamily, which consists mostly of large, ground-dwelling species. In contrast, members of Trechinae and Harpalinae exhibit a greater degree of ecological diversity (Kotze et al., 2011).

These beetles inhabit every continent except extreme desert regions. The highest species richness is found in tropical areas, yet research in these ecosystems remains relatively scarce, as most studies have focused on populations in the Northern Hemisphere (Avgın & Luff, 2010).

In Europe, *Carabus auronitens* (Fabricius, 1792) is frequently encountered in temperate forests, while in North America, *Pterostichus melanarius* (Illiger, 1798) is one of the most widespread species in agricultural environments (Niemelä, 2001). In colder regions, species such as *Nebria* spp. have adapted to harsh climatic conditions by reducing metabolic activity and developing resistance to low temperatures (Kotze et al., 2011).

Populations in temperate ecosystems display exceptional ecological flexibility, allowing them to thrive in a wide variety of habitats, from cultivated lands to mountain forests and wetlands. Studies on habitat fragmentation suggest that landscape changes significantly influence species distribution. While some are negatively impacted by habitat loss, others, particularly generalists, may benefit from environmental alterations (Niemelä, 2001; Thomas et al., 2002).

Ecological Factors Influencing Carabid Populations

Carabid populations are shaped by a range of ecological factors that influence their distribution, abundance, and ecological roles within agroecosystems.

Abiotic elements such as temperature, humidity, and habitat structure, alongside biotic interactions with other organisms, contribute to population dynamics and determine the success of these species across different environments (Murdoch, 1966).

The intensification of agricultural practices has led to substantial habitat alterations, impacting both species diversity and community composition. Research suggests that intensively managed farmland experiences a decline in larger carabid species, while opportunistic ones, better adapted to environmental disturbances, tend to thrive (Cole et al., 2002). In contrast, semi-natural areas and undisturbed habitats support higher biodiversity, providing essential resources for survival and reproduction (Gill & Garg, 2014).

Anthropogenic landscape modifications, including deforestation, urban expansion, and agricultural land conversion, reduce habitat connectivity, affecting the dispersal of carabid species.

Studies indicate that generalist species are more capable of persisting in fragmented environments, whereas specialists often experience significant population declines (Niemelä, 2001).

These shifts have direct consequences on the stability of carabid communities and their ability to contribute to biological control within agroecosystems (Murdoch, 1966).

Climatic variations further influence the life cycles and behavior of carabids, altering reproductive rates and feeding activity.

Research shows that higher temperatures enhance the activity of predatory species, while humidity plays a crucial role in sustaining populations in arid regions (Murdoch, 1966).

Additionally, climate change may lead to range expansions for certain species, potentially disrupting trophic structures and ecological balance in newly colonized areas (Koivula, 2011) (Figure 1).

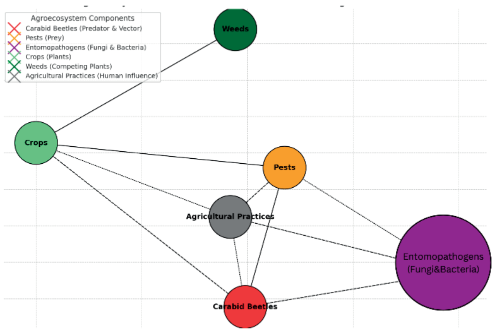

Figure 1. Ecological network of an agroecosystem highlighting carabid beetles as predators and vectors of entomopathogens. Solid lines represent direct trophic interactions, while dashed lines indicate indirect effects. Carabid beetles regulate pest populations through predation and by vectoring entomopathogenic fungi and bacteria. Agricultural practices influence these interactions by altering vegetation structure, pest dynamics, and carabid behavior. Integrating biological control strategies can enhance ecosystem stability and reduce reliance on chemical inputs
Source: Adapted from De Heij, S. E., & Willenborg, C. J. (2020)

Multitrophic Interactions and the Role of Carabids in Agroecosystems

In agroecosystems, multitrophic interactions shape the complex relationships between predators, parasites, pathogens, and herbivores, directly influencing the structure and stability of food webs (Ivanković Tatalović, 2023). Ground beetles play a crucial role in these interactions, impacting pest population dynamics and enhancing biological control efficiency through their diverse ecological relationships (Ivanković Tatalović, 2023; De Heij & Willenborg, 2020).

As polyphagous predators, these beetles feed on a wide range of prey, including herbivorous insects, other predatory species, and even entomopathogens (Kamenova et al., 2017). Research suggests that carabid communities can be divided into two trophic groups: some species specialize in resources associated with cultivated plants, while others have a more flexible diet that adjusts to seasonal fluctuations in food availability (Kamenova et al., 2017). This dietary variation influences pest control efficiency depending on agricultural management practices and resource availability (Gill & Garg, 2014).

Carabids contribute to the natural regulation of pest populations, helping to maintain ecological balance and reducing the reliance on chemical pesticides (Winder et al., 2001). For instance, *Pterostichus melanarius* is known for its ability to significantly lower populations of aphids and lepidopteran larvae in cereal crops (Symondson et al., 2006). Moreover, studies emphasize that the effectiveness of these beetles as biocontrol agents is closely linked to the complexity of the food web and habitat characteristics (Gill & Garg, 2014; De Heij & Willenborg, 2020).

A key aspect of multitrophic interactions is the relationship between carabids and entomopathogens. These predators can act as vectors, aiding in the spread of fungal spores to susceptible hosts, but they can also reduce the effectiveness of entomopathogens by preying on infected insects before spores are released (Roy & Pell, 2000). Additionally, the presence of alternative prey may alter the efficiency of carabids as biocontrol agents, as they tend to diversify their diet and prioritize more accessible food sources, leading to seasonal variations in pest suppression (Symondson et al., 2006; De Heij & Willenborg, 2020).

The outcomes of these interactions are strongly influenced by agroecosystem conditions and agricultural practices. Pesticide applications, for example, can negatively impact both carabids and entomopathogens, disrupting natural food webs and reducing biocontrol efficiency (Ivanković Tatalović, 2023). On the other hand, habitat management strategies such as ecological field margins and uncultivated areas help support stable beetle populations and enhance their role in pest suppression (De Heij & Willenborg, 2020; Winder et al., 2001). In this context, conserving carabid diversity in agricultural landscapes emerges as a key strategy for maintaining ecosystem services and promoting sustainable farming practices.

Carabids as Predatory Insects and Biological Control Agents

Carabids are widely recognized as effective predators that contribute to the regulation of herbivorous insect populations in agricultural environments. Their role in pest suppression makes them essential components of sustainable farming systems.

Many species within this group exhibit polyphagous feeding behavior, preying on a broad range of agricultural pests, including aphids, lepidopteran larvae, and coleopteran eggs. Research indicates that larger species such as *Carabus nemoralis* (O.F.Müller, 1764) and *Carabus granulatus* (Linnaeus, 1758) are particularly effective in controlling slugs and harmful beetle larvae (SaccoKromp, 1999; Sacco-Martret de Préville et al., 2024).

Table1. Carabid Species and Their Contribution to Biological Pest Control in Agroecosystems. Source: Kromp, 1999; Lang et al., 1999; Symondson et al., 2002; Hanson et al., 2016

Species	Description and comments	Selected references
Pterostichus melanarius	Commonly found in agricultural fields, has been extensively studied for its effectiveness in biological control, playing a crucial role in reducing aphid populations and other pests in wheat crops	Hanson et al., 2016
Harpalus rufipes	A key species in biological control, capable of reducing both harmful insects and weeds. Research indicates that it feeds on invasive plant seeds, helping to limit their spread in agricultural fields.	Kromp, 1999
Poecilus cupreus	An active predator in corn and soybean fields, playing a crucial role in regulating caterpillar and lepidopteran larval populations. Studies confirm its significant impact on pest control in cereal crops.	Lang et al., 1999
Carabus nemoralis	A large-sized species, highly effective in preying on slugs and harmful larvae in horticultural crops. Commonly found in orchards and gardens, it plays a vital role in natural biological control.	Symondson et al., 2002

In wheat and maize fields, *Pterostichus melanarius* (Illiger, 1798) and *Poecilus cupreus* (Linnaeus, 1758) are among the most prevalent predators targeting aphids and caterpillars. Experimental studies have demonstrated that these species significantly reduce populations of Cicadellidae and Thysanoptera, thereby mitigating crop damage caused by these pests (Lang et al., 1999) (Table 1).

Maintaining a diverse habitat has been shown to support stable carabid communities, enhancing their effectiveness in biological control. According to Hanson et al. (2016), variations in agricultural land use influence the distribution and abundance of these beetles, which can directly impact their ability to suppress pest populations.

Predation Mechanisms and Impact on Pest Populations

Ground beetles are opportunistic predators that employ various hunting strategies depending on habitat conditions, resource availability, and prey characteristics. Research indicates that larger species, such as *Carabus auratus* (Linnaeus, 1761) and *Pterostichus melanarius*, can effectively control pest populations in agroecosystems by preying on larger insects (Rouabah et al., 2013).

Carabids can be classified based on their body size and feeding preferences into surface-active predators, soil hunters, and specialists in seed or larval consumption. For instance, *Poecilus cupreus* and *Harpalus rufipes* (Degeer, 1774) are ecologically adaptable species with a significant impact on aphid and lepidopteran larvae populations (Williams et al., 2010). By consuming eggs, larvae, and adult insects, these beetles play a key role in natural pest suppression. Studies have demonstrated that large predatory species, including *Pterostichus melanarius*, can substantially reduce aphid and caterpillar numbers, providing an effective form of biological control (Hummel et al., 2012).

An important factor influencing predation efficiency is the relationship between beetle size and prey size. Rouabah et al. (2013) found that larger species are capable of consuming a broader range of pests, including sizable beetle larvae and caterpillars, which contributes to a significant decline in these pest populations. Predator-prey interactions are not solely determined by beetle abundance but are also influenced by competition among different predator groups. Studies in organic farming systems suggest that carabids compete for food resources with other natural enemies, such as predatory spiders and hemipterans (Kromp, 1989).

Additionally, intra-guild competition among carabids can affect their efficiency as biological control agents. Experimental research has shown that the presence of dominant and highly aggressive species, such as *Carabus auratus*, may lead smaller species like *Bembidion lampros* (Herbst, 1784) to alter their foraging strategies and reduce feeding activity to avoid direct encounters (Williams et al., 2010).

The functional diversity of carabids plays a crucial role in enhancing biological control efficiency. Experimental studies suggest that the coexistence of multiple species with varying sizes can improve predation success due to trophic complementarity, where different species target various pest developmental stages or trophic levels (Rouabah et al., 2013). Maintaining high carabid diversity in agroecosystems is essential for effective pest control and reducing dependence on chemical pesticides. Sustainable agricultural practices, such as maintaining vegetated field margins and implementing crop rotation, can help maximize the benefits provided by these insects in pest suppression (Hummel et al., 2012).

Comparative Efficiency of Carabids Versus

For a long time, conventional agricultural systems have relied heavily on insecticides to manage pest populations. However, research indicates that the presence of carabids in organic farming and no-till systems can effectively suppress pests without intensive chemical use, achieving agronomic results comparable to traditional pesticide applications (Prasifka et al., 2007; Koss et al., 2005). Their predatory activity contributes to reducing weed seeds and harmful insect populations, reinforcing the sustainability of biological control approaches (Prasifka et al., 2007).

The widespread use of broad-spectrum insecticides significantly affects the abundance and diversity of carabids and other natural predators, which may lead to secondary outbreaks of resistant herbivorous insect species (Kennedy et al., 2001). Additionally, exposure to chemical treatments such as pyrethroids initially increases predatory activity but eventually results in population declines due to high mortality rates and sublethal effects, including reduced reproductive success and diminished biocontrol efficiency (Prasifka et al., 2007; Koss et al., 2005).

A comparative assessment of fields treated with selective insecticides, broad-spectrum pesticides, and organic biocontrol strategies found that areas managed using selective insecticides and organic methods supported higher densities of carabids and other beneficial predators. This increase in natural enemy populations led to more effective pest suppression than in fields exposed to broad-spectrum chemicals (Koss et al., 2005). Consequently, integrating carabids into pest management strategies offers significant benefits, including lower pest densities, enhanced biodiversity conservation, and reduced reliance on synthetic insecticides. Long-term sustainability can be achieved through habitat management and natural predator conservation, providing efficient pest control while preserving soil health and agroecosystem biodiversity (Prasad & Snyder, 2004).

Ground beetles as Vectors of Entomopathogens

Carabid beetles host diverse bacterial communities that influence their physiology and ecological role. Research has identified specific bacterial strains within the digestive tracts of species such as Harpalus pensylvanicus and Anisodactylus sanctaecrucis. These microorganisms, including Serratia, Burkholderia, Hafnia, Phenylbacterium, Caedibacter, Spiroplasma, Enterobacter, and Weissella, contribute to nutrient digestion and trophic interactions within agroecosystems (Lundgren et al., 2007).

One notable entomopathogenic bacterium associated with carabids is Photorhabdus luminescens (Thomas & Poinar, 1979; Boemare et al., 1993), a symbiotic microorganism of entomopathogenic nematodes. This bacterium exhibits strong insecticidal properties, targeting key agricultural pests, and may indirectly influence carabid predation activity in farming systems (Muhammad et al., 2022). Entomopathogenic fungi also play a crucial role in regulating insect populations, with several species identified in association with carabids. *Beauveria bassiana* is among the most commonly reported fungal pathogens affecting these beetles, having been isolated from species such as *Bembidion lampros* and *Agonum dorsale* (Pontoppidan, 1763). While adult beetles show lower infection rates, larvae tend to be more susceptible (Riedel & Steenberg, 1998). Another relevant fungal pathogen, *Metarhizium anisopliae*, has been detected in agricultural soils and in carabids collected from pesticide-free fields. Frequently used in biological pest control, this fungus can be passively transported by carabids, potentially facilitating its spread to other insect species within agroecosystems (Steenberg et al., 1995).

Further studies have also recorded the presence of other fungal pathogens, such as *Paecilomyces farinosus* (Holmsk.; A.H.S.Br. & G.Sm.) and L*ecanicillium lecanii (*Zimm.; Zare & W. Gams, 2001). While their direct impact on carabid populations remains less understood, these fungi are known to affect agricultural pests and may contribute to ecological pest regulation (Steenberg et al., 1995).

A comprehensive review of carabid-fungus interactions, analyzing 200 years of literature, identified 3,378 unique associations between 1,776 carabid species and 676 fungal taxa. The findings suggest that most interactions involve ectoparasitic fungi from the order Laboulbeniales, whereas entomopathogenic fungi such as *Beauveria* and *Metarhizium* are less frequently recorded (Pozsgai et al., 2021).

Mechanisms of Entomopathogen Dispersal

Carabid beetles contribute significantly to the dissemination of entomopathogens by passively carrying fungal spores (*Beauveria bassiana*, *Metarhizium anisopliae*) and bacterial cells on their body surfaces, particularly on the cuticle and locomotory appendages. These spores can be transferred to other insects or soil substrates, facilitating pathogen spread within the agroecosystem (Steenberg et al., 1995; Meyling & Hajek, 2009).

Field studies indicate that fungal spores adhering to carabid cuticles can remain viable for extended periods, and their contact with other insects may lead to infection, exerting continuous pressure on pest populations (Meyling & Hajek, 2009) (Figure 2).

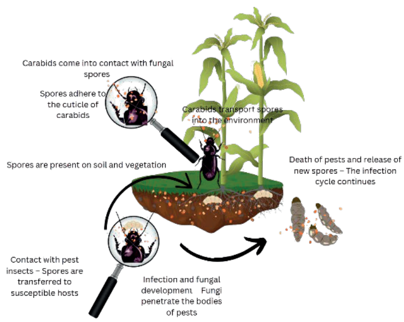

Figure 2. Carabid Beetles as Predators and Entomopathogen Vectors in Agroecosystems. Source: Adapted from Steenberg et al. (1995) and Meyling & Hajek (2009), own elaboration

Another significant pathway for entomopathogen spread is through the ingestion of infected prey. Carabid beetles can consume diseased insects and later excrete viable spores, facilitating pathogen dispersal within the agroecosystem (Wang-Peng et al., 2018). For instance, the consumption of prey infected with fungi such as *Beauveria brongniartii* (Sacc.; Petch, 1926) contributes to its propagation, as spores are expelled into the environment, a process documented in *Poecilus* species (Traugott et al., 2005).

Additionally, carabids can serve as intermediate hosts for entomopathogenic nematodes (*Steinernema*, *Heterorhabditis*), acting as vectors by coming into direct contact with contaminated soil or by consuming infected prey (Labaude & Griffin, 2018). Research indicates that certain nematodes have developed adaptive mechanisms that allow them to survive passage through the digestive tract of carabids, eventually being excreted into the soil, where they can seek out and infect new hosts (Jones et al., 2015).

Factors Influencing the Vector Efficiency of Carabids

The effectiveness of carabid beetles as vectors of entomopathogens is shaped by abiotic conditions such as temperature, humidity, and soil composition. Extreme temperatures and low moisture levels can negatively impact both fungal spore viability and the activity of entomopathogenic nematodes, thereby reducing the role of carabids in pathogen dissemination (Matuska-Łyżwa et al., 2024). Additionally, sandy soils enhance beetle mobility but decrease fungal spore persistence, whereas clay-rich soils retain moisture, creating favorable conditions for pathogen survival (Kamata, 2000; Tscharntke et al., 2007). Population density is another critical factor influencing vector efficiency, as a higher number of individuals increases the likelihood of encounters with infected insects and contributes to pathogen spread within the agroecosystem (Rosenheim, 1995). However, not all carabid species demonstrate equal efficiency in this process. For instance, *Poecilus cupreus* and *Harpalus rufipes* are recognized as effective vectors due to their high mobility and feeding behavior (Cividanes, 2021). The presence of other predators in agroecosystems can also affect the role of carabids in entomopathogen transmission. Intraguild predation, where carabids compete with other natural enemies for resources, may alter feeding strategies and influence the efficiency of pathogen dispersal (Kamata, 2000). Furthermore, habitat fragmentation impacts carabid distribution and their effectiveness in biological control, with more diverse agricultural landscapes supporting stable populations and enhancing their potential as pathogen vectors (Tscharntke et al., 2007).

Persistence and ecological impact of entomopathogens in carabid beetles

Entomopathogenic fungal spores can remain viable in the soil for extended periods, exerting continuous infection pressure on pest populations. Carabid beetles play a role in the redistribution of these spores, transporting them across considerable distances during their nocturnal activity, which enhances their ecological spread within agroecosystems (Meyling & Hajek, 2009).

Although most entomopathogens have minimal impact on adult carabids, some research suggests that prolonged exposure to fungi such as *Beauveria bassiana* may lead to decreased mobility and survival rates in certain species. These effects could alter population dynamics and potentially reduce their effectiveness as biological control agents (Steenberg et al., 1995).

Agricultural Practices and Their Influence on Carabid Vector Efficiency

The application of pesticides can significantly impact the ability of carabid beetles to transport and spread entomopathogens. Recent studies indicate that certain insecticides not only reduce carabid diversity but may also disrupt the transmission of entomopathogenic fungi by altering beetle behavior (Menalled et al., 2007; Matuska-Łyżwa et al., 2024).

Effects of Conservation Practices on Carabid-Mediated Pathogen Dispersal

Agricultural practices aimed at biodiversity conservation, such as implementing ecological field margins and crop rotation, can enhance the efficiency of carabids as vectors of entomopathogens. Cividanes (2021) highlights that agricultural landscapes incorporating natural habitats help sustain stable carabid populations, ultimately improving their role in pathogen dissemination.

Integrating Carabid Beetles into Integrated Pest Management (IPM)

Research highlights that sustaining carabid beetle populations in agricultural landscapes requires targeted strategies, such as ecological field margins and habitat management (Jowett et al., 2022).

A crucial factor in successfully incorporating carabids into IPM is the establishment of suitable habitats, including field borders and ecological corridors, which can significantly enhance their biocontrol efficiency. Studies indicate that these conservation measures lead to increased carabid density and diversity, contributing to a reduction in pest populations (Ameixa & Kindlmann, 2008).

Adopting an IPM approach that integrates carabid beetles can minimize reliance on insecticides and help prevent the development of pesticide resistance in pest species. Proper habitat management has been shown to improve the effectiveness of these beetles in controlling agricultural pests, particularly in cereal and vegetable crops (Labrie et al., 2003).

Certain agricultural practices for crop production and pest management can support beneficial organisms in maize fields. Research shows that conserving crop residue and reducing tillage enhance the survival of ground-dwelling predators, such as ground beetles and spiders, which naturally control maize pests (Chiriloaie-Palade et al., 2024).

Farmer perception of carabid benefits is another key factor influencing the success of IPM. Research suggests that farmers who recognize the ecological role of these beetles are more likely to adopt conservation-friendly practices, such as reduced tillage and the implementation of ecological field margins (Jowett et al., 2022).

The effectiveness of carabids as biocontrol agents varies depending on agricultural practices. For instance, the application of broad-spectrum pesticides can negatively impact their populations, diminishing their pest suppression capabilities. Conversely, IPM strategies that prioritize habitat conservation can enhance agricultural sustainability by maintaining robust carabid communities (Legrand et al., 2011).

Incorporating carabid beetles into IPM presents a viable approach to reducing pesticide dependence while maintaining ecological balance in agroecosystems. Conservation efforts and farmer engagement in biodiversity-friendly practices can position carabids as a key component of sustainable pest control strategies (Warner et al., 2000).

Challenges and Future Perspectives

Gaps in Current Knowledge

Although numerous studies have highlighted the role of carabid beetles in biological control, significant knowledge gaps remain regarding the specific mechanisms through which they influence pest population dynamics. Macfadyen et al. (2019) emphasize that there is a lack of direct studies correlating carabid abundance with actual reductions in pest densities, making it challenging to integrate them into evidence-based Integrated Pest Management (IPM) programs. Additionally, the extent to which ecological factors affect their efficiency as biocontrol agents is not yet fully quantified (Holland & Luff, 2000).

Another underexplored aspect is the relationship between carabids and entomopathogens. While interactions between these organisms may play a crucial role in spreading entomopathogenic diseases, research on the specific transmission mechanisms and their impact on pest suppression remains limited (Jowett et al., 2022). Furthermore, the incomplete taxonomic classification of certain carabid species in agroecosystems complicates efforts to determine the precise role of individual species in pest regulation (Macfadyen et al., 2019).

Long-Term Ecological Impact and Future Research Directions

Shifts in agricultural practices, such as intensive monocropping and widespread pesticide application, have negatively affected carabid beetle abundance and diversity, diminishing their effectiveness as natural pest regulators. The conversion of natural habitats into intensively farmed land has led to the loss of essential ecological refuges needed for maintaining stable beetle populations (Holland & Luff, 2000). Furthermore, climate change is altering carabid distribution and predatory efficiency, highlighting the need for Integrated Pest Management (IPM) strategies to adapt to evolving environmental conditions (Macfadyen et al., 2019).

To optimize the role of carabids in IPM, future research should focus on agroecological approaches such as ecological field margins and crop diversification, which have the potential to enhance their pest control efficiency (Jowett et al., 2022). Additionally, developing precise monitoring techniques using advanced technologies, such as DNA analysis of carabid gut contents, could provide insights into prey composition and feeding dynamics (Macfadyen et al., 2019). Long-term studies are also essential to assess the sustainability of carabids as biocontrol agents across different agroecosystems (Holland & Luff, 2000).

CONCLUSIONS

This study examined the complex role of carabid beetles as vectors of entomopathogens, highlighting their ecological importance and potential applications in integrated pest management (IPM). The findings suggest that these beetles not only regulate pest populations through predation but also play a role in spreading entomopathogens, thereby enhancing biological control efficiency. However, their effectiveness is shaped by various factors, including habitat conditions, pesticide exposure, and environmental variables.

To successfully integrate carabids into IPM systems, it is essential to implement strategies that sustain their presence and activity in agricultural landscapes. Agroecological approaches such as preserving semi-natural habitats, minimizing chemical inputs, and fostering biodiversity can contribute to maintaining stable carabid populations and optimizing their role in pest suppression. Effective habitat management is particularly important in maximizing their ecological benefits while reducing dependence on conventional pest control methods.

Further research is needed to deepen the understanding of the interactions between carabids, pests, and entomopathogens. Investigating how these beetles contribute to pathogen transmission could support the development of more targeted and sustainable pest management strategies. Additionally, adapting biocontrol methods to climate change and specific agroecosystem conditions may improve the long-term viability of these approaches.

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THE INFLUENCE OF BIOTIC FACTORS ON THE PRODUCTION AND QUALITY OF SOME NEW WHEAT LINES IN THE 2023-2024 AGRICULTURAL YEAR

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Abstract

Powdery mildew is caused by Blumeria graminis f. sp. tritici and is one of the most important diseases affecting wheat crops. The fungus develops at temperatures of 15-22 °C and appears on all plant organs, but most often on leaves. Given the economic importance of wheat, within the Agricultural Research and Development Station Pitesti, in order to conduct research on improving this species, the response of the studied genotypes to the attack of this pathogen is monitored, among other things. In this paper, we present the results obtained under the conditions of the 2023-2024 agricultural year. The aim of our study was to evaluate the response of five genotypes to the attack of powdery mildew (production level and quality indices, respectively protein and gluten content). The studied material was represented by the Trivale variety, as a control variant, and 4 new winter wheat genotypes created within the wheat breeding laboratory, lines A4-10, A44-13, A95-13 and A57-14. The experimental factors were: genotype, seed treatment and type of fertilization. The results obtained showed that line A4-10 presented better tolerance to powdery mildew.

Key words: *Blumeria graminis, fertilization, genotype, gluten, protein.*

INTRODUCTION

opulation growth also increases the demand for agricultural products, and wheat is one of the most important food species for a significant part of the global population. Agricultural producers face numerous obstacles in achieving agricultural production, and one of the major challenges is the control of fungal diseases (Dodhia et al., 2021). Powdery mildew is one of the main diseases of wheat, present in all cultivation areas and produces quantitative and qualitative losses through grain shrinkage (Cotuna et al., 2015). It is known that agricultural practices, such as monoculture, high plant density, and high atmospheric humidity favor the occurrence of *Blumeria graminis* disease. High levels of atmospheric humidity are an important factor in the occurrence of powdery mildew on wheat plants, a very common phenomenon in spring. In cereals, powdery mildew causes symptoms on leaves, stems and ears (Köhl et al., 2019). *Blumeria graminis* f. sp. *tritici* is a globally important pathogen that can evolve rapidly, destroying wheat resistance genes (Kloppe, 2023). The pathogen's rapid spread and adaptation are amplified by its short life cycle, the ease with which airborne spores can spread over long distances, and the possibility of sexual recombination leading to the generation of new virulent races (Jankovics et al., 2015). Therefore, it is essential to identify new resistance genes and develop new sustainable solutions to prevent the attack of this pathogen on wheat (Ren et al., 2024). Thus, breeding plays a particularly important role in meeting the needs of agricultural producers from this perspective, as the creation of resistant varieties is the most economically and ecologically relevant way to reduce or control powdery mildew attack (Li et al., 2023). The creation of new varieties should not only aim to increase production potential, but also to increase the plasticity of the species towards environmental factors (Vlasenko et al., 2019), especially towards those pathogens that cause significant damage to wheat production and its quality.
The aim of the study that is the subject of this paper was to evaluate the response of five wheat

genotypes compared to a recognized variety in terms of response to powdery mildew attack. The effects on production and quality indices (protein and gluten content) were monitored.

MATERIALS AND METHODS

The study was conducted in one of the experimental fields of the Agricultural Research and Development Station Pitești between September 2023 and June 2024, with winter wheat being cultivated in Romania. The soil is acidic, has a low content of nutrients, being poorly supplied with nitrogen (Nt=0.130% mg/kg), poorly fertile, with a humus content in the arable horizon of 1.96% and a high content of mobile aluminum ions of about 30 ppm, which leads to the blocking of mobile phosphorus. Therefore, it is necessary to periodically apply calcium-based amendments (CaCO3).

To see the influence of environmental factors on plant evolution, temperatures and precipitation were recorded between September 2023 and June 2024. The organization of the experiment was carried out according to the randomized block method, trifactorial type AxBxC in four repetitions. The experimental factors were: factor A - genotype (A1 - Trivale variety - control, A2 - line A4-10, A3 - line A44-10, A4 - line A95-13, A5 - line A57-14), factor B - seed treatment with two graduations, B1 - untreated, B2 - treated, factor C - mineral fertilization with two graduations, C1 - N:120, P:96, K0, C2 - N:160, P: 96, K0. For seed treatment, the insect-fungicide Austral Plus from Syngenta was used. The dose used was 5L/ton of seeds. During the crop's vegetation, the systemic herbicide Axial One from Syngenta was used to combat weeds, 1L/ha. Disease and pest control during vegetation was achieved by performing treatments in two phases: a) at the end of twinning, simultaneously with herbicide application, with the fungicide Verben from Corteva, 1L/ha (Proquinazid 50 g/L and Protioconazole 200 g/L) and the insecticide Mospilan 20 SG (Agro Pataki) 0.1 L/ha (acetamiprid 200g/kg) and b) at the end of flowering with Nativo Pro 325SC from Bayer (protioconazole 175 g/L+trifloxystrobin 150 g/L) 0.8 L/ha. The frequency of attack by powdery mildew on wheat plants was determined with a metric frame (1 m x 1 m). The frequency of attack (F%) represents the ratio between the number of plants or plant organs attacked (n) and the total number of plants or organs analyzed (N), expressed as a percentage, according to the formula: F(%) = (n x 100)/N. Protein content (P%) and gluten content (G%) were determined using the Inframatic IM 9500 device.

Statistical data processing was performed with the PoliFact statistical calculation program (Ivan, 2001).

RESULTS AND DISCUSSIONS

The temperatures recorded in the months of this agricultural year, except for May when a negative deviation of -0.5°C was recorded, had positive thermal deviations, with values ranging between +1.72°C in November and +6.7°C in February (Figure 1). The average thermal deviation of the entire period was +3.27°C, compared to the 40-year multiannual average of 8.54°C.

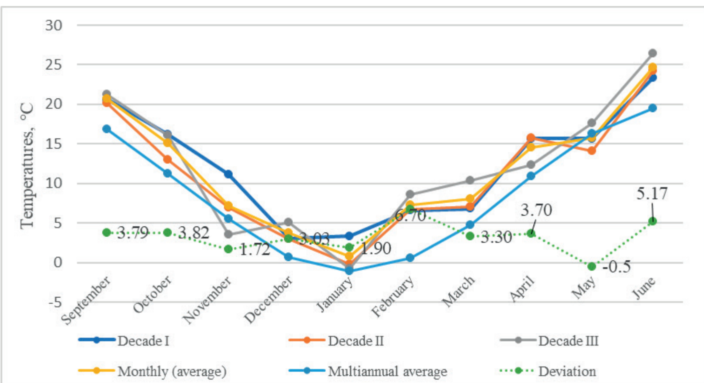

Figure 1. Average monthly temperatures recorded during September 2023-June 2024 at ARDS Pitești

During the analyzed period, a precipitation deficit was recorded in the months of September (-10.3 mm), October (-41.1), December (-46.0 mm), February (-24.4 mm), April (-10.8 mm), May (-65.2 mm) and June (-78.1), the values being below the multiannual average of the mentioned months (Figure 2). In January, there was an excess of 28.5 mm, compared to the multiannual average for this month. During the period in which the study was conducted, September 2023-June 2024, a total of 334.2 mm of precipitation was recorded, so that, compared to the multiannual average for 40 years, which was 542.3 mm, a deficit of -208.1 mm was recorded.

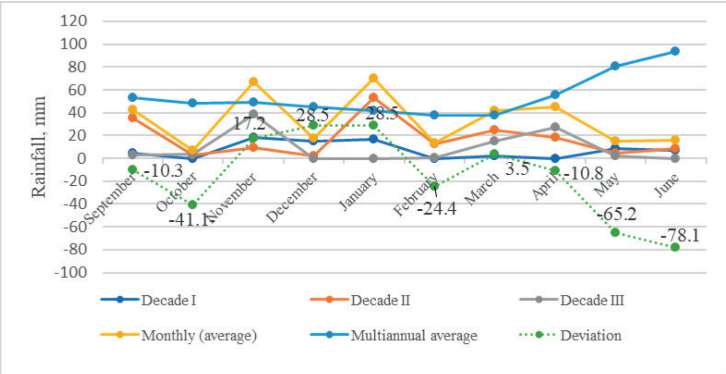

Figure 2. Average monthly rainfall recorded during september 2023-June 2024 at ARDS Piteşti

Regarding the frequency of powdery mildew attack, Table 1 presents the data resulting from the analysis of the interaction of the studied factors, fertilization with 120 kg/ha N a.s. being taken as a control in each case. Thus, in the Trivale variety, the frequency of attack was very significantly positively influenced in the case of fertilization with 160 kg N a.s./ha compared to fertilization with 120 kg N a.s./ha. The wheat genotype A4-10 recorded a significantly positive frequency of powdery mildew attack in the case of fertilization with 160 kg N/ha, compared to the variant in which 120 kg N a.s./ha were applied; in the case of the genotypes A44-13 and A95-13, a very significantly positive frequency of attack is observed in the variants fertilized with 160 kg N a.s./ha, compared to those fertilized with 120 kg N a.s./ha, and the frequency of powdery mildew attack in the case of the genotype A57-14, recorded a distinctly significant positive difference in the variants fertilized with 160 kg N a.s./ha compared to those fertilized with 120 kg N a.s./ha. This highlights that the level of N fertilization increases the sensitivity of plants in general, but some genotypes show a certain tolerance, as is the case for A4-10 and A57-14.

Table 1. Interactions between fertilized variants and genotype on the frequency of powdery mildew (%)

Fertilizer rates	Genotype	F (%)	%	Difference	Significance
120 kg N/ha	Trivale	12.68	100.0	0.00	Mt.
160 kg N/ha		15.91	125.5	3.23	***
120 kg N/ha	A4-10	3.22	100.0	0.00	Mt.
160 kg N/ha		4.26	132.3	1.04	*
120 kg N/ha	A44-13	16.39	100.0	0.00	Mt.
160 kg N/ha		18.18	110.9	1.79	***
120 kg N/ha	A95-13	12.46	100.0	0.00	Mt.
160 kg N/ha		15.77	126.6	3.31	***
120 kg N/ha	A57-14	3.83	100.0	0.00	Mt.
160 kg N/ha		5.48	143.1	1.65	**
LSD (p 5%)				0.94	
LSD (p 1%)				1.26	
LSD (p 0.1%)				1.68	

Data on production and the influence of the interaction between the studied genotype and the applied fertilization level are presented in Table 2. Thus, the production achieved in lines A4-10, A44-13 and A95-13, in the variants fertilized with 120 kg N a.s./ha, recorded very significant positive differences, compared to the Trivale control. But, the production achieved in line A57-14, in the variants fertilized with 120 kg N a.s./ha, recorded a distinctly significant negative difference compared to the Trivale control. As in the case of fertilization with 120 kg N a.s./ha, in the variants fertilized with 160 kg N a.s./ha, the production obtained in lines A4-10, A44-13, and A95-13 recorded a very significant positive difference compared to the Trivale control, and the production achieved in line A57-14 recorded a distinctly significant negative difference compared to the same control.

Table 2. Interactions between genotype and fertilized variants on production

Fertilizer rates	Genotype	Production (kg/ha)	%	Difference	Significance
120 kg N/ha	Trivale	5936.75	100.0	0.00	Mt.
	A4-10	6378.00	107.4	441.25	***
	A44-13	6260.38	105.5	323.63	***
	A95-13	6267.75	105.6	331.00	***
	A57-14	5654.25	96.9	-282.50	000
160 kg N/ha	Trivale	6128.75	100.0	0.00	Mt.
	A4-10	6585.75	107.5	457.00	***
	A44-13	6822.75	111.3	694.00	***
	A95-13	6663.38	108.7	534.63	***
	A57-14	5888.75	103.1	-240.00	000
LSD (p 5%)				98.93	
LSD (p 1%)				137.55	
LSD (p 0.1%)				191.49	

Table 3 presents the interaction between the fertilization factor and genotype on the protein content of the studied variants. Thus, in the case of using the dose of 120 kg N d.a./ha, the genotypes A4-10, A44-13 and A95-13 recorded very significant negative differences compared to the Trivale control, while the genotype A57-14 recorded a very significant positive difference regarding the protein content compared to the Trivale control. Also, when using the dose of 160 kg N a.s./ha, it is observed that the winter wheat genotypes A4-10, A44-13, and A95-13 recorded very significantly negative differences compared to the Trivale control, unlike the A57-14 line which recorded a very significantly positive difference in protein content, compared to the same control.

Table 3. Interactions between fertilized variants and genotype on protein content (%)

Fertilizer rates	Genotype	Protein (%)	%	Difference	Significance
120 kg N/ha	Trivale	14.39	100.0	0.00	Mt.
	A4-10	13.85	98.3	-0.54	000
	A44-13	13.78	104.9	-0.61	000
	A95-13	13.81	103.0	-0.58	000
	A57-14	15.56	108.2	1.18	***
160 kg N/ha	Trivale	14.50	100.0	0.00	Mt.
	A4-10	13.96	98.0	-0.54	000
	A44-13	13.91	104.3	-0.59	000
	A95-13	13.94	100.9	-0.56	000
	A57-14	15.66	107.3	1.16	***
LSD (p 5%)				0.27	
LSD (p 1%)				0.37	
LSD (p 0.1%)				0.52	

Table 4 presents the interaction between the use of fertilization doses and wheat genotypes on gluten content. It can be seen that in the case of using a dose of 120 kg N a.s./ha, the genotypes A44-13, A95-13 and A57-14 recorded very significant positive differences in gluten content compared to the Trivale control, while the wheat genotype A4-10 did not record significant differences in gluten content, compared to the same control. When using the dose of 160 kg N a.s./ha, very significant positive differences were recorded regarding gluten content in genotypes A44-13 and A57-14, compared to the Trivale control, while genotype A95-13 recorded distinctly significant positive differences, compared to the same control. Genotype A4-10 did not record significant differences regarding gluten content even when using the dose of 160 kg N a.s./ha.

Table 4. Interactions between fertilized variants and genotype on gluten content (%)

Fertilizer rates	Genotype	Gluten (%)	%	Difference	Significance
120 kg N/ha	Trivale	28.40	100.0	0.00	Mt.
	A4-10	28.35	99.8	-0.05	-
	A44-13	30.56	107.6	2.16	***
	A95-13	29.95	105.5	1.55	***
	A57-14	31.74	111.8	3.34	***
160 kg N/ha	Trivale	28.50	100.0	0.00	Mt.
	A4-10	28.48	99.9	-0.02	-
	A44-13	30.55	107.2	2.05	***
	A95-13	29.39	103.1	0.89	**
	A57-14	31.83	111.7	3.33	***
	LSD (p 5%)			0.50	
	LSD (p 1%)			0.70	
	LSD (p 0.1%)			0.97	

CONCLUSIONS

The analysis of the interaction between fertilization with different doses of nitrogen and genotype on the frequency of powdery mildew attack revealed that it was very significantly positively influenced in the case of using the dose of 160 kg N a.s./ha, compared to the use of the dose of 120 kg N a.s./ha considered as the control. In some cases, nitrogen fertilization increases plant sensitivity, but some genotypes show better tolerance, as was the case of the genotypes A4-10 and A57-14.

When using both doses, fertilization with 120 kg N a.s./ha and 160 kg N a.s./ha, the interaction between the studied genotype and the applied fertilization level influenced the production very significantly positively in the wheat genotypes A4-10, A44-13, A95-13, compared to the Trivale genotype, considered the control, while the production of the wheat genotype A57-14 recorded a very significantly negative difference, compared to the same control.

Due to the negative correlation between production and protein content, it was observed that when using both doses, fertilization with 120 kg N a.s./ha and 160 kg N a.s./ha, the wheat genotypes A4-10, A44-13, A95-13 recorded very significant negative differences in protein content, compared to the control genotype Trivale, while the wheat genotype A57-14 recorded a very significant positive difference in protein content, compared to the same control.

From the interaction between the use of both fertilization doses, 120 kg N a.s./ha and 160 kg N a.s./ha, and genotypes on gluten content, it was found that the wheat genotypes A4-10 and A57-14 recorded very significant positive differences, compared to the Trivale control.

The interaction between fertilization doses and the frequency of attack caused by powdery mildew did not produce significant changes in the production, protein content and gluten content recorded in winter wheat genotypes in the 2023-2024

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THE EVALUATION OF THE BIOMASS QUALITY OF *Lolium perenne* 'MĂGURA' AND *Phleum pratense* 'TIROM' GROWN UNDER THE CONDITIONS OF MOLDOVA

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Abstract

The primary objective of this study was to assess the quality indicators of green biomass harvested from monocultures of the perennial grasses Lolium perenne 'Măgura' and Phleum pratense 'Tirom', grown in the experimental field of the NBGI MSU in Chişinău. The study of the biochemical composition and nutritional value of the dry matter of green mass harvested from the investigated perennial grasses were defined by the following indicators: 86-95 g/kg crude protein, 97-111 g/kg ash, 336-391 g/kg CF, 35.5-41.7 g/kg ADF, 633-734 g/kg NDF, 22-26 g/kg ADL, 65-181g/kg TSS, 9.22-9.92 MJ/kg metabolizable energy, and the net energy for lactation was calculated as 5.24-5.95 MJ/kg. The analyzed green biomass substrates intended for biogas production contained 33.3–39.1% cellulose (Cel), 27.8–31.7% hemicellulose (HC), and a carbon-to-nitrogen (C/N) ratio of 32–36. The estimated biochemical methane potential ranged from 324 to 339 L/kg of dry matter (DM). The Romanian cultivars of perennial grasses — Lolium perenne 'Măgura' and Phleum pratense 'Tirom'— are versatile crops that can serve both as organic forage for livestock and as feedstock for biogas production facilities.

Key words: *biochemical biomethane potential, biochemical composition, green mass, Lolium perenne 'Măgura', nutritional value, Phleum pretense 'Tirom'.*

INTRODUCTION

The Poaceae family, comprising 777 genera and 11,461 recognized species, ranks among the largest and most economically significant plant families worldwide. According to *The Plant List*, the genus *Phleum* L. includes 18 accepted species, while the genus *Lolium* L. includes 11. In the native flora of the Republic of Moldova, four species of *Phleum* and two species of *Lolium* have been recorded (Negru, 2007). In contrast, Romania hosts seven *Phleum* species and four *Lolium* species (Marușca, 1999).
The species *Lolium perenne* L. is characterized by high productivity and economic value, and have been researched in scientific centers as crops with multiple uses (Mut et al., 2017; Chornolata et al., 2018; Janković et al., 2018; Amaleviciute-Volunge et al., 2020; Karbivska et al., 2020; Wang et al., 2020; Olszewska, 2021; Rancāne et al., 2021; Ravindran et al., 2022; Sosnowski et al., 2022; Ţîţei, 2023; Becker et al., 2023; Coşman et al., 2023; Ţîţei et al., 2023; Bozhanska et al., 2024; Bužinskienė, 2024; Czubaszek et al., 2024).
The Catalogue of Plant Varieties of the Republic of Moldova does not include any registered grass cultivars.
However, the Official Catalogue of Agricultural Plant Varieties of Romania lists two cultivars of *Phleum pratense* and twelve cultivars of *Lolium perenne*.
Romanian-developed cultivars yield between 37–65 t/ha of fresh biomass or 9.3–17 t/ha of hay (Marușca et al., 2011).
This research primarily aimed at assessing the quality parameters of *Lolium perenne* and *Phleum pratense* green biomass for use as livestock forage and as feedstock for biogas production in Moldova.

MATERIALS AND METHODS

The cultivars 'Măgura' of perennial ryegrass (*Lolium perenne*) and 'Tirom' of timothy grass (*Phleum pratense*), developed at the Research and Development Institute for Grasslands in Braşov, Romania, and cultivated in monoculture within the experimental plots of the "Al. Ciubotaru" National Botanical Garden (Institute) in Chişinău, Moldova, were used as the subjects of this study. Sampling was conducted during the third growing season, with the first cut performed at the early flowering stage. The harvested biomass was chopped into 1.5–2.0 cm segments using a laboratory forage chopper. Dry matter content was determined by drying the samples at 105 °C, to a constant weight.

For chemical analysis, the chopped samples were further dried in a forced-air oven at 60 °C, then ground using a beater mill equipped with a 1 mm mesh sieve. Key biochemical parameters — crude protein, acid detergent fiber, neutral detergent fiber, acid detergent lignin, total soluble sugars, and ash content were analyzed using near-infrared spectroscopy (NIRS) with a PERTEN DA 7200 NIR analyzer. Cellulose, hemicellulose, digestible dry matter, relative feed value, metabolizable energy, digestible energy and net energy for lactation were calculated using established standard procedures.

The carbon content of the biomass substrates was estimated using an empirical formula proposed by Badger et al. (1979), while the biochemical methane potential (BMP) was calculated based on the methodology outlined by Dandikas et al. (2015).

RESULTS AND DISCUSSIONS

Forage quality is determined by a range of characteristics, with nutrient content influenced by factors such as grass species or cultivars, growth stage, and soil conditions. Based on the analysis of quality indicators for the harvested green biomass of the studied *Lolium perenne* and *Phleum pratense* cultivars (Table 1), it is noteworthy that the dry matter contained 86-95 g/kg of crude protein, 97-111 g/kg of ash, 336-391 g/kg of crude fiber, 35.5-41.7 g/kg of acid detergent fiber, 633-734 g/kg of neutral detergent fiber, 22-26 g/kg of acid detergent lignin, and 65-181 g/kg of total soluble sugars. The green biomass of *Lolium perenne* is characterized by a lower content of minerals and structural carbohydrates, but a higher concentration of total soluble sugars compared to that of *Phleum pratense*. In contrast, *Phleum pratense* green fodder contains a more optimal level of crude protein. The reduced structural carbohydrate content in *Lolium perenne* enhances its digestibility, resulting in higher relative feed value, metabolizable energy, as well as net energy for lactation.

Numerous studies have evaluated the forage quality of *Lolium perenne* and *Phleum pratense*, highlighting considerable variability in their biochemical composition depending on growth conditions, cultivar, and harvest stage.

Burlacu et al. (2002) reported that at the flowering stage, *Lolium perenne* forage contained 250 g/kg dry matter, 8.6% crude protein, 32.7% crude fiber, 7.2% ash, and 18.3 MJ/kg gross energy; in comparison, *Phleum pratense* had 265 g/kg dry matter, 8.7% crude protein, 32.5 crude fiber, 7.8% ash, 18.4 MJ/kg gross energy. Hetta et al. (2003) observed that first-cut *Phleum pratense* contained 182 g/kg dry matter, 12.4% crude protein, 54.5% neutral detergent fiber and 11 MJ/kg metabolizable energy. Tomić et al. (2007) recorded crude protein contents in *Phleum pratense* ranging from 8.36% to 13.95% and crude fiber from 26.92% to 29.36%, while *Lolium perenne* had 9.70-13.40% crude protein and 30.72% crude fiber. Mahnert et al. (2005) reported that perennial ryegrass had 176-256 g/kg dry matter with 11.9-14.7% crude protein, 24.8-29.1% crude fiber and 9.4-9.9% ash. Dewhurst et al. (2009) found early-flowering *Lolium perenne* dry matter to contain 22.9% crude protein, 11.3% ash, 32.0% neutral detergent fiber, 19.3% acid detergent fiber, 80% matter digestibility. Surmen et al. (2013) observed that *Lolium perenne* lines contained 9.43-12.09% crude protein, 55.31-58.52% neutral detergent fiber, 37.24-40.36% acid detergent fiber, 49.24-53.27% total digestible nutrients, and a relative feed value of 92.19-98.57. Under moderate drought, Küchenmeister et al. (2014) found that *Lolium perenne* had 9.0% crude protein, 52.7% neutral detergent fiber, and 28.6% acid detergent fiber, while under severe drought, it had 11.5%

crude protein, 59.0% neutral detergent fiber, and 33.1% acid detergent fiber.

Wang et al. (2014) reported that *Phleum pratense* contained 124-133 g/kg dry matter, 13.6-17.4% crude protein, 58.1% neutral detergent fiber, 32.3-36.0% acid detergent fiber. Tran and Lebas (2015) documented a wide range of *Phleum pratense* dry matter composition: 7.6-22.3% crude protein, 49.5-63.5% neutral detergent fiber, 25.4-44.7% acid detergent fiber 1.4-3.2% lignin, 4.9-11.8% ash, with 58.0-76.9% digestible dry matter and 11.6 MJ/kg digestible energy. Marușca et al. (2016) found *Lolium perenne* forage to contain 14-17% crude protein and 24-28% crude fiber. Mut et al. (2017) noted that genotypes of *Lolium perenne* had 7.20-21.00% crude protein, 50.80-82.70% neutral detergent fiber, 21.58-43.90% acid detergent fiber. Chornolata et al. (2018) characterized *Phleum pratense* as having 16.05% crude protein, 23.10% crude fiber, 50.45% neutral detergent fiber, 24.82% acid detergent fiber, 14.60% cellulose, 1.88% starch, 15.80% hemicellulose, and 6.10% lignin. Janković et al. (2018) reported *Phleum pratense* dry matter had 13.20-14.52% crude protein and 24.30-26.98% crude fiber. Amaleviciute-Volunge et al. (2020) found that *Lolium perenne* fresh biomass contained 10.28% crude protein, 6.67% ash, 32.8% acid detergent fiber, 54.75% neutral detergent fiber, 3.97% acid detergent lignin, and 57.9% digestible dry matter, in comparison, *Festuca arundinacea* had higher crude protein (15.06%), more ash (7.52%), and better digestibility (62.5%). Karbivska et al. (2020) documented the forage quality of *Lolium perenne* dry matter as 11.4% crude protein, 29.6% crude fiber, 58% digestible dry matter., 0.72 fodder units/kg, 8.2 MJ/kg metabolizable energy, and 109 g digestible protein per nutritive unit; for *Phleum pratense* they reported 10.7% crude protein, 28.8% crude fiber, 7.4% ash, 58% digestible dry matter 0.70 fodder units/kg, 8.1 MJ/kg metabolizable energy, 109 g digestible protein per fodder unit. Reiné et al. (2020) compared the species and found *Lolium perenne* to have 371 g/kg dry matter with 6.8% crude protein, 5.8% ash, 63.6% neutral detergent fiber, 2.8% acid detergent lignin, 32.7% acid detergent fiber, and 63.8 digestible dry matter, while *Phleum pratense* had 377 g/kg dry matter, 7.6% crude protein, 3.9% ash, 34.0% acid detergent fiber, 68.5% neutral detergent fiber, 4.0% acid detergent lignin, and 62.4% digestible dry matter. Wang et al. (2020) found *Lolium perenne* herbage had 93.4-94.0% organic matter, 3.20–3.98% nitrogen, 38.8-41.4% neutral detergent fiber, and 784-830 g/kg digestible dry matter. Olszewska (2021) observed that cultivars contained 13.0-14.34% crude protein. Rancāne et al. (2021) evaluated 19 tetraploid genotypes of *Lolium perenne* and reported 6.99-10.68% crude protein, 19.92-25.11% acid detergent fiber, 38.79-46.74% neutral detergent fiber, 693.4-733.8 g/kg digestible dry matter, relative feed value of 138-175, and 6.71-7.02 MJ/kg net energy for lactation.

Table 1. Comparative biochemical and nutritional characteristics of *Lolium perenne* and *Phleum pratense* green mass

Indices	*Lolium perenne* 'Măgura'	*Phleum pratense* 'Tirom'
Crude protein, g/kg DM	86	95
Crude fiber, g/kg DM	336	372
Minerals, g/kg DM	97	111
Acid detergent fiber, g/kg DM	355	417
Neutral detergent fiber, g/kg DM	633	734
Acid detergent lignin, g/kg DM	22	26
Cellulose, g/kg DM	333	391
Hemicellulose, g/kg DM	278	317
Total soluble sugars	181	65
Digestible dry matter, g/kg DM	620	586
Relative feed value	90	72
Digestible energy, MJ/ kg DM	12.09	11.23
Metabolizable energy, MJ/ kg DM	9.92	9.22
Net energy for lactation, MJ/ kg DM	5.95	5.24

According to Ţîţei et al. (2022), the composition of green mass of timothy grass included 10.4-12.4% crude protein, 28.9-35.1% crude fiber, 7.5-8.5% ash, 49.5-58.9% neutral detergent fiber, 31.4-36.8% acid detergent fiber, 3.6-4.1% acid detergent lignin, 56.9-61.4% digestible dry matter, with a relative feed value of 95-121, metabolizable energy of 9.78-10.38 MJ/kg, and 5.81-6.42 MJ/kg net energy for lactation. Sosnowski et al. (2022) reported that the net energy for lactation in *Lolium perenne* forage ranged from 5.80 to 6.12 MJ/kg. According to Becker et al. (2023), the forage value of *Lolium perenne* was characterized by 17.7% crude protein and 6.4 MJ/kg net energy for lactation, while *Phleum pratense* contained 18.7% crude protein and 6.1 MJ/kg net energy for lactation. Coşman et al. (2023) observed that the nutrient composition of *Lolium perenne* ranged from 212.9 to 298.7g/kg dry matter, with 6.88-10.25% crude protein, 28.49-31.75% crude fiber, and 8.84-11.91% ash. Petkova et al. (2023) found that *Lolium perenne* dry matter contained 10.31% crude protein, 39.66% crude fiber, and 6.04% ash. Similarly, Ţîţei (2023) reported that the dry matter of harvested perennial ryegrass had 10.74% crude protein, 29.95% crude fiber. Bozhanska et al. (2024) highlighted that *Lolium perenne* forage contained 9.06% crude protein, 4.53% ash, 41.30% crude fiber whereas *Phleum pratense* had slightly higher values for crude protein (9.49%) with 4.01% ash, and 40.56% crude fiber.

Table 2. Estimated biochemical methane potential of the analyzed *Lolium perenne* and *Phleum pratense* substrates

Indices	*Lolium perenne* 'Măgura'	*Phleum pratense* 'Tirom'
Crude protein, g/kg DM	86.00	95.00
Nitrogen, g/kg DM	13.76	15.20
Ash, g/kg DM	97.00	111.00
Carbon, g/kg DM	501.67	493.89
Ratio carbon/nitrogen	36.46	32.49
Acid detergent lignin, g/kg DM	22.00	26.00
Hemicellulose, g/kg DM	278.00	317.00
Biogas potential, L/kg VS	686.00	684.00
Biomethane potential, L/kg VS	367.00	364.00
Biomethane potential, L/kg DM	338.74	323.60

Biogas is a renewable energy source that significantly contributes to reducing fossil fuel emissions across sectors such as transportation, heating, and electricity generation. Its utilization plays a key role in climate change mitigation and achieving renewable energy targets. In recent years, the direct production of biogas from harvested and chopped green biomass has garnered considerable interest in Europe.

Our study focused on the quality indices of green biomass substrates from *Lolium perenne* (perennial ryegrass) and *Phleum pratense* (timothy grass), and their potential for biomethane production, as presented in Table 2. The nitrogen content in the analyzed substrates ranged from 13.76 to 15.20 g/kg, while carbon content varied between 493.89 and 501.67 g/kg. The carbon-to-nitrogen (C/N) ratio ranged from 32.49 to 36.46. Acid detergent lignin content was between 22.00 and 26.00 g/kg, hemicellulose content ranged from 278.00 to 317.00 g/kg, and the biochemical methane potential (BMP) varied from 323.6 to 338.74 L/kg DM. *Lolium perenne* exhibited the highest biomethane potential among the tested substrates.

Previous studies have reported varying results. Mähnert et al. (2002) found that *Lolium perenne* fresh mass co-substrate had a dry matter (DM) content of 176 g/kg with 90.1% organic dry matter (ODM), producing 859 L/kg volatile solids (VS) of biogas over 28 days. *Phleum pratense* had a DM content of 148 g/kg, 90.1% ODM, and gas yields between 733-828 L/kg VS. Mähnert et al. (2005) reported a methane yield of 0.36 m^3/kg VS for *Lolium perenne*.

Kaiser and Gronauer (2007) reported specific methane yields for five ryegrass cultivars ranging from 198-443 L/kg VS, or 2,500-5,800 m^3/ha. For timothy grass, yields ranged from 345-375 L/kg VS, or 4,500-4,800 m^3/ha. Ebeling et al. (2013) observed that the methane yield of *Lolium perenne* varied from 320 to 335

L/kg VS depending on harvest timing and fertilization levels.

Żurek and Martyniak (2020) reported a biogas yield of 611.9 L/kg VS with 54.8% methane content for *Lolium perenne*, while according to Amalevičiūtė-Volungė et al. (2021), methane yields were 205.7 L/kg VS.

Ravindran et al. (2022) found that biorefined press cake from perennial ryegrass had a DM content of 390 g/kg, containing 4.67% ash, 682.2 g/kg VS, 2.74 g/kg nitrogen, 47.81% carbon, a C/N ratio of 19, and a biomethane yield of 487 L/kg VS. Bužinskienė (2024) reported that perennial ryegrass biomass substrates yielded 270-410 L/kg or 2,500-6,150 m^3/ha, while timothy grass yielded 151-322 L/kg or 1,362-5,800 m^3/ha. Czubaszek et al. (2024) noted that substrates from grass verges, predominantly composed of perennial ryegrass, contained 380 g/kg DM, 682.2 g/kg VS, 20.76 g/kg nitrogen, and 378.8g/kg total organic carbon, with a biogas yield of 715.05 L/kg VS and methane content of 59.7%.

CONCLUSIONS

The Romanian cultivars *Lolium perenne* 'Măgura' and *Phleum pratense* 'Tirom' demonstrate favorable biochemical composition and nutritional value in their dry matter, with key parameters including 86-95 g/kg crude protein, 97-111 g/kg ash, and 336-391 g/kg crude fiber. Their fiber fractions ranged from 35.5-41.7 g/kg acid detergent fiber, 633-734 g/kg neutral detergent fiber, and 22-26 g/kg acid detergent lignin, while total soluble sugars varied between 65-181 g/kg. Energy values were also substantial, with metabolizable energy of 9.22-9.92 MJ/kg and net energy for lactation of 5.24-5.95 MJ/kg.

The biochemical methane potential of the green biomass from these cultivars ranged from 324 to 339 L/kg dry matter, indicating a solid capacity for renewable energy production. Among the two, *Lolium perenne* 'Măgura' exhibited superior nutritional quality and a higher energy yield.

Both cultivars are well-suited for use in grassland restoration projects, the establishment of temporary pastures (either in monoculture or mixed with other perennial species), and for agroecological purposes such as inter-row planting in orchards and vineyards. Their harvested biomass can serve dual purposes: as valuable forage for livestock and as an efficient substrate for biogas production in the renewable energy sector.

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EFFECTIVE INSECTICIDE APPLICATION TO PREVENT ECONOMIC LOSSES FOR UKRAINE CAUSED BY WESTERN CORN ROOTWORM (*Diabrotica virgifera virgifera* Le Conte) SPREADING

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Abstract

The first individuals of western corn rootworms (Diabrotica virgifera virgifera Le Conte) were noticed in the border zone of Transcarpathia region in 2001. These insect pests had inhabited only two regions of the country for a long period of time. However, the gradual spread of Diabrotica species from the places of the first detection to the South and West of Ukraine has been noticed recently. The main aim of the research is to study the spreading tendency of the western corn rootworms (WCR) in the Ukrainian forest-steppe zone and to create the preventative system for identifying insect pest localization and extermination. According to the research results, it has been determined that the population of insect pests is increasing every year. There is a plant-feeding insect adaptation due to corn cultivation increase, poor crop rotation and climate change. The research priority and relevance has been done on the basis of the interest in insect pest identification, studying of their biological spread peculiarities under the consideration of modern conditions and improving of the insect pest control preventative methods.

Key words: *Diabrotica, crop rotation, insect, effective insecticide.*

INTRODUCTION

In recent years, there has been dangerous and massive insect pest spreading of western corn rootworms (*Diabrotica virgifera* Le Conte) for corn crops in Ukraine. As a result of harmful insect pest influence, there are losses not only for corn producers in the USA but for other countries as well (Adamchuk, 2008).

D. v. virgifera species (WCR) are originally from Northern America. They are considered to be the most economically harmful insect pests for corn production. They cause approximately 1,000 million dollar losses. It includes annual expenses of preventative methods and yield losses. That is why these insect pests are called "billion dollar bugs" (Krysan & Miller,1986).

The main area of *D. v. Virgifera* inhabitancy is considered to be Mexico and central regions of Southern America. In 1909, *Diabrotica* species were discovered in the USA. The insect pests spread gradually from the East of Colorado to Southwest Nebraska. Since 1955, there has been a massive bug spreading. A bit at a time they have become one of the most dangerous insect pests of corn crops not only in the USA but in Canada as well (Hornovska & Gorodetskyi, 2023).

From 1985 to 1989 the spreading of the western corn rootworms had occurred more quickly in western, central and north-central parts of Virginia (the USA) with only 39% of the corn cultivated area than in the eastern and south-western parts with 92% of crop cultivated area being used in crop rotation from 1989 to 1992 (Andreyanova, 2010).

In 1992, the western corn rootworms were detected for the first time in Europe (close to the international airport of Belgrade, Surcin, Serbia). Based on scientists' idea, the beetles could have been brought in by accident with the help of American aviation and military equipment. At first the damage caused by insect pest was mistakenly taken by *Agrotis segetum* Schift species (Meinke et al., 2009).

The origin of these insect pests is still unknown. Having appeared in Europe, they spread quickly in the Danube basin. The previous potential spreading study, conducted by researches in Croatia, France, Germany, has shown that these species live and spread where the corn crops are cultivated in Europe (Spencer et al., 2009).

Their inhabited area was discovered in Croatia (1995), Hungary and Romania (1996), Bosnia and Herzegovina (1997), Bulgaria and Italy (1998), Slovakia and Switzerland (2000), Ukraine (2001), Austria, France, the Czech Republic (2002), Slovenia, Belgium, Holland and Great Britain (2003), Poland (2005) (Wesseler & Fall, 2010).

In 1997, the inhabited area by beetles included the territory over 5300km² in Serbia. In 1998, the beetles were identified in Bulgaria and Italy (close to the international airport of Morocco, Polo). In 2000, the western corn rootworms appeared in Slovakia, Switzerland (Lugano) and also in Italy (close to the airport on the outskirts of Milan) (Butkalyuk, 2003).

In 2001, for the first time, they were detected in the region of Transcarpathia and it also was confirmed by their inhabited area in Hungary (Adamchuk, 2008; Hornovska & Gorodetskyi, 2023).

Due to their quick migration and ability to fly for a long distance, it is possible to make a conclusion that the insect pests have penetrated to the territory of Ukraine from the inhabited area of these countries (Grabovskyi et al., 2023).

On the basis of scientists' data (Sivcev, 1996; Edwards, 1999), inhabiting the area, the insect pests can be found in pheromone traps in three and more years (Edwards, 1999; Sivcev et al., 1996).

It is explained by the fact that the beetle fertilized female inhabits the area. Therefore, the monitoring and forecast are conducted due to the female pheromone traps used for male catch (Andreyanova, 2010).

Analyzing the fact that the period from the first appearance of the western corn rootworms to their identification is long enough and it is possible to draw a conclusion that they can be brought not only from the nearest countries, but these species have started their population growth on the territory of Ukraine (Rudenko, 2014).

The inhabited area by these species is constantly increasing each year. The western corn rootworms are found on corn crops in Transcarpathia, Lviv, Ivano-Frankivsk, Ternopil, Chernivtsi, Vinnytsia, Zhytomyr, Khmelnytskyi, Volyn regions. They were detected for the first time in Cherkasy region in 2017 (Stankevych & Hornovska, 2022).

In accordance with Sikura et al (2011) the attention should be paid to the rapid spreading of new corn insect pest species in Europe that have made EPPO add them to the control list of the most dangerous quarantine insect pests (A-2) that are limited in spreading in Europe.

In Ukraine, the western corn rootworms have been added to the list of controlled insect pests and to the second list of quarantine insect pests that are limited in spreading (Hornovska & Khakhula, 2020).

One of the main factors contributing to the insect pest infestation and further spreading is the presence of a host plant (corn) suitable for their development. Growing corn in monoculture conditions will provide the beetles with a permanent food base. As a result, it will lead to their significant spreading and harm in Ukraine.

Crop rotation is mentioned as the most effective preventative measure for *Diabrotica* species in all domestic and international literary sources (Hornovska & Gorodetskyi, 2023; Rudenko, 2014).

Following the crop rotation is enough to decrease the insect pest population and their damage: coming back to three or four year corn crop rotation in the field. However, using the corn crop rotation randomly can cause quick insect pest adaptation (Prymak et al., 2022). There is a reason to use three year crop rotation and rotation more than three years in the following way: corn, soybean, oat. Thus, Europe suggests using the following crop rotation: corn, soybeans, sunflower and cereals (wheat, oat, barley) (Prymak et al., 2023).

The constant international and trade-economic relation expansion contributes to the import volume increase and agricultural product export. It creates conditions for the penetration of quarantine harmful organisms into Ukraine. Their quarantine status and significant economic influence can't be always known and predictable for Ukraine (Andreyanova, 2010).

Aggressive insect pest species are dangerous for agricultural production, especially for agricultural companies that produce corn crops. The western corn rootworms have become a new problem for European famers that produce corn crops. The damage caused by (*Diabrotica virgifera virgifera* Le Conte) can be immense and alike to losses that American famers have (Adamchuk, 2008).

The amount of countries, where the phytophagous insects appear, is increasing yearly. The *Diabrotica* species inhabit broad areas and can be found in countries with subtropical or subcontinental and mild climate (mild winter and hot summer). The constant movement of all types of vehicles from other countries to Ukraine and inside the country can distribute insect pest spreading and the appearance of new infestation localizations. Also, insect pest spreading is distributed by the species ability to migrate for a long distance naturally. During the growing season they can reach distance of 40-100 km (Eben, 2022).

Since February 24, 2022, this issue has become of great importance after starting of the Russian federation a war against Ukraine. The insect pest penetration and spreading on the wheels of vehicles, tank tracks, military equipment and food supply for the army have created a dangerous situation. It will be possible to determine the infestation volume and to start preventative actions against quarantine species after de-occupation and demining of surveyed territories, farmlands of different use. It has relation to occupied regions of Donetsk, Lugansk, Kherson, Zaporizhzhia (Stankevych & Hornovska, 2022).

It is important to mention that from 2017 to 2020 phytophagous insects had been detected in new regions. They increased their spreading on the territories of 15 regions in comparison with 2015, 2016 (on the territories of 9 regions). The phytophagous have been identified in the regions of Sumy, Kyiv, Kropyvnytskyi (Kirovohrad), Vinnytsia, Cherkasy, Chernihiv (Hornovska & Gorodetskyi, 2023).

According to the 1st of January, 2018, the beetles were spread over the area of 88, 9 thousand hectares. At the beginning of October of the same year infested area increased by 20 thousand hectares. The new infestation localization was detected on the territory of 12 regions (Stankevych & Hornovska, 2022).

According to the 1st of January, 2024, the WCR spread in 16 regions of Ukraine. The regions of Mykolaiv and Chernivtsi turned out to be the most infested. They can be found on the area of 28524, 03 and 55287, 13 hectares. The region of Dnipro was less infested – 257 hectares. The dynamics of the insect pest spreading was visible in Ukraine (from 861667, 49 hectares in 2015 to 144167, 75 hectares in 2022) (Saliienko & Fedorenko, 2024).

The real threat connected with the dangerous corn insect pest spreading has appeared in Ukraine. The losses caused by them can be significant and alike to losses of the USA and other countries. The literacy resources suggest crop rotation as the most effective method against *Diabrotica* species. Though, some authors insist on combination of several methods: steady crop rotation, insecticide use, resistant corn variety cultivation (Marchioro & Krechemer et al., 2018).

The modern tendency for the insect pest prevention is focused on the biological control of population quantity as it influences the environment ecological state (Sikura, 2010).

The aim of the research is to analyze the western corn rootworm (*Diabrotica virgifera virgifera* Le Conte) spreading in 2024 on the territory of Ukrainian forest-steppe zone, to make a conclusion from the phytosanitary monitoring, to create preventative measures of insect pest spreading, localization identification and extermination in case of detection.

MATERIALS AND METHODS

The research purpose is to make a conclusion from the monitoring results of the western corn rootworm (*Diabrotica virgifera virgifera* Le Conte) phytosanitary spread control conducted in 2024 on the territory of the forest-steppe zone of Ukraine. The basis of the research is phytosanitary monitoring of various region territories of Ukraine where *Diabrotica* species are widespread. The agrocenosis phytosanitary state monitoring is a main conventional method of the research (Sikura et al.,2011; Stankevych, Zabrodina, 2016).

The corn crops had been examined three times in each month (at the beginning, in the middle and at the end) for the studied period. The monitoring was carried out by the route examination with the use of artificial sex pheromones in the corn crops.

The traps for *Diabrotica* detection were located in the corn fields with amount of 1 trap per 5 hectares. Yellow glue traps were exposed in amount of 1, 3, 6, 9, 12 samples independently from the area of corn crop during the massive imago flight. All variants of samples were used three times.

The traps were installed on the level of corncob covering their stalks. The traps were changed every week and the pheromone capsules once in two weeks. Counting and sampling of *Diabrotica* imago were carried out in the traps once in seven days. The pheromone capsules were changed in 4-5 weeks.

The constant soil digging and examination of plants were done for insect pest early detection. Since the blooming period, the beetles had been found on leaves, stalks, silk, young corncobs The *Diabrotica* species were caught by yellow, blue and transparent glue traps with and without an attractant. The traps of triangular and round shapes were used as well as traps of a panel type that turned out to be the most effective for beetle catching. Metoksifenetanol 4 was used as an attractant.

The visual root system examination for larva and egg identification was done equally by soil digging method in the fields. Basically, the edges and the middles of the fields were examined near dying, turned yellow and stunted plants.

The digging area had the form of an "envelop". The digging in the form of a "snake" was carried out in narrow and long field areas (Baca et al., 1995).

The examination of the pheromone traps with insect pest samples on filter paper was performed. Then they were placed in a test tube with a fixed label for the further identification of their species. Insect pest species were identified using text books and pest atlases.

Phytosanitary experts mention that in recent years, pheromone monitoring has become an important and profitable method for the timely detection, spread control and assessment of the insect pest number dynamics in comparison with the method of visual crop examination (Adamchuk, 2008; Stankevych, Zabrodina, 2016).

312 samples consisting of 216 adult insects and 112 larvae had been collected for the period of the research. The western corn rootworms were calculated only in the fields where the corn crop had been cultivated as a monoculture for three and more years. Strong attention was paid to the fields bordering with the highways and railway lines. The insect pests were detected on all stages of their development. Stunted and turned yellow crops were the signs of insect pest inhabitancy.

RESULTS AND DISCUSSIONS

The western corn rootworm harmfulness and amount are changing in Ukraine. In 2024, 427 infestation localizations of western corn rootworms (*Diabrotica virgifera virgifera* Le Conte) were recorded in Ukraine. The quarantine regime was suggested for *Diabrotica* species in 16 regions. In 2024, the inhabited area by insect pests was 130603, 5383 hectares. Since 2015 to 2024, there had been the increasing tendency (by 1,6 times) of insect pest inhabitancy on the territory of Ukraine (Figure 1).

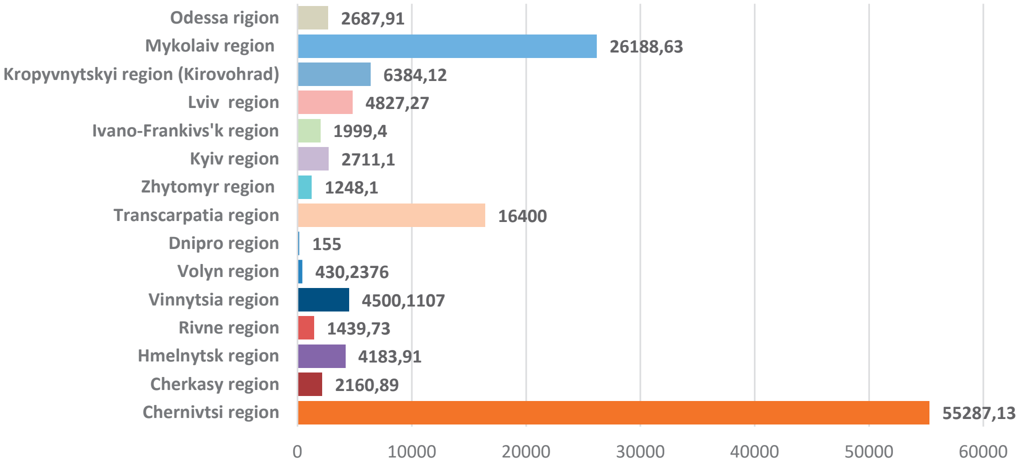

Figure 1. The inhabited area by western corn rootworms (*Diabrotica virgifera virgifera* Le Conte)

Analyzing conducted study, it is possible to make a conclusion that there is a tendency for increasing of the inhabited area by insect pests on the territory of Ukraine. The researches have a forecast that the insect pests will inhabit all corn crop agrocenosis in five years.

To analyze the seasonal flight dynamics of *Diabrotica* adult species, there was a need to conduct the observation of the flight season (at the beginning, in the middle and at the end) and to identify the general flight duration. As a result of these observations there was an opportunity to create the system for optimizing the pest control and extermination.

In 2024, the research expedition was carried out in the forest-steppe zone where the corn crops had been cultivated. The sites for studying were located at the husbandries of Kyiv region. There was a corn crop area of 5 hectares.

Starting from the last 10 days of May, the signal pheromone traps were hung on the experimented sites with corn crops. In the first 10 days of July, the first beetles of *Diabrotica* species were found in traps. When the insect pest flight was started, the pheromone traps were hung in the middle of July and yellow glue traps – in October. They were exploited to study the beetle dynamics flight using different amount of traps. In the period of massive imago flight, yellow traps in the amount of 1, 3, 6, 9, 12 were installed without dependency on the sown corn crop area. The pheromone traps in the amount of 1, 3, 5 were located for imago monitoring in the corn crops. All trap variants had three replicates.

The calculation results of the found amount of adult western corn rootworms in the traps are shown in the (Table 1).

Table 1. The number of the adult western corn rootworms (*Diabrotica virgifera virgifera* Le Conte) found in the pheromone and yellow traps.

Calculation date	The average number of the found adult beetles by the pheromone traps			The average number of the found adult beetles by the yellow glue traps				
	1	3	5	1	3	6	9	12
15.07	86±8	77±10	98±4	-	-	-		-
22.07	169±13	185±7	183±11	8±3	7±6	16±5	10±4	7±3
29.07	180±9	194±15	186±17	40±6	45±8	69±5	46±7	43±5
05.08	68±6	73±5	78±9	30±5	34±7	40±6	36±8	35±10
12.08	162±27	140±14	148±12	36±8	43±6	57±7	40±5	44±8
19.08	110±23	125±21	109±5	35±7	38±12	52±12	38±9	36±6
26.08	105±34	98±17	95±14	29±5	32±8	40±7	35±8	32±5
02.09	85±12	78±8	81±6	-	-	-	-	-
07.09	64±10	70±7	69±6	-	-	-	-	-
14.09	45±8	48±7	52±9	-	-	-	-	-
21.09	18±4	21±3	25±3	-	-	-	-	-
28.09	0,5	0,9	0,6	-	-	-	-	-
Adults detected on average, specimens/traps	91.0±14	92.5±10.4	93.7±8.1	14.8±2.8	16.6±3.9	22.8±3.5	17.1±3.4	16.4±3.1

It was established during the research that all pheromone trap variants in different quantities showed almost the same beginning and duration of the *Diabrotica* species flight period.

All pheromone traps were used in different amounts in the experiment. Analyzing the data in the table 1, it was possible to come to conclusion that almost the same number of beetles was found in the pheromone traps on the date of registration.

The beginning of the *Diabrotica* imago flight was observed on the 15th of July in 2024 when the massive amount of the male beetles started coming out from the soil to search the females for mating.

The massive flight of beetles lasted from the third decade of July to the end of August. It was that period that indicated active feeding, mating and the female egg laying of *Diabrotica* species (Figures 2, 3).

Figure 2. Feeding of *Diabrotica* imago (*Diabrotica virgifera virgifera* Le Conte)

Figure 3. Corn crop laying caused by the larvae of *Diabrotica* species

As stated by this observation the leap of the imago flight was in the first decade of September. The end of the flight was in the last decade of September.

The leap of the Diabrotica flight had been recorded from 22.07.2024 to 12.08.2024.

The biggest amount of imago beetle was found in pheromone traps (29.07.2024: 194 samples per trap). That is why it was suggested using insecticides exactly in this period. 5 insecticides for western corn rootworms were used for the experiment.

The experiment was carried out randomly four times with repetition in accordance with the scheme:

1. Insect pest control of the site;
2. Coragen CS (chlorantraniliprole, 200 g/l), 0.15 l/ha;
3. Karate Zeon 050 CS (lambda-cyhalothrin, 50 g/l), 0.3 l/ha;
4. Engio 247 CS (thiamethoxam, 141 g/l + lambda-cyhalothrin, 106 g/l), 0.18 l/ha;
5. Avaunt EC (indoxacarb, 150 g/l), 0.17 l/ha;
6. Danadym stable, EC (dimethoate, 400 g/l), 1.0 l/ha.

The insecticides were applied in the stage of early corn maturity, which corresponds to the international scale of growth and development of grain crops (BBCH). A portable selective sprayer Pulverexper was used to apply chemicals. The effective dose of solution was 200 l/ha.

To compare the insecticide effectiveness, statistically reliable indices (the number of pests in traps) were used for each calculation.

The effectiveness of the chemicals was measured in 1-5 days.

The effectiveness of insecticides against diabrotic adults depends on the seasonal dynamics of their flight - at the beginning and during the mass flight of beetles, when their number is the highest. Therefore, the effectiveness of chemicals against adults was determined during their mass flight.

The studies showed high technical efficiency of the studied chemical insecticides against the western corn borer.

The most effective insecticide was Avaunt, EC. (indoxacarb, 150 g/l) with an application dose of 0.17 l/ha. This chemical showed 96.97% decrease of the amount of *Diabrotica* species in comparison with the control samples, Coragen, CS - 94.36%, Karate Zeon 050, CS with. - 96.78%, Engio 247, CS - 94.01%, Danadym stable, EC - 93.32% (Table 2).

The highest efficacy of the products was observed on the 3rd day after their application. This is due to the fact that the pest is in direct contact with the insecticide.

Table 2. Effectiveness of insecticides when used against imago of *Diabrotica virgifera virgifera*

Variant	Consumption rate l, kg/ha	Number of imago on ... day of accounting, units/100 plants			Technical efficiency, %.
		before processing	on the 3rd day	on the 5rd day	
		2024			
Control		39.5	49.0	42.0	-
Coragen CS (chlorantraniliprole, 200 g/l)	0.15	47.00	8.8	2.65	94.36
Karate Zeon 050 CS (lambda-cyhalothrin, 50 g/l)	0.3	47.27	2.25	1.52	96.78
Engio 247 CS (thiamethoxam, 141 g/l + lambda-cyhalothrin, 106 g/l)	0.18	46.25	6.6	2.77	94.01
Avaunt EC (indoxacarb, 150 g/l)	0.17	48.90	2.28	1.48	96.97
Danadym stable, EC (dimethoate, 400 g/l)	1.0	49.22	6.54	3.29	93.32

The decrease of *Diabroticia* amount in the comparison with the control samples was established in the range of 50-75% in 6-8 days after the application of insecticides.

It was found out that the larvae, being obligate monophages and feeding only on the roots of corn, were especially harmful for corn crops. It was observed during the research that first of all, the larvae ate root hairs, then thin roots and later

– large and tap roots. At the same time, they introduced root rot pathogens.

As a result, damaged plants turned yellow, stunted, withered and young plants died quite often.

It was noticed that mature plants had laid out easily during strong wind and rainfall. The plant stems became in the form of a “duck neck”. At the same time mechanized harvesting was impossible. But for beetle feeding on the generative organs, the seed amount decreased in the corncob. That was the reason of yield declining (Figure 4).

Figure. 4 Damaged roots by western corn rootworms (*Diabrotica virgifera virgifera* Le Conte)

The biggest damage caused by the western corn rootworms was observed in the fields without crop rotation. But for steady corn cultivation, the population of beetles increased.

That is why the crop rotation, including cereals, perennial grasses, clover, alfalfa, is an effective agricultural measure against the western corn rootworms.

Thus, in case of their overcoming the economic damages (in the amount of 2 larvae per plant), the soil insecticide use is effective to control larvae of the western corn rootworms. The insecticides can be used before and during sowing or during the growing season.

CONCLUSIONS

It has been found out during the chemical use and the seasonal imago flight determination that the application of 1 pheromone trap per 5 hectares of corn crops is enough to monitor the inhabited forest-steppe zone by imago of the western corn rootworms.

In order to decline the probable appearance of the WCR in the corn crops, an important action is to carry out the control by detecting larvae and examining the root system, leaf surface, silk. To determine the early stages of beetle development there is a need to dig the soil.

The main preventative measure against imago and larvae is the insecticide use.

It is highly recommended to use insecticides that have shown great effectiveness: Avaunt, k.e. and Karate Zeon 050 CS, hp, with technical efficiency of 96.97% and 96.78%, respectively.

They are mentioned in the list of approved pesticides and agrochemicals of Ukraine (2023).

The declined amount of Diabrotica is observed (in the rate of 50-75% and lower than on control sites) in 6-8 days after pesticide using. This is the evidence of a long-lasting protection effect of the insecticide.

The most effective and approved insecticides by the research are recommended to corn crop production regions with *Diabrotica* species spreading.

The use of the recommended insecticides at the first appearance of diabrotica will allow farmers to maintain corn yields, thereby increasing the economic efficiency of growing the crop.

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ROMANIAN FARMERS' PERCEPTION ON THE IMPORTANCE OF POLLINATORS

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Abstract

To analyse the perception of Romanian farmers regarding the importance of pollinators, a survey was conducted between February and August 2024. A total of 122 farmers (small, medium and big) growing rapeseed and sunflower crops were selected from 13 counties of Romania. The main goal of this study was to assess farmers' perception on pollinators, and how perceptions are linked with management practices. Basically, in this study, farmers' perception towards the pollination of two oilseed crops, namely sunflower and rapeseed, were assessed. The farmers showed interest in pollinators; over 90% of respondents considered pollinating insects to be essential for their crops. The majority of interviewed farmers recognize the importance of pollinators, but also exhibit uncertainty about the extent of native pollinators' contribution to yield and how to best manage them. The majority of farmers growing sunflower and rapeseed consider pollination services necessary to improve the productivity of their crops and they are concerned about the pollination of their crops, but however there are some farmers who perceive limited benefits from bees, or from investing in pollinator-friendly practices. Among big farmers, it was noticed that majority of the farmers perceive the honey bees to be the primary species for the pollination activity. The good perception of big farmers on importance of pollination could change the view of small farmers through the example power, considered to be the safest form of education.

Key words: *farmer, perception, pollination, native pollinators, honey bees, oilseed crops, survey.*

INTRODUCTION

For pollen transport, plants depend on various vectors, such us wind, water, and animal pollinators like bats, moths, hoverflies, birds, bees, butterflies, wasps, thrips, and beetles, animal-based pollination contributing to approximately one-third of the total human dietary supply (Khalifa et al., 2021). In agricultural ecosystems, pollinators' diversity increases the quality and quantity of crop yield, but the pollinator groups are also useful in monitoring environmental pollution, aid in pest and disease control, and provide cultural and aesthetic value (Katumo et al., 2022).
Over 1,300 species of plants are believed to be grown worldwide for their fruits, spices, medicines etc., about 75% of these species being pollinated by animals (Diwan et al., 2022). Fruit, vegetable or seed production from 87 of the leading global food crops is dependent upon animal pollination, while 28 crops do not rely upon animal pollination, which is showing the importance of the animal pollination, while 60% of global production comes from crops that do not depend on animal pollination, 35% comes from crops that depend on pollinators, and 5% are unevaluated (Klein et al., 2007).
Among the different groups of pollinators, bees play a crucial role in maintaining the health and balance of ecosystems. They are essential pollinators, facilitating the reproduction of a wide variety of plants, including many food crops. However, preserving the delicate balance in pollination services for sustainable agroecosystems poses a substantial challenge (Sindhu & Shivalingaiah, 2023). Despite their obvious importance, bees face numerous threats that endanger their survival. Agriculture plays an ambivalent role in bee health; it provides essential food resources for bees, but it can endanger bee populations through the use of pesticides and monoculture practices. To support the fight against possible

crises that could arise due to the pollinators' population decline or species extinction, it is important that everyone values them and collaborates to maintain and even increase their populations.

Native bees (including honey bees) are the most important pollinators because of their diversity and specificity to many flowering plant species. Their population decline is a serious global concern due to their potential impact on ecosystems and human well-being. There are over 20,000 bee species worldwide (Locklear, 2023), of which 2,138 species are in Europe (Ghisbain et al., 2023). Although native wild pollinators are not efficient enough to replace honey bees in pollination, they are considered extremely important for ensuring the pollination of numerous wild or cultivated plant species or in certain weather conditions, when the efficiency of honey bees is reduced (Hanes et al., 2013).

On the other hand, little is known about the perceptions and knowledge base of the main stakeholders (farmers and beekeepers) within this system and how they make management decisions. In particular, little is known about the extent to which farmers perceive pollination service deficits (yield reductions due to inadequate pollination) and how they respond to these deficits (Hanes et al., 2013). While there is evidence that beekeepers consider crop and environmental risks when locating their beehives, particularly in Europe, there is limited research on how various environmental factors influence honeybee health, as well as the competition with other wild bee species. Studies highlight the need for a better understanding of how various factors, such as climate, food availability, and human activities, affect both managed and wild honeybee populations. Lack of knowledge hinders participants' intentions and behaviour to engage in critical environmental practices (Locklear, 2023).

There are financial supports for agri-environment schemes (AES) that encourage farmers to incorporate biodiversity-friendly farming practices. However, the implementation of biodiversity-friendly techniques and strategies into farming practices remains a central challenge for conservation and sustainability, in part because of information gaps and the lack of transdisciplinary communication and collaboration between science and practice (Maas et al., 2021).

Little is known about how different assessments and perspectives on biodiversity, ecosystem services, and conservation among agricultural stakeholders from science and practice influence AES implementation strategies (Maas et al., 2021).

Farmer perception of pollinators, particularly non-bee insects, is a rarely studied area, though it's crucial for understanding and supporting pollination services in agriculture. In practice, whether pollinating insects are declining or not is hard to prove for most wild pollinators taxa because there is a lack of systematic and standardised monitoring of species diversity and populations abundances (Vanbergen et al., 2014). While farmers, generally, they recognize bees as important pollinators, their awareness and appreciation of the roles of non-bee pollinators (like flies, beetles, and moths) are often limited, despite the significant contribution these insects can make to crop yields. There are few studies on farmers' knowledge of and attitudes towards the biodiversity of their land.

Pollinator conservation has become a key challenge to achieve sustainable agricultural landscapes and safeguard food supplies. Considering the potential negative effects of pollinating insects decline, international efforts have been developed to promote agri-environmental measures and pollinator-friendly management practices. However, little effort has been devoted to assess the farmers' perceptions and knowledge about pollinators, or to assess the farmers' role in enhancing pollination (Hevia et al., 2020). Some studies suggest that providing farmers with more information about pollinators, their role in crop production, and effective management practices can further increase their engagement and adoption of pollinator-friendly practices.

Pollinator declines and dependence on insect pollination, particularly in fruit and vegetable crops, create a pressing need to understand growers' interactions with pollinators and factors affecting pollination strategies (Hanes et al., 2013). Many farmers recognize the importance of pollinating insects for their livelihoods and actively take steps to protect and support them on their farms. Local and traditional knowledge can provide some solutions to the current challenge of pollinators' decline, but there is no integration and analysis of this knowledge for its practical use (IPBES, 2016).

The few studies addressing farmer's knowledge reveal that local information about pollinators and pollination varies significantly based on regional, national, and crop-specific contexts (Osterman et al., 2021). This variation stems from differences in pollinator diversity, foraging behaviours, and the specific pollination needs of different crops, which influence farmer's management practices and perceptions. Understanding how farmers perceive pollinators is crucial for developing effective conservation strategies. Farmers' knowledge, attitudes, and practices directly influence pollinating insect populations and pollination services they provide. By understanding farmers' perceptions, one could tailor outreach programs and management recommendations to increase the adoption of pollinator-friendly practices.

A study focusing on university staff and students (Locklear, 2023), found that while most individuals recognize the importance of pollinators like bees, there's a significant gap in knowledge regarding their diversity and specific roles in the ecosystem. Specifically, a substantial number of participants struggled to identify different bee species, even though they acknowledged bees' vital role in pollination. This suggests a need for improved educational initiatives that go beyond simply stating the importance of pollinators and delve into their ecology and conservation.

On-farm experiences significantly influence farmers' knowledge, perceptions, and management practices related to pollinating insects. Farmers' direct observations and interactions with pollinators on their farms shape their understanding of the importance of different pollinator types, their contribution to crop yields, and the effectiveness of various management practices aimed at enhancing pollination services. This knowledge, in turn, impacts their decisions on how to manage their farms to support pollinators and optimize pollination (Osterman et al., 2021). In essence, on-farm experiences act as a crucial link between scientific understanding of pollinators and practical on-the-ground management.

The main goal of this study was to assess farmers' perception on pollinators, and how perceptions are linked with management practices. Basically, this study evaluated farmers' perceptions on pollination of two oilseed crops, namely sunflower and rapeseed.

In fact, these two crops have a special significance in Romania due to their contribution to the national economy, being the cornerstones of the agricultural sector. Romania occupies a prominent position in the EU, both in terms of the cultivated area with oilseed crops and the production of oilseeds. In addition, the sunflower and rapeseed crops are an important source of nectar and pollen for wild bee species and managed honeybees. Pollination is an important bio-input for enhancing the productivity of these two oilseed crops. In the case of sunflower (*Helianthus annuus* L.) there are strong evidences that insufficient pollination can significantly minimize the grain yield. According to the existing data regarding the efficiency of pollination performed by honey bees, it was found that an increase of about 36.2% in sunflower grain yield is due to honey bees (Sanduleac, 1960). Rapeseed (*Brassica napus* ssp. *napus* L.) is a crop that benefit by a mixed pollination system (self-pollination and cross-pollination). Yellow flowers with sticky pollen and high volume of nectar play an important role in attracting different pollinators (Ion, 2024). With the ongoing global decline in wild and managed pollinators, there is a growing concern that their negative impact will become pronounced on oilseed crops.

By interviewing farmers, context-dependent preferences can be identified which can help identify effective practical management strategies that farmers are willing and able to implement (Osterman et al., 2021).

The hope is that by understanding the farmers' perception, preferences and knowledge gaps regarding the interrelations between honeybees and crop pollination can be identified, as well as potential collaborations between farmers and beekeepers and effective actions can be formulated (Breeze et al., 2019).

Understanding farmers' perception on pollinators' importance in oilseed crops can help develop strategies to reduce the negative attitudes and influence the change in attitudes and opinions towards the adoption of environmentally friendly farming practices by farmers (Munyuli, 2011).

MATERIALS AND METHODS

As it was mentioned in the above section, oilseed crops, namely sunflower and rapeseed, are ones of the most important and dynamic crops of Romanian agriculture, both for farmers and beekeepers.
The study areas were selected from the agricultural landscape dominated by sunflower and rapeseed crops highly (Figure 1). More precisely, the respondents came from the areas favourable for sunflower and rapeseed cultivation in Romania.

Figure 1. Romanian map with the marked areas showing distribution of surveys

Farmers growing sunflower and rapeseed, from 13 counties responded to the performed survey by filling a structured questionnaire designed to assess knowledge, attitude and perception of pollinators' importance (Table 1). In total, 122 of completed questionnaires were collected.
The survey was conducted between February and August 2024. The questionnaires were distributed to farmers at the end of the professional courses organised in different counties by the UnivAgx Association in association with the farmer cooperatives of Romania.
The first part of the questionnaire was referring to the personal data of the respondent (age, gender and educational level), location (county), and acreage of the farm (number of hectares). The following section, assessed the implemented strategies by farmers to improve the pollination services (if any) and their willingness to receive beehives.

Table 1. Counties and number of farmers who responded to the performed survey

No.	County	No. of respondents
1	Argeș	19
2	Bacău	3
3	Brăila	21
4	Buzău	1
5	Călărași	1
6	Constanța	12
7	Galați	18
8	Ialomița	1
9	Ilfov	1
10	Iași	11
11	Neamț	5
12	Olt	2
13	Teleorman	5
Total		*100*
1	Farmers who did not declare the county	3
2	Farmers who did not declare the identification data	19
Total		*22*

Furthermore, a series of other questions have been addressed to gain further insights about their beliefs regarding pollination service increases or its perceived limitations. The last part included a series of open questions in order to examine the farmers' perceptions towards the adoption of policy incentives for the provision of pollination services in agricultural systems. Farmers were asked if they are willing to adopt their crop management as to attract more beekeepers, as well as to identify practices for the provision of wild pollinators within their farmlands. Additionally, there were 5 questions to assess the attitude of farmers towards pollination services, respectively related to (1) farmers' perception of pollination services, (2) current status of pollination services, (3) the farmer-beekeeper relationship, (4) how it is determined the number of beehives per hectare for pollination of rapeseed and sunflower crops, and 5) current agricultural practices in relation to pollination.

RESULTS AND DISCUSSIONS

The socio-economic characteristics of the farmers

Of the 100 farmers who responded to the survey with identification data (filling their name and county), 84 farmers declared the area they manage (Table 2). Together they manage a total of 87,788 ha of land. On average, a farmer owns 1,045 ha, with variations from 37 ha up to 6,800 ha. Of all farmers, 26.2% manage large farms (between 1000 and 6,800 ha), 27.4% manage medium-sized farms (between 300 and 999 ha), and 46,4% manage small farms (under 300 ha). The large farms count 80% of the total area of the studied farms.

Of the 94 of farmers who declared their gender, 89.4% are men and 10.6% are women (Table 3).

While farming has historically been perceived as men-dominated, there is a growing trend of women participation and leadership in the agricultural sector. Some regions show a higher proportion of women farmers. Although men still hold a disproportionate number of leadership positions and farm ownership, women are increasingly taking on roles as primary producers, farm managers, and entrepreneurs.

Table 2. Number of farmers per county who declared the area they manage and their farms size

County	Argeș	Bacău	Brăila	Buzău	Constanța	Galați	Ialomița	Ilfov	Iași	Neamț	Olt	Teleorman	Total
No. of farmers per county who declared their farm area	19	1	18	1	11	14	1	1	9	3	2	4	**84**
Surface (ha) of farms with more than 1,000 ha (big farms) and number of farmers	1800	-	1000	6200	-	5200	-	5000	6800			5000	**22 farmers (26.2%)**
	1550	-	-	-	-	4000	-	-	6500	-	-	5000	
	1000	-	-	-	-	1300	-	-	6000	-	-	2200	
	1000	-	-	-	-	1182	-	-	2100	-	-	2000	
	-	-	-	-	-	-	-	-	2000	-	-	-	
	-	-	-	-	-	-	-	-	1850	-	-	-	
	-	-		-	-	-	-	-	1800	-	-	-	
	70,482 ha												
Surface (ha) of farms with 300-999 ha (medium farms) and number of farmers	930	-	550	-	500	530	-	-	720	-	-	-	**23 farmers (27.4%)**
	800	-	400	-	500	433	-	-	320	-	-	-	
	800	-	400	-	460	400	-	-	-	-	-	-	
	750	-	300	-	326	-	-	-	-	-	-	-	
	600	-	300	-	-	-	-	-	-	-	-	-	
	500	-	-	-	-	-	-	-	-	-	-	-	
	400	-	-	-	-	-	-	-	-	-	-	-	
	315	-	-	-	-	-	-	-	-	-	-	-	
	314	-	-	-	-	-	-	-	-	-	-	-	
	11,548 ha												
Surface (ha) of farms with less than 300 ha (small farms) and number of farmers	250	280	270	-	280	150	220	-	-	140	170	-	**39 farmers (46.4%)**
	220	-	260	-	260	150	-	-	-	60	87	-	
	200	-	250	-	210	113	-	-	-	50	-	-	
	160	-	170	-	195	110	-	-	-		-	-	
	157	-	150	-	172	92	-	-	-		-	-	
	37	-	125	-	80	40	-	-	-	-	-	-	
	-	-	120	-	60	38	-	-	-	-	-	-	
	-	-	115	-			-	-	-	-	-	-	
	-	-	100	-	-	-	-	-	-	-	-	-	
	-	-	80	-	-	-	-	-	-	-	-	-	
	-	-	77	-	-	-	-	-	-	-	-	-	
	-	-	60	-	-	-	-	-	-	-	-	-	
	5,758 ha												

Women own and operate farms of various sizes, including large ones, though they are more likely to operate smaller farms.

Indeed, the average size of farms operated by women is often smaller than those operated by men.

Table 3. Number of farmers who declared their gender

County	No. of farmers who declared their gender		
	Total, out of which:	Men	Women
Argeș	19	15	4
Bacău	3	3	0
Brăila	20	18	2
Buzău	1	1	0
Constanța	12	10	2
Galați	15	14	1
Ialomița	1	1	0
Ilfov	1	1	0
Iași	11	10	1
Neamț	5	5	0
Olt	2	2	0
Teleorman	4	4	0
Total	*94*	*84 (89.4%)*	*10 (10.6%)*

Farmers perception regarding pollination services

1. Farmers' perception on pollination services and collaboration with beekeepers:

- 60% of interviewed farmers believe that pollinating insects are necessary for agricultural crops in their area and they believe that the yield of these crops does not depend only on the cultivation technology and climatic conditions (these farmers know the importance of pollination);
- 33% of interviewed farmers believe that pollinating insects are necessary for agricultural crops in their area, but they believe that the yield of these crops depends only on the applied cultivation technology and climatic conditions (these farmers know the role of pollinating insects but do not know the importance of pollination);
- 8% of the interviewed farmers did not give a concrete answer, respectively they answered "I don't know" or "Yes" to the question of whether pollinating insects are necessary for agricultural crops in their area, and thus they contradicted the answer at the question of whether the yield of these crops depends only on the cultivation technology used and climatic conditions.

Farmers' perceptions in the oilseed crops industry are complex, with most recognizing the importance of pollinating insects, but also exhibiting uncertainty about the extent of native pollinators' contribution to yield and how to best manage them. A large majority of farmers (over 90%) acknowledge that pollinating insects are crucial for crop production.

The perceptions of farmers in the oilseed crop industry span a wide range of views on sustainability, profitability, and the impact of farming practices. These perceptions are influenced by factors like farm size, experience, access to information, and the specific context of their farming operations.

2. Current status of pollination services.

Regarding the importance of pollination services, the results highlight that the large majority of farmers consider pollination services necessary to improve the productivity of their crops (Table 4).

Table 4. Farmers' answers regarding the yield decrease in the absence of pollination insects

Yield decrease in the absence of pollination insects	Number of respondent farmers	Farmers answer at the question of whether the recorded losses by sunflower and rapeseed crops in the absence of pollination are negligible		
		No	I don't know	Yes
More than 50%	26	15 (57.7%)	6 (23.1%)	5 (16.2%)
Less than 50%	70	43 (61.4%)	12 (17.2%)	15 (21.4%)
Uncertain answer	4	-	-	-
Total	100	58 (58%)	18 (18%)	20 (20%)

70% of the interviewed farmers (70 farmers) responded that in the absence of pollinating insects, the yield of sunflower and rapeseed crops would decrease by less than 50%, but when asked whether the losses recorded in the absence of pollination were negligible, 61.4% of them answered "no".

26% of the interviewed farmers (26 farmers) claimed that in the absence of pollinating insects, the yield of sunflower and rapeseed crops would decrease by over 50%, but when asked whether the losses recorded in the absence of pollination were negligible, 57.7% of them answered "no". Some oilseed crop growers perceive limited benefits from bees, or from investing in pollinator-friendly practices, due to a combination of factors, such as dominance of managed bees, a lack of understanding about the contribution of wild pollinating insects, and the perception that managed bees are sufficient for pollination. Additionally, some farmers may not see a direct, immediate, or significant increase in crop yield from pollinator-friendly practices. There are more reasons:

- Dominance of managed bees: many farmers rely heavily on managed bees, particularly honeybees, for pollination services; these are often seen as a more reliable and controllable option, and farmers may not fully appreciate the role of wild insects in crop pollination.
- Limited understanding of importance of the wild pollinating insects: some farmers may not be aware of the diversity and importance of wild pollinating insects in their local ecosystem; they may not realize that wild bees can contribute significantly to pollination, especially in certain crops or regions.
- Focus on managed bees: there can be a tendency to focus on the benefits of managed bees (e.g., honey production, pollination services) while overlooking the potential benefits of other pollinators; this can lead to a perception that managed bees are the primary or even sole solution for pollination needs.
- Perceived insufficient yield increase: some farmers may not see a substantial and immediate increase in crop yield from investing in pollinator-friendly practices or from relying on wild pollinating insects; this can lead to the perception that the benefits are limited or not worth the effort or cost.

3. The farmer-beekeeper relationship

The obtained results highlight that farmers, who recognized a deficit in pollination services, adopt multiple management strategies to address it. To the question "Are you concerned about the pollination of rapeseed and/or sunflower crops?", the answers were as follows (Table 5):

- 80.8% of the interviewed farmers (76 farmers) responded that they are concerned about the pollination of rapeseed and sunflower crops, of which 85.5% have good and fairly good relations with beekeepers (65 farmers). There was only one case of conflicting relationship.
- 11.7% of the interviewed farmers (11 farmers) responded that they are not concerned about pollination of rapeseed and sunflower crops, but 81.8% of them responded that they have good and fairly good relationships with beekeepers.
- 6.4% of the interviewed farmers (6 farmers) did not answer whether they are concerned about the pollination of rapeseed and sunflower crops, but all of them stated that they have good and fairly good relationships with beekeepers.

Table 5. Farmers′ answers regarding concerns on pollination of rapeseed and/or sunflower crops as well as regarding the relationship with beekeepers

Are you concerned about the pollination of rapeseed and/or sunflower crops?	Number of respondents	How is the relationship between you (farmer) and beekeepers?				
		Very Good	Good	Fairly good	Conflictual	No answer
Yes	76 (80.8%)	1	53	12	1	9
No	11 (11.7%)	-	4	5	-	2
No answer	6 (6.4%)	-	4	2	-	-
Farmer with his own apiary	1 (1.1%)	-	-	-	-	-
Total	*94, out of which:*	***1***	***61***	***19***	***1***	***11***

Among the interviewed farmers, there is one farmer who owns his own beehives.

The relationship between farmers and beekeepers is a symbiotic one, crucial for both agricultural production and the health of bee populations.

Farmers rely on bees for pollination, which is vital for crop yields. Beekeepers, in turn, benefit from the access to various nectar and pollen sources provided by farms, which contributes to honey production and overall hive health.

Some farmers and beekeepers developed long-term partnerships, with the beekeeper managing hives on or near the farm, ensuring a consistent pollination service and a healthy bee population. Long-term partnerships between farmers and beekeepers should become increasingly common, as they are beneficial for both parties. These partnerships, often involving pollination services and honey production, can lead to improved agricultural practices, enhanced environmental sustainability, and increased economic opportunities for both farmers and beekeepers. Partnerships farmer - beekeeper can strengthen rural communities by creating new economic opportunities and promoting collaboration among stakeholders.

4. Determining the number of beehives per hectare for pollination of rapeseed and sunflower crops

For this category, the answers to two questions were correlated, namely; "What do you think is the number of beehives per hectare for pollination of rapeseed and sunflower crops?" and "How is the number of beehives/ha calculated?", the results being the following:

- 15% of the interviewed farmers (14 farmers) turn to beekeepers and know the correct answer. The majority of farmers who turn to beekeepers know the optimal number of hives per unit area (9 out of 14 farmers who turn to beekeepers answered that the number of hives for pollination is between 2 and 6), while 5 out of the 9 farmers who turn to beekeepers declared that they have no idea.
- 45% of the interviewed farmers (42 farmers) calculate the number of honeybee colonies/ha based on the beekeepers' indications. From this category, it is noted that almost half of the farmers (45%) have no idea about the necessary number of honeybee colonies for pollination (19 farmers out of 42).
- 22% of the interviewed farmers (21 farmers) leave the pollination process to chance, although a good portion of them (28%, i.e. 6 farmers out of the 21 interviewed) have solid knowledge about the number of beehives needed per unit area.
- 18% of the interviewed farmers (17 farmers) did not answer any questions.

Farmers often rely on beekeepers to calculate the optimal number of honeybee colonies needed for pollinating their crops. This is because the number of required beehives varies based on several factors, including the specific crop, its attractiveness to bees, the presence of other pollinators, and environmental conditions. Beekeepers, with their expertise in honeybee behaviour and colony health, can assess these factors and recommend the appropriate hive density to maximize pollination and crop yield

Farmers and beekeepers should collaborate to find a balance that benefits both their industries and the environment. This collaboration is crucial for sustainable agriculture and the health of pollinator populations.

The beekeeper's expertise in honeybee behaviour and colony management, combined with the farmer's knowledge of the crop and local conditions, ensures optimal pollination and healthy harvests. By working together, they can optimize crop yields, protect bees from harmful pesticides, and ensure the long-term viability of both farming and beekeeping. Regular communication between farmers and beekeepers is essential to understand each other's needs and concerns.

5. Agricultural practices

For this category, the results were the following: 90% of large farmers (20 farmers) responded that they are willing to modify agricultural practices on their farm to protect bees and half of them consider pollination to be an important production factor in the same way as other agricultural inputs and rank pollination with a score of 10 as a production factor (on a scale from 1 to 10).

Farmers are increasingly interested in practices that support native pollinators. This interest stems from the recognition that healthy pollinator populations are crucial for crop production and ecosystem health.

Generally, farmers understand the importance of native pollinating insects, especially as a form of insurance against honeybee colony losses and poor weather conditions. While native pollinating insects are acknowledged as crucial, particularly in providing pollination services during unfavourable weather conditions when honeybees are less effective, there's a strong reliance on rented honeybee colonies for pollination. This reliance is partly due to a lack of clarity regarding the impact of native pollinating insects on yield and difficulty in monitoring their populations.

Farmers are interested to adopt strategies like planting native flowering plants, creating pollinator-friendly habitats, and implementing practices that support native pollinators, such as wildflower strips and hedgerows, but require more knowledge and resources to implement them effectively.

Farmer' decisions about implementing pollinator-friendly practices are heavily influenced by the perceived costs associated with these practices, even when they understand the ecological benefits. These costs can include direct financial investments in things like planting bee-friendly cover crops or restricting pesticide use, as well as opportunity costs, such as using land for pollinator habitat instead of more profitable crops. Growers also weigh the potential benefits of increased yields or reduced pollination service costs against these costs.

CONCLUSIONS

The farmers showed interest in pollinators; over 90% of respondents considered pollinating insects to be essential for their crops. The majority of interviewed farmers recognize the importance of pollinators, but also exhibit uncertainty about the extent of native pollinators' contribution to yield and how to best manage them.

The majority of farmers growing sunflower and rapeseed consider pollination services necessary to improve the productivity of their crops and they are concerned about the pollination of their crops, but however there are some farmers who perceive limited benefits from bees, or from investing in pollinator-friendly practices.

Among big farmers, it was noticed that majority of the farmers perceive the honey bees (*Apis mellifera*) to be the primary species for the pollination activity. The good perception of big farmers on importance of pollination could change the view of small farmers through the example power, considered to be the safest form of education.

Farmers often rely on beekeepers to establish the optimal number of honeybee colonies needed for pollinating their crops.

The performed survey led to the identification of the following needs:

- Bridging the Knowledge Gap: Scientists and extension experts need to provide farmers with more information on pollination importance, native pollinator ecology and management practices.
- Demonstrating Value: More research is needed to quantify the contribution of native pollinators to yield and to develop effective methods to monitoring their populations.
- Promoting Sustainable Practices: Encouraging the adoption of pollinator-friendly practices is crucial for long-term sustainability.

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EXPERIMENTAL RESEARCH FOR PROMOTING A TECHNICAL EQUIPMENT FOR NARROW STRIP TILLAGE AND DIRECT SEEDING IN THE GRASS COVER OF A GRASS MIXTURE

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Abstract

Considering the imperative of improving the quality of life in the long term and under the pressure of increasing consumer demands for plant and animal agricultural products, there are scientific concerns in Western Europe and developed countries on other continents regarding rational grassland utilization technology. This paper presents the results of experimental research conducted under operational conditions on grassland quality restoration technology in the context of climate change, through narrow strip tillage and direct seeding into the grass cover of a grass mixture or even a single species, while preserving the existing vegetation entirely or to a certain extent.

***Key words**: tillage, direct seeding, meadows.*

INTRODUCTION

Grasslands play a crucial role in capturing and storing carbon, which is why nowadays approximately one-third of the global terrestrial carbon stocks are found in soils, with plant diversity playing an essential role in storing and maintaining this carbon (Bai & Cotrufo, 2022).

Degradation of grasslands generated by climate change, including rising temperatures and decreasing precipitation, contributes to increased CO_2 emissions (Puche et al., 2023).

It is estimated that approximately half of the global natural grasslands are degraded in various forms by overgrazing, climate change, deforestation, or conversion to agricultural land (Bardgett et al., 2021).

Overseeding of grasslands plays a crucial role in capturing and storing carbon. By introducing fast-growing and highly resilient grass and legume species, organic matter production is stimulated, deeper and denser root systems help sequester carbon in the soil for the long term, and a denser vegetation cover protects the soil from erosion while improving its structure.

Overseeding is a sustainable and effective strategy for combating climate change, maintaining soil health, and increasing agricultural productivity (Barszczewski & Horaczek, 2024; Cardarelli et al., 2020; Bondaruk et al., 2020).

Grasslands suitable for overseeding include degraded areas where yields have decreased by approximately 10-90%, depending on the intensity of soil erosion and the degree of degradation, intensively used lands through grazing or mowing, fertile soils with sparse vegetation, as well as areas invaded by weeds or unproductive species that reduce their forage value (Durant & Doublet, 2022).

Overseeding can be performed using various types of agricultural machinery, depending on the land area, soil type, and plant species used. For instance, the grassland regeneration machine developed at National Institute of Research-Development for Machines and Installations Designed for Agriculture and Food Industry-INMA Bucharest, who has the oldest and the most prestigious researching activity in the domain of agricultural machinery and mechanization technologies in Romania, performs multiple operations in a single pass, including narrow strip tillage, direct seeding of a grass mixture, or even a single species, into the existing grass cover while preserving it either entirely or partially, and light soil compaction

over the seeds to ensure proper contact for optimal germination (Manea et al., 2017).
The Institute of Research-Development for Grasslands Brașov - ICDP Brașov, which coordinates the project *“ADER 15.3.2 - Research on the development and promotion of mechanized technologies for the reconstruction and maintenance of permanent grasslands, environmental protection, and biodiversity conservation using new specific equipment”*, has conducted, in collaboration with INMA Bucharest, field experiments (during the autumn season) on an experimental model of a grassland overseeding machine for degraded grasslands. The purpose of these tests was to ensure that the equipment operates according to technical specifications (https://www.madr.ro/attachments/article/504/ADER-1532-Faza-1.pdf).

MATERIALS AND METHODS

At Institute of Research-Development for Grasslands - ICDP Brașov, a technology has been developed for improving degraded grasslands through the direct overseeding of seeds into the soil using an experimental model of overseeding machine for degraded grasslands, without affecting the existing vegetation cover. This approach contributes to increasing forage production and conserving biodiversity (Mocanu et al., 2025).
The experimental model of overseeding machine for degraded grasslands (Fig. 1) consists of the following main components: assembled frame, furrow-opening equipment, seeding equipment, motion transmission mechanism for the seeding equipment, and furrow-compacting wheels (Marușca, 2008).

Figure 1. Experimental model of overseeding machine for degraded grasslands

During the experiments to determine operational indices, a TL 100A New Holland tractor, available at ICDPP Brașov, was used, with the experimental model of overseeding machine for degraded grasslands coupled to it.
When the tractor-overseeding machine unit is in operation, the rimmed discs of the furrow-opening sections vertically and longitudinally fracture the old sod, the coulters create the furrow and place the forage plant seeds at the desired depth, as distributed by the machine’s seeding equipment, while the compacting wheels ensure proper seed-to-soil contact along each seeded row (Mocanu & Hermenean, 2008).
Table 1 presents the main technical characteristics of the experimental model of overseeding machine for degraded grasslands (Marușca, 2008).

Table 1. The main technical characteristics of the equipment used in the experiment

Characteristic	UM	Value
Working width	m	2.55
Working depth	cm	0.5...4
Number of processed rows	pcs.	17
Minimum row spacing	cm	15
Terrain contour following range per processed row	cm	± 10
Volume of the perennial grass seed compartment	dm^3	55
Seeding rate adjustment for perennial grasses	-	Northon gearbox with 72 steps
Seeding rate adjustment for perennial legumes	-	adjustable grooved cylinder length
Mass	kg	1175

The tests were conducted on the experimental fields of ICDP Brașov in accordance with a specific testing procedure.
The average climatic data recorded at the experimental research location were as follows: in August 2024, the average daily temperature was 13°C, and the average precipitation was 74 mm; in September 2024, the average daily temperature was 9°C, and the average precipitation was 56 mm.
The determination of operational indices was carried out through field experimental research on a grassland plot characterized by low herbaceous vegetation cover, poor floristic composition, and a low percentage of clover, alfalfa, and ryegrass.
For the seeds used in the experiments, the following physical and mechanical characteristics were determined: purity degree, thousand-seed weight, hectoliter mass, and

moisture content. The measurements were performed using an electronic balance with an accuracy of ±0.1 grams, in three repetitions. The average values obtained are presented in Table 2.

Table 2. Physical and mechanical characteristics of the seeds used in the experiments

Seed name	Degree of purity %	Thousand-seed weight g	Hectoliter mass g/dm^3	Moisture content %
Trifolium repens	99.02	0.62	70.63	9.5
Lolium perenne	97.32	1.72	28.05	8.8

The additional equipment used to determine the soil characteristics of the experimental field included: a FIELDSCOUT SC 900 digital electronic penetrometer for measuring soil penetration resistance (Kumar, 2023) a portable Extech MO750 soil moisture meter for the direct measurement of soil water content, expressed as a percentage of soil volume (Hilal et al., 2022).
Table 3 presents the characteristics of the experimental field, where the tests were conducted using the experimental model of overseeding machine for degraded grasslands, coupled with the TL 100A New Holland tractor.

Table 3 Characteristics of the experimental field

No.	Characteristic	UM	Value
1	Soil type	-	podzolic
2	Degree of soil coverage with vegetation	%	82
3	Average plant height	cm	4.8
4	Plant mass	g/m2	46
5	Soil moisture at 0…10 cm depth	%	11.8
6	Soil penetration resistance at 10 cm depth	MPa	2750

The calculation of the slippage (δ) of TL 100A New Holland tractor's drive wheels which occurs due to the traction power transmission process, was performed using Equation (1). The slippage was determined by measuring the average working speed (v_l) over a specific distance (s) and counting the number of loaded (n_s) and unloaded (n_g) wheel rotations during the tests.

$$\delta = \frac{(n_s - n_g)}{n_s} \times 100, \%$$ (Persu et al., 2020) (1)

The traction force was determined using the tensometric method, which involved the use of strain gauges, a data acquisition system, and a computer equipped with specialized software packages. The stored data were retrieved and processed using specialized software, after which the average values of the traction force were determined by eliminating transition regimes recorded during the experimental research (Persu et al., 2020).
The traction power (P_{tr}) was calculated based on the travel speed (v_l) and the traction force (F_{tr}) (Marin et al., 2012).
The determination of operational indices, including effective working time, time losses, actual working capacity, and fuel consumption per hectare, for the experimental model of overseeding machine for degraded grasslands coupled with the TL 100A New Holland tractor, allowed for the evaluation of its performance and efficiency in the agricultural process. This assessment aimed to ensure maximum economic benefits under the specific conditions of each farm (Hunt, 2008).
The fuel consumption per hectare was determined by directly measuring the volume of fuel consumed. This process involved: completely filling the fuel tank before the overseeding operation, performing the overseeding task on a determined area, and refilling the tank after the operation to measure the volume of fuel added (Voicu et al., 2020).

RESULTS AND DISCUSSIONS

The determination of soil penetration resistance provided an accurate assessment of soil compaction. The average value of 1374 kPa, measured in situ, indicated that it was close to the upper permissible limit - as values above 2000 kPa significantly hinder root penetration, compromising plant growth.
By analyzing soil moisture in the experimental area, the presence of water in the soil was confirmed, which is beneficial for seed germination and emergence processes, particularly for *Trifolium repens* and *Lolium perenne*.
The soil penetration resistance of 1394 kPa (approximately 1.39 MPa) combined with a volumetric moisture content of 17% in podzolic soil suggests relatively high compaction, which could hinder the root development of *Trifolium repens* and *Lolium perenne* seeds. This compaction, along with low moisture levels, may negatively impact germination, as seeds could struggle to penetrate the soil, leading to uneven emergence and reduced plant development.

To improve germination conditions, a lighter compaction roller was used on the overseeding machine during the overseeding process to prevent excessive soil compaction.
Table 4 presents the average values of soil penetration resistance and soil moisture obtained in the 0...10 cm layer.

Tabel 4 Average values of soil characteristics in the experimental field

No.	Characteristic	UM	Value
1	Average soil penetration resistance	kPa	1374
2	Average soil moisture	%	17

Table 5 presents the average values of energy indices (average working speed and wheel slip of the tractor in the unit) recorded for two speed levels of the tractor during uphill and downhill travel. Slip of the tractor's drive wheels uphill increased in second gear, because the tractor needed more torque at the wheels, as a result of the increase in speed and forward resistance and downhill it was reduced in both cases, due to the lower mechanical load on the transmission.
By using the second gear, a moderate increase in working speed is achieved, with a relatively small impact on slip of the tractor's drive wheels, especially downhill. Conversely uphill, increased of tractor's drive wheels slip may indicate a possible reduction in energy efficiency, caused by difficult terrain conditions.

Table 5 Average working speed and wheel slip of the tractor unit

Speed level	Average working speed uphill, (km/h)	Average working speed downhill, (km/h)	Slip of the tractor's drive wheels uphill (%)	Slip of the tractor's drive wheels downhill (%)
V_1	6.08	6.24	18.79	14.25
V_2	6.79	6.95	19.39	14.95

Table 6 presents the average values of traction force (F_{tr}) and traction power (P_{tr}) recorded for two speed levels of the tractor unit during uphill and downhill travel.

Table 6 Average values of traction force and traction power

	Average traction force	Average traction power	
	uphill	uphill	
	[daN]	[CP]	[kW]
V_1	379.29	8.54	6.28
V_2	423.74	10.66	7.84
	downhill	downhill	
V_1	334.84	7.74	5.69
V_2	389.23	10.02	7.37

An example of the data acquisition visualization panel used for determining the traction force is shown in Figure 2.

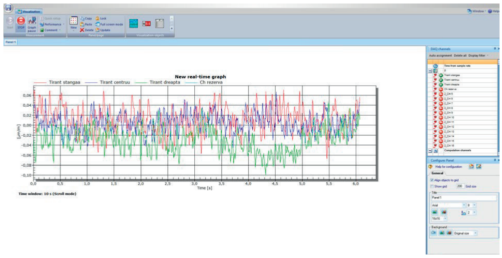

Figure 2. View of the data acquisition process for measuring traction resistance

In Figure 3, a graphical representation is shown of the variation of the determined indices at two speed levels for the experimental model of overseeding machine for degraded grasslands coupled with the TL 100A New Holland tractor on the ICDP Brașov experimental field during uphill travel.

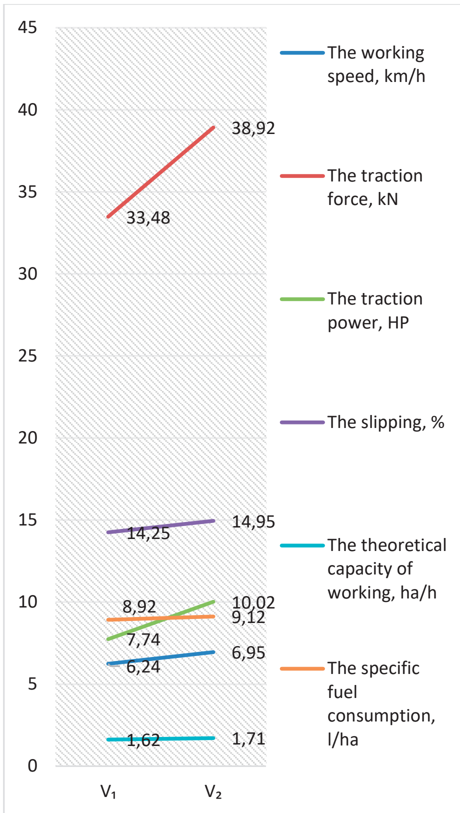

Figure 3. Graphical representation of the variation of the determined indices during uphill travel

In Figure 4, a graphical representation is shown of the variation of the determined indices at two speed levels for the experimental model of overseeding machine for degraded grasslands coupled with the TL 100A New Holland tractor on the ICDP Brașov experimental field during downhill travel.

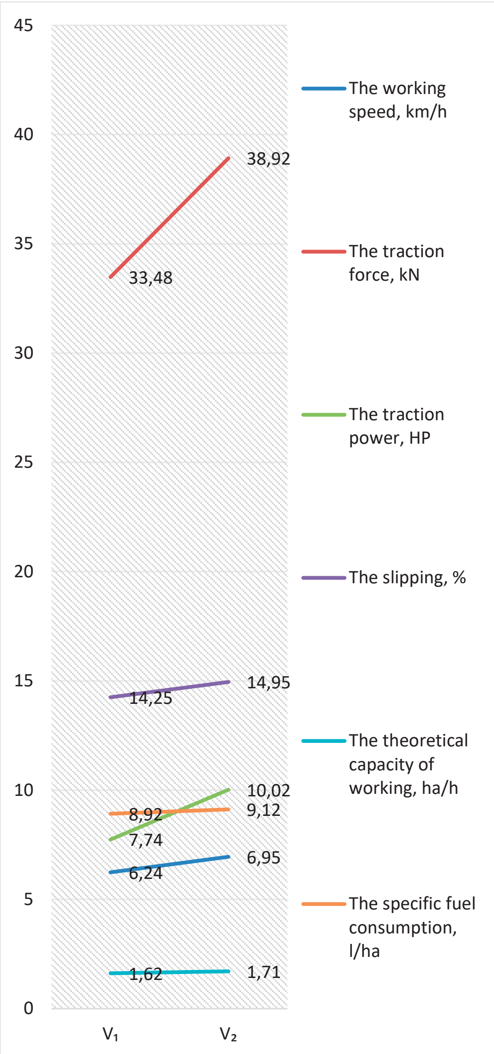

Figure 4. Graphical representation of the variation of the determined indices during downhill travel

Table 7 presents the results of the operational indices during the working process of the experimental model of overseeding machine for degraded grasslands, coupled with the TL 100A New Holland tractor, on the ICDP Brașov experimental field over an area of 1 hectare.

Table 7. Technological parameters during work coupled with TL 100A New Holland

Specification	**UM**	**Value**
Fertilization type	-	NPK
Average displacement speed	km/h	1.52
Surface	ha	1
Real working time, T_1	min	39.2
Working hourly capacity at real time, W_{ef}	ha/h	1.53
Capacity of working per hour at shift time W_{07}	ha/h	1.32
Coefficient of technological safety, K_{41}	-	0.99
Coefficient of technological safety, K_{42}	-	1.0
Coefficient of reliability, K_4	-	0.93
Coefficient of using the shift time, K_{07}	-	0.86
Fuel consumption per hectare	l/ha	9.10

During operational experiments conducted over an area of 1 hectare, the experimental model performed well throughout the testing period. Under these conditions, it achieved: an operational safety coefficient of 0.99, a performance-based hourly working capacity of 1.32 ha/h, and a fuel consumption rate of 9.10 l/ha.

Figure 5 presents two aspects from the operational experiments conducted on the experimental field owned by ICDP Brașov.

Figure 5. Aspects from the operational experiments conducted in field conditions

CONCLUSIONS

From an operational perspective, the overseeding machine for degraded grasslands successfully met the technological parameters, which are in compliance with the agrotechnical requirements for the overseeding work.

During the tests, the experimental model of overseeding machine for degraded grasslands, coupled with the TL 100A New Holland tractor, demonstrated good performance, achieving an average working capacity of 1.53 ha/h and an average fuel consumption of 9.10 l/ha.

The experimental research confirmed the functionality of the technical solutions implemented in the design of the experimental model, ensuring that a competitive piece of equipment can be offered to stakeholders. This equipment is designed to improve degraded permanent grasslands with low-inputs through surface measures.

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THE PRODUCTIVITY AND QUALITI OF *Arrhenatherum elatius* GRASSLANDS FROM THE ORHEI NATIONAL PARK, REPUBLIC OF MOLDOVA

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Abstract

This article presents new findings on the productivity of grasslands in Orhei National Park (Republic of Moldova). The research was conducted during 2021-2022 on a grassland dominated by Arrhenatherum elatius, located in the village of Neculaeuca, Orhei district. Hay yield ranged from 4.13 to 5.63 t/ha. The dry matter of the hay contained 107-142 g/kg CP, 343-373 g/kg CF, 93-106 g/kg ash, 373-395 g/kg ADF, 595-648 g/kg NDF, 43-44 g/kg ADL, 57-85 g/kg TSS, 330-351 g/kg Cel, and 22 g/kg HC. The nutritive and energy values were as follows: 58.1-61.9% DMD, RFV = 83-97, 11.53-12.21 MJ/kg DE, 9.47-10.03 MJ/kg ME, and 5.49-5.74 MJ/kg NEl. In addition to its fodder value, the hay substrate demonstrated an optimal lignin and hemicellulose content for anaerobic digestion, with an estimated biochemical methane potential ranging from 313 to 362 l/kg VS.

Key words: *Arrhenatherum elatius grasslands, biochemical methane potential, fodder values, hay productivity, Orhei National Park.*

INTRODUCTION

Grasslands hold significant ecological importance, as they comprise a substantial portion of terrestrial habitats. In Europe, grasslands cover approximately 1.8 million hectares, accounting for about 40% of the land surface. Natural grasslands play a crucial role in conserving European phytodiversity. They also provide essential ecosystem services, including carbon sequestration, erosion control, and habitat for a wide range of plant and animal species (Dengler et al., 2014). The sustainable management of grassland biomass has become a growing challenge across Europe. While the primary function of grasslands remains the production of forage for livestock, recent studies have shown that grassland biomass also serves as a valuable substrate for biofuel production and biorefining processes (Dindová et al. 2019; Von Cossel et al. 2019; Carni et al. 2021; Schaub et al. 2025).

Arrhenatherion meadows are among the most common grassland plant communities and serve as an important resource for the ecological restoration of species-rich grasslands. In Europe, these communities are often among the most biodiverse ecosystems, both at the microhabitat scale and across broader landscapes, underscoring the need for their protection and sustainable management. To preserve and enhance plant biodiversity, hay mowing followed by hay removal is generally recommended over grazing. Typically, *Arrhenatherum elatius* meadows are mown two to three times per year. Additionally, these meadows are frequently used as seed sources in grassland restoration efforts (Graiss et al. 2013, Scotton, 2016).

Currently, *Arrhenatheretum elatioris* meadows developed in a typical form are becoming more and more rare. *Arrhenatheretum elatioris* grassland are studied in different scientific centres and universities, and implemented with multiple uses in different regions of the Earth (D'Ottavio & Ziliotto, 2003; Wyłupek, 2006; Kryszak & Kryszak, 2007; Goliński & Goliński, 2013; Meserszmit et al. 2024).

Orhei National Park was founded in 2013 and operates under the Law on the Fund of Natural Areas Protected by the State and the National Strategy for the Conservation of Biological Diversity, it covers 33.8 thousand hectares and includes the territory of 18 communes from four districts – Orhei, Straseni, Calarasi and Criuleni located in the Central Zone- Codrii being the first park of its kind in the Republic of Moldova. With a unique relief in Europe, Orhei National Park offers important scientific, educational and tourist opportunities, while contributing to stopping the degradation of forest ecosystems, illegal logging, deterioration of pastures and forests, sustainable management of natural ecosystems, as well as promoting organic agriculture and ecotourism. The variety of local natural conditions of relief and climate determined the diversity of the flora in the Orhei National Park, where over 700 species of flora belonging to 334 genera and 80 families from the *Pteridophyta* (0.2%) and *Magnoliophyta* (98.0%) phyla were inventoried, 52 rare plant species with varying conservation statuses were highlighted according to the International Union for Conservation of Nature IUCN. The plant species have a valuable phytoeconomic potential with multiple utility, such as fodder, food, ornamental, medicinal, melliferous, etc.

The aim of this research was to evaluate the hay productivity of *Arrhenatherum elatius* grasslands, as well as the economic value of the hay as livestock feed and as a substrate for biomethane production.

MATERIALS AND METHODS

The research was conducted during 2021-2022 on an *Arrhenatherum elatius* grassland located in the village of Neculaeuca, Orhei District, within Orhei National Park, Republic of Moldova. Samples were collected from the first hay cut. Mowing was performed at the inflorescence emergence stage of the dominant species, tall oat grass (*Arrhenatherum elatius*). The harvested phytomass was dried directly in the field. Hay yield was determined by weighing the total dry phytomass from plots measuring 10 m² (5 × 2 m), with five replications. The dry matter content was assessed by oven-drying samples at 105 °C until a constant weight was achieved. For chemical analysis, hay samples were chopped into pieces 1.5-2.0 cm in length, dried in a forced-air oven at 60 °C, and then milled using a beater mill equipped with a 1 mm sieve. The primary biochemical parameters – crude protein (CP), ash, acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL), and total soluble sugars (TSS) – were determined using near-infrared spectroscopy (NIRS) with a Perten DA 7200 analyzer at the Research and Development Institute for Grasslands, Brașov, Romania. The concentrations of hemicellulose (HC), cellulose (Cel), digestible dry matter (DDM) digestible energy (DE), metabolizable energy (ME), net energy for lactation (NEl) and relative feed value (RFV) were calculated following standard procedures.

The carbon content of the substrates was determined using an empirical equation according to Badger et al. (1979). The biochemical methane potential was calculated according to the equations of Dandikas et al. (2015).

RESULTS AND DISCUSSIONS

It is well established that forage yield and quality are primarily influenced by the floristic composition of the grassland, soil characteristics, precipitation patterns and distribution, temperature conditions, and light availability. Hay is a low-cost source of roughage and essential nutrients, playing a critical role in maintaining livestock health and productivity, particularly from autumn through mid-spring. The prepared hay from *Arrhenatherum elatius* grasslands contained 87.8–90.4% dry matter. The results of the hay quality analysis from *Arrhenatherum elatius* grasslands are presented in Table 1. The hay dry matter contained 107-142g/kg CP, 343-373 g/kg CF, 93-106 g/kg ash, 373- 395g/kg ADF, 595-648 g/kg NDF, 43-44 g/kg ADL,57-85 g/kg TSS, 330-351 g/kg Cel, 22 g/kg HC, with nutritive and energy value 58.1-61.9% DMD, RFV=83-97, 11.53-12.21 MJ/kg DE, 9.47-10.03 MJ/kg ME, 5.74-5.49 MJ/kg NEl. The hay prepared during the 2021 growing season was characterized by a higher content of crude protein and minerals, and a lower concentration of total soluble sugars, cellulose, and hemicellulose, which contributed to improved digestibility and a higher energy concentration compared to the hay prepared in the 2022 growing season.

In the specialized literature, there are various data on the forage quality of *Arrhenatherum elatius* plants. Medvedev & Smetannikova (1981) mentioned that *Arrhenatherum elatius* hay contained 7.6-12.7% CP, 1.6-3.4% EE, 23.2-32.0% CF, 36.0-50.0% NFE, 7.0-10.0% ash. D'Ottavio & Ziliotto (2003) reported that forage from grassland with most abundant species *Arrhenatherum elatius*, *Agropyron repens*, *Dactylis glomerata*, *Lolium perenne*, *Trisetum flavescens*, *Trifolium pratense*, *Trifolium repens* and *Taraxacum officinale* achieved at 1st cut dry matter yield 6.48 t/ha with 5.08% CP, 1.54% EE, 6.09% ash, 35.66% CF, 61.34% NDF, 35.43% ADF, 6.09% ADL, 3.19 MJ/kg NEL, while from the 2nd cut – 3.66 t/ha DM, 7.19% CP, 2.19% EE, 7.46% ash, 31.10% CF, 59.58% NDF, 31.35% ADF, 5.166.09% ADL, and 4.60 MJ/kg NEL. Wyłupek (2006) mentioned that content of some nutrients in selected *Arrhenatheretum elatioris* phytocenoses biomass was 11.37% CP, 1.22 g/kg P, 7.1 g/kg K, 2.04 g/kg Mg, 3.36 g/kg Ca. Heinsoo et al. (2010) found that the nutritive value of forage from mesic meadows was 9.4% CP, 59.7% NDF, 6.1% ash, 18 MJ/kg GCV. Skládanka et al. (2008, 2010) found that the forage dry matter from *Arrhenatherum elatius* plants contained 7.92-9.49% CP, 29.34-30.25% CF, 55.48-61.20% NDF, 35.9% ADF, 71.80-78.0% OMD and 5.46 MJ/kg NEl. Tomić et al. (2005) reported that the grass quality of *Arrhenatherum elatius* grown the pasture associations was 6.28% CP, 30.07% CF, 8.11% ash. Cop et al. (2009) reported that dry matter from *Arrhenatherum elatius* grassland contained 11.74-15.91% CP, 24.48-29.67% CF, and 5.4-6.0 MJ/kg NEl. Grygierzec (2012) reported that hay from the *Arrhenatheretum elatioris typicum* community meadow contained 111-121 g/kg CP, 61.6-63.2 g/kg ash, 510-512 g/kg NFE, 129.9-130.1 g/kg EE, 446-474 g/kg NDF, 338-340 g/kg ADF, 62 g/kg ADL, and 268-283 g/kg cellulose. Goliński and Goliński (2013) noted that biomass from semi-natural grasslands, primarily represented by the *Arrhenatherion* alliance, had 308 g/kg dry matter, with 10.35% CP, 6.36% ash, 50.98% NDF, 31.61% ADF. Von Cossel et al. (2019) reported that the first-cut biomass from *Arrhenatherion* grasslands was characterized by 240-297 g/kg DM with 7.0-8.1% ash, 4.7-5.7% lignin, 29.3-31.9% Cel, 20.7-25.2% HC and 1.4-1.7 % N. Meserszmit et al. (2021) mentioned that the herbage from *Arrhenatherum elatius* and *Dactylis glomerata* plant community contained 8.00% CP, 3.17% EE, 57.30% NDF, 16.56% HC, 29.47% Cel, 11.24% lignin, 7.73% ash. Reiné et al. (2020) remarked that *Arrhenatherum elatius* contained 421 g/kg DM with 7.6% CP, 4.5% ash, 1.6% EE, 66.5% NDF, 35.2% ADF, 3.0% ADL, 61.5% DDM, 0.13% P, 0.50% Ca. Țîței (2024) found that the hay prepared from tall oatgrass plants grown in monoculture contained 77 g/kg CP, 414 g/kg CF, 80 g/kg ash, 436 g/kg ADF, 740 g/kg NDF, 40 g/kg ADL, 98 g/kg TSS, 396 g/kg Cel and 304 g/kg HC, with nutritive and energy value of 54.1% DDM, 10.82 MJ/kg DE, 8.88 MJ/kg ME and 5.02 MJ/kg NEl.

The use of phytomass from grassland as substrate for biogas production has recently become of major interest in Europe. The results regarding the quality indices of studied hay substrates for anaerobic digestion and the its potential for obtaining biomethane are shown in Table 2. We found that in the investigated hay substrates, according to the C/N ratio, which constituted 21.86-29.43, the amount of acid detergent lignin (43-44 g/kg) and hemicellulose (222-25 3g/kg) met the established standards and biochemical methane potential achieved 303-306 l/kg DM. Several literature sources describe the methane yield of the biomass substrates from *Arrhenatheretum elatioris* grasslands. According to Prochnow et al. (2009) biomethane yield varied from 155 to 293 l/kg VS. Ebeling et al. (2013) reported that dependent of harvest dates and levels of fertilizer application the specific methane yield Goliński and Goliński (2013) reported a methane yield of 338 l/kg VS from substrates originating from semi-natural grasslands dominated by the *Arrhenatherion* alliance. Herrmann et al. (2013) found that methane yields of grassland biomass decreased substantially with later harvest, from up to 309 l/ kg organic dry matter in May to below 60 l/ kg organic dry matter in February, in correlation with increasing crude fibre contents. Boob et al. (2019) found that the methane yield of the biomass from *Arrhenatherion* grasslands was 300 l/kg VS. Von Cossel et al. (2019) remarked that methane yield of the first-cut biomass substrate from *Arrhenatherion* grasslands ranged from 289 to 297 l/kg VS. Meserszmit et al. (2021) mentioned that the methane yield of herbage from *Arrhenatherum elatius* and *Dactylis glomerata* was 249 l/kg VS. Ababii et al. (2019) revealed that biomethane production potential of hay substrates from *Festuca arundinacea was* 346 l/kg VS, but of substrate from *Arrhenatherum elatius* hay *343* l/kg VS. In our previous study, Miron et al. (2023) found that the best methane yield from hay substrates collected from grasslands dominated by *Poa pratensis* ranged from 282 to 314 l/kg VS. Brandhorst et al. (2024) observed that the specific methane yield of substrates from orchard meadows decreased linearly with delayed cutting dates, from dates from 0.325 to 0.237 m^3/kg ODM.

Table 1. The biochemical composition and the nutritive value of the hay from *Arrhenatherum elatius* grasslands

Indices	growing seasons	
	2021	2022
Crude protein, g/kg DM	142	107
Minerals, g/kg DM	106	93
Crude fibre, g/kg DM	347	373
Acid detergent fibre, g/kg DM ,	373	395
Neutral detergent fibre, g/kg DM	595	648
Acid detergent lignin, g/kg DM	43	44
Total soluble sugars, g/kg DM	57	85
Cellulose, g/kg DM	330	351
Hemicellulose, g/kg DM	222	253
Digestible dry matter, g/kg DM	619	581
Relative feed value	97	83
Digestible energy, MJ/ kg	12.21	11.53
Metabolizable energy, MJ/ kg	10.03	9.47
Net energy for lactation, MJ/ kg	5.74	5.49

Table 2. The biochemical biomethane production potential of the investigated hay substrates from *Arrhenatherum elatius* grasslands

Indices	growing seasons	
	2021	*2022*
Crude protein, g/kg DM	142.00	107.00
Nitrogen, g/kg DM	22.72	17.12
Minerals, g/kg DM	106.00	93.00
Organic matter, g/kg	894.00	907.00
Carbon, g/kg DM	496.67	503.89
Ratio carbon/nitrogen	21.86	29.43
Acid detergent lignin, g/kg DM	43.00	44.00
Hemicellulose, g/kg DM	222.00	253.00
Biomethane potential, L/kg VS	342	334
Biomethane potential, L/kg DM	306	303

Table 3. The economic value of the investigated hay from *Arrhenatherum elatius grasslands*

Indices	growing seasons	
	2021	*2022*
Hay yield, t/ha	5.63	4.13
Dry matter hay yield, t/ha	4.94	3.73
Crude protein, kg/ha	702	400
Digestible protein, kg/ha	421	240
Metabolizable energy, GJ/ ha	49.5	35.3
Net energy for lactation, GJ/ ha	28.4	20.5
Biomethane yield, m^3/ha L/kg VS	1512	1130

Grassland yield and its quality indices are economically significant aspects of relevant forage and energy production. The economic value of hay collected from *Arrhenatherum elatius* grasslands is presented in Table 3. The estimated economic potential of hay from the first cut is reflected in the following indicators: 400-700 kg/ha/year of crude protein, 240-421 kg/ha/year of digestible protein, 35.3-49.5 GJ/ha/year of metabolizable energy, and 20.5-28.4 GJ/ha/year of net energy for lactation. The biomethane yield potential from hay substrates is estimated at 1130-1512 m^3/ha/year.

Several literature sources have described the productivity of *Arrhenatheretum elatioris* grasslands. Wyłupek (2006) found that average yield of dry mass from the spring regrowth of semi-natural *Arrhenatheretum elatioris* was 3.14 t/ha. Kryszak & Kryszak (2007) reported that yield of meadows with *Arrhenatheretum elatioris dactylidosum glomeratae* were 7.5-9.0 t/ha hay, while in meadows which *Arrhenatheretum elatioris brizosum mediae* sub-association were low productivity 1.5-2.5 t/ha dry matter. Cop et al. (2009) mentioned that annual productivity of non-fertilized *Arrhenatherum elatius* grassland was 5.17-6.15 t/ha dry matter. Grygierzec (2012) mentioned that total yields of dry mass from the *Arrhenatheretum elatioris* meadow fluctuated from 4.92 to 6.57 t/ha, whereas hay yield from the first cut ranged from 3.14 to 4.26 t/ha. Tomić et al. (2018) revealed that the hay productivity of nonfertilized *Arrhenhenatherum elatius* grasslands was 3.50 t/ha, but fertilization treatments applied 5.98-8.31 t/ha. Meserszmit et al. (2024) mentioned that *Arrhenatherum elatius* and *Dactylis glomerata* plant community achieved yield 3.55-3.77 t/ha and methane yield 1564-1649 m^3 /ha. Miron et al. (2023) found that methane productivity of *Elymus repens* grassland ranged from 1217 to 2273 m^3/ha, grassland with *Poa pratensis* – 433 to 998 m^3/ha, while with *Festuca valesiaca* – 409 to 941 m^3/ha, respectively. Brandhorst et al. (2024) mentioned that cumulated area-related methane yields of the orchard meadows ranged from 818 m^3/ha to 1036 m^3 /ha.

CONCLUSIONS

In Orhei National Park the hay productivity of grassland with *Arrhenatheretum elatioris* varied from 4.13 to 5.63 t/ha with 107-142 g/kg CP, 343-373 g/kg CF, 93-106 g/kg ash, 43-44 g/kg ADL,57-85 g/kg TSS, 330-351 g/kg Cel, 22 g/kg HC, 58.1-61.9% DMD, RFV=83-97, 9.47-10.03 MJ/kg ME, 5.74- 5.49 MJ/kg NEl.

The economic potential of collected hays from first cut are 400-700 kg/ha/year of crude protein or 240-421kg/ha/year of digestible protein, 35.3-49.5 GJ/ha/year of metabolizable energy, and 20.5-28.4 GJ/ha/year of net energy for lactation.

The biomethane yield potential from hays substrates 1130-1512 m^3/ha/year.

The hay collected from the studied *Arrhenatheretum elatioris* grasslands can be used as fodder for livestock, also as energy biomass for biomethane production.

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REGENERATION DYNAMICS OF THE BEECH FOREST IN THE UPPER BASIN OF THE NAIBA VALLEY, GODEANU MOUNTAINS

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Abstract

The imbalances produced by climate change, wind blows, excessive drought, increasingly high temperatures, excessive drought, the lack of precipitation or their decreasing amount can greatly affect the synchrony of regeneration and, therefore, the stability of beech forests in Romania and around the world. Studies on the dynamics of regeneration of beech stands are based research regarding: the structure of the stand, the dynamics of the tree population, the characteristics of gap formation, the distribution and characteristics of the shrub layer the vitality of the individuals within the analyzed beech populations, the distribution and characteristics of the seed. In order for natural regeneration to take place in the seedbed, it is necessary to have enough mature trees, able to bear fruit abundantly and seed the entire surface. Following the research carried out in Naiba Valley, it was found that there are favorable conditions for the natural regeneration of the beech groves and the dynamics of the regeneration is good in relation to the current eco-pedo-climatic conditions due to appropriate and adapted conservation measures in this area.

Key words*: beech forest, Naiba Valley, regeneration, dynamics, plant community.*

INTRODUCTION

Carpathian beech forests are of particular importance from an ecological point of view and have considerable economic value.

The problem of the natural regeneration of the edifying species of these types of forests European beech (*Fagus sylvatica* L.) is very topical and an important concern of specialists in the field in recent years.

A regeneration with good results involves studies on the main factors that directly or indirectly influence this process. The value of the genetic resources, the morpho-anatomical characters of the naturally regenerated saplings, the ecological factors such as tolerance to shade and increased temperatures, drought, are of particular importance to the degree of beech regeneration.

The beech forest can beconsidered a complex phytocenotic structure whose dynamics, physiognomy, composition, state of health, vitality, conservation and regeneration are in close correlation with the current eco-pedo-climatic conditions and at the same time with the degree of anthropogenic impact exerted.

The natural regeneration of beech forests in Romania is a very important step to ensure their long-term sustainability.

Regeneration of forests in accordance with climate change and the anthropogenic impact resulting from socio-economic activities, represent challenges and opportunities regardingas good as possible forest management.

Seed germination is closely correlated with soil conditions, climate factors, and at the same time subsequent seedling growth will become more and more dependent on soil characteristics and prevailing microclimatic conditions. The growth rate of seedlings at early stages is an important factor affecting their survival (Doniță et al., 2007; Chiriță et al., 1977).

The objectives of this article are based on the analysis of the current state of regeneration of the beech forests in the Naiba Valley in order to determine the correct regeneration mechanisms and the proposal of research strategies to improve regeneration in these beech forests.

MATERIALS AND METHODS

Study area:

The research area is located in the south-western part of Romania, in the upper basin of Naiba Valley, an integral part of the "Domogled-Valea Cernei" National Park

(Figure 1).From a physical-geographical point of view, the researched territory belongs to the Godeanu Mountains, the group of mountains that is part of the Romanian Southern Carpathians. The Godeanu Mountains, whose main peak forms the basin between the Cerna and Râului Mare rivers, have a maximum altitude of 2.291m at the Gugu Peak.

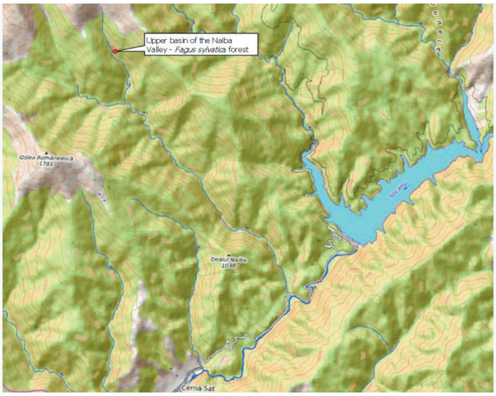

Figure 1. Map of the studied territory

Data Collection:

The data were collected from 20 experimental areas with a size of 400 m^2 (40 x 10 m), installed in comparable stands in terms of vegetation conditions, structural and physiognomic features.

Phytosociological studies were based on the methods of research characteristic of Central European phyto-sociological School and on the principles of Braun-Blanquet (1932).

To identify the taxa and infrataxa it was analyzed Romanian Flora, vol. I (Săvulescu coord., 1966), Flora Europaea (Tutin et al., 1968-1993). The plant communities were identified according to the characteristic, edifying, dominant and differential species.

Their identification and coenotaxonomic classification were made using synthesis works of Coldea (1975, 1991, 1997), Sanda et al. (1997), as well as Oberdorfer (1992), Mucina et al. (1993, 2016) and Rodwell (2002).

To determine the dynamics of beech regeneration within the identified plant community, 20 sample areas smaller than 100 m^2 (10 x 10 m), in forest of the upper basin of the Naiba Valley, were also selected, located in each described phytocoenosis, where analyses were made regarding the dynamics of the seedling, the germination rate, as well as the morphological state, viability, vitality and abundance-dominance of beech seedlings.

The vitality, viability and germination power of the analyzed beech species were determined through direct observations of the morphology of vegetative and reproductive organs as well as the analysis of the population size of individuals in the established sample areas.

In order to determine the level of regeneration of a beech tree, we must pay special attention to the following aspects: the degree of fruiting, the distribution and characteristics of the seed, the survival and seedling population dynamics and gap formation (Doniță et al., 2007; Chiriță et al., 1977).

RESULTS AND DISCUSSIONS

Ecological and cenological characterization of the species *Fagus sylvatica* L. in the investigated territory

Following field research in the beech forests in the upper basin of the Naiba Valley, an integral part of the "Domogled-Valea Cernei" National Park, it was found that they belong to the following plant community:*Hieracio rotundati-Fagetum* (Vida 1963) Tauber 1987 (Syn.: *Deschampsio flexuosae-Fagetum* Sóo 1962) (Boșcaiu, 1971; Niculescu, 2020) (Figure 2). From a conservation point of view, this plant community has a special importance in the edification of the following types of natural habitats: 9110 - *Luzulo-Fagetum* beech forests (RO habitat type code: R4102, R4105, R4106, R4107, R4110; CLAS. PAL.: 41.11) this being interwoven at the lower limit with the habitats 91V0 - Dacian Beech forest (Symphyto-*Fagion*) (RO habitat type code: R4101, R4103, R4104, R4108, R4109, R4116) and 91K0 - Illyrian *Fagus sylvatica* forests (*Aremonio-Fagion*) (RO habitat type code: R4112-4115, R4121; CLAS. PAL.: 41.1C) (Gafta and Mountford, coord. 2008).

The edifying species for the plant community that builds this habitat are *Fagus sylvatica* L. and *Hieracium transylvanicum* Heuff., alongside which there is a nucleus of well-defined species belonging in particular to the order FAGETALIA SYLVATICAE Pawl. 1928 and the alliance SYMPHYTO-FAGION Vida 1959.

The research we conducted in this forest habitat has highlighted the presence of well-structured phytocoenoses from a floristic, physiognomic and coenotic point of view, thus we can mention the following species in the grassy layer that have constancy and high abundance-dominance: *Luzula luzuloides* (Lam) Dandy & Wilmott, *Calamagrostis arundinacea* (L.) Roth, *Vaccinium myrtillus* L., *Galium odoratum* (L.) Scop., *G. schultesii* Vest, *Oxalis acetosella* L., *Dentaria glandulosa* W. et K., *D. bulbifera* L., *Deschampsia flexuosa* (L.) Trin., *Veronica officinalis* L., *Pteridium aquilinum* (L.) Kuhn., *Carex pilosa* Scop., *Mycelis muralis* (L.) Dumort, *Poa nemoralis* L., *Athyrium filix-femina* (L.) Roth., *Dryopteris filix-mas* (L.) Schott., *Viola reichenbachiana* Jord., *Rubus idaeus* L., *Glechoma hederacea* L., *Leucanthemum waldsteinii* (Schultz Bip.) Pouzar, *Actaea spicata* L., *Asarum europaeum* L., *Helleborus purpurascens* Waldst. et Kit., *Euphorbia amygdaloides* L., *Melica uniflora* L., *Stachys sylvatica* L., *Geranium robertianum* L., *Mercurialis perennis* L., *Polytrichum formosum* (Hedw.) G.L. Smith. In the grassy layer of the studied phytocoenoses within these forests, a series of orchid species are also found, including: *Neottia nidus-avis* (L.) L. C. M. Rich., *Cephalanthera rubra* (L.) Rich. *Platanthera bifolia* (L.) L. C. M. Rich., *P. chloranta* (Custer) Rchb., *Neottia cordata* (L.) Rich., *Cephalanthera damasonium* (Mill.) Druce (1906), *Epipactis helleborine* (L.) Crantz.

The forest floor is also covered with a layer of grasses with a maximum coverage of 35%.

The shrub layer is extremely rare. The main species in this layer is *Rubus idaeus* which is found in relatively high abundance-dominance AD=2-3, and which has highly developed populations in some phytocoenoses where they can reach abundance-dominance of up to 2-3 according to field studies.

Regeneration dynamics of the beech forest in the study area

At the basis of a very good regeneration, with a high dynamic in the beech forests of the researched territory, the treatment of successive cuttings (preparatory cutting,seeding cutting, development cutting, final cutting) which was applied for a longer time before carrying out these studies, was of particular importance

Figure 2. The plant community of *Fagus sylvatica* on Naiba Valley

The seed from a tree is of particular importance in its natural regeneration, by forming new individuals. In order for natural regeneration to take place from the seedbed, it is necessary to have enough mature trees, able to bear fruit abundantly and seed the entire surface.

In the studied area located in the Naiba Valley basin, the soil is valuable according to field observations and data from the Baia de Arama Forest District Management and presents favorable conditions for growth and development.of the study species.

Following studies conducted through direct morphological observations of the vegetative organs of individuals from each sample area, it was found that the beech trees in the Naiba Valley have an active (vigorous) state of vegetation. Following the biomonitoring of this forest ecosystem built by *Fagus sylvatica*, it was found that the health and vitality of the trees that make up the stand is good.

Through the morphological and physiological analyzes carried out in the 20 sample areas established for the edification of the plant community in the *Fagus sylvatica* stands on Naiba Valley, it was found that the phenomena of damage, drying and breakage of the beech trees have a minimal value. Physiological and morpho-anatomical state of the leaf sheath is good. Thus, the phytocoenoses of *Fagus sylvatica*, *Hieracio rotundati-Fagetum*(Vida

1963) Tauber 1987 (Syn.: *Deschampsio flexuosae-Fagetum*Sóo 1962), were found to be characterized by: poor drying, isolated breaks, isolated fellings, weak game damage. Regarding *Fagus sylvatica*, the edifying species of the arboretum on Naiba Valley, it was observed after the studies carried out that fruits and seeds are formed throughout the crown of the tree, which also loves moonlight.

Fruiting of *Fagus sylvatica* trees depends on: trees maturity, differentiation and formation of flower buds, flowering and pollination, seed formation, seed maturation and ripening.

The *Fagus sylvatica* seed is abundant, the species fructifies very well under natural conditions, in several subplots, this may have a special role in the phenomenon of natural regeneration of these valuable trees. The seed inventoried in 20 sample areas within each phytocenosis that builds the plant community and including the natural habitat has a high density, a high germination power and increased viability. The density of seedlings was between 25-35/100 individuals m^2 (Figure 2, Figure 3).

Figure 3. Seeds development in the beech forests on the Naiba Valley

Figure 4. Beech seedlings within the analyzed phytocenoses on the Naiba Valley

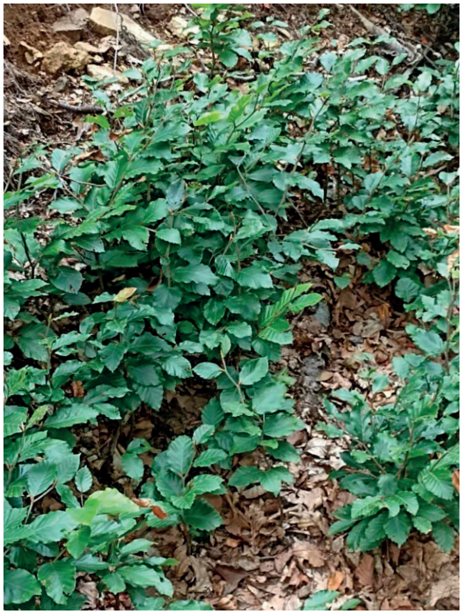

Figure 5. Evolution of beech seedlings in the Naiba Valley (at the upper forest limit of the beech forest, altitude 1450 m)

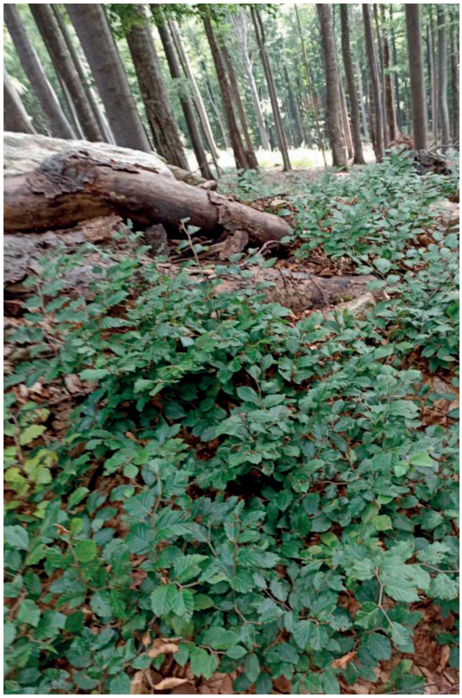

Figure 6. Natural regeneration of beech in a phytocoenosis in which dead wood was also preserved on the Naiba Valley

Figure 7. Natural regeneration of beech in the Habitat 9110 - *Luzulo-Fagetum* beech forests on the Naiba Valley

CONCLUSIONS

In conclusions, in the perspective of climate changes that greatly influence the quality and structure, the physiognomy, the conservation value but also the syndynamic global biodiversity, the degree of natural regeneration of virgin beech forests is of great importance for the research of forest ecosystems both from the perspective of protection and management, in order to ensure a sustainable natural capital.
Following the research carried out, it was found that the forest in which studies were conducted is prepared for abundant fruiting of the valuable species, and the eco-pedo-climatic conditions are favorable for the germination of seeds fallen on the forest floor.
In order to favor the development of the seedling in the first years after installation, it is recommended in the studied area to extract broken, dried trees within the limits of the accepted norms regarding the maintenance of dead wood in a protected area.
Throughout the Carpathian Chain in Romania and beyond, the conservation of beech forests is of particular importance from a biological, silvicultural, pedological, conservation, economic and social point of view.
In order for them to have a very good state of conservation, a harmonious and favorable evolution, silvicultural actions and works must be planned and implemented in such a way as to respect ecological criteria and conservation measures through the implementation of adequate management systems in the phenomenon of natural forest regeneration must have priority.

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THE INHIBITORY EFFECT OF LAVENDER COMPOST EXTRACTS ON VARIOUS PATHOGENS

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Abstract

Different types of composts have gained attention for their potential applications in sustainable agriculture and for their inhibitory effects against plant pathogens. This study investigates the suppressive effects of lavender compost extracts on common plant and soil pathogens and evaluates the composts' microbial composition. Pathogenic fungi, including Sclerotinia sclerotiorum, Fusarium oxysporum, Botrytis cinerea, and Alternaria alternata, were subjected to in vitro assays with varying concentrations of the extracts. The results revealed inhibitory effects due to compost microbiota; several fungal genera (Aspergillus, Penicillium, Trichoderma) and bacteria were detected. Microbial analysis of the compost extracts indicated the presence of beneficial microorganisms, such as Trichoderma spp., which may contribute to plant pathogen suppression. These findings suggest that lavender compost extracts can be a natural, eco-friendly alternative for managing plant pathogens and promoting sustainable agricultural practices. Further studies regarding the mechanisms underlying their biocontrol properties are recommended.

Key words*: lavender compost, plant pathogen suppression.*

INTRODUCTION

Through the European Green Deal (EGD) elaborated by the European Commission (EC), the European Union (EU) sets the path to a green transition, aiming to reach climate neutrality by 2050. Some of the EGD's main goals refer to circular economy (CE), a healthier environment, and more sustainable farming. Strategies such as the Zero Pollution Action Plan, Farm to Fork and Biodiversity are the key deliverables of EGD. In 2018, the legislative framework on waste (Directive 2008/98/EU) was updated, setting ambitious recycling targets of 60% by 2030 and 65% by 2035 (Directive 2018/851/EU).
The EU is reshaping food production and consumption in Europe to minimize the environmental impact of food systems. Consequently, in 2022, EC proposed a new regulation on the sustainable use of plant protection products (EC, 2022). The goal is to reduce the use of chemical pesticides by 50% and decrease the use of more hazardous pesticides to 50% by 2030, in alignment with the EU's Farm to Fork and Biodiversity strategies.
In recent years, the application of compost has attracted significant attention due to its potential contributions to sustainable agriculture. The application of compost serves various purposes, including enriching soil quality, enhancing plant growth (Garcia-Gil et al., 2000; Albiach et al., 2001; Whalen et al., 2003; Bastida et al., 2007; Iovieno et al., 2009; Bellino et al., 2015), reducing the need for chemical fertilizers and pesticides, promoting soil microbial activity (Bellino et al., 2015; Emmerling et al., 2010; Ros et al., 2006; Reimer et al., 2023), and acting as a biocontrol agent (Veeken et al., 2005; Trillas et al., 2006; De Corato, 2020; Greff et al., 2023).
Among the various compost types tested by other authors, lavender compost has been less analyzed, as lavender waste is not easily compostable due to its physicochemical composition (Lesage-Meessen et al., 2018). However, it is important to consider whether the composted lavender waste has the capacity to suppress certain soil and plant pathogens, thereby serving as an effective component within plant protection strategies, considering that the essential oils of this plant exert some

antifungal activity (Widmer & Laurent, 2006; Erdoğan et al., 2016).

Notable pathogens, such as *Sclerotinia sclerotiorum*, *Fusarium oxysporum*, *Botrytis cinerea*, and *Alternaria alternata*, are recognized for their capacity to induce substantial crop losses and compromise plant health.

Sclerotinia sclerotiorum is an ascomycetous fungal pathogen with a notably broad host range, infecting over 400 plant species across multiple families, including Brassicaceae, Fabaceae, Solanaceae, Asteraceae and Apiaceae (Boland & Hall, 1994; Bolton et al., 2006; Jiang et al., 2013). The fungus survives between growing seasons by forming sclerotia, which can germinate in two ways depending on the environmental conditions: myceliogenically and carpogenically (Steadman, 1979; Roper et al., 2010; Jiang et al., 2013). Infection typically causes soft rot symptoms and rapid plant death. The fungus produces abundant aerial hyphae on infected tissues, hence the name white mold. At later stages, new sclerotia form on lesions, contaminating soil, plant debris, or seeds (Jiang et al., 2013).

Fusarium oxysporum is a widely represented anamorphic species in soils and the rhizosphere of plants worldwide (Burgess, 1981; Gordon & Martyn, 1997; Fravel et al., 2003). All strains are saprophytic, capable of surviving on organic matter, though they differ in pathogenicity: some induce vascular wilts or root rots, while others remain nonpathogenic (Garrett, 1970; Olivain & Alabouvette, 1997; Fravel et al., 2003). The pathogenic strains are classified into more than 120 formae speciales and races, based on host range and cultivar specificity (Fravel et al., 2003). These pathogens show high host specificity, and new races can emerge that overcome host resistance, making disease management challenging (Fravel et al., 2003).

Traditional management strategies rely on resistant cultivars (e.g., tomato varieties resistant to *F. oxysporum* f. sp. *lycopersici*). However, breeding is difficult for some crops, and new pathogen races often overcome resistance. Chemical soil fumigation, historically with broad-spectrum agents such as methyl bromide, is environmentally damaging. Research efforts have increasingly focused on biological control strategies, particularly the use of nonpathogenic *F. oxysporum* strains as sustainable alternatives (Fravel et al., 2003), along with the exploration of essential oils from aromatic and medicinal plants, which have shown disease-suppressive properties (Wogiatzi et al., 2009; Ahmad et al., 2020).

Botrytis cinerea is one of the most recognized plant pathogenic fungi, commonly causing gray mold on soft fruits such as strawberries (Elad et al., 2004; Williamson et al., 2007; Pearson & Bailey, 2013). Its symptoms include abundant aerial hyphae and massive sporulation, producing gray conidiospores. While often a postharvest issue, it is also an important pathogen of growing plants, with a host range exceeding 235 species as early as 1968 (Macfarlane, 1968; Pearson & Bailey, 2013). The fungus is widespread globally, thriving particularly in humid environments such as tropical regions, dense canopies, and protected cropping systems. It infects multiple plant tissues (petals, leaves, stems, tubers) by germinating conidia that penetrate directly or through wounds. Pathogenesis is aided by cell wall–degrading enzymes (Urbanek & Zalewska-Sobczak, 2003; Pearson & Bailey, 2013) and toxins (Choquer et al., 2007; Pearson & Bailey, 2013). After colonizing host tissues, the fungus sporulates and spreads to new hosts. Importantly, infection is not always immediately symptomatic: *B. cinerea* can enter a latent phase, remaining hidden until host physiology favours fungal growth (Jaspers et al., 2012; Pearson & Bailey, 2013).

Alternaria alternata is a fungus with a broad host range, acting as both a pathogen of economically important crops and an asymptomatic endophyte (Zamora et al., 2008; Woudenberg et al., 2013; Woudenberg et al., 2015; Armitage et al., 2015; Lawrence et al., 2016; El Gobashy et al., 2018; Armitage et al., 2020; DeMers, 2022). It causes leaf spots, rots, and blights on over 380 plant species, with yield losses up to 79% in tomato and significant postharvest damage due to latent infections (Abbo et al., 2014; Dube, 2014; Troncoso-Rojas & Tiznado-Hernández, 2014; Tozlu et al., 2018). Conventional control through fungicides is effective but raises concerns about cost, resistance, and environmental impact (Chaerani & Voorrips, 2006; Heydari & Pessarakli, 2010; Tozlu et al., 2018). As a result, research has focused on sustainable alternatives such as fungal and bacterial biocontrol agents (Benhamou & Chet, 1993; Tozlu et al., 2017; Gao et al., 2017; Tozlu et al., 2018) and natural compounds, with cassia and thyme oils showing strong antifungal activity. These strategies highlight the potential of eco-friendly approaches for the management of *A. alternata* diseases (Feng & Zheng, 2007; Troncoso-Rojas & Tiznado-Hernández, 2014).

On the other hand, there are also beneficial fungi, such as *Trichoderma* spp., which was also included in our study.

Trichoderma spp. are fungi commonly found in soils, rotting wood, and plant tissues, with some strains isolated from unusual habitats such as shellfish and termites (Samuels, 1996; Druzhinina et al., 2006; Harman et al., 2004; Blaszczyk et al., 2014). They are fast-growing, produce abundant conidia, and are widely known for their antagonism against plant pathogens through mycoparasitism, antibiosis, competition, and induction of systemic resistance (Howell, 2003; Benitez et al., 2004; Schuster & Schmoll, 2010; Blaszczyk et al., 2014). Beyond disease suppression, *Trichoderma* spp. enhances plant growth, nutrient uptake, and yield (Vinale et al., 2008; Carvalho et al., 2018; Zin & Badaluddin, 2020), while also improving composting processes and facilitating soil detoxification (Rai et al., 2016; Carvalho et al., 2018; Zin & Badaluddin, 2020). Their industrial importance lies in the production of antifungal enzymes, which are synergistic in pathogen control and useful in engineering resistant plants (Kubicek et al., 2001; Blaszczyk et al., 2014; Zin & Badaluddin, 2020). Field studies in crops like soybean, cucumber, and tomato confirm their value as sustainable biocontrol agents (Almeida et al., 2001; Ethur et al., 2008; Carvalho et al., 2018).

Aligned with the regulations and goals of the EU, this study investigates the inhibitory effects of lavender compost extracts on these prevalent plant and soil pathogens. Through the execution of *in vitro* assays utilizing varying concentrations of the extracts, we seek to assess the suppressive capabilities and microbial composition of the compost. Identifying beneficial microorganisms, such as *Trichoderma* spp., present in the compost extracts accentuates the potential of lavender compost as a natural and environmentally sustainable alternative for the management of plant pathogens.

This paper further explores the mechanisms underlying the bio-control properties of lavender compost extracts and their implications for sustainable agricultural practices.

MATERIALS AND METHODS

Composts used

This study analyzed four compost samples: one from compost that underwent fermentation in 2023 (C1) and three from compost produced in 2024 (C2, C3, and C4). The samples consisted of co-composted manure (sheep and goat), lavender waste (including undistilled lavender biomass and distilled lavender stalks), and wheat straw (C2). Details of the compost recipes are provided in Table 1.

Table 1. Recipes of the composts used

C1 (2023)	C2 (2024)	C3 (2024)	C4 (2024)
500 kg of sheep manure	±33% (kg/kg) of sheep manure	±33% (kg/kg) of sheep manure	±33% (kg/kg) of sheep manure
200 kg mix of undistilled and distilled lavender stalks	±33% (kg/kg) goat manure	±33% (kg/kg) goat manure	±33% (kg/kg) goat manure
6 kg compost activator	±33% (kg/kg) wheat straws	±33% (kg/kg) distilled lavender stalks	±33% (kg/kg) undistilled lavender biomass

Counting of microbial load in compost teas

To determine the cultivable microbial load, the samples were brought to the laboratory for analysis within 24 hours of collection. Serial decimal dilutions were prepared from the compost to quantify the microbial load and perform quantitative microbiological analyses. For the first dilutions (10^{-1}), 10 grams of compost were processed and infused in 90 ml of sterile distilled water for 1 hour at room temperature, under agitation at 150 rpm. Subsequently, serial decimal dilutions were made up to 10^{-5}.

For the quantitative microbiological tests, different agar media were used, such as: (i) Plate Count Agar (PCA) medium for determining cultivable bacterial load, (ii) Rose Bengal Chloramphenicol Agar (RBC) medium for isolating and counting fungal load, and (iii)

Eosin Methylene Blue (EMB) Agar according to Levine's recipe for isolating, counting, and differentiating bacteria from the Enterobacteriaceae family (Dușa et al., 2022, 2023).
For testing, the lawn inoculation method was used on the surface of the agar, with 100 µl suspension from the 10^{-2} to 10^{-5} dilutions, depending on the type of analysis and the culture medium used. After processing, the samples were inoculated on PCA medium to determine the total cultivable bacterial load and incubated at 28°C. In contrast, those inoculated on EMB medium for determining enterobacterial load were incubated at 36°C. The samples inoculated on RBC medium, processed to determine fungal load, were incubated at 26°C for 7 days to ensure the thermal conditions were suitable for the development of this category of microorganisms and sufficient time for fungal colony growth.

In vitro suppression assay

Fungi used in this assay were *S. sclerotiorum*, *Trichoderma* spp., *F. oxysporum*, *B. cinerea,* and *A. alternata* (Figure 1), which were maintained on potato dextrose agar (PDA) medium at 20°C until the incubation test.
Compost teas (CT) with a 20% concentration were made for the experiment. For the CTs, compost samples were mixed with distilled water (20 g of compost and 180 mL of distilled water). The mixture was homogenized for 10 minutes and left to rest for 24 hours. The aqueous extract was passed through a filter paper; the filtrate was centrifuged at 4000 rpm for 20 minutes.

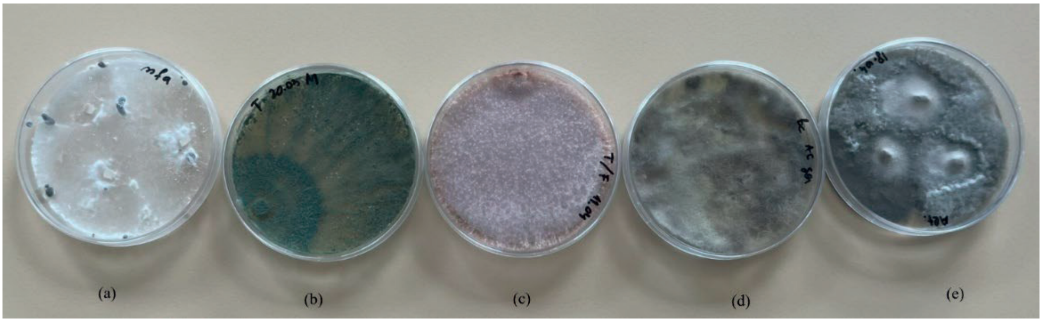


Figure 1. Fungi used for the incubation (a) *S. sclerotiorum*, (b) *Trichoderma* spp., (c) *F. oxysporum*, (c) *B. cinerea,* (d) *A. alternata*

The CTs were incorporated into the PDA medium to provide final concentrations of 5%, 2.5%, 1.25%, and 0.625%, resulting in 16 variants. The control consisted of only the PDA without CT. On each petri plate, 5 mL of medium with combined CTs was poured. One disc (0.5 cm) of mycelium of each fungus was placed centrally on the PDA medium. All plates were incubated at 25°C for 6 days.
The same test was repeated 2 weeks later with autoclaved, sterilized CTs.
The assay was performed as a completely randomized design with three repetitions.

RESULTS AND DISCUSSIONS

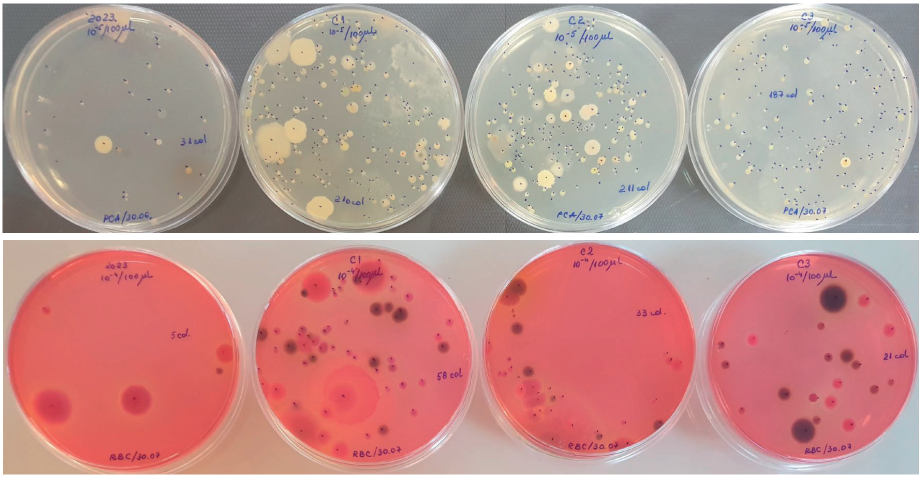
Counting of microbial load in compost teas

Microbiological tests revealed the cultivable microbial load present in the compost by microbial categories: bacteria and fungi. Table 2 shows that the microbial load in C1 is high, and there are no notable differences in microbial load between C2, C3, and C4. Composts C2, C3, and C4 each have a cultivable bacterial load of 108 CFU/g and a fungal load of 106 CFU/g. However, a slight decrease can be observed from C2 to C4 in both microbial categories analyzed (bacteria and fungi).
Substantial differences can be observed between C1 and C2, C3, and C4. The compost from 2023 has a microbial load 10 times lower than the composts from 2024, both in terms of bacteria and fungi (Figure 2). The analysis of enterobacteria presence in compost samples by plating dilutions (10^{-1} - 10^{-4}) on a semi-selective EMB medium suggests the absence of these bacteria categories in the compost or a load below the detection limit of 102 CFU/g.

Table 2. Microbial abundance and composition of microbial communities in composts

Substrate	Counting	Incubation period (days)	Composts			
			2023	2024		
			C1	C2	C3	C4
			(CFU/g)			
PCA	Cultivable bacteria	3	2.14×10^7	5.84×10^8	2.16×10^8	1.2×10^8
RBC	Fungi	7	2.6×10^5	4.9×10^6	3.3×10^6	2.1×10^6
EMB	Enterobacteriaceae	3-5	B.D.L.	B.D.L.	B.D.L.	B.D.L.

Legend: PCA = Plate Count Agar medium, RBC = Rose-Bengal Chloramphenicol agar medium, EMB = Eosin Methylene Blue agar medium, CFU = colony forming units, g = gram, B.D.L. = below the detection limit (102 CFU/ml)



A

B

Figure 2. Bacterial (A) and fungal (B) load in the analyzed compost samples

CTs fungal suppressiveness

Experiments were conducted to evaluate the potential of different CTs in inhibiting the growth of various fungal pathogens. The results from Petri plate assays (Figures 3, 4, 5, and 6) indicated that the degree of mycelial growth reduction varied according to both the type of CT used and the concentration applied.

The results revealed considerable variability in the degree of fungal growth suppression, which depended on both the specific compost tea tested and its applied concentration. While some CTs exhibited notable inhibitory effects at certain concentrations, others demonstrated more limited activity. These observations highlighted the absence of a clear or consistent pattern of fungal inhibition across the tested samples and concentrations, making it difficult to draw definitive conclusions regarding the efficacy of any of the CTs tested.

Although the findings were not precise enough to establish firm trends, the experiments underscored the complex interactions between CT composition and fungal suppression. It is worth noting that current research is increasingly interested in the use of aromatic and medicinal plant extracts and composts for their potential to suppress or inhibit the growth of specific plant and soil pathogens, suggesting a direction for further investigations in this field (De Corato et al., 2019; De Corato, 2020; Paraschiv (Jafri) et al., 2023).

In contrast, when the compost teas were sterilised, they failed to exhibit any inhibitory effects on fungal growth. This outcome highlights that sterilised CTs were ineffective in suppressing fungal development under the conditions evaluated (data not shown).

Sclerotinia sclerotiorum

Experiments involving plates inoculated with *S. sclerotiorum* demonstrated that compost samples C3 and C4 were effective in inhibiting the growth of this pathogen. Notably, C4 exhibited the strongest suppressive effect among the tested samples. In the case of C3, the presence of *Trichoderma* species played a competitive role, actively preventing the development of *S. sclerotiorum* on the plates. Conversely, the results for compost samples C1 and C2 were inconclusive, as a clear pattern of inhibition could not be established based on the observations (Figure 3).

Previous studies have demonstrated that *Origanum* spp. and *Mentha* spp. are effective in suppressing the growth of *S. sclerotiorum* (Soylu et al., 2007; Boligłowa et al., 2009; Wogiatzi et al., 2009). Since all three plants are classified within the same botanical family (*Lamiaceae*), it is reasonable to infer that lavender may also demonstrate a comparable suppressive effect against this pathogen (Greff et al., 2023).

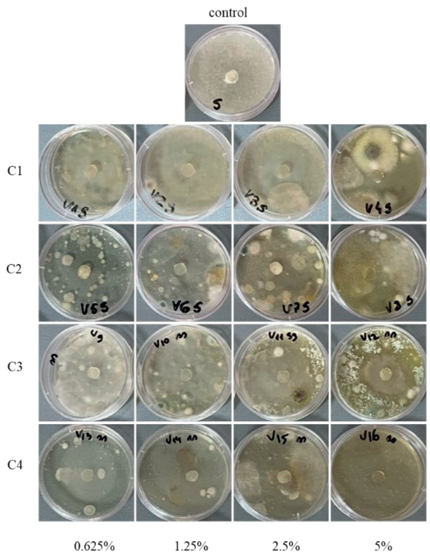


Figure 3. *Sclerotinia sclerotiorum*

Fusarium oxysporum

Figure 4 illustrates the suppressive potential of different CTs against *F. oxysporum*. At the lowest concentration tested, compost samples C1 and C2 demonstrated a modest inhibitory effect on fungal growth. However, as the concentration of these CTs increased, the composition of the microbial community within the compost also changed, with additional microorganisms emerging. This shift made it difficult to accurately assess the suppressive action of C1 and C2 at higher concentrations, as the presence of other bacteria and fungi may have interfered with the results.

In contrast, CTs derived from C3 and C4 displayed the highest suppressive effects at the highest concentrations applied. Notably, compost sample C3 exhibited an early presence of *Trichoderma* spp., which originated from the compost itself and may have contributed to its enhanced inhibitory activity against *F. oxysporum*.

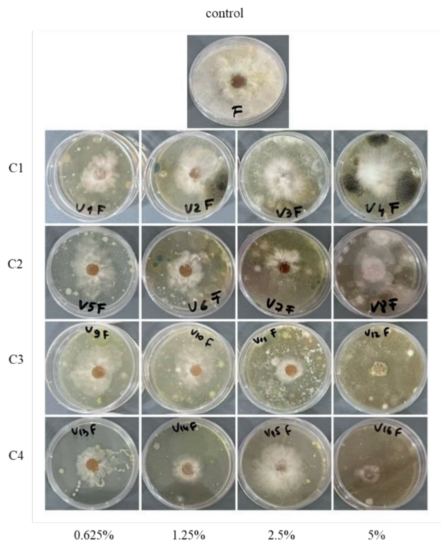


Figure 4. *Fusarium oxysporum*

Similar to the results obtained for *S. sclerotiorum*, additional members of the *Lamiaceae* family have also demonstrated suppressive effects against *F. oxysporum*. Extracts from *Thymus pallescens* (Moutassem et al., 2019) and *Salvia officinalis* (Ahmad & Matsubara, 2020) were tested and found to be effective in inhibiting the growth of *F. oxysporum* (Wogiatzi et al., 2009; Moutassem et al., 2019; Ahmad & Matsubara, 2020). These findings reinforce the potential of the plants within the *Lamiaceae* family to serve as valuable resources for the biocontrol of plant pathogens.

Botrytis cinerea

Composts C1 and C2 were found to contain a variety of microorganisms, including bacteria and fungi such as *Aspergillus* and *Penicillium*. The presence of these microorganisms interfered with the ability to accurately assess the suppressive effects of these compost teas against *B. cinerea*. As a result, the evaluation of their antifungal activity was complicated by microbial interactions within the samples.

In contrast, CTs derived from C3 and C4 consistently exhibited suppressive effects against *B. cinerea*, regardless of the concentration applied. The CT from C3 contained its own strains of *Trichoderma* spp., which were present across all tested concentrations, further contributing to its suppressive properties. Notably, C4 displayed the most substantial antifungal activity at the highest tested concentration of 5%, demonstrating its effectiveness in inhibiting fungal growth (Figure 5).

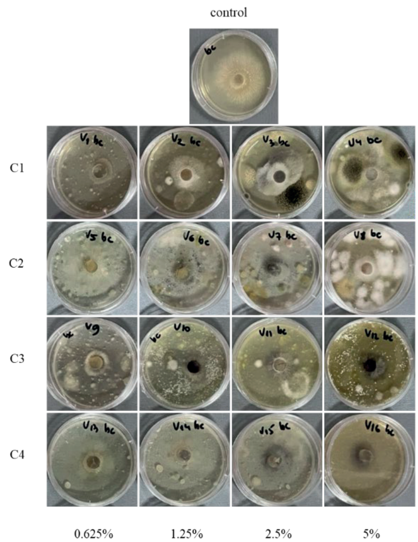


Figure 5. *Botrytis cinerea*

Alternaria alternata

At the lowest concentration tested (0.625%), CTs derived from samples C1 and C4 demonstrated the highest suppressive effects on the growth of *A. alternata*. As the concentrations of C1 and C2 CTs increased, there was a noticeable emergence of additional bacterial and fungal species within these samples, indicating shifts in microbial community composition that accompanied the higher application rates. Notably, *Trichoderma* spp. appeared in C3 at higher concentrations, suggesting a dynamic change in the microbial profile influenced by the strength of the CT.

Among all the CTs evaluated, C4 consistently exhibited the strongest antifungal activity against *A. alternata*, regardless of the concentration applied. This finding underscores the superior inhibitory potential of C4 in controlling fungal pathogens, as illustrated in Figure 6.

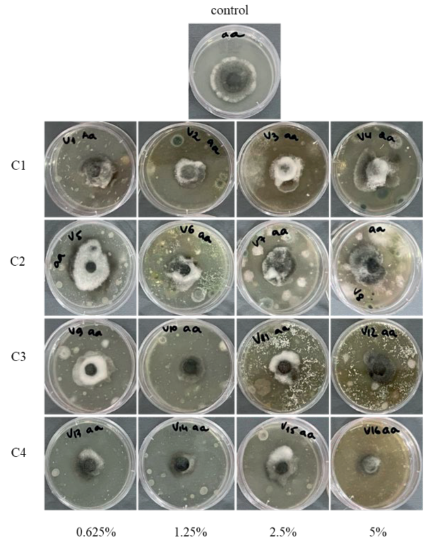


Figure 6. *Alternaria alternata*

***Trichoderma* spp.**

The evaluation of CTs against *Trichoderma* spp. revealed diverse differences in suppressive capabilities among the compost samples. Specifically, composts C1 and C2, which previously exhibited limited suppressive effects on other fungal species, were also found to have no inhibitory impact on *Trichoderma* spp. This observation is clearly illustrated in Figure 7, where the absence of suppression by C1 and C2 is noticed.

The compost C3 presented a unique challenge for assessment, as it naturally contained its own *Trichoderma* strains. The presence of these endogenous strains complicated the evaluation of any potential inhibitory effects exerted by the CT, making it difficult to draw definitive conclusions regarding its suppressive properties against *Trichoderma* spp.

In contrast, the compost C4 demonstrated a consistent and effective ability to inhibit fungal growth, including *Trichoderma* spp. The suppressive effect of C4 was observed across all tested fungal species, highlighting its superior antifungal activity and its potential role in biocontrol strategies.

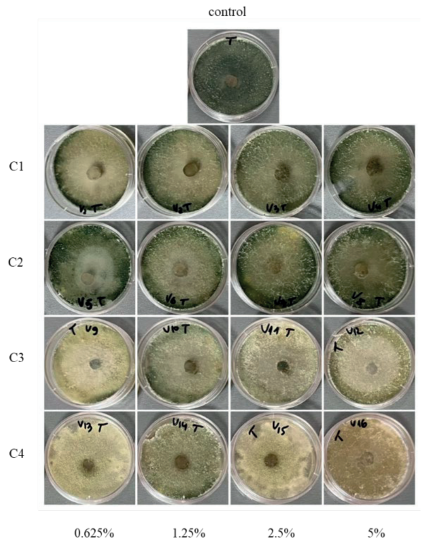


Figure 7. *Trichoderma* spp.

CONCLUSIONS

The microbiological analysis of compost samples from 2024 demonstrated a markedly higher microbial load compared to those from 2023, with bacterial and fungal counts of 108 CFU/g and 106 CFU/g, respectively, indicating enhanced microbial activity in the more recent compost. In contrast, in the 2023 composts, the microbial load was 10 times lower, with 107 bacterial CFU/g and 105 fungal CFU/g detected, respectively. Importantly, enterobacteria were undetected in all samples, with counts below the threshold of 102 CFU/g, suggesting the composts are microbiologically safe regarding this group of bacteria.

The suppressive effects of the CTs varied notably among the samples and target organisms. C4 emerged as the most effective CT, consistently displaying high inhibitory activity against *A. alternata* and *Trichoderma* spp, across all concentrations tested. This highlights the potential of C4 as a promising candidate for biocontrol strategies in managing fungal pathogens. The appearance of additional microbial species at higher CT concentrations, particularly in C1 and C2, indicates that increasing the strength of CTs can alter microbial community composition, possibly enhancing or diminishing suppressive effects.

Notably, C1 and C2 showed limited to no suppression of *Trichoderma* spp. At the same time, the assessment of C3 was complicated by the natural presence of endogenous *Trichoderma* strains, making it challenging to isolate the effects of CT application. Overall, C4's broad-spectrum antifungal activity suggests that specific compost formulations can be optimised to maximise disease suppression. The absence of enterobacteria further supports the microbiological safety of these composts for agricultural use.

Collectively, these findings highlight the significance of compost composition and microbial dynamics in developing effective and safe biocontrol agents. Optimising compost and CT formulations could play a significant role in sustainable disease management and soil health improvement strategies. However, further studies regarding the mechanisms underlying compost biocontrol properties should be continued.

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EVALUATION OF THE PRODUCTIVITY OF PERMANENT MESOPHILIC GRASSLANDS FROM CODRU-MOMA MOUNTAINS (NW ROMANIA)

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Abstract

The paper presents a case study for evaluating the productivity of permanent grasslands of Festuco rubrae-Agrostetum capillaris Horvat 1951, Trifolio-Lolietum perennis Krippelová 1967, Anthoxantho-Agrostetum capillaris Sillinger 1933 and Nardo-Festucetum rubrae fallax, from Codru-Moma Mountains, based on floristic relevées. Following the floristic study and the assessment of the participation weight of the component species in each type of grassland, the production of green mass and animal load was determined for each type of grassland studied. Among the studied plant associations, the highest productivity was found in Trifolio-Lolietum perennis Krippelová 1967, with productions of 18.15 t/ha of green mass and a capacity of 1.59 livestock/ha, Festuco rubrae-Agrostetum capillaris Horvat 1951 with productions of 11.64 t/ha of green mass and a capacity of 1.02 livestock/ha loading with animals. The lowest productions were evaluated in the grasslands of Nardo-Festucetum rubrae fallax, with productions of 3.86 t/ha of green mass and a capacity of 0.37 livestock/ha. The data provided by the present study are useful in characterizing the pastoral quality of these grasslands in the context of the improvement and rational use of the pastoral fund.

Key words*: grasslands, evaluation, green mass production, pastoral value, carrying capacity.*

INTRODUCTION

Grasslands are an essential element of sustainable farming systems that meet the demands of healthy, high-quality food (White et al., 2000). In addition grasslands have an important function in rural development and the environment, reflected by the conservation of biodiversity, improvement of soil fertility, hydrological balance, carbon storage, landscape quality and important cultural heritage (Wick et al., 2016).

Herbage mass production and forage quality of the plant species are the most important factors for grazing livestock performance and carrying capacity (Novák, 2004; Carlier et al., 2009; Soder et al., 2007).

The effects of grazing intensity on forage value as well as floristic diversity differ depending on the type of grazing animals and the ecological context (Liu et al. 2015; Schmitz & Isselstein, 2020). Some studies have found that cessation of grazing can lead to a decrease in the floristic diversity of pastures (Janišová et al., 2020; Bohner et al., 2019), while some research finds an increase in plant richness (Ford et al., 2012).

Methods for assessing grassland productivity that involve cutting and weighing phytomass are accurate but it requires a lot of time and high costs (Angerer, 2012; Peratoner & Pötsch, 2019).

In agronomic practice, pasture biomass can be assessed using the vegetation study method (visual estimation), which is considered comparable to the analytical method. (Novák, 2004; Angerer, 2012; Marușca, 2019; Peratoner & Pötsch, 2019).

The most widely used method for visual assessment of vegetation characteristics is the floristic relevées, which highlights the coverage of each species through the abundance-dominance class. (Cristea et al., 2004; Marușca, 2019; Peratoner & Pötsch, 2019). The estimation of species abundance-dominance is carried out using the Braun-Blanquet scale (1964), which has a variation range that differs between classes, namely: 4.5% between +-1 class, 12.5% between 1-2 class, 20% between 2-

3 class, 25% between 3-4 class and 25% between 4-5 class.
The large difference (25%) between 3-4 class and 4-5 class, 20% between 2-3 class leads to an inappropriate estimate for the quantitative assessment of biomass (Marușca, 2019). To eliminate these estimation differences, another quantitative participation evaluation scale was developed, using the constancy (K) for each species (Marușca, 2019; Marușca et al., 2019, 2020).
This new method for quantitative biomass assessment was used in the present study mainly pursuing the following objectives:
1. Determining the pastoral value and green mass production of mesophilic grasslands in the Codru Moma Mountains, with determination of grazing capacity;
2. Comparative study of the main pastoral parameters (pastoral value, green mass production, grazing capacity) corresponding to the *Festuco rubrae-Agrostetum capillaris* Horvat 1951 association, from the Codru Moma Mountains with the situation in the neighboring mountainous areas (Bihor Mountains, Pădurea Craiului Mountains, Vlădeasa Mountains).

MATERIALS AND METHODS

The study is located in the Codru-Moma Mountains, which are part of the Apuseni Mountains group, being situated in their western part and presenting themselves in the form of a well-defined promontory, oriented in the NW-SE direction. The territory of the Codru-Moma Mountains is located in the Bihor county and Arad county, being comprised between 46º20'-46º41' north latitude and 22º06'- 22º32' east longitude (Buz, 1980).
Within the described limits, Codru-Moma Mountains occupy an area of about 675 km², having the shape of a parallelogram with a length of about 39 km and a width of 17 km (Figure 1). Mesophile grassland vegetation represents about 10% of the total area of the territory, being spread mainly in the area of the Vașcău Plateau.
In order to assess the productivity of mesophilic grasslands in the Codru Moma Mountains, the study of flora and vegetation conducted by Pășcuț (2012) was taken into account.

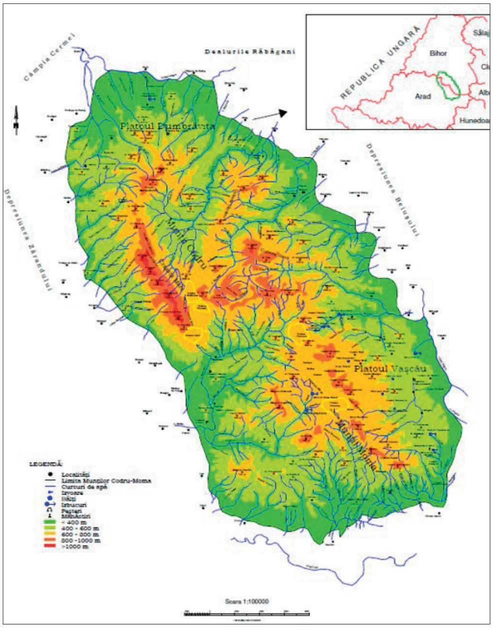

Figure 1. Geographical location of Codru Moma Mountains

The botanical nomenclature used for the identified species is in accordance with the work developed by Ciocârlan (2009). In the study of the floristic composition of mesophilous grasslands, relevées with an area of 100 m² were used.
In order to highlight the floristic similarity of the phytocoenoses corresponding to grasslands studied, the Jaccard index was used as a calculation model, determined with the help of the Past 5 statistical program.
The scientific name of the association and the name of the author were matched taking into account the International Code of Phytosociological Nomenclature, Edition 3 (Weber et al., 2000). The classification of the grassland association into higher syntaxonomic units, alliance, order and class was established on the basis of a group of characteristic species indicated in various specialized works, especially those developed by Pott (1995), Mucina (1997), Chifu (2004-2006).
Appreciation of abundance-dominance (AD) of species from the herbaceous layer in the grasslands of Codru Moma Mountains was performed on the Braun-Blanquet scale, described by Cristea et al. (2004).

Conversion of AD assessment notes into percentages (Table 1) according to constancy classes (K) was made after the model initiated by Marușca (2019).

Table 1. Appreciation of participation (P%) from synthetic floristic surveys, depending on the abundance-dominance scale intervals (AD) and average constancy (K%) for phytocoenoses of permanent grasslands (Marușca, 2019)

AD Scale Br. – Bl.	AD according to K (%)				
	V (81 – 100)	IV (61 – 80)	III (41 – 60)	II (21 – 40)	I (<20)
5	**87.5****	**61.3**	**43.8**	**26.3**	**8.8**
4 - 5	75.0	52.5	37.5	22.5	7.5
3 - 5	62.5	43.8	31.3	18.8	6.3
2 - 5	52.5	36.8	26.3	15.8	5.3
1 - 5	46.3	32.4	23.2	13.9	4.6
+ - 5	44.0	30.8	22.0	13.2	4.4
4	**62.5***	**43.8**	**31.3**	**18.8**	**6.3**
3 – 4	50.0	35.0	25.0	15.0	5.0
2 – 4	40.0	28.0	20.0	12.0	4.0
1 – 4	33.8	23.7	16.9	10.1	3.4
+ - 4	31.5	22.1	15.8	9.5	3.2
3	**37.5***	**26.3**	**18.9**	**11.3**	**3.8**
2 – 3	27.5	19.3	13.8	8.3	2.8
1 – 3	21.3	14.9	10.7	6.4	2.1
+ - 3	19.0	13.3	9.5	5.7	1.9
2	**17.5***	**12.3**	**8.8**	**5.3**	**1.8**
1 – 2	11.3	7.9	5.7	3.4	1.1
+ - 2	9.0	6.3	4.5	2.7	0.9
1	**5.0***	**3.5**	**2.5**	**1.5**	**0.5**
+ - 1	2.8	2.0	1.4	0.8	0.3
+	**0.5***	**0.4**	**0.3**	**0.2**	**0.1**

*)Appreciation scale transformation A+D, Braun-Blanquet in percentage, after Cristea et al. (2004).

After conversion into percentages of participation, according to the assessment scale, the indicators of forage quality (F4 - F9) and harmfulness (F1 - F3) are entered for each species in the floristic table, together with the indicators of useful forage phytomass (M1- M9) and harmful phytomass (M0 for F1 - F3) (Marușca, 2019; Marușca et al., 2020).

The fodder value indices (F) after Kovacs (1979), Păcurar & Rotar (2014), Marușca (2019) are the following:

Feed value indices (F): 1 = toxic to animals and humans; 2 = harmful to animal products; 3 = harmful to the vegetal layer; 4 = weak fodder (ballast species); 5 = mediocre fodder (former F1); 6 = medium forage (formerly F2); 7 = good fodder (former F3); 8 = very good fodder (former F4); 9 = excellent fodder (former F5); X = specis of unknown feed value.

The relevées thus prepared with the participation in % of the species in the vegetal layer with the mention of the fodder quality indices (F) and those of useful phytomass (M) make possible the calculation of the pastoral value (VP) according to the formula:

$$VP = \sum P(\%)\frac{F}{9}$$

in which: VP - pastoral value indicator (0-100) according to which the forage quality of a grassland is assessed: 0-5 degraded grassland; 5-15 very weak; 15-25 weak; 25-40 mediocre; 40-60 medium; 60-80 good; 80-100 very good.

F - has values between 4 and 9.

For the evaluation of the net fodder production, a new indirect method of determination was applied based on the floristic relevées and production indices (M) of the fodder species (F4-F9) from the vegetal layer of the grasslands (Marușca 2019; Marușca et al., 2020).

The average green mass production index (IM) of permanent grassland phytocoenoses was calculated with the following formula:

$$IM = \sum P(\%)\frac{M}{100}$$

in which: M - has values between 1-9 only for values of F between 4 - and 9.

After establishing the average green feed mass index (IM) the corresponding interval of the IM value is searched from Table 2 and multiplied by the coefficient of transformation into green mass production (CMV), resulting the production in tonnes per hectare, and finally the coefficient of appreciation for this indicator (Marușca 2019; Marușca et al., 2020).

The green mass production of phytocoenoses is very heterogeneous, starting with 0.2 t/ha (very weak) and can reach over 30 t/ha (excellent) on well-managed and well-exploited permanent grasslands.

Based on these data, the optimal load with animals or carrying capacity (CP) expressed in livestock units (LU) per hectare are further established using the formula:

$$CP(UVM/ha) = \frac{MV(kg/ha)}{NzxZp}$$

in which: Nz - the daily requirement of grass for 1 livestock unit (LU), 65 kg (50 kg + 30% (15 kg) seasonal climate fluctuations and unconsumed debris)

Zp = number of grazing days (season)

The duration of the optimal grazing season for the Codru Moma Mountains is on average 175 days at the altitude of 400-600 m and 160 days at the altitude of 600-800 m.

Table 2. Production indices for feed species and estimating the useful yield per hectar of permanent unfertilized grasslands (Marușca, 2019; Marușca et al., 2020)

Average production indices green mass forage species (IM)	Coefficients of transformation in green mass production (CMV)	Green mass production estimate (MV) (t/ha)	Appreciation of production value
0.1 – 0.5	x 1.8	0.18 – 0.90	Very weak
0.6 – 1.0	x 1.9	1.14 – 1.90	
1.1 – 1.5	x 2.0	2.20 – 3.00	Weak
1.6 – 2.0	x 2.1	3.36 – 4.20	
2.1 – 2.5	x 2.2	4.62 – 5.50	Weak - Medium
2.6 – 3.0	x 2.3	5.98 – 6.90	
3.1 – 3.5	x 2.4	7.44 – 8.40	Medium
3.6 – 4.0	x 2.5	9.00 – 10.00	
4.1 – 4.5	x 2.6	10.66 – 11.70	Middle - Good
4.6 – 5.0	x 2.7	12.42 – 13.50	
5.1 – 5.5	x 2.8	14.28 – 15.40	Good
5.6 – 6.0	x 2.9	16.24 – 17.40	
6.1 – 6.5	x 3.0	18.30 – 19.50	Good - Very good
6.6 – 7.0	x 3.1	20.46 – 21.70	
7.1 – 7.5	x 3.2	22.72 – 24.00	Very good
7.6 – 8.0	x 3.3	25.08 – 26.40	
8.1 – 8.5	x 3.4	27.54 – 28.90	Excellent
8.6 – 9.0	x 3.5	30.10 – 31.50	

RESULTS AND DISCUSSIONS

Grassy vegetation occupies an area of 10.150 ha in the Codru Moma Mountains, representing 15% of the total area. Mesophilic grasslands occupy 10% of the total area, in which the grasslands represented by *Festuca rubra* L. and *Agrostis capillaris* L. predominate (Figure 2).

Four mesophilic grassland associations from the Codru Moma Mountains were studied, which are classified from a coenotaxonomic point of view as follows:

Molinio-Arrhenatheretea class (R. Tüxen 1937);
Arrhenatheretalia order (R. Tüxen 1931);
Cynosurion alliance (R. Tüxen 1947);
Festuco rubrae-Agrostetum capillaris association (Horvat 1951);
Trifolio-Lolietum perennis association (Krippelová 1967);
Anthoxantho-Agrostetum capillaris association (Sillinger 1933);
Nardo-Callunetea class (Preising 1949);
Nardetalia order (Oberdorfer 1949);
Potentillo-Nardion alliance (Simon 1959);
Nardo-Festucetum rubrae fallax association (Pușcaru et al. 1959).

Figure 2. *Festuco rubrae-Agrostetum capillaris* Horvat 1951, in Ponoraș Glade (Codru Moma Mountains)

The mesophilic grasslands of the *Festuco rubrae-Agrostetum capillaris* association grow on deep, humus-rich districambosol soils, occurring in the upper hilly area at altitudes of 480-880 m, on flat terrain and with slopes of up to 15º, with different exposures. On lands with higher soil moisture, compacted and with a low nutrient content, *Festuca rubra* L. dominates, while on lands with looser soil, *Agrostis capillaris* L. dominates.

The floristic composition of these grasslands is very varied, correspond to 157 species (Table 3). A total of 25 releveés were studied, carried out between 2008 and 2024. The largest number of relevées were carried out in the Vașcău Plateau, Ponoraș Glade, Brătcoaia Glade, Izoiu Glade.

These grasslands are characterized by the presence of a high percentage (76.4%) of species from *Poaceae*, of which the largest share is: *Agrostis capillaris* L., *Festuca rubra* L., *Holcus lanatus* L., *Phleum pratense* L., *Danthonia decumbens* (L.) DC., *Cynosurus cristatus* L., *Dactylis glomerata* L., *Brachypodium pinnatum* (L.) Beauv. The *Fabaceae* species have a reduced coverage (2.2%), being represented by a greater constancy of *Lotus corniculatus* L., *Trifolium repens* L., *Trifolium pratense* L., *Trifolium campestre* Schreber (Table 3). In these grasslands, some forage species from other families also appear with great frequency, *Achillea millefolium* L., *Centaurea phrygia* L., *Potentilla erecta* (L.) Räusch., *Hieracium pilosella* L., *Leucanthemum vulgare* Lam., *Plantago lanceolata* L., *Rumex acetosa* L., *Thymus glabrescens* Willd., *Thymus pulegioides* L. A number of species harmful to livestock products also enter these grasslands, *Carduus acanthoides* L., *Carduus nutans* L., *Rumex acetosella* L. and toxic and harmful plants *Stellaria graminea* L., *Hypericum perforatum* L., *Pteridium aquilinum* (L.) Kuhn, *Euphorbia cyparissias* L., *Hypericum maculatum* Crantz.

The meadows belonging to the *Trifolio-Lolietum perennis* association are spread in the Codru-Moma Mountains, at altitudes of 380-420 m, on almost flat (0-5°), tamped and compacted lands (Table 3). They are the grasslans with the highest pastoral value in which the *Poaceae* species have a high share (61.6%), and the *Fabaceae* species are present in a proportion of 29.9%. The species with the highest forage value are represented by *Lolium perenne* L., *Trifolium repens* L., *Lotus corniculatus* L., *Dactylis glomerata* L., *Poa pratensis* L.

Grasslands of *Agrostis capillaris* L. and *Anthoxanthum odoratum* L. are characterized by a fairly wide ecological amplitude, occupying stations from the understory of the common sedge to the understory of the beech. They are spread on lands with varying slopes (5-18°), on different exposures, at altitudes of 300-620 m (Table 3). The physiognomy of these grasslands is given by *Poaceae* species (76%), with *Fabaceae* species having a reduced presence (1.9%). The useful phytomass of these grasslands is represented by species with high forage value such as *Agrostis capillaris* L., *Anthoxanthum odoratum* L., *Briza media* L., *Cynosurus cristatus* L., *Festuca pratensis* Hudson, *Lotus corniculatus* L., *Trifolium repens* L., *Achillea millefolium* L., *Plantago lanceolata* L.

Grasslands of *Festuca rubra* L. and *Nardus stricta* L. are spread at altitudes of 600-800 m, on terrains with a slope of 5-25° and varied exposures (Table 3). They are present on soils of the districambosol type, well-drained, moderately acidic and of medium trophicity. They are the least productive grasslands with a lack of *Fabaceae* species and a low presence of forage *Poaceae* species (33.3%).

Dendrogram of mesophilous grasslands in the Codru Moma Mountains highlights a relative uniformity of their floristic composition, with pronounced similarity of the grasslands represented by the *Festuco rubrae-Agrostetum capillaris* (1) and *Anthoxantho-Agrostetum capillaris* (3) associations (Figure 3).

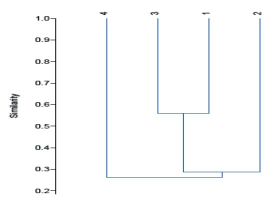

Figure 3. Jaccard similarity index from the grasslans studied in the Codru Moma Mountains, where: 1 - *Festuco rubrae-Agrostetum capillaris*, 2 - *Trifolio-Lolietum perennis*, 3 - *Anthoxantho-Agrostetum capillaris*, 4 - *Nardo-Festucetum rubrae fallax*

Table 3. The floristic composition of the mesophilous grasslands in the Codru Moma Mountains, Bihor county (where: F - Fodder quality indices; M - Production indices; K – constancy)

Plant association	*Festuco rubrae-Agrostetum capillaris*			*Trifolio-Lolietum perennis*			*Anthoxantho-Agrostetum capillaris*			*Nardo-Festucetum rubrae fallax*			Indicators	
		ADm	K		ADm	K		ADm	K		ADm	K		
No. of relevées	25			8			13			9				
Altitude (m)	480-880			380-420			300-620			600-800				
The coverage of grass layer (%)	99,6			98,8			99,8			99,6				
Exposition	V, E, NE, NV			E, SE			S, E, N, SV, SE			N, S, V, SV				
Slope (degree) (º)	0-15			0-5			5-18			5-25				
Area (m²)	100			100			100			100			F	M
0	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*
Poaceae														
Agrostis capillaris	40	2-4	V	2	+-1	IV	40	2-4	V	0.4	+	IV	7	5
Agrostis gigantean	0.1	+	I	·	·	·	·	·	·	1.4	+-1	III	7	7
Anthoxanthum odoratum	0.1	+	I	9	+-2	I	33.8	1-4	V	0.2	+	II	5	3
Brachypodium pinnatum	0.2	+	II	·	·	·	·	·	·	·	·	·	5	7
Briza media	0.1	+	I	·	·	·	0.3	+	III	0.3	+	III	5	2
Cynosurus cristatus	0.3	+	III	·	·	·	0.3	+	III	·	·	·	7	4
Dactylis glomerata	0.2	+	II	0.2	+	II	0.2	+	II	·	·	·	9	8
Danthonia decumbens	0.3	+	III	·	·	·	·	·	·	0.4	+	IV	5	4
Deschampsia flexuosa	0.1	+	I	·	·	·	·	·	·	9	+-2	V	4	3
Festuca pratensis	0.1	+	I	·	·	·	0.4	+	IV	·	·	·	9	8
Festuca rubra	33.8	1-4	V	0.2	+	II	0.2	+	II	21.3	1-3	V	7	6
Festuca rupicola	0.1	+	I	·	·	·	0.1	+	I	·	·	·	5	5
Festuca valesiaca	0.1	+	I	·	·	·	·	·	·	·	·	·	5	3
Holcus lanatus	0.3	+	III	·	·	·	0.3	+-1	I	0.3	+	III	6	6
Lolium perenne	·	·	·	50	3-4	V	·	·	·	·	·	·	9	8
Poa annua	0.1	+	I	·	·	·	·	·	·	·	·	·	7	2
Poa pratensis	0.1	+	I	0.2	+	II	0.2	+	II	·	·	·	8	6
Phleum montanum	0.1	+	I	·	·	·	·	·	·	·	·	·	6	5
Phleum pratense	0.3	+	III	·	·	·	0.2	+	II	·	·	·	9	8
Fabaceae														
Anthyllis vulneraria	0.1	+	I	·	·	·	0.1	+	I	·	·	·	6	5
Lathyrus pratensis	0.1	+	I	0.1	+	I	·	·	·	·	·	·	7	6
Lotus corniculatus	0.5	+	V	2	+-1	IV	0.5	+	V	·	·	·	8	6
Medicago lupulina	0.1	+	I	0.1	+	I	0.1	+	I	·	·	·	8	3
Trifolium campestre	0.2	+	II	0.1	+	I	0.2	+	II	·	·	·	7	2
Trifolium hybridum	0.1	+	I	0.1	+	I	0.2	+	II	·	·	·	8	6
Trifolium medium	0.1	+	I	·	·	·	·	·	·	·	·	·	6	4
Trifolium montanum	0.1	+	I	·	·	·	·	·	·	·	·	·	7	4
Trifolium pannonicum	0.1	+	I	·	·	·	·	·	·	·	·	·	7	5
Trifolium pratense	0.3	+	III	·	·	·	0.2	+	II	·	·	·	8	7
Trifolium repens	0.3	+	III	27.5	2-3	V	0.4	+	IV	·	·	·	8	5
Vicia cracca	0.1	+	I	·	·	·	0.2	+	II	·	·	·	7	6
Vicia tetrasperma	0.1	+	I	·	·	·	·	·	·	·	·	·	6	3
Forage species from other botanical families														
Achillea millefolium	0.5	+	V	0.5	+	V	0.5	+	V	0.3	+	III	6	4
Antennaria dioica	·	·	·	·	·	·	·	·	·	0.1	+	I	4	2
Bellis perennis	0.1	+	I	·	·	·	0.2	+	II	·	·	·	5	1
Carex pallescens	0.1	+	I	·	·	·	·	·	·	0.2	+	II	4	3
Carum carvi	0.1	+	I	0.1	+	I	0.8	+-1	II	·	·	·	6	3
Centaurea phrygia	0.4	+	IV	·	·	·	0.3	+	III	0.3	+	III	4	6
Cichorium intybus	0.1	+	I	0.2	+	II	0.3	+	III	·	·	·	5	6
Convolvulus arvensis	0.1	+	I	·	·	·	0.1	+	I	·	·	·	7	6
Daucus carota	0.1	+	I	0.1	+	I	0.8	+-1	II	·	·	·	6	5
Filipendula vulgaris	0.1	+	I	·	·	·	·	·	·	·	·	·	5	4
Fragaria vesca	0.1	+	I	·	·	·	0.2	+	II	·	·	·	5	1
Fragaria viridis	0.2	+	II	·	·	·	0.2	+	II	·	·	·	4	1
Galium verum	0.2	+	II	·	·	·	0.3	+	III	0.2	+	II	5	4
Helianthemum nummularium	0.1	+	I	·	·	·	·	·	·	·	·	·	4	2
Hieracium pilosella	0.3	+	III	·	·	·	0.4	+	IV	0.3	+	III	4	1
Knautia arvensis	0.2	+	II	0.1	+	I	0.2	+	II	·	·	·	4	4
Leontodon autumnalis	0.2	+	II	·	·	·	1.4	+-1	III	·	·	·	5	3
Leontodon hispidus	0.2	+	II	0.2	+	II	0.3	+	III	·	·	·	5	3
Leucanthemum vulgare	0.3	+	III	·	·	·	0.2	+	II	0.2	+	II	5	5
Luzula campestris	0.1	+	I	·	·	·	0.1	+	I	0.4	+	IV	4	2
Lysimachia vulgaris	0.1	+	I	·	·	·	0.1	+	I	0.2	+	II	4	7
Origanum vulgare	0.1	+	I	·	·	·	·	·	·	·	·	·	4	4

0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Plantago lanceolata	0.3	+	III	2	+-1	IV	0.4	+	IV	0.2	+	II	6	1
Plantago major	·	·	·	0.1	+	I	0.2	+	II	·	·	·	5	3
Plantago media	0.1	+	I	0.8	+-1	II	·	·	·	·	·	·	6	2
Polygala vulgaris	0.2	+	II	0.2	+	II	0.3	+	III	0.2	+	II	4	1
Potentilla erecta	0.4	+	IV	·	·	·	0.2	+	II	0.5	+	V	5	2
Prunella laciniata	0.1	+	I	0.1	+	I	0.1	+	I	·	·	·	4	2
Prunella vulgaris	0.1	+	I	0.4	+	IV	0.4	+	IV	·	·	·	4	2
Ranunculus polyanthemos	0.2	+	II	·	·	·	0.1	+	I	·	·	·	4	4
Rumex acetosa	0.3	+	III	0.1	+	I	0.1	+	I	0.3	+	III	4	5
Rumex obtusifolius	·	·	·	0.1	+	I	·	·	·	·	·	·	4	6
Salvia pratensis	·	·	·	0.1	+	I	·	·	·	·	·	·	4	4
Sanguisorba officinalis	0.1	+	I	·	·	·	·	·	·	·	·	·	7	5
Scabiosa ochroleuca	0.2	+	II	·	·	·	0.1	+	I	·	·	·	4	4
Taraxacum officinale	·	·	·	0.2	+	II	0.1	+	I	·	·	·	7	3
Thymus glabrescens	0.3	+	III	·	·	·	0.3	+	III	0.2	+	II	4	2
Thymus pulegioides	0.3	+	III	·	·	·	0.3	+	III	·	·	·	4	2
Tragopogon pratensis	·	·	·	·	·	·	0.1	+	I	·	·	·	5	5
Verbena officinalis	·	·	·	0.1	+	I	·	·	·	·	·	·	4	4
Veronica chamaedrys	0.1	+	I	·	·	·	0.1	+	I	·	·	·	4	2
Viola canina	0.3	+-1	I	·	·	·	·	·	·	1.4	+-1	III	4	1
Plant species not consumed or with a low degree of consumability (F3), (M0)														
Acinos arvensis	0.1	+	I	·	·	·	0.1	+	I	·	·	·	3	0
Agrimonia eupatoria	0.1	+	I	·	·	·	0.2	+	II	·	·	·	3	0
Ajuga reptans	·	·	·	·	·	·	0.1	+	I	·	·	·	3	0
Asperula cynanchica	0.1	+	I	·	·	·	0.2	+	II	·	·	·	3	0
Betula pendula	0.2	+	II	·	·	·	·	·	·	0.2	+	II	3	0
Blechnum spicant	·	·	·	·	·	·	·	·	·	0.1	+	I	3	0
Calamagrostis arundinacea	0.1	+	I	·	·	·	·	·	·	0.2	+	II	3	0
Calamagrostis epigeios	0.3	+-1	I	·	·	·	0.2	+	II	·	·	·	3	0
Campanula glomerata	0.2	+	II	·	·	·	0.1	+	I	·	·	·	3	0
Campanula patula	0.4	+	IV	·	·	·	0.2	+	II	0.3	+	III	3	0
Campanula persicifolia	0.1	+	I	·	·	·	·	·	·	·	·	·	3	0
Cardamine pratensis	0.1	+	I	·	·	·	·	·	·	·	·	·	3	0
Carex ovalis	·	·	·	·	·	·	·	·	·	0.1	+	I	3	0
Carex tomentosa	0.1	+	I	·	·	·	0.1	+	I	·	·	·	3	0
Carlina acaulis	0.2	+	II	0.1	+	I	0.2	+	II	·	·	·	3	0
Carlina biebersteinii	0.1	+	I	·	·	·	0.2	+	II	·	·	·	3	0
Carlina vulgaris	0.1	+	I	·	·	·	0.2	+	II	·	·	·	3	0
Centaurea nigrescens	0.1	+	I	0.1	+	I	0.8	+-1	II	·	·	·	3	0
Centaurium erythraea	0.1	+	I	·	·	·	0.3	+	III	·	·	·	3	0
Cerastium holosteoides	0.2	+	II	·	·	·	0.1	+	I	·	·	·	3	0
Cirsium canum	0.1	+	I	·	·	·	·	·	·	·	·	·	3	0
Clinopodium vulgare	0.1	+	I	·	·	·	·	·	·	·	·	·	3	0
Corylus avellana	0.1	+	I	·	·	·	·	·	·	·	·	·	3	0
Crataegus monogyna	0.3	+	III	0.1	+	I	0.3	+	III	·	·	·	3	0
Cruciata glabra	0.3	+	III	·	·	·	·	·	·	0.2	+	II	3	0
Cytisus nigricans	0.1	+	I	·	·	·	·	·	·	0.2	+	II	3	0
Dactylorhiza maculata	0.1	+	I	·	·	·	·	·	·	·	·	·	3	0
Dactylorhiza sambucina	0.1	+	I	·	·	·	0.1	+	I	·	·	·	3	0
Deschampsia caespitosa	·	·	·	·	·	·	·	·	·	0.3	+	III	3	0
Dianthus armeria	0.1	+	I	·	·	·	·	·	·	·	·	·	3	0
Dianthus carthusianorum	0.2	+	II	·	·	·	0.1	+	I	0.2	+	II	3	0
Dianthus spiculifolius	0.1	+	I	·	·	·	·	·	·	·	·	·	3	0
Dichanthium ischaemum	·	·	·	·	·	·	0.1	+	I	·	·	·	3	0
Dorycnium pentaphyllum	0.1	+	I	·	·	·	·	·	·	·	·	·	3	0
Erigeron annuus	0.1	+	I	·	·	·	0.3	+	III	·	·	·	3	0
Eryngium campestre	·	·	·	0.2	+	II	0.2	+	II	·	·	·	3	0
Euphrasia officinalis	0.1	+	I	·	·	·	0.1	+	I	·	·	·	3	0
Euphrasia stricta	0.2	+	II	·	·	·	0.1	+	I	·	·	·	3	0
Filago arvensis	0.1	+	I	·	·	·	·	·	·	·	·	·	3	0
Galium album	0.1	+	I	·	·	·	·	·	·	·	·	·	3	0
Galium mollugo	0.3	+	III	·	·	·	0.3	+	III	0.2	+	II	3	0
Genista tinctoria	·	·	·	·	·	·	0.1	+	I	·	·	·	3	0
Genistella sagittalis	0.1	+	I	·	·	·	0.2	+	II	1.4	+-1	III	3	0
Gentiana asclepiadea	0.2	+	II	·	·	·	·	·	·	0.2	+	II	3	0
Gentiana cruciata	0.2	+	II	·	·	·	0.1	+	I	0.2	+	II	3	0
Gladiolus imbricatus	0.1	+	I	·	·	·	·	·	·	·	·	·	3	0
Gnaphalium sylvaticum	0.1	+	I	·	·	·	·	·	·	0.2	+	II	3	0
Hieracium umbellatum	·	·	·	·	·	·	·	·	·	0.1	+	I	3	0

0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Hypochaeris radicata	0.1	+	I	·	·	·	0.2	+	II	·	·	·	3	0
Juncus conglomeratus	·	·	·	0.1	+	I	0.2	+	II	·	·	·	3	0
Juncus effusus	0.2	+	II	·	·	·	0.1	+	I	·	·	·	3	0
Juncus tenuis	0.1	+	I	·	·	·	·	·	·	·	·	·	3	0
Juniperus communis	0.3	+	III	·	·	·	0.2	+	II	0.2	+	II	3	0
Linaria vulgaris	0.1	+	I	·	·	·	·	·	·	·	·	·	3	0
Linum catharticum	0.2	+	II	·	·	·	0.1	+	I	·	·	·	3	0
Luzula luzuloides	0.1	+	I	·	·	·	·	·	·	0.3	+	III	3	0
Lycopodium clavatum	·	·	·	·	·	·	·	·	·	0.1	+	I	3	0
Lysimachia nummularia	0.1	+	I	·	·	·	·	·	·	·	·	·	3	0
Malus sylvestris	0.2	+	II	·	·	·	0.2	+	II	·	·	·	3	0
Mentha pulegium	·	·	·	0.2	+	II	·	·	·	·	·	·	3	0
Molinia caerulea	0.1	+	I	·	·	·	·	·	·	·	·	·	3	0
Nardus stricta	0.3	+	III	·	·	·	0.2	+	II	46.3	1-5	V	3	0
Orchis morio	0.1	+	I	·	·	·	·	·	·	·	·	·	3	0
Ononis spinosa	·	·	·	·	·	·	0.2	+	II	·	·	·	3	0
Peucedanum oreoselinum	0.2	+	II	·	·	·	·	·	·	·	·	·	3	0
Populus tremula	0.1	+	I	·	·	·	·	·	·	·	·	·	3	0
Prunus spinosa	0.2	+	II	·	·	·	0.2	+	II	·	·	·	3	0
Pyrus pyraster	0.2	+	II	·	·	·	0.2	+	II	·	·	·	3	0
Rhinanthus minor	0.1	+	I	·	·	·	0.1	+	I	·	·	·	3	0
Rosa canina	0.3	+	III	0.2	+	II	0.4	+	IV	·	·	·	3	0
Rubus sulcatus	0.2	+	II	0.1	+	I	0.3	+	III	·	·	·	3	0
Rumex crispus	0.1	+	I	0.1	+	I	·	·	·	·	·	·	3	0
Salix capraea	0.1	+	I	·	·	·	·	·	·	·	·	·	3	0
Sambucus ebulus	0.1	+	I	·	·	·	·	·	·	·	·	·	3	0
Senecio germanicus	0.1	+	I	·	·	·	·	·	·	·	·	·	3	0
Seseli osseum	0.3	+	III	·	·	·	0.2	+	II	0.3	+	III	3	0
Seseli peucedanoides	0.1	+	I	·	·	·	·	·	·	·	·	·	3	0
Setaria pumila	·	·	·	0.1	+	I	0.1	+	I	·	·	·	3	0
Sorbus aucuparia	·	·	·	·	·	·	·	·	·	0.2	+	II	3	0
Stachys officinalis	0.2	+	II	·	·	·	0.2	+	II	0.2	+	II	3	0
Stachys recta	0.1	+	I	·	·	·	·	·	·	·	·	·	3	0
Succisa pratensis	0.2	+	II	·	·	·	·	·	·	0.3	+	III	3	0
Teucrium chamaedrys	0.2	+	II	·	·	·	0.2	+	II	·	·	·	3	0
Thymus comosus	0.1	+	I	·	·	·	·	·	·	·	·	·	3	0
Veronica officinalis	0.2	+	II	·	·	·	0.2	+	II	0.3	+	III	3	0
Veronica teucrium	0.2	+	II	·	·	·	·	·	·	·	·	·	3	0
Viola arvensis	0.1	+	I	·	·	·	·	·	·	·	·	·	3	0
Viola tricolor	0.1	+	I	·	·	·	0.1	+	I	0.2	+	II	3	0
Vaccinium myrtillus	0.1	+	I	·	·	·	·	·	·	2.7	+-2	II	3	0
Vaccinium vitis-idaea	·	·	·	·	·	·	·	·	·	4.5	+-2	III	3	0
Vulpia myuros	0.1	+	I	·	·	·	0.2	+	II	·	·	·	3	0
Plants that harm livestock products (F2), (M0)														
Carduus acanthoides	0.1	+	I	·	·	·	0.2	+	II	·	·	·	2	0
Carduus nutans	0.1	+	I	0.1	+	I	0.2	+	II	·	·	·	2	0
Rumex acetosella	0.1	+	I	·	·	·	·	·	·	·	·	·	2	0
Toxic and harmful plants (F1), (M0)														
Aconitum vulparia	0.1	+	I	·	·	·	·	·	·	·	·	·	1	0
Colchicum autumnale	0.1	+	I	·	·	·	0.1	+	I	·	·	·	1	0
Coronilla varia	0.1	+	I	·	·	·	·	·	·	·	·	·	1	0
Euphorbia cyparissias	0.2	+	II	0.1	+	I	2	+-1	IV	·	·	·	1	0
Euphorbia villosa	0.1	+	I	·	·	·	·	·	·	·	·	·	1	0
Hypericum maculatum	0.2	+	II	·	·	·	0.1	+	I	0.4	+	IV	1	0
Hypericum perforatum	0.3	+	III	·	·	·	0.2	+	II	·	·	·	1	0
Hypericum tetrapterum	0.1	+	I	·	·	·	0.1	+	I	·	·	·	1	0
Pteridium aquilinum	0.8	+-1	II	·	·	·	0.8	+-1	II	·	·	·	1	0
Ranunculus repens	·	·	·	0.1	+	I	·	·	·	·	·	·	1	0
Stellaria graminea	0.3	+	III	0.2	+	II	0.2	+	II	·	·	·	1	0
Veratrum album	0.1	+	I	·	·	·	·	·	·	0.2	+	II	1	0
Verbascum nigrum	0.1	+	I	·	·	·	·	·	·	·	·	·	1	0
Verbascum phlomoides	·	·	·	0.1	+	I	·	·	·	·	·	·	1	0

The highest green fodder mass (MV) production was evaluated in the *Trifolio-Lolietum perennis* association of 18.15 t/ha, with a very good pastoral value (84.20 VP) and a grazing capacity of 1.59 large cattle livestock unit (LU)/ha. At the opposite pole are the *Nardo-Festucetum rubrae fallax* grasslands with 3.86 t/ha green fodder mass (MV) production, 24.85 pastoral value (VP) and a grazing capacity of only 0.37 large cattle livestock unit (LU)/ha (Table 4).

Table 4. Productivity of plant associations from mesophilous grasslans (Codru Moma Mountains)

Plant associations	Season duration (days)	Pastoral value (VP)	Useful phytomass index (IM)	Green mass production (MV) (t/ha)	Livestock units (LU)
Festuco rubrae-Agrostetum capillaris	175	63.40	4.48	11.64	1.02
Trifolio-Lolietum perennis	175	84.20	6.05	18.15	1.59
Anthoxantho-Agrostetum capillaris	175	57.80	3.53	8.47	0.74
Nardo-Festucetum rubrae fallax	160	24.85	1.84	3.86	0.37

In order to have an idea of the productivity of *F. rubra* L. and *A. capillaris* L. meadows in the Codru Moma Mountains, we considered it appropriate to compare them with similar grasslands in the neighboring Mountains. In this sense, we compared the productivity of these grasslands with those in Vlădeasa Mountains (Marușca et al., 2021, Coldea et al., 2008), Bihor Mountains (Togor, 2016) and Pădurea Craiului Mountains (Groza, 2008).

To carry out the comparative study of grassland productivity, a number of 46 relevées from the Vlădeasa Mountains, located at altitudes of 580-1300 m, totaling 109 species, were processed; 16 relevées from the Bihor Mountains, located at altitudes of 700-1360 m, with a total number of 144 species and 21 relevées from Pădurea Craiului Mountains, existing at an altitude of 300-720 m, with a total of 145 species.

The grasslands of *F. rubra* L. and *A. capillaris* L. that are present in Codru Moma Mountains are similar in terms of floristic diversity to those from Pădurea Craiului Mountains and different from those that are present in Vlădeasa Mountains and Bihor Mountains (Figure 4.). This is mainly due to the climatic and pedological conditions in which this type of grassland grows in the neighboring massifs.

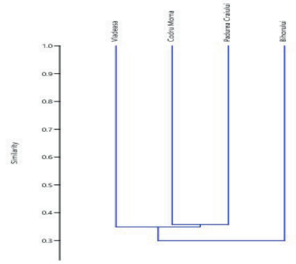

Figure 4. Jaccard similarity index from the grasslands in the regions surrounding Codru Moma Mountains

In terms of green mass productivity, the grasslands in the 4 geographical areas are similar with higher production in Pădurea Craiului Mountains (12.99 t/ha MV) and lower in Vlădeasa Mountains (9.05 t/ha MV). The highest grazing capacity is registred in the grasslands located in Bihor Mountains (1.22 LU) and the lower values in Vlădeasa Mountains (0.96 LU) (Table 5).

Table 5. Evaluation the productivity of the *Festuco rubrae-Agrostetum capillaris* association grasslands in the regions surrounding the Codru Moma Mountains

Geographical region	Season duration (days)	Pastoral value (VP)	Useful phytomass index (IM)	Green mass production (MV) (t/ha)	Livestock units (LU)
Vlădeasa Mountains	145	55.70	3.62	9.05	0.96
Bihorului Mountains	145	64.50	4.43	11.52	1.22
Pădurea Craiului Mountains	175	67.58	4.81	12.99	1.14
Codru Moma Mountains	175	63.40	4.48	11.64	1.02

CONCLUSIONS

The permanent grasslands from Codru Moma Mountains have a great variability from a phytocoenological and agroproductive point of view, with mesophilic plant associations, trampled places and calcareous hills.

The highest productivity (pastoral value and fodder production) can be observed within the grasslands belonging to *Trifolio-Lolietum perennis* association, with 84.20 pastoral value (VP), 18.15 t/ha green fodder mass and 1.59 large cattle livestock unit (LU) and *Festuco rubrae-Agrostetum capillaris* association, with

63.40 pastoral value (VP), 11.64 t/ha green fodder mass (MV) which allows an optimal loading of 1.02 large cattle livestock unit (LU)/ha in 175 days of grazing days.

The lowest productivity is evaluated in *Nardo-Festucetum rubrae fallax* association with 24.85 pastoral value (VP), 3.86 t/ha green fodder mass (MV), and 0.37 large cattle livestock unit (LU)/ha and in *Anthoxantho-Agrostetum capillaris* with 57.80 pastoral value (VP), 8.47 t/ha green fodder mass (MV) which barely allow 0.74 large cattle livestock unit (LU)/ha in a grazing season.

F. rubra L. and *A. capillaris* L. grasslands are the most widespread in Codru Moma Mountains and the neighboring massifs. The pastoral value of these grasslands in the studied mountain area is 55.70-67.58 (VP), with a green fodder mass productivity of 9.05-12.99 t/ha (MV) and a grazing capacity of 0.96-1.22 large cattle livestock unit (LU)/ha.

The data on the productivity of the grasslands determined through the new method of evaluation based on floristic surveys are used to prepare pastoral arrangements and research on the evolution over time of this main indicator.

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RESULTS REGARDING THE CONTROL OF THE PATHOGEN *Erisiphe pisi* (de Candolle) IN THE PEA CROP UNDER THE CONDITIONS AT ARDS PITEȘTI-ALBOTA

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Abstract

Erisiphe pisi (de Candolle) is the pathogen that causes powdery mildew in peas, one of the most important fungal diseases. The objective of the research was to estimate the level of the powdery mildew attack in the Alvesta and Nicoleta pea genotypes, under the experimental conditions at ARDS Piteşti-Albota, in the period 2023-2024. The effect of azoxystrobin and azoxystrobin + difenoconazole molecules in controlling the pathogen was determined. The frequency (F %), intensity (I %), degree of attack (G.A %) of the disease and effectiveness (E %) of the treatments were calculated. The results showed that the azoxystrobin + difenoconazole variant had the lowest attack degree 1.47%, followed by the azoxystrobin variant with 2.26% in the Alvesta genotype. In the Nicoleta variety, higher values of the degree of attack were recorded compared to the Alvesta variety. The effectiveness had the highest value, of 82.92%, when applying the azoxystrobin + difenoconazole treatment, for the Alvesta genotype.

Key words*: Erisiphe pisi, pea, attack degree, variety, disease, effectiveness.*

INTRODUCTION

Pea (*Pisum sativum* L.) is a widely distributed leguminous that is frequently affected by various biotic and abiotic stress factors during the growing season (Ali et al., 1994; Wang et al., 2007; Sun et al., 2019). Powdery mildew is one of the most damaging legume diseases, which is transmitted by air (Rubiales et al., 2015; Santos et al., 2020). *Erysiphe pisi*, belongs to the order Erysiphe's, which forms an important group of plant pathogens, producing powdery mildew in over 10,000 species of angiosperms, which include field crops and wild plants of economic and ecological importance (Ale-Agha et al., 2008; Parthasarathy et al., 2017). The development of the disease is favored by humidity above 70% with temperatures between 15-25°C during the flowering and pod filling period. Thus, under these conditions, the infection can completely colonize the plant within 5-6 days and the disease spreads rapidly to adjacent areas. Symptoms of the attack manifest themselves on leaves, stipules, stems, pods and berries. Whitish-dusty spots appear on the leaf surfaces, which are made up of the mycelium and fruiting bodies of the fungus. As the fungus develops, the whitish layer darkens and becomes gray, and the affected tissues turn brown and necrotic (https://www.anfdf.ro/sanatate/ghid/ghid_leguminoase_2021.pdf).

These fungi have complex life cycles and are biotrophic in nature. Due to this characteristic, they are difficult to study under laboratory conditions (Singh et al., 1988; Smith et al., 1996; Parthasarathy et al., 2017). Worldwide, powdery mildew caused by *Erysiphe pisi* (de Candolle) can reduce the yield and quality of pea crops (Gritton et al., 1975; Peng et al., 1991; Smith et al., 1996; Sun et al., 2019). Severe infections can lead to a yield reduction of up to 80% (Smith et al., 1996; Ghafoor et al., 2012; Sun et al., 2019). *Erysiphe pisi,* the agent causing powdery mildew in peas, can cause infection of the aerial parts of the plants (Singh, 2000; Bahadur et al., 2008). This pathogen negatively affects the total biomass, the number of pods per plant, the number of grains per pod, the number of nodes and the height of the plants (Gritton et al., 1975; Attanayake et al., 2010). In order to manage the disease, the use of chemicals is the most commonly used method when resistance is absent in the varieties available on the market. The high cost of fungicides makes their use uneconomical (Kapoor et al., 1995). The measures for the prevention and management of pathogens consider an integrated control based on the cultivated genotype, and where appropriate, treatments are applied and their effectiveness is also necessary to calculate (Toth & Cristea, 2018; Jaloba et al., 2019; Toth & Cristea, 2020; Podea et al., 2024). In this paper, data are presented on the behavior of two pea varieties, Alvesta and Nicoleta, to powdery mildew attack, with different treatments.

MATERIALS AND METHODS

The research aimed to identify and establish the attack caused by the pathogen *Erysiphe pisi* (de Candolle), under different treatment conditions on 2 pea varieties, Alvesta and Nicoleta. The research was carried out in the experimental field of the ARDS Pitești-Albota in 2023, 2024. To achieve the proposed objectives, a bifactorial experiment with 4 repetitions was set up.

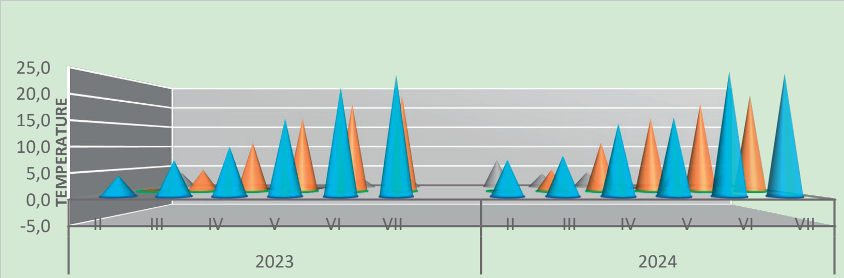
Factor A. pea varieties (Alvesta and Nicoleta).
Factor B - treatments: V1 control variant, V2 *azoxystrobin*; V3 *azoxystrobin+ difenoconazole*. The treatments were applied during the vegetation period when the first inflorescences appeared. The control variant was not treated. The frequency (F %), attack intensity (I %), attack rate (A.D %) and efficacy (E %) of the applied treatments were determined. They were calculated according to the formulas: Frequency (F %) $= \frac{n}{N}\ x\ 100$, where N = number of plants observed (%), n = number of plants with specific symptoms. The intensity was noted in percentages and calculated according to the formula: Intensity (I %) = Σ $\frac{ixf}{n}$,, where I = given percentage, f = number of plants/organs with the respective percentage, n = total number of plants/organs attacked. Based on the data obtained by calculating the frequency and intensity, the degree of attack was calculated: A.D (%) = $\frac{F\%\ x\ I\ \%}{100}$, where A.D = degree of attack (%), F = frequency (%), I = intensity (%).

The effectiveness of the treatments was determined according to the formula E % = [Gam - Gav/ Gam] x 100 (%) (Abbott's formula), where Gam = the degree of attack on the control variant, Gav = the degree of attack on the treated variant.

Table 1. The variants used in the experiment

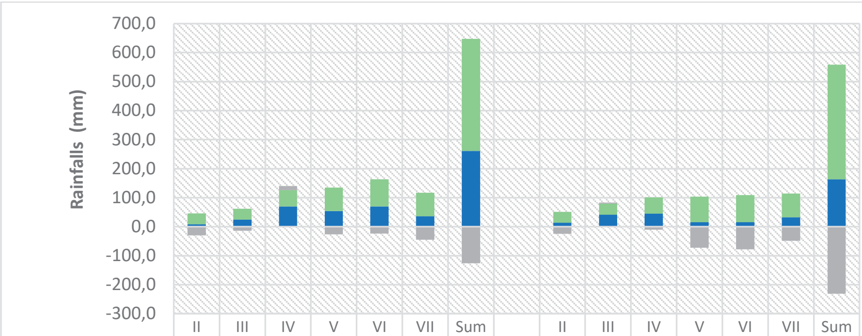
Var.	Product	Active ingredient	Rate (l, t, kg/ha)
1	Untreated	-	
2	Zoxis	azoxystrobin 250 g/l	1 l/ha
3	Ortiva Top	azoxystrobin (200 g/l) + difenoconazole (125 g/l)	1 l /ha

The climatic conditions were registered at the weather station located in the station during the period February-July (the crop vegetation period). The meteorological data characterize the 2 years as years with high temperatures, with positive deviations from the multiannual average, except for the months of April and May when negative deviations of - 0.9°C, - 0.7°C , and - 0.5°C were registered. In 2023, the average temperature of the period was 13.86 °C, exceeding the multiannual average of 12.25 °C by 1.61°C. The year 2024 registered an average temperature of 15.9°C, 3.65°C higher than the multiannual average (Figure 1). The weather data of the pea growing season describe the 2 years as dry with low, unevenly distributed precipitation. Regarding rainfall during the research period, in 2023 an amount of 260.7 mm was registered, with a deficit of – 126.1 mm. Negative deviations from the multiannual amount were registered during the 6 months, with the exception of April, which brought an increase of 13.9 mm compared to the multiannual amount of 55.9 mm. The amount of rainfall in 2024 was lower compared to 2023, totalling 163.4 mm, with a - 231.4 mm. March was the only month in which a positive deviation of 3.5 mm was registered (Figure 2).



	2023							2024					
	II	III	IV	V	VI	VII		II	III	IV	V	VI	VII
Monthly average	4,2	7,2	10,0	15,6	21,8	24,4		7,31	8,11	14,6	15,8	25	24,6
Multiannual average	0,6	4,8	10,9	16,3	19,5	21,4		0,6	4,8	10,9	16,3	19,5	21,4
Deviation	3,6	2,4	-0,9	-0,7	2,3	3,0		6,7	3,3	3,7	-0,5	5,5	3,2

Figure 1. The monthly average temperature in the period February-July 2023 and 2024 (ARDS Albota weather station)



	II	III	IV	V	VI	VII	Sum		II	III	IV	V	VI	VII	Sum
Deviation	-29,8	-14,0	13,9	-26,7	-24,0	-45,5	-126,		-24,4	3,5	-10,8	-73,2	-78,1	-48,4	-231
Multiannual sum	37,7	38,0	55,9	80,5	93,6	81,1	386,8		37,7	38	55,9	88,5	93,6	81,1	394,8
Monthly sum	7,9	24,0	69,8	54,0	69,6	35,6	260,7		13,3	41,5	45,1	15,3	15,5	32,7	163,4
	2023								2024						

Figure 2. The evolution of rainfall in the period February - July 2023 and 2024 (ARDS Albota weather station)

RESULTS AND DISCUSSIONS

Pea is a leguminous that is affected by numerous pathogens that induce severe diseases, including powdery mildew (*Erysiphe pisi*).

The study of pathogen attack on pea crops is of major importance in determining the need for chemical treatments during the growing season. An important role in obtaining a high yield is played by the crop's disease protection system (Zotov & Badarin, 2015; Gulnar et al., 2022).

The first symptoms of powdery mildew attack appeared in the second decade of May, first on the leaves, then they covered the entire plant.

The determinations were carried out under natural contamination conditions (Figure 3).

The first fungicide treatment was applied at the appearance of the first inflorescences (T1), and the second treatment after approximately 14 days (only for the Ortiva Top product). The Zoxis product was applied only once.

Figure 3. Powdery mildew attack on peas (original)

The results obtained in 2023, regarding the frequency of attacks, registered values between 26 and 61%. Alvesta genotype obtained the lowest frequency value (26.0%) in the variant with the application of treatment with *difenoconazole* + *azoxystrobin*, followed by the variant with the application of treatment with a*zoxystrobin* of 32.0%, with very significantly negative differences.

Attack intensity ranged from 9.2 to 21.2%. The lowest degree of attack was also registered with the *azoxystrobin* + *difenoconazole* variant of 2.40%.

In the Nicoleta variety, the attack frequency was between 37% and 61% with an intensity between 9.8-20.2%, resulting in an attack degree with values between 3.62 and 12.30%.

The attack produced by powdery mildew registered very significant negative differences in both varieties tested (Table 2).

Table 2. The behavior of pea varieties to the attack caused by powdery mildew in the year 2023

Variety	Variants tested	The pathogen / disease/ powdery mildew *Erysiphe pisi (de Candolle)*								
		F%	Dif.	Sem.	I %	Dif.	Sem.	AD%	Dif.	Sem.
Alvesta	Control variant	53.0	-	Mt.	21.2	-	Mt.	11.23	-	Mt.
	Azoxystrobin	32.0	-21.0	000	11.3	-9.88	000	3.61	-7.62	000
	Azoxystrobin+ difenoconazole	26.0	-27.0	000	9.2	-12.05	000	2.40	-8.83	000
Nicoleta	Control variant	61.0	-	Mt.	20.2	-	Mt.	12.30	-	Mt.
	Azoxystrobin	41.0	-20.0	000	13.9	- 6.20	000	5.70	-5.53	000
	Azoxystrobin+ difenoconazole	37.0	-24.0	000	9.8	-10.45	000	3.62	-7.61	000
LSD 5%		**8,12**			**2.34**			**2.21**		
LSD 1%		**11,40**			**3.29**			**3.10**		
LSD 0.1%		**16,10**			**4.65**			**4.38**		

F(%) - Frequency, I(%) - Intensity, AD (%) - Attack degree, Dif. - Difference, Semen. - Semification

Table 3 shows results regarding the frequency, intensity of the attack and the degree of attack produced by powdery mildew in the year 2024. In the Alvesta variety, the attack frequency registered average values between 16 and 46%. The highest frequency of 46% occurred in the control variant, followed by the variant with the administration of *azoxystrobin* treatment with 21% in which the intensity was 10.75% and the degree of attack obtained 2.26%. The lowest value of 16% of the frequency was registered when *azoxystrobin +difenoconazole* products were administered, the intensity was 9.20 and the AD% obtained was 1.47%. The frequency of attacked plants in the Nicoleta variety varied between 23 and 55 %. In the control version, the attack frequency had the highest value, 55% with an intensity of 19.08, obtaining a AD% of 10.50%. The application of the *azoxystrobin* treatment thus influenced the mealybug attack: the resulting frequency was 30%, the intensity 10.38% which resulted in a AD of 3.11%. In the variant to which the two molecules of *azoxystrobin +difenoconazole* were applied, the frequency of attacked plants was the lowest (23%) compared to the rest of the variants, the intensity of 10.25% in which the degree of attack also registered the lowest value, namely 2.35%. The application of the two treatments against powdery mildew to the 2 studied varieties registered very significantly negative differences compared to the control variants.

Table 2. The behavior of pea varieties to the attack caused by powdery mildew in the year 2024
F(%) - Frequency, I(%)- Intensity, AD (%) - Attack degree, Dif-Difference, Sem-Semification

Variety	Variants tested	The pathogen / disease/ powdery mildew *Erysiphe pisi (de Candolle)*								
		F%	Dif.	Sem.	I %	Dif.	Sem.	AD%	Dif.	Sem.
Alvesta	Control variant	46.00	-	Mt.	18.50	-	Mt.	8.51	-	Mt.
	Azoxystrobin	21.00	-25.00	000	10.75	-7.75	000	2.26	-6.19	000
	Azoxystrobin+ difenoconazole	16.00	-30.00	000	9.20	-9.30	000	1.47	-7.00	000
Nicoleta	Control variant	55.00	-	Mt.	19.08	-	Mt.	10.50	-	Mt.
	Azoxystrobin	30.00	-25.00	000	10.38	-8.70	000	3.11	-7.50	000
	Azoxystrobin+ difenoconazole	23.00	-32.00	000	10.25	-8.83	000	2.35	-8.28	000
LSD 5%		**8.82**			**3.82**			**2.58**		
LSD 1%		**12.38**			**5.36**			**3.63**		
LSD 0,1%		**17.47**			**7.57**			**5.12**		

Determining the effectiveness of products in combating plant pathogens is a permanent concern in research and application activity (Buzatu et al., 2018; Jaloba et al., 2019; Alexandru et al., 2019; Chiriac &, Cristea 2021). Regarding the effectiveness of the products applied in the period 2023, 2024 (Figure 4) against the pathogen *Erysiphe pisi*, values between 53.31 and 82.92% were obtained. The application of the *azoxystrobin* treatment to the Alvesta variety in 2023 ensured an effectiveness of over 67%, while an effectiveness of 53% was registered for the Nicoleta genotype. In 2024, the same treatment reduced the attack of the pathogen ensuring an effectiveness of 73.41% for the Alvesta variety and 70.75% for Nicoleta. The treatment with the substances azoxystrobin +difenoconazole in the year 2023 had an effectiveness of the Alvesta variety of 78.37%, Nicoleta 70.43% and the year 2024 brought an effectiveness of 82.92% (Alvesta) and 78.11% for Nicoleta.

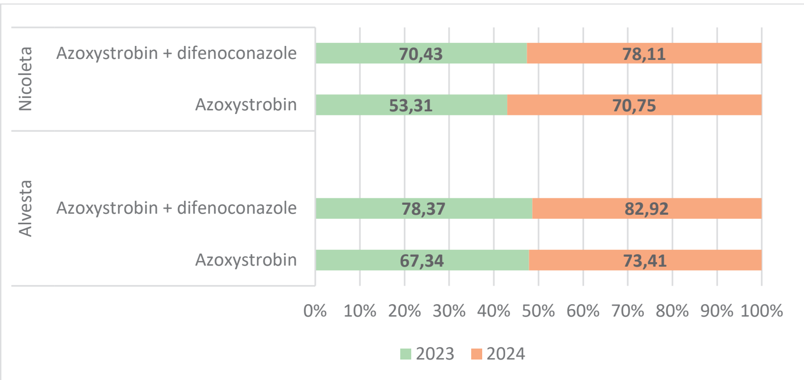


Figure 4. The effectiveness (%) of treatments in the period 2023 and 2024

CONCLUSIONS

Application of a treatment scheme with approved products to ensure disease control is necessary to obtain high yields. In the experimental conditions at ARDS Pitesti-Albota, the powdery mildew attack on the 2 pea varieties manifested itself differently during the researched period. In the Alvesta variety, the attack values were lower compared to the Nicoleta genotype in the 2 years of the study. The type of treatment applied against powdery mildew influenced the degree of attack by the fungus. In the year 2023 the AD% of the pathogen recorded higher values in the 2 genotypes, compared to the year 2024. The application of the molecules *azoxystrobin* + *difenoconazole* had the best effectiveness against powdery mildew.

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PERFORMANCE AND QUALITY OF HEMP MICROGREENS UNDER SUBSTRATE AND WATERING CONDITIONS

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Abstract

Microgreens are a sustainable and innovative food source, highly valued for their nutritional content, short cultivation time, and potential to address global food security challenges. The experiment was conducted in 2024 in a controlled environment growth chamber using monoecious hemp seeds. It evaluated the effects of three substrates (perlite, peat and vermicompost) and two types of watering (distilled water and water from a recirculating aquaculture system – RAS water) on the biometric traits, yield, and nutritional quality of hemp microgreens. The results showed that the Peat x RAS water variant produced the highest fresh matter yield (12.75 g/100 cm²) and the largest leaf area index (LAI – 332.25 cm²/100 cm²), as well as the highest protein content (23.72%). The Vermicompost x distilled water combination resulted in the highest total fiber content (18.62%), while the Perlite x distilled water variant had the highest content of total soluble solids (7.2°BX). These findings highlight the essential role of substrate and watering in optimizing biometry, yield, and nutritional properties, further establishing hemp microgreens as a sustainable and innovative choice for modern diets.

Key words: *hemp microgreens, biometric traits, matter yield, nutritional quality.*

INTRODUCTION

Microgreens are tender, immature greens that are generally larger than sprouts and smaller than baby greens, recognized as 'functional food' (Lenzi et al., 2019). They have gained significant attention recently due to their high nutraceutical value and ability to meet dietary nutrient adequacy (Di Gioia et al., 2021). This is attributed to their rich bioactive phytochemical content, which includes polyphenols, vitamins, minerals, and proteins (Dayakar Rao et al., 2017). Microgreens are considered specialty crops for their intense flavors, attractive colors, and rich bioactive compounds, making them highly valued in culinary applications (Lenzi et al., 2019).

Different species of microgreens contain varying levels of essential nutrients and bioactive compounds, making them a valuable addition to a health-promoting diet. For instance, amaranth microgreens contain chlorophyll a (0.25 mg/g), chlorophyll b (0.20 mg/g), carotenoids (0.023 mg/g), anthocyanins (9 mg/100 g), and ascorbic acid (0.031 mg/g), which contribute to their antioxidant activity (Sarker and Oba, 2019; Rocchetti et al., 2020). Red beet microgreens are rich in polyphenols (313.8 mg/100 g), betaxanthins (432.7 mg/100 g), and betacyanins (226.7 mg/100 g), offering both antioxidant and gastrointestinal benefits (Rocchetti et al., 2020). Quinoa microgreens are notable for their tocopherols (65 µg/g), β-carotene (738 µg/g), and fatty acids, including α-linolenic acid (35.1%) and linolenic acid (11.36%), which enhance their antioxidant properties (Pathan and Siddiqui, 2022). Spinach microgreens contain chlorophylls (44 µg/g), lutein (54.2 µg/g), β-carotene (44 µg/g), phenols (632.3 µg/g), and ascorbic acid (130.5

µg/g), contributing to their strong antioxidant activity (Petropoulos et al., 2021).

The cultivation of microgreens involves selecting appropriate seeds, growth methods, and substrates to optimize their nutritional value and yield (Gunjal et al., 2024). These young, immature greens are typically harvested between 7 and 21 days after germination, and in some cases up to 28 days, depending on the species, cultivar, and growing conditions. At this developmental stage, microgreens are characterized by high concentrations of vitamins, minerals, antioxidants etc. (Kyriacou et al., 2019; Rouphael et al., 2021; Gunjal et al., 2024; Popa et al., 2024). Scientific literature indicates that microgreens typically contain higher levels of essential phytonutrients compared to their mature counterparts (El-Nakhel et al., 2020; Pannico et al., 2020; Paraschivu et al., 2021). Various growing methods, including indoor, outdoor, and controlled environments like greenhouses, are employed to enhance their growth (Di Gioia and Santamaria, 2015). In terms of value addition, microgreens are increasingly being incorporated into various food products, such as functional beverages, gluten-free baked goods, and ready-to-eat chutney powders, to enhance their nutritional profile and appeal (Sharma et al., 2021; Kaur et al., 2022; Nivedha and Lakshmy Priya, 2018). These applications not only improve the sensory qualities of the products but also provide significant health benefits due to the high content of bioactive compounds in microgreens (Gunjal et al., 2024). Substrate selection is a critical factor influencing the growth, yield, and nutritional quality of microgreens. Different substrates affect water retention, aeration, nutrient availability, and root development, ultimately impacting plant metabolism and biochemical composition. Organic substrates, such as peat and vermicompost, are known to enhance nutrient accumulation, whereas inert media like perlite may influence secondary metabolite synthesis, as previously highlighted by Kyriacou et al. (2019). Understanding the role of substrates in optimizing microgreen production is essential for maximizing both nutritional value and consumer acceptance in sustainable agricultural systems.

Hemp (*Cannabis sativa* L.) is a versatile multi-purpose crop cultivated in various agro-ecological conditions and processed for multiple uses, including textile fibers, paper, paint, biofuels, timber, biodegradable plastics, hempcrete, human food and animal feed, as well as for medicinal purposes (Popa et al., 2021; Adam and Isopescu, 2022; Pannico et al., 2022). Hemp plants synthesize hundreds of biologically active secondary metabolites, including terpenoids, cannabinoids, glycosidic compounds, polyphenols, fatty acids, simple acids, amino acids, enzymes, steroids, pigments, and vitamins (Kuddus et al., 2013). These findings suggest that hemp microgreens can be a valuable addition to the diet, offering significant health benefits through their rich content of bioactive compounds.

Given the growing interest in optimizing microgreen production for enhanced nutritional and visual quality, this study aims to evaluate the impact of different growing substrates-perlite, peat, and vermicompost-combined with two watering regimes (distilled water and RAS water) on the yield, nutritional composition, biometric traits, and color parameters of hemp (*Cannabis sativa* L.) microgreens. Specifically, the research seeks to determine how substrate choice influences key nutritional components such as protein, fiber, ash content, total soluble solids, and oxalic acid concentration, as well as biometric traits and color attributes (L, a, b values).

MATERIALS AND METHODS

Biological Material and Experimental Design

The research was conducted at University of Life Sciences Iasi (IULS), using monoecious hemp seeds provided by Agricultural Research and Development Station Secuieni (ARDS Secuieni), the owner of the biological material. Monoecious hemp microgreens were cultivated under controlled conditions to evaluate the influence of different substrates and watering regimes on yield, nutritional composition, biometric traits, and color parameters. The experiment was organized using a bifactorial design (3 × 2), involving three substrate types (perlite, peat and vermicompost) and two watering regimes (distilled water and water from a recirculating aquaculture system - RAS water).

Substrate and Watering Treatments

In this experiment, perlite was used as a growing substrate due to its properties as an inert medium with very good aeration and high drainage capacity, promoting microgreens root development and preventing excess moisture.

The choice of peat was based on its high water retention capacity and moderate aeration, providing a stable growing environment that enhances nutrient availability and sustains root hydration throughout microgreens development.

Vermicompost was selected as a growing substrate due to its biologically active nature, resulting from the decomposition of organic matter by earthworms, which enhances microbial diversity and ensures a steady supply of essential nutrients, thereby promoting optimal microgreens growth and development.

The experimental variants were watered with distilled water and RAS water. The latter was sourced from a recirculating aquaponic system and contains natural nutrients, including nitrogen, phosphorus, and potassium, which may influence the metabolism and growth of microgreens.

Microgreens Growth Conditions

The seeds were uniformly sown in trays filled with the selected substrates. Subsequently, the trays were placed in a growth chamber under controlled environmental conditions: temperature 22 ± 2°C, relative humidity 70%, and a photoperiod of 16 h light / 8 h dark (Figure 1). Watering was performed manually once daily to maintain a consistent substrate moisture level. The microgreens were harvested 15 days after sowing.

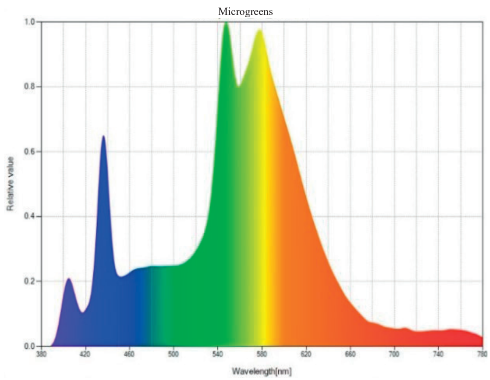


Figure 1. Spectral composition of light in the climate-controlled chamber

Yield and Biometric Traits

At harvest, the following measurements were taken for microgreens: fresh matter yield (g/100 cm²), dry matter yield (g/100 cm²), microgreens length (cm), and leaf area index (LAI).

Fresh matter yield (g/100 cm²) was determined using an analytical balance.

Dry matter yield (g/100 cm²) was determined after drying samples in an oven at 60°C for 48 hours.

Microgreens length (cm) was measured using a ruler.

Leaf Area Index (LAI) was measured using the LI-3100C area meter (LI-COR, Lincoln, NE, USA), with results expressed in cm² per 100 cm².

Nutritional and Biochemical Analysis

The nutritional composition of the microgreens, including **protein, ash, neutral detergent fiber (NDF), acid detergent fiber (ADF), fiber** and **energy** content, was analyzed using a Near-Infrared Reflectance (NIR) DA 7250 Analyzer (Perten, Sweden), which enables a rapid and precise assessment of macronutrient content. All compounds were expressed as percentages, except for energy, which was reported in $MJ \cdot kg^{-1}$.

Titratable acidity (TA) was determined using the titrimetric method. Following the homogenization of hemp microgreens samples in distilled water, titration was performed with NaOH until reaching a pH of 8.1. The results were expressed as a percentage of oxalic acid.

Total Soluble Solids (TSS) were quantified using a digital refractometer, with results expressed in °Brix, in accordance with Irimia (2013) and the OECD standards (2018).

Color Parameters

Leaf color was assessed using a HunterLab colorimeter, applying the CIELAB scale, which measures lightness (L*), where higher values indicate a lighter green shade and lower values correspond to darker tones; the red-green axis (a*), where negative values denote increased greenness; and the yellow-blue axis (b*), where higher values represent a more yellowish hue.

Statistical Analysis

All data were analyzed using one-way and two-way ANOVA to evaluate the effects of substrate and watering treatment. Post-hoc comparisons were performed using Tukey's HSD test ($p < 0.05$) to identify significant differences among variants. Results are presented as mean ± standard error (SE). Statistical analyses were conducted using SPSS v.25 (IBM Corp.).

Additionally, Principal Component Analysis (PCA) and Pearson correlation analysis were applied to assess the effects of substrate type variation and watering regime on the yield, biometric traits, nutritional composition and color parameters of hemp microgreens.

RESULTS AND DISCUSSIONS

Substrate type significantly influenced the yield and biometric traits of hemp microgreens (Table 1). Microgreens grown on peat exhibited the highest fresh matter yield (12.27 ± 0.4 g·cm^{-2}), dry matter yield (1.55 ± 0.09 g·cm^{-2}), and length (8.78 ± 0.32 cm). In contrast, those cultivated on vermicompost recorded the lowest values for fresh matter yield (4.97 ± 0.46 g·cm^{-2}) and dry matter yield (0.65 ± 0.03 g·cm^{-2}). However, length did not significantly differ between microgreens grown on vermicompost (7.04 ± 0.55 cm) and those cultivated on perlite (6.68 ± 0.15 cm), while peat resulted in a significantly higher length (8.78 ± 0.32 cm).

The Leaf Area Index (LAI) was also significantly higher in peat-grown microgreens (307.73 ± 30.52 cm²·cm^{-2}) compared to those cultivated on perlite (209.09 ± 9.01 cm²·cm^{-2}) and vermicompost (129.14 ± 8.17 cm²·cm^{-2}). These findings confirm that peat provided the most favorable conditions for biomass accumulation in hemp microgreens, while vermicompost resulted in significantly lower fresh matter yield and dry matter yield, despite similar length to perlite.

Watering regime had a limited effect on the biometric traits of hemp microgreens, while its influence on yield was significant only for dry matter yield. Differences in fresh matter yield, length, and LAI did not reach statistical significance (Table 1). Microgreens watered with RAS water exhibited slightly higher fresh matter yield (9.41 ± 0.22 g·cm^{-2}) and dry matter yield (1.13 ± 0.02 g·cm^{-2}) compared to those watered with distilled water (8.47 ± 0.38 g·cm^{-2} fresh matter yield, 0.92 ± 0.05 g·cm^{-2} dry matter yield). Among these parameters, only dry matter yield was significantly influenced by the watering regime, with higher values recorded under RAS water application.

Length did not differ significantly between watering regimes, with values ranging from 7.39 ± 0.33 cm in microgreens watered with RAS water to 7.62 ± 0.08 cm in those watered with distilled water. Similarly, LAI was slightly higher in microgreens watered with RAS water (228.09 ± 5.05 cm²·cm^{-2}) compared to those receiving distilled water (202.54 ± 11.83 cm²·cm^{-2}), but the difference was not statistically significant.

These results indicate that the watering regime had a significant effect on dry matter yield but did not significantly influence fresh matter yield and biometric traits.

Table 1. Effect of substrate and watering regime on yield and biometric traits of hemp microgreens

Treatment	Fresh matter yield (g·cm^{-2})	Dry matter yield (g·cm^{-2})	Length (cm)	LAI (cm^2·cm^{-2})
Substrate				
Perlite	9.58 ± 0.23 b	0.88 ± 0.03 b	6.68 ± 0.15 b	209.09 ± 9.01 b
Peat	12.27 ± 0.40 a	1.55 ± 0.09 a	8.78 ± 0.32 a	307.73 ± 30.52 a
Vermicompost	4.97 ± 0.46 c	0.65 ± 0.03 b	7.04 ± 0.55 b	129.14 ± 8.17 b
Significance	*	*	*	*
Watering				
Distilled water	8.47 ± 0.38	0.92 ± 0.05	7.62 ± 0.08	202.54 ± 11.83
RAS water	9.41 ± 0.22	1.13 ± 0.02	7.39 ± 0.33	228.09 ± 5.05
Significance	ns	*	ns	ns

Values are presented as mean ± standard error (SE). Within each column, * - statistically significant difference, ns - no statistically significant difference, values associated to different letters are significantly different according to Tukey's test at $p<0.05$. LAI - Leaf Area Index; RAS water - water from recirculating aquaculture system.

The combined influence of substrate and watering regime significantly affected the biometric traits and yield of hemp microgreens (Table 2). The highest fresh matter yield was observed in microgreens grown on peat with RAS water (12.75 ± 0.38 g), followed by those on peat with distilled water (11.8 ± 0.74 g). The lowest fresh matter yield values were recorded in microgreens cultivated on vermicompost, regardless of watering regime (4.57 ± 0.34 g – 5.37 ± 0.59 g). Similarly, dry matter yield followed a comparable trend, with the highest

values in peat-grown microgreens (1.7 ± 0.07 g) and the lowest in vermicompost (0.59 ± 0.02 g).
Regarding biometric traits, microgreens cultivated on peat exhibited the greatest length (8.83 ± 0.35 cm), while those grown on perlite had the shortest (6.2 ± 0.38 cm). Vermicompost-grown microgreens had intermediate values (6.94 ± 0.24 cm – 7.13 ± 0.9 cm), without significant differences compared to perlite or peat.
Leaf area index (LAI) was also highest in peat-grown microgreens watered with RAS water (332.25 ± 34.98), while vermicompost treatments resulted in the lowest values (120.86 ± 15.52 – 137.44 ± 4.34).
These results indicate that peat substrates, particularly in combination with RAS water, promoted the highest fresh and dry matter yield, as well as greater leaf area development in hemp microgreens. However, length and LAI were primarily influenced by substrate type rather than the watering regime, as differences between watering treatments within the same substrate were not statistically significant.

Table 2. Combined effect of substrate and watering regime on yield and biometric traits of hemp microgreens

Treatment	Fresh matter yield ($g \cdot cm^{-2}$)	Dry matter yield ($g \cdot cm^{-2}$)	Length (cm)	LAI ($cm^2 \cdot cm^{-2}$)
Perlite x Distilled water	9.05 ± 0.53 c	0.78 ± 0.04 bc	7.17 ± 0.17 ab	203.58 ± 9.08 bc
Perlite x RAS water	10.11 ± 0.32 bc	0.98 ± 0.04 b	6.20 ± 0.38 b	214.59 ± 21.14 abc
Peat x Distilled water	11.80 ± 0.74 ab	1.40 ± 0.17 a	8.73 ± 0.33 a	283.19 ± 44.30 ab
Peat x RAS water	12.75 ± 0.38 a	1.70 ± 0.07 a	8.83 ± 0.35 a	332.25 ± 34.98 a
Vermicompost x Distilled water	4.57 ± 0.34 d	0.59 ± 0.02 c	6.94 ± 0.24 ab	120.86 ± 15.52 c
Vermicompost x RAS water	5.37 ± 0.59 d	0.72 ± 0.05 bc	7.13 ± 0.90 ab	137.44 ± 4.34 c
Significance	*	*	*	*

Values are presented as mean ± standard error (SE). Within each column, * - statistically significant difference, values associated to different letters are significantly different according to Tukey's test at $p<0.05$. LAI - Leaf Area Index; RAS water - water from recirculating aquaculture system.

Substrate type had a significant influence on the nutritional composition of hemp microgreens (Table 3). Microgreens grown on peat exhibited the highest protein content (22.72 ± 0.09%), followed by those on vermicompost (21.47 ± 0.22%) and perlite (20.11 ± 0.17%). Ash content was highest in microgreens grown on perlite (13.88 ± 0.20%) and lowest in vermicompost (13.11 ± 0.14%). Regarding fiber content, peat-grown microgreens showed the highest NDF content (25.25 ± 0.17%), whereas perlite-grown microgreens had the lowest (21.17 ± 0.80%). Acid detergent fiber (ADF) did not significantly differ among substrates. Fiber content was highest in microgreens grown on vermicompost (16.76 ± 0.24%) and lowest on peat (15.95 ± 0.14%). Energy content was highest in perlite-grown microgreens (11.27 ± 0.04 $MJ \cdot kg^{-1}$), with peat showing the lowest values (11.02 ± 0.04 $MJ \cdot kg^{-1}$). These results suggest that peat substrates provide optimal conditions for protein and NDF accumulation, whereas perlite favors higher ash and energy content.
The watering regime significantly influenced certain nutritional properties of hemp microgreens (Table 3). Microgreens watered with RAS water showed significantly higher protein content (22.29 ± 0.18%) compared to those watered with distilled water (20.57 ± 0.04%). Ash, NDF, and ADF contents were not significantly influenced by watering regime, despite slight numerical differences between treatments. Fiber was significantly greater under distilled water conditions (17.30 ± 0.12%) compared to RAS water (15.57 ± 0.10%). Energy content was also not significantly influenced by the watering regime.

Table 3. Effect of substrate and watering regime on the nutritional composition of hemp microgreens

Treatment	Protein (%)	Ash (%)	NDF (%)	ADF (%)	Fiber (%)	Energy (MJ·kg^{-1})
Substrate						
Perlite	20.11 ± 0.17 c	13.88 ± 0.20 a	21.17 ± 0.80 b	40.17 ± 0.11	16.60 ± 0.07 a	11.27 ± 0.04 a
Peat	22.72 ± 0.09 a	13.83 ± 0.06 a	25.25 ± 0.17 a	40.08 ± 0.15	15.95 ± 0.14 b	11.02 ± 0.04 b
Vermicompost	21.47 ± 0.22 b	13.11 ± 0.14 b	23.33 ± 0.88 ab	40.42 ± 0.15	16.76 ± 0.24 a	11.13 ± 0.05 ab
Significance	*	*	*	ns	*	*
Watering						
Distilled water	20.57 ± 0.04	13.41 ± 0.14	24.06 ± 0.83	40.44 ± 0.14	17.30 ± 0.12	11.10 ± 0.03
RAS water	22.29 ± 0.18	13.80 ± 0.12	22.45 ± 0.53	40.00 ± 0.12	15.57 ± 0.10	11.17 ± 0.04
Significance	*	ns	ns	ns	*	ns

Values are presented as mean ± standard error (SE). Within each column, * - statistically significant difference, ns - no statistically significant difference, values associated to different letters are significantly different according to Tukey's test at p<0.05. NDF - Neutral Detergent Fiber; ADF - Acid Detergent Fiber; RAS water - water from recirculating aquaculture system.

The combined influence of substrate and watering regime significantly affected the nutritional composition of hemp microgreens (Table 4). Protein content varied significantly across treatments, with the highest values recorded in microgreens cultivated on peat with RAS water (23.72 ± 0.19%) and vermicompost with RAS water (23.13 ± 0.43%). In contrast, the lowest protein content was observed in microgreens grown on vermicompost with distilled water (19.80 ± 0.12%) and perlite with RAS water (20.03 ± 0.33%).

Ash content also varied significantly, with the highest value recorded in peat with RAS water (14.17 ± 0.13%) and the lowest value in vermicompost with distilled water (12.90 ± 0.19%).

The highest NDF values were observed in microgreens cultivated on peat with distilled water (25.83 ± 0.17%), while the lowest values occurred in those grown on perlite with distilled water (20.67 ± 1.02%). ADF content was significantly influenced, with the highest value found in vermicompost with distilled water (41.00 ± 0.26%) and the lowest in vermicompost with RAS water (39.83 ± 0.17%). The highest fiber content was observed in microgreens cultivated on vermicompost with distilled water (18.62 ± 0.38%), while the lowest value was recorded in those grown on vermicompost with RAS water (14.90 ± 0.12%).

Energy content ranged from 10.95 ± 0.05 MJ·kg^{-1} (vermicompost with distilled water) to 11.30 ± 0.06 MJ·kg^{-1} (perlite with distilled water and vermicompost with RAS water), confirming the significant combined impact of substrate and watering regime on the nutritional quality of hemp microgreens.

Table 4. Combined effect of substrate and watering regime on the nutritional composition of hemp microgreens

Treatment	Protein (%)	Ash (%)	NDF (%)	ADF (%)	Fiber (%)	Energy (MJ·kg^{-1})
Perlite x Distilled water	20.18 ± 0.09 c	13.83 ± 0.29 ab	20.67 ± 1.02 c	40.33 ± 0.21 ab	16.77 ± 0.08 b	11.30 ± 0.04 a
Perlite x RAS water	20.03 ± 0.33 c	13.92 ± 0.17 ab	21.67 ± 0.99 bc	40.00 ± 0.02 b	16.43 ± 0.13 b	11.24 ± 0.06 ab
Peat x Distilled water	21.72 ± 0.23 b	13.50 ± 0.14 abc	25.83 ± 0.17 a	40.00 ± 0.02 b	16.52 ± 0.09 b	11.05 ± 0.05 bc
Peat x RAS water	23.72 ± 0.19 a	14.17 ± 0.13 a	24.67 ± 0.33 ab	40.17 ± 0.31 ab	15.38 ± 0.22 c	10.98 ± 0.06 c
Vermicompost x Distilled water	19.80 ± 0.12 c	12.90 ± 0.19 c	25.67 ± 1.48 a	41.00 ± 0.26 a	18.62 ± 0.38 a	10.95 ± 0.05 c
Vermicompost x RAS water	23.13 ± 0.43 a	13.32 ± 0.12 bc	21.00 ± 0.89 bc	39.83 ± 0.17 b	14.90 ± 0.12 c	11.30 ± 0.06 a
Significance	*	*	*	*	*	*

Values are presented as mean ± standard error (SE). Within each column, * - statistically significant difference, values associated to different letters are significantly different according to Tukey's test at p<0.05. NDF - Neutral Detergent Fiber; ADF - Acid Detergent Fiber; RAS water - water from recirculating aquaculture system.

The type of substrate significantly affected the titratable acidity (TA) and total soluble solids (TSS) content of hemp microgreens (Table 5). The highest TA values, expressed as % oxalic acid, were recorded in microgreens grown on perlite (0.26 ± 0.01%), followed by peat (0.22 ± 0.01%), with the lowest values observed on vermicompost (0.18 ± 0.01%). These findings indicate that perlite may enhance oxalate synthesis, while vermicompost helps to reduce oxalic acid accumulation. TSS content was highest in microgreens grown on perlite and vermicompost (6.50 ± 0.01 °Bx), significantly higher compared to peat (5.60 ± 0.05 °Bx).

These results indicate that vermicompost, despite lowering oxalic acid content, maintained TSS levels comparable to perlite, suggesting its suitability for simultaneously improving nutritional quality and reducing oxalate content.

The watering regime significantly influenced both titratable acidity and TSS content (Table 5). Microgreens watered with distilled water had significantly higher TA (0.27 ± 0.01%) compared to those watered with RAS water (0.17 ± 0.00%), indicating that RAS water provides nutrients that effectively reduce oxalic acid accumulation in hemp microgreens. Similarly, TSS was slightly higher under distilled water conditions (6.40 ± 0.03 °Bx) compared to RAS water (6.00 ± 0.01 °Bx), with significant differences observed between watering regimes.

Table 5. Effect of substrate and watering regime on titratable acidity and total soluble solids of hemp microgreens

Treatment	TA (% oxalic acid)	TSS (°Brix)
Substrate		
Perlite	0.26 ± 0.01 a	6.50 ± 0.01 a
Peat	0.22 ± 0.01 b	5.60 ± 0.05 b
Vermicompost	0.18 ± 0.01 c	6.50 ± 0.01 a
Significance	*	*
Watering		
Distilled water	0.27 ± 0.01	6.40 ± 0.03
RAS water	0.17 ± 0.00	6.00 ± 0.01
Significance	*	*

Values are presented as mean ± standard error (SE). Within each column, * - statistically significant difference, values associated to different letters are significantly different according to Tukey's test at p<0.05. TA - Titratable acidity; TSS - Total Soluble Solids; RAS water - water from recirculating aquaculture system.

The combined influence of substrate and watering regime significantly affected the oxalic acid and total soluble solids (TSS) content of hemp microgreens (Table 6). The highest oxalic acid concentration was recorded in microgreens grown on perlite with distilled water (0.33 ± 0.01%), while the lowest levels (0.16 ± 0.01%) occurred in microgreens grown on peat and vermicompost substrates with RAS water. These results suggest that RAS water significantly reduced oxalic acid accumulation in all tested substrates, with organic substrates (peat and vermicompost) showing the greatest reduction, potentially enhancing nutritional safety.

Total soluble solids (TSS) content was highest in microgreens grown on perlite with distilled water (7.20 ± 0.06 °Bx) and lowest in those grown on peat with distilled water (5.00 ± 0.06 °Bx).

These results suggest that the use of RAS water had a significant effect in reducing oxalic acid accumulation in hemp microgreens, regardless of substrate. However, the lowest oxalic acid values were obtained with organic substrates (peat and vermicompost) combined with RAS water, which could represent an effective solution for producing microgreens with reduced oxalate content.

Table 6. Combined effect of substrate and watering regime on titratable acidity and total soluble solids of hemp microgreens

Treatment	TA (% oxalic acid)	TSS (°Brix)
Perlite x Distilled water	0.33 ± 0.01 a	7.20 ± 0.06 a
Perlite x RAS water	0.19 ± 0.01 c	5.80 ± 0.06 c
Peat x Distilled water	0.28 ± 0.01 b	5.00 ± 0.06 d
Peat x RAS water	0.16 ± 0.01 d	6.20 ± 0.06 b
Vermicompost x Distilled water	0.19 ± 0.01 c	7.00 ± 0.06 a
Vermicompost x RAS water	0.16 ± 0.01 d	6.00 ± 0.06 bc
Significance	*	*

Values are presented as mean ± standard error (SE). Within each column, * - statistically significant difference, values associated to different letters are significantly different according to Tukey's test at p<0.05. TA - Titratable acidity; TSS - Total Soluble Solids; RAS water - water from recirculating aquaculture system.

Substrate type significantly influenced only the b* (yellowness-blueness) color attribute of hemp microgreens (Table 7). Microgreens grown on peat exhibited the highest b* value (19.34 ± 0.47), followed by vermicompost (18.19 ± 0.82), while perlite resulted in significantly lower b* values (16.84 ± 0.32). This indicates that peat promotes a more vibrant and yellowish-green coloration. However, substrate type had no statistically significant effect on the L* (lightness) and a* (redness-greenness) color attributes.

Watering regime did not significantly influence any of the color parameters (L*, a*, and b*) evaluated (Table 7). Microgreens watered with distilled water and those receiving RAS water exhibited comparable values for all color attributes. Specifically, lightness (L*) was nearly identical between distilled water (37.35 ± 1.77) and RAS water (37.62 ± 1.88). Similarly, no significant differences were observed for redness-greenness (a*), with values of -5.35 ± 0.27 for distilled water and -5.28 ± 0.40 for RAS water. The yellowness-blueness parameter (b*) also remained stable across watering treatments, at 18.14 ± 0.78 for distilled water and 18.10 ± 0.16 for RAS water. These results indicate that watering regime does not significantly affect the visual appearance of hemp microgreens.

Table 7. Effect of substrate and watering regime on the color parameters of hemp microgreens

Treatment	L*	a*	b*
Substrate			
Perlite	33.87 ± 2.10	-4.23 ± 0.72	16.84 ± 0.32 b
Peat	39.65 ± 0.92	-6.01 ± 0.42	19.34 ± 0.47 a
Vermicompost	38.95 ± 2.51	-5.72 ± 0.31	18.19 ± 0.82 ab
Significance	ns	ns	*
Watering			
Distilled water	37.35 ± 1.77	-5.35 ± 0.27	18.14 ± 0.78
RAS water	37.62 ± 1.88	-5.28 ± 0.40	18.10 ± 0.16
Significance	ns	ns	ns

Values are presented as mean ± standard error (SE). Within each column, * - statistically significant difference, ns - no statistically significant difference, values associated to different letters are significantly different according to Tukey's test at p<0.05. L* - lightness-darkness; a* - redness-greenness; b* - yellowness-blueness; RAS water - water from recirculating aquaculture system.

The combined influence of substrate and watering regime significantly affected the L* (lightness-darkness) and b* (yellowness-blueness) color attributes of hemp microgreens (Table 8).

Table 8. Combined effect of substrate and watering regime on the color parameters of hemp microgreens

Treatment	L*	a*	b*
Perlite x Distilled water	36.22 ± 1.09 ab	-4.37 ± 0.32	17.98 ± 0.54 ab
Perlite x RAS water	31.51 ± 3.38 b	-4.08 ± 1.12	15.70 ± 0.30 b
Peat x Distilled water	37.12 ± 0.94 ab	-6.19 ± 0.42	18.59 ± 0.54 ab
Peat x RAS water	42.18 ± 0.94 a	-5.83 ± 0.72	20.10 ± 0.82 a
Vermicompost x Distilled water	38.72 ± 3.71 ab	-5.48 ± 0.62	17.85 ± 1.43 ab
Vermicompost x RAS water	39.17 ± 1.65 a	-5.94 ± 0.23	18.52 ± 0.79 ab
Significance	*	ns	*

Values are presented as mean ± standard error (SE). Within each column, * - statistically significant difference, ns - no statistically significant difference, values aTastați ecuația aici.ssociated to different letters are significantly different according to Tukey's test at p<0.05. L* - lightness-darkness; a* - redness-greenness; b* - yellowness-blueness; RAS water - water from recirculating aquaculture system.

The highest L* value, indicating a lighter green coloration, was observed in microgreens grown on peat with RAS water (42.18 ± 0.94), while the lowest L* value, corresponding to darker green shades, was recorded in microgreens grown on perlite with RAS water (31.51 ± 3.38). The yellowness-blueness parameter (b* values) also varied significantly, with peat-grown microgreens watered with RAS water having the highest value (20.10 ± 0.82), indicating a more pronounced yellowish hue. The lowest b* value was recorded in microgreens grown on perlite with RAS water (15.70 ± 0.30).

In contrast, substrate and watering regime interactions had no statistically significant influence on the redness-greenness (a* values). These results highlight that substrate and watering regime interactions predominantly affect the brightness and yellowish coloration of hemp microgreens.

Substrate selection plays a crucial role in determining the yield, nutritional composition, biometric traits, and color parameters of hemp (*Cannabis sativa* L.) microgreens, as evidenced by the results of the PCA analysis applied to the data obtained.

The PCA analysis returned five principal components (PCs), four of which had eigenvalues greater than 1, indicating that these components explain most of the variability in the dataset. The first two principal components (PC1 and PC2) together explain 68.28% of the total variation in the data, with PC1 having an eigenvalue of 6.39 and explaining 42.59% of the variation, and PC2 having an eigenvalue of 3.85 and contributing 25.69% (Table 9). These results suggest that the first two principal components are the most relevant for describing the differences between experimental variants.

Table 9. Eigenvalues of the correlation matrix showing affinity of different PCA against the traits of hemp microgreens

PC	Eigenvalue	Percentage of variance (%)	Cumulative percentage (%)
1	6.39	42.59	42.59
2	3.85	25.69	68.28
3	2.38	15.86	84.14
4	1.40	9.32	93.46
5	0.98	6.54	100.00

The graphical representation of PC1 and PC2 from the PCA analysis indicates that cultivation in peat with RAS water is most favorable for growth and protein accumulation, while perlite results in higher energy content and titratable acidity (TA). Conversely, vermicompost with distilled water is associated with increased fiber, ADF, and total soluble solids (TSS) content (Figure 2). These findings align with previous studies highlighting that organic substrates provide better nutrient retention and microbial interactions, leading to improved biomass and nutrient accumulation in microgreens (Lenzi et al., 2019; Di Gioia et al., 2021). Particularly, peat promoted higher microgreens length and fresh matter yield, reinforcing the idea that organic substrates optimize water retention and aeration, creating a more favorable microenvironment for microgreens development (Gunjal et al., 2024).

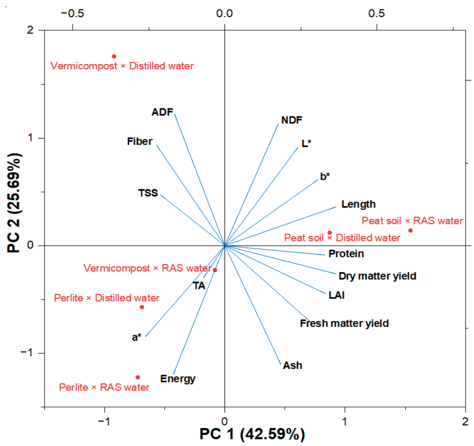


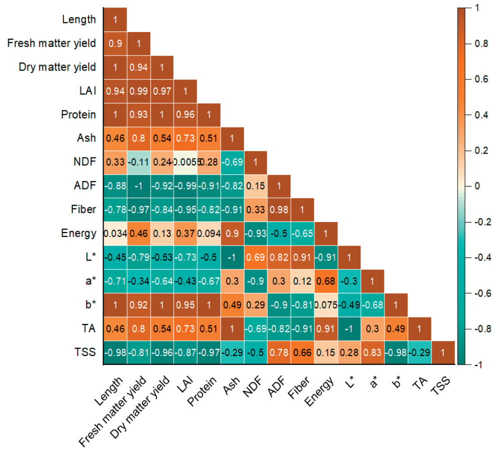
Figure 2. PCA plot showing the association of treatments with hemp microgreens traits

Watering with RAS water significantly increased protein content while reducing oxalic acid levels, a desirable outcome given the anti-nutritional effects of oxalates. These results are also highlighted by the Pearson correlation diagram, which indicates a strong negative relationship. In contrast, watering with distilled water resulted in a strong positive correlation between protein content and oxalate levels (Figure 3). This supports previous findings that nutrient-rich water sources, including those derived from aquaponic systems, enhance nitrogen availability, leading to improved protein synthesis and reduced anti-nutritional compounds in microgreens (Pannico et al., 2022; Kyriacou et al., 2019). Additionally, the results suggest that RAS water provides bioavailable nutrients that optimize metabolic activity, a phenomenon also observed in studies evaluating the impact of hydroponic nutrient solutions on microgreens (Di Gioia and Santamaria, 2015).

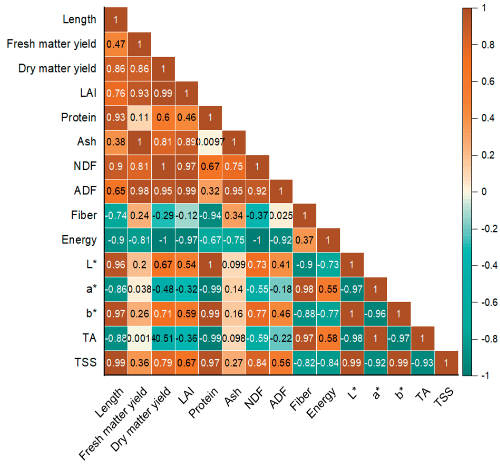
The color parameters (L*, a*, b*) varied significantly based on substrate selection, with peat-grown microgreens exhibiting higher L* values, indicating a lighter green hue, while perlite resulted in darker shades. This is consistent with reports stating that substrate composition influences chlorophyll accumulation and carotenoid content, both of which contribute to plant coloration (Meas et al., 2020; Pannico et al., 2022). In the PCA biplot (Figure 2), L*, a*, and b* are positioned prominently along PC1, indicating their significant contribution to variability. The b* values, indicating the yellow-blue spectrum, were highest in peat-grown microgreens, suggesting increased carotenoid accumulation (Petropoulos et al., 2021). The results of this study reveal that peat-based substrates can enhance marketability by producing visually attractive, nutrient-rich microgreens. In contrast, darker microgreens grown on perlite substrate may indicate increased chlorophyll content, potentially enhancing antioxidant properties. However, while substrate type had clear impacts on microgreens characteristics, the watering regime showed a more limited effect. Specifically, watering significantly influenced nutritional composition, but its impact on yield was significant only for dry matter, with limited effects on biometric traits and color parameters. This indicates that substrate characteristics exert a more dominant influence on microgreens structure, supporting previous research suggesting that substrate composition primarily determines microgreen quality (Di Gioia et al., 2021; Sharma et al., 2021). The observed trends in biometric traits and yield align with previous findings showing that peat-based substrates support greater biomass accumulation compared to inert substrates such as perlite (Lenzi et al., 2019).

Overall, the study highlights the importance of selecting appropriate substrates and watering strategies to optimize the nutritional value, yield characteristics, and visual appeal of hemp microgreens. Organic substrates such as peat and vermicompost, combined with nutrient-rich watering sources, provide a promising strategy for enhancing microgreens yield and bioactive compound content (Kyriacou et al., 2019; Pannico et al., 2022).

Future research should focus on optimizing light conditions and biofortification strategies to further enhance hemp microgreens' nutritional quality, as previous studies have demonstrated the significant impact of spectral modifications on microgreens phytochemistry (Lobiuc et al., 2017; Meas et al., 2020; Petropoulos et al., 2021).



(a)



(b)

Figure 3. Pearson correlation diagram illustrating the effects of (a) distilled water and (b) RAS water on the linear relationships between the traits of hemp microgreens

CONCLUSIONS

The organic substrate, particularly peat, promoted the greatest biomass development, as reflected by higher fresh and dry matter yield, microgreens length, and leaf area index (LAI), compared to perlite and vermicompost substrates. Therefore, peat can be recommended as the optimal substrate for hemp microgreens cultivation.

Using water from the recirculating aquaculture system (RAS) significantly increased the protein content of microgreens and reduced oxalic acid accumulation, a beneficial aspect from a food safety perspective.

The most favorable substrate-water combination for maximizing yield and nutritional quality (high protein content and low oxalic acid) proved to be peat substrate watered with RAS water.

Color parameters were predominantly influenced by substrate type, with peat producing lighter and more vibrant-colored microgreens (higher L and b values), which is an important attribute for enhancing the commercial appeal of the final product.

Utilizing organic substrates (peat or vermicompost) in combination with nutrient-rich water from recirculating aquaponic systems (RAS) provides a sustainable and efficient approach for enhancing yield, nutritional value, and visual attractiveness of hemp microgreens.

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RESEARCH ON THE MANAGEMENT OF CARBON DIOXIDE EMISSIONS AND SEQUESTRATION BY DIFFERENT CROPS, DEPENDING ON THE AGRICULTURAL TECHNOLOGIES APPLIED

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Abstract

The carbon footprint calculation was carried out based on the technological data of each crop sown 2023 and 2024 in 3 farms: Terra Nostra Farm Băilești - organic technology, Trăistaru Farm Băilești - conventional technology and Sonnenhof Farm Wolpertshausen - Germany - Demeter organic technology. At Terra Nostra Farm, the calculation was carried out for corn, wheat, soybean and alfalfa crops. At Trăistaru Farm, corn, wheat and sunflower crops were analyzed. Lentil + camelina, spelt wheat, mustard, coriander, alfalfa crops were studied at the Farm in Germany. The carbon emitted through works and inputs from the crops at the Farm in Germany were grouped as follows: lentils + camelina, eco mustard, coriander - values around 500 kg CO_2/ha and eco spelt and eco alfalfa - values around 1000 kg CO_2/ha. There were no differences in the carbon sequestered by corn depending on the technology applied. In wheat, a yield of 3000 kg/ha obtained at the Terra Nostra Farm sequestered a much smaller amount of carbon than the 5500-6000 kg/ha yields from the German Farm.

***Key words**: carbon footprint; conventional technology; organic technology Demeter; carbon dioxide emissions; carbon sequestered.*

INTRODUCTION

Climate change resulting in global warming stems, in part, from the accumulation of greenhouse gases in the atmosphere, mainly from anthropogenic emissions and land use change (IPCC, 2013). The most important greenhouse gas, carbon dioxide, contributes approximately 60% of the total greenhouse gas effect on global warming (Rastogi et al., 2002), thus the consequences on the atmosphere show an increase from 280 ppm in the pre-industrial period to 400 ppm in the year 2013 (Rastogi et al., 2002; Rapp, 2014). A forecast of various international studies has shown that, depending on the calculation methods used, it can be considered that by the year 2100, CO_2 could increase between 490-1370 ppm (Keidel et al., 2015).

Human-produced greenhouse gas emissions must be reduced to limit global warming.

The most important greenhouse gases (GHGs) were established by the Kyoto Protocol (Japan), following the International Conference on Climate Change in 1997. The first mandatory period was 2005-2012, and the second was 2013-2020. In 2021, the Paris Climate Conference organized by the UNO took place, which continued the foundations laid in Kyoto with the objective of limiting global warming to below 2°C with a target of 1.5°C.

The most important greenhouse gases, according to the Kyoto Protocol, are: CH_4 - methane; CO_2 - carbon dioxide; PFC - perfluorinated carbon dioxide; HFC - hydrogen-containing fluorocarbons; NF_3 - nitrogen trifluoride; SF_6 - sulphur hexafluoride; N_2O - nitrous oxide.

The impact of all the gases mentioned above on the climate is reflected in the greenhouse effect, which influences global climate change. Soil organic carbon is of particular importance for ecosystems (Batjes, 1996; Nadelhoffer, 2004). It is known globally that soils, especially in forest areas, contain three times more carbon than exists in the atmosphere, but compared to the total terrestrial reserve in the biosphere, soils contain four and a half times more (Lajtha et al., 2014), which represents a higher carbon content than the atmosphere and plants combined (Jobbagy et al., 2000).

The flow of carbon dioxide from the soil of ecosystems plays a special role, carbon dioxide being released from the soil through the process of respiration or the so-called movement of carbon dioxide in the soil (Raich and Schlesinger, 1992; Lu et al., 2017). An important part of soil respiration is represented by autotrophic respiration (FCO_2) through roots and mycorrhizae, which is strongly reflected in plant metabolism (Berger et al., 2010). Autotrophic respiration shows interannual and intra-annually differences and the type of vegetation, climatic conditions and soil characteristics are factors that determine the variability of autotrophic respiration (Epron et al., 2012). The amount of CO_2 produced by root respiration is determined by the biomass of the roots and their respiration rate (Epron et al., 2001).

The agricultural sector is a significant contributor to global carbon emissions, through the production and use of agricultural machinery, crop protection chemicals such as herbicides, insecticides and fungicides, and fertilizers. Organic farming has a positive impact on the environment, because it promotes the responsible and sustainable use of energy and natural resources and preserving biodiversity (Bonciu, 2022; 2023; Bonciu et al., 2022; De Souza, 2022; Paunescu et al., 2023).

The proportion of the nation's global carbon footprint due to agriculture is approximately 8%, of which 75% is directly related to fertilizer use (Choudrie et al., 2008).

Interest remains predominantly towards models of carbon sequestration in agricultural ecosystems, where we have greater intervention possibilities (Berca, 2021).

Carbon sequestration in the terrestrial ecosystem, in our case in agroecosystems, begins with a priority of refraining from creating and releasing as few CO_2 emissions into the atmosphere as possible and, if possible, reducing them completely (Topham, 2011; Pietantozzi, 2003).

The first measure is to retain CO_2 in the soil and cultivated plants. For this, we take into account that by reducing work with agricultural machinery, we will reduce emissions. Burning 1 litre of diesel leads to emissions of 2.7-2.9 kg of CO_2. With the classic soil work system, a minimum of 70 litres of diesel/ha = 203 kg of CO_2/ha is consumed. If the conservative work system is used, consumption is reduced to 40 litres = 120 kg of CO_2. If the “No-tillage” system is applied, consumption is reduced only during sowing = 20 l/ha, i.e. a maximum of 60 kg of CO_2/ha, 3 times less than in the first case. About 180 kg of CO_2 remained in the soil CO_2 pool.

Studies on carbon sequestration in different crops have been carried out by numerous researchers and the results have been very different (Jarechi and Lal., 2010; Ferreira et al., 2012; Freitas et al., 2014). Thus, in maize, Jarechi and Lal (2010) at a production of 4.33 t/ha established a carbon input brought with biological residues of 1.95 t/ha; Ferreira (2012) at a production of 8.90 t/ha established a carbon input brought with biological residues of 4.40 t/ha; Freitas (2014) at a production of 5.50 t/ha established a carbon input of 2.72 t/ha. In wheat, the carbon input highlighted at productions of 2.69 t/ha, 5.50 t/ha was 1.82 t/ha, respectively 2.35 in the conception of the first two authors. In soybean, at productions of 2.27 t/ha, 3.22 t/ha and 4.52 t/ha, the carbon brought through biological residues was 1.02 t/ha (Jarechi and Lal, 2010), 1.23 t/ha (Ferreira et al., 2012), respectively 4.3 t/ha (Freitas et al., 2014). In sunflower, at a production of 3.14 t/ha, the carbon from plant residues was 1.10 t/ha (Freitas et al., 2014).

INRA published in July 2019 a summary on carbon sequestration. The main conclusions stated were: the transition to direct sowing remains a topic “still controversial” in the carbon issue. If we consider the entire soil profile, the transition from ploughing to direct sowing would have no effect on the total C stock in the soil. There is, however, a positive result because organic matter is often transformed into stable humus on “poor” C soil. The net gain is an average of +59 kg C/ha/year. Since 1 t of dry matter represents between 400 and 500 kg of carbon on average, the increase in production has a significant quantitative carbon capture effect (Hypolite, 2021).

The choice of crops will influence the carbon emission (peas < sunflower < winter cereals < maize) but also their productive performance.

MATERIALS AND METHODS

Based on the technological sheets for each crop, from each farm analysed (Terra Nostra Ecoland organic-biodynamic Demeter Farm Băilești, Trăistaru Conventional Farm and Sonnenhof organic-biodynamic Demeter Farm), the tables with the work performed and diesel consumption were completed according to the model presented by Berca (Berca, 2021).

Based on the biomass (sample taken from each crop), the harvest index but also the defining parameters of the stored carbon, the carbon sequestered by the crops studied in 2023 and 2024 was calculated (Table 1).

Table 1. Defining parameters of carbon stored by different crops, in different parts of the plant (crop). Biomass and carbon stocks, excluding storable crops

No. Crt.	Crops	Number of tests performed	Biomass (t/ha)				Carbon stock (t/ha)				Substance Organic Carbon SOC (humus C) t/ha
			Total plant	Roots (R)	Overground (S)	R/S	Total plant	Roots (R)	Overground (S)	R/S	
1	Perennial herbs	29	19.80	10.70	9.10	1.17	6.80	3.60	3.20	1.12	50
2	Maize	60	11.20	5.20	6.00	0.87	6.30	2.56	3.74	0.68	80 (asol. soybean)
3	Sorghum	26	9.50	1.90	7.60	0.25	4.20	1.10	3.10	0.35	40
4	Autumn wheat	57	9.40	2.10	7.30	0.29	2.40	0.80	1.60	0.50	40
5	Triticale	3	9.60	3.20	6.40	0.50	-	-	-	0.58	-
6	Soybean	52	4.10	1.10	3.00	0.37	3.00	1.10	1.90	0.58	80 (asol. maize)
7	Peas	18	3.80	1.50	2.30	0.65	1.10	0.30	0.80	0.38	38
8	Sunflower	8	3.90	1.50	2.40	0.63	1.20	0.40	0.80	0.50	30
9	Rapeseed	12	4.20	1.20	3.00	0.40	1.80	0.30	1.50	0.20	30

Source: Mathew I. et al., 2017 - What crop type for atmospheric carbon sequestration: Results from a global data analysis. Agriculture, Ecosystems & Environment, 243: 34-46.

Starting from the values obtained for each of the calculated indicators, a graphic representation was made for the carbon footprint of the crops on each farm and then a comparison was made between farms for the common crops.

The calculations were made for: carbon emitted from field operations and from inputs, carbon emitted through burned diesel, carbon sequestered by the crop, carbon fixed from the atmosphere, the amount of humus C brought into the soil.

For the Terra Nostra Ecoland organic-biodynamic Demeter Farm in Băilești, the calculation was made for maize, wheat, soybean and alfalfa crops, they were treated with Demeter products.

At the Trăistaru Conventional Farm, maize, wheat and sunflower crops were analysed.

The crops of lentils + camelina, spelt wheat, mustard, coriander, alfalfa crops were studied at the organic-biodynamic Demeter Farm in Wolpertshausen - Germany, they were treated with Demeter products.

For each crop, the following scheme was followed according to the Berca model (2021):

K=0.2 - coefficient calculated for agricultural crops

1. Carbon emitted by field operations = (carbon emitted by field operations + carbon emitted by field operations) x k = carbon emitted x t harvest = carbon emitted by field operations.

TOTAL CARBON EMITTED (kg/ha) = carbon emitted by field operations + carbon emitted by inputs.

Calculated in CO_2 (kg/ha) = TOTAL CARBON EMITTED x 3.67 (transformation coefficient) = kg equivalent CO_2 /ha.

The carbon emitted depending on the inputs used was that established by Lal (2004) and mentioned by Hillier et al. (2009):

- for nitrogen fertilizers - 2.96 kg carbon emitted/ha;

- for potassium fertilizers - 0.2 kg carbon emitted/ha;
- for plant protection with herbicides - 6.3 kg carbon emitted/ha;
- for plant protection with insecticides - 0.36 kg carbon emitted/ha;
- for plant protection with fungicides - 3.16 kg carbon emitted/ha.

2. Calculation by the amount of diesel burned/ha

TOTAL CO_2 Kg/ha = TOTAL DIESEL BURNED * 2.7 (transformation coefficient)

TOTAL CO_2 (kg/ha): carbon emitted through inputs x 3.67 (multiplication coefficient) = kg/ha + TOTAL CO_2 by the amount of diesel burned = kg CO_2 equivalent/ha

3. CARBON SEQUESTERED BY CROP (according to Mathew et al., 2017) – the biomass is established by the sample taken from the field and is calculated according to the defining parameters of the carbon deposited by different crops, in different parts of the plant (crops). Biomass and carbon stocks, excluding storable crops.

4. CARBON FIXED IN THE AIR:

To calculate the carbon fixed in the atmosphere, we started from the biomass production (overground and underground part):

- t grains x 0.45 (45% carbon) = t C (stored quantity removed from the system);
- t straw, stalks, stems, grasses x 0.44 (44% carbon) = t C;
- t roots x 0.43 (43% carbon) = t C.

TOTAL : t residual biomass (straw, etc. + roots) = t C.

30% of this carbon is returned to the atmosphere through respiratory processes and other losses.

Knowing that 1 t C = 0.34 t humus C, it is shown that 1 ha of crop brings a net quantity of t humus C to the soil.

RESULTS AND DISCUSSIONS

At the organic-biodynamic Demeter Băilești farm, on average over the 2 years, the highest amount of CO_2, expressed in kg/ha, was recorded by the alfalfa crop at an average production of 8500 kg/ha and the lowest by soybean at an average production of 1000 kg/ha, the amplitude being 306.99 kg/ha CO_2 (Figure 1). Very close to the maximum value of CO_2 was the amount of carbon dioxide emitted by the maize crop.

The amount of CO_2 emitted by the burned diesel is very close in value regardless of the crop, which suggests that the field operations do not differ much and the consumption is very close. Also, from the comparison with the carbon emitted by field operations and inputs, it was observed that the latter is quantitatively higher in maize and alfalfa compared to wheat and soybean (Figure 2).

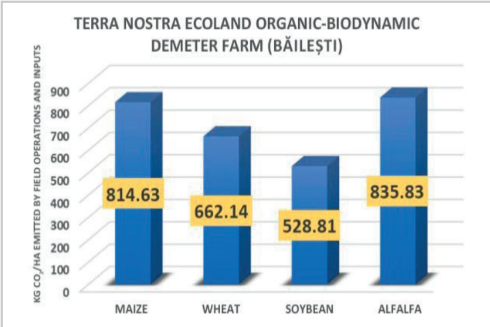

Figure 1. Carbon emitted through field operations and inputs to crops at Terra Nostra Ecoland organic-biodynamic Demeter Farm (Băilești) - average for 2023+2024

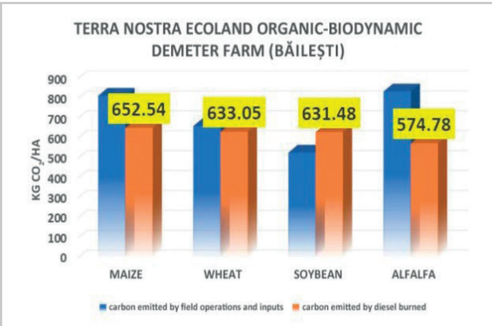

Figure 2. Carbon emitted through field operations and inputs compared to that emitted through diesel burned in crops at Terra Nostra Ecoland organic-biodynamic Demeter Farm (Băilești) - average for 2023+2024

Based on the analysis of global data on atmospheric carbon sequestration carried out by Mathew and his collaborators in 2017, starting from biomass samples and the harvest index calculated for each crop, the carbon sequestered by them was established (Figure 3).

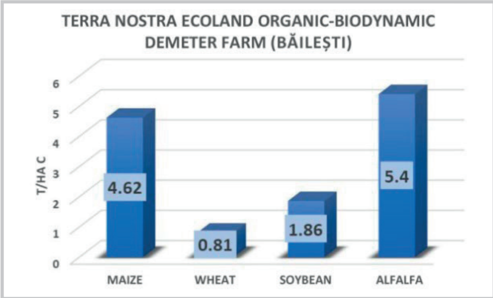

Figure 3. Carbon sequestered by crops at Terra Nostra Ecoland organic-biodynamic Demeter Farm (Băilești) - average for 2023+2024

For maize, the harvest index was 0.50, for wheat 0.55 and for soybean 0.38. On average, over the two years, organic - biodynamic Demeter maize had the highest amount of carbon sequestered, followed by alfalfa, wheat and soybean.

After the crop is stored and removed from the system, carbon fixed from the atmosphere by the aboveground and underground parts remains. Of this, 30% is lost to the air through respiration processes. Logically, the values of carbon lost through respiration are proportional to the amount of carbon fixed by each crop (Figure 4).

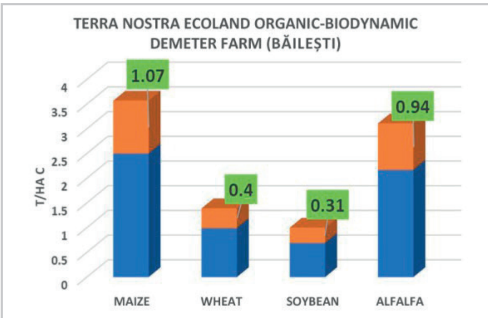

Figure 4. Carbon fixed from the atmosphere by crops at Terra Nostra Ecoland organic-biodynamic Demeter Farm (Băilești) / carbon released through respiration - average for 2023+2024

At the Trăistaru Conventional Farm, also located in Băilești, the carbon emitted through field operations and inputs had the highest values for maize and wheat compared to that emitted at the other farms studied. Also, the differences between the first two crops (maize and wheat) were insignificant.

For sunflower, we did not have a comparison period, but we can mention that this is a more environmentally friendly crop, the amount of carbon emitted being less than half of the other two crops (Figure 5).

It was observed that the burned diesel emits carbon close to the level emitted by the field operations and inputs to the conventional technology applied to this Farm (Figure 6).

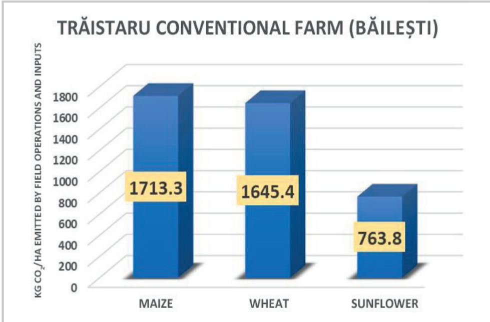

Figure 5. Carbon emitted through field operations and inputs to crops at the Trăistaru conventional Farm (Băilești) - average for 2023+2024

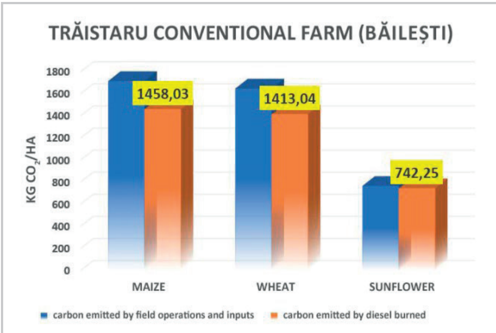

Figure 6. Carbon emitted through field operations and inputs compared to that emitted through diesel burned in crops at the Trăistaru Conventional Farm (Băilești) - average for 2023+2024

The carbon emitted in large quantities when conventional technology is applied is mainly due to the inputs that are very large and diverse (NP fertilizers, fungicides, herbicides, insecticides, foliar fertilizers). While in conventional the carbon emitted by these has values around 330 kg/ha, in organic-biodynamic Demeter the values are almost identical - 126 kg/ha.

The carbon sequestered by crops is high in maize, especially due to biomass (Figure 7). According to data from Mathew et al. (2017), at a biomass of 11.2 t/ha, the carbon stock is 6.3 t/ha. In our case, at a biomass of 7.69 t/ha, the carbon stock

was 4.32 t/ha. Biomass does not include storable crops.
Carbon lost through respiration on conventional farms is approximately equal in maize and wheat, but almost halved in sunflowers (Figure 8).

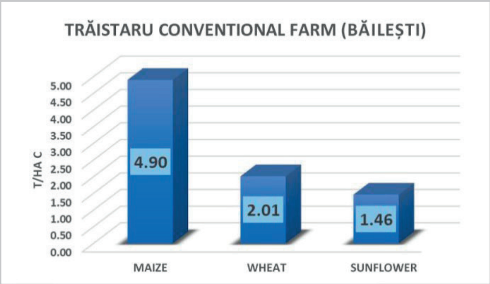

Figure 7. Carbon sequestered by crops at Trăistaru conventional Farm (Băilești) - average for 2023+2024

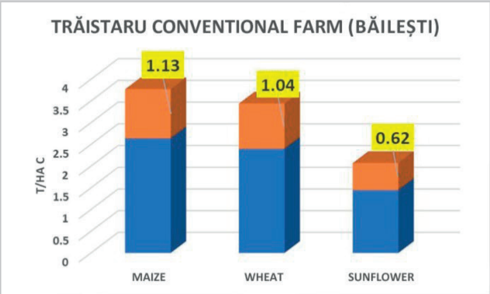

Figure 8. Carbon fixed from the atmosphere by crops at the Trăistaru conventional Farm (Băilești) / carbon released through respiration - average for 2023+2024

The carbon emitted through field operations and inputs (only organic-biodynamic Demeter products) from the crops sown at the Farm in Germany were grouped as follows: organic-biodynamic Demeter lentils + camelina, organic-biodynamic Demeter mustard, organic-biodynamic Demeter coriander – values around 500 kg CO_2/ha and for organic-biodynamic Demeter spelt wheat and organic-biodynamic Demeter alfalfa – values around 1000 kg CO_2/ha. This is due to the much higher average productions of wheat (5750 kg/ha) and alfalfa (9250 kg/ha), the transport of the production to these requiring a higher consumption of diesel (Figure 9).
With the exception of alfalfa, all crops had a similar diesel consumption and implicitly the carbon emitted by its combustion was practically equal (Figure 10).

At the Sonnenhof organic-biodynamic Demeter farm, the largest amount of carbon sequestered was in the alfalfa crop, which also recorded a high production compared to the other crops, with a value practically 4 times higher (Figure 11).
In descending order, it was followed by organic-biodynamic Demeter coriander, lentils+camelina, mustard and spelt wheat, also in organic-biodynamic Demeter system.

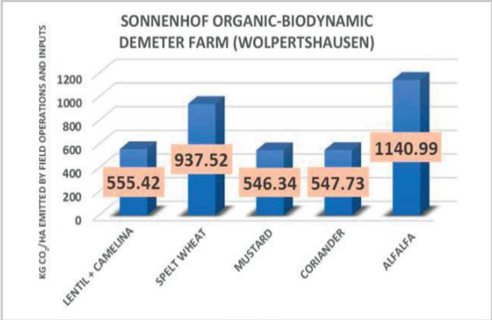

Figure 9. Carbon emitted through field operations and inputs to crops at Sonnenhof organic-biodynamic Demeter Farm Wolpertshausen - average for 2023+2024

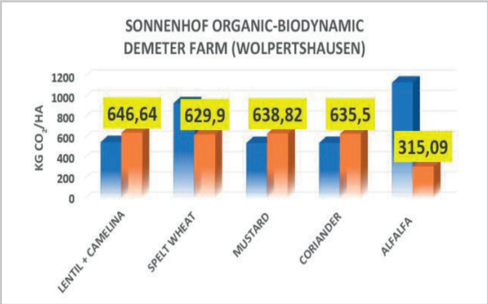

Figure 10. Carbon emitted through field operations and inputs compared to that emitted through diesel burned at Sonnenhof organic-biodynamic Demeter Farm Wolpertshausen crops - average for 2023+2024

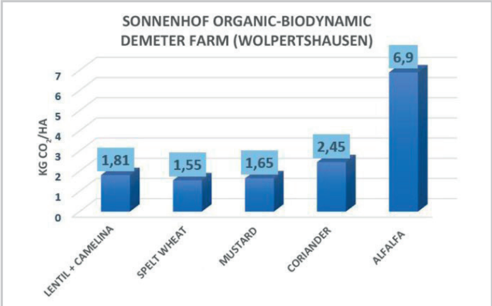

Figure 11. Carbon sequestered by crops at Sonnenhof

organic-biodynamic Demeter Farm Germany - average for 2023+2024

The carbon released through respiration, representing 30% of that fixed in the atmosphere, presents a decreasing sequence modified from the previous one, mustard being the last (Figure 12).

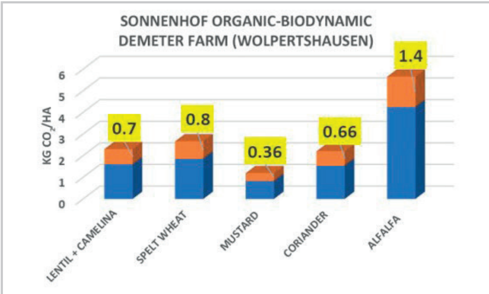

Figure 12. Carbon fixed from the atmosphere by crops at Sonnenhof organic-biodynamic Demeter Farm Germany / carbon released through respiration - average for 2023+2024

Comparatively, the carbon emitted through field operations and inputs to the maize crop, between the organic-biodynamic - technology applied at the Terra Nostra Ecoland Farm in Băilești and the conventional technology applied at the Claudiu Trăistaru Farm also located in Băilești, shows us that in the latter, the amount is practically double. In the wheat crop, in the conventional system, the carbon emitted is almost triple compared to the organic-biodynamic Demeter technology applied at Băilești and represents approximately 70% of the carbon emitted in the spelt wheat cultivated in the organic-biodynamic Demeter system at the Farm in Wolpertshausen - Germany (Figure 13).

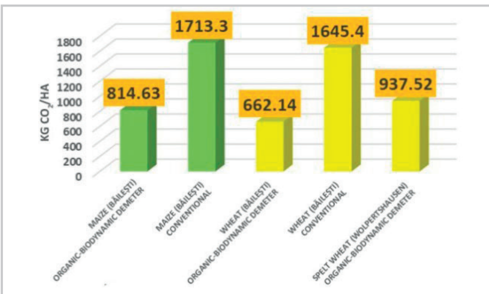

Figure 13. Carbon emitted through field operations and inputs to maize and wheat crops depending on the technology applied to the farms studied - average for 2023+2024

Regarding the carbon emitted calculated based on the burned diesel, the conventional amounts are equally high, the proportion being over 200% for both the maize and wheat crops. The only difference is that for the wheat crop, the carbon emitted at the Terra Nostra Ecoland Farm in Băilești - Romania and the Sonnenhof Farm in Wolpertshausen - Germany is practically equal in terms of quantity (Figure 14).

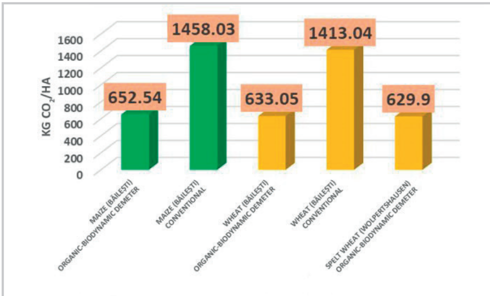

Figure 14. Carbon emitted through diesel burned in maize and wheat crops depending on the technology applied on the farms studied - average for 2023+2024

There were no differences in the carbon sequestered by maize crops depending on the technology applied, since the biomass was similar and the yields were equal – 5000 kg/ha. In contrast, for wheat, a crop with an average yield of 3000 kg/ha and the corresponding biomass obtained at Terra Nostra Ecoland Farm sequestered a much smaller amount of carbon than the crops from the conventional Traistaru farm and Sonnenhof German Farm where the yields were 5500 and 5750 kg/ha, respectively (Figure 15).

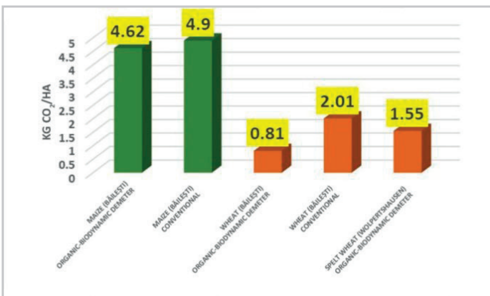

Figure 15. Carbon sequestered by maize and wheat crops on the studied farms - average for 2023+2024

CONCLUSIONS

By comparison, the carbon emitted through field operations and inputs to the maize crop, between the organic-biodynamic Demeter technology applied at the Terra Nostra Ecoland Farm in Băilești and the conventional technology applied at Claudiu Trăistaru Farm also located in Băilești, shows us that in the latter, the quantity is practically double.

In the wheat crop, in the conventional system, the carbon emitted is almost triple compared to the organic-biodynamic Demeter technology applied at Băilești and represents approximately 70% of the carbon emitted by the spelt wheat cultivated in the organic-biodynamic Demeter system at the Sonnenhof Farm in Germany. Regarding the carbon emitted calculated based on the burned diesel, in the conventional system the resulting quantities are equally high, the proportion being over 200% for both the maize crop and the wheat crop.

The only difference is that in the wheat crop, the carbon emitted at the Terra Nostra Ecoland Farm and the Sonnenhof Farm in Germany is practically equal in terms of quantity. There were no differences in the carbon sequestered by maize crops depending on the technology applied, because the biomass was similar and the yields were equal - 4750-5000 kg/ha.

In contrast, in wheat, a crop with a production of 3000 kg/ha and the corresponding biomass obtained at the Terra Nostra Ecoland Farm sequestered a much smaller amount of carbon than the crops from the conventional and from the Sonnenhof farm in Germany where the yields were 5500-5750 kg/ha.

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WASTE FROM THE WINE AND ETHYL ALCOHOL PRODUCTION INDUSTRY - AN IMPORTANT SOURCE FOR INCREASING SOIL FERTILITY AND CROP PRODUCTIVITY

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Abstract

The Republic of Moldova generate approximately 100 thousand tons of wastes each year. These contains 28 thousand tons of organic matter, 180 tons of nitrogen, 82 tons of phosphorus and 257 tons of potassium. Fertilization with waste from the production of alcoholic beverages led to a significant increase in the content of organic matter by 0.17-0.41%, nitric nitrogen - 0.45-5.80 mg/kg, mobile phosphorus - 0.22-0.68 mg/100 g and exchangeable potassium by 7-16 mg/100 g of soil. The application of solid wine yeasts ensures a significant increase in grape (Sauvignon) production on average over eight years of 1.4-2.4 t/ha. The average harvest increase when incorporating vinasse over eight years was 1.0-1.1 t/ha. The cereal mash applied to the soil determined the achievement of average increases in plant production over ten years of 1.100-1.700 kg/ha cereal units or 50-65% compared to the unfertilized variant. Based on the research, innovative technologies for the valorisation of waste from the production of alcoholic beverages as local organic fertilizers are developed.

Key words*: grapevine, field crops, solid wine lees, vinasse, grain mash.*

INTRODUCTION

At the current stage in the Republic of Moldova, the ecological state, in most natural ecosystems of the environment is deplorable. Pollution with organic waste of different origin represents a major danger, both for human life and for the environment (Duca & Covaliov, 2001; Duca & Ţugui, 2006).

The Moldova`s primary natural resource is its soil, particularly chernozems, which require ongoing care and attention from the government, specialized institutions and every individual resident. Although there are various types of waste that should not be disposed chaotically in landfills, the country lacks a facility for their processing or recycling. Among these is waste from the wine industry, which is estimated to hundreds of tons annually and represents a significant source of environmental pollution. When these wastes reach the soil in large quantities, they percolate into water bodies, leading to eutrophication and disrupting the balance of these ecosystems (Duca, 2011).

The main purpose of the research was to assess the chemical composition and fertilizer potential of alcoholic waste (wine yeast, vinasse and cereal dregs), to utilize them as fertilizers.

MATERIALS AND METHODS

The research focused on the soil (cambic / leached chernozem), grapevine, field crops and by-products from the production of alcoholic beverages (wine yeast, vinasse and grain dregs), which were applied in two field experiments (Table 1) conducted at the Technological-Experimental Station "Codru" in the commune of Codru, Chisinau Municipality. The experiments were carried out during 2011-2022. Waste was applied manually each year.

Table 1. Field experiments with waste

Wineries waste		Cereal waste	
Variant	*Dose*	*Variant*	*Dose*
Control	unfertilized	Control	unfertilized
Wine yeast (N_{100})	13 t/ha	Grain mash (N_{120})	47 m^3/ha
Wine yeast (N_{200})	26 t/ha	Grain mash (N_{240})	94 m^3/ha
Vinasse (K_{450})	300 m^3/ha	-	-
Vinasse (K_{900})	600 m^3/ha	-	-

Prior to the application of the tested waste materials, the following parameters were analyzed: moisture content, humus content, mineral nitrogen, phosphorus and potassium and the ionic composition of the aqueous extract. Soil samples analyses were conducted using established classical methods.

Solid wine yeast is a by-product of the wine industry, generated during the fermentation of grape juice by yeast. Typically, approximately 10-15% of the volume of juice undergoing fermentation results in the formation of wine yeast, which is in a semi-solid state and has a dry matter content of 12-13% (Plămădeală et al., 2016).

Vinasse is a waste product generated during the distillation of wine to produce wine distillate. However, the treatment and ecological management of the liquid remaining after distillation lack effective technological solutions for its recovery or neutralization in accordance with environmental standards.

Grain mash. In ethyl alcohol production plants, grain mash (wheat, barley, maize), potato mash and molasses are formed as a by-product. In most cases, these products are discarded, causing a polluting impact on the environment.

RESULTS AND DISCUSSIONS

Solid wine yeast. Currently, in wine factories, depending on the existing equipment, ethyl alcohol is extracted from the yeast by distillation. At the same time, the yeast can be dehydrated on filter presses. With or without dehydration, the yeast is evacuated or discharged as waste. The research carried out on the possibility of using them as nutritional additives in animal feed is quite interesting (Chiseliţa, 2010; Duca, 2011).

Wine yeasts are characterized by an acidic status. The average pH value is 3.5. Humidity varies from 42.0 to 58.9%, averaging 48.0%. The chemical composition demonstrates that solid yeasts constitute an important source of organic matter for the soil and nutrients for plants. Calculated from the mass with natural moisture, the organic matter content constitutes an average of 46.8% (Table 2).

Among the primary elements, total potassium predominates with a share of 2.5%, followed by total nitrogen - 1.5%, total phosphorus - 0.70%.

Table 2. Chemical composition of solid wine yeasts, reported to the natural humidity mass (2011-2022), n=12

Index	x	Min.	Max.
pH	3.5	3.2	3.7
Humidity, %	48.0	42.0	58.9
Organic matter, %	46.8	38.3	50.3
Ash, %	5.3	2.8	8.8
Carbon, %	23.4	19.2	25.5
Total nitrogen, %	1.5	0.8	1.8
$N\text{-}NO_3$, mg/100 g	1.6	0.7	2.8
$N\text{-}NH_4$, mg/100g	32.9	26.9	51.7
Total phosphorus, %	0.7	0.6	0.8
Total potassium, %	2.5	2.3	2.7

Compared to conventional manure, solid wine yeasts contain 2.7 times more nitrogen, 1.6 times more phosphorus, 2.4 times more potassium and 2.7 times more organic matter. On average, 1 tone of solid wine yeasts with natural moisture contains 47 kg of NPK, with a ratio of these elements of 1:0.5:1.7, which approximately corresponds to the nutritional needs of the main cultivated plants.

Vinasse. The amount of vinasse represents 75-85% of the volume of wines subjected to distillation. Vinasse is an opalescent or slightly cloudy liquid, of a golden-crimson color, with a specific smell of heat treatment and a sour taste and contains all the compounds originally found in wine: organic and mineral compounds, proteins, coloring compounds, etc. It is characterized by an acidic status. The average pH value is 3.4 units (Table 3).

The dry residue varies from 7.5 to 24.7 g/l, averaging 15.2 g/l. The organic matter content averages 13.3%, varying from 6.3% to 21.7%.

Mineral compounds average 1.9 g/l. Of the primary elements, total potassium predominates in the composition of vinasse with an average value of 0.12%. The total nitrogen and phosphorus content averages 0.02%. Of the total nitrogen content, ammonia constitutes approximately 34%. In the aqueous extract, monovalent potassium cations (579 mg/l) and sodium (172 mg/l) predominate. The concentration of bivalent calcium and magnesium cations averages 106 mg/l and 84 mg/l. Among the anions predominate sulphates. Their concentration ranges from 79 mg/l to 280 mg/l with an average value of 155 mg/l. The chlorine content varies from 69 to 122 mg/l, averaging 90 mg/l (Table 3).

Table 3. Chemical composition of vinasse (2011-2022), n=12

Index	x	Min.	Max.
pH	3.4	3.0	3.7
Dry residue, g/l	15.2	7.5	24.7
Fixed residue, g/l	1.9	1.2	2.9
Organic matter, g/l	13.3	6.3	21.7
Total nitrogen, %	0.02	0.007	0.05
Total phosphorus, %	0.02	0.006	0.039
Total potassium, %	0.12	0.048	0.157
$N-NH_4$, mg/l	67.0	52.0	86.0
$N-NO_3$, mg/l	9.3	0.31	23.8
Ca^{+2}, mg/l	106.0	72.0	120.0
Mg^{+2}, mg/l	84.0	49.0	146.0
Na^{+}, mg/l	172.0	125.0	210.0
K^{+}, mg/l	579.0	335.0	1127.0
Cl^{+}, mg/l	90.0	69.0	122.0
SO_4^{-2}, mg/l	155.0	79.0	280.0

Grain mash (Cereal porridge). The grain dregs are characterized by an acidic medium. The pH value is 3.4-4.2 units and they have a varied content of primary elements: total nitrogen - 0.21-0.33%, total phosphorus - 0.06-0.19%, total potassium - 0.09-0.13%.

Among the cations, the monovalent ones of potassium and sodium predominate (783 mg/l and 450 mg/l). The concentration of the bivalent cations of magnesium and calcium is on average 97 mg/l and 234 mg/l. Among the anions predominate sulphates. Their concentration varies between 188 mg/l and 533 mg/l, the average being 367 mg/l.

The average chlorine content is 299 mg/l (Table 4).

Table 4. Chemical composition of grain mash from ethylic alcohol production enterprises (2012-2022), n=12

Index	x	Min.	Max.
pH	3.7	3.4	4.2
Dry residue, g/l	66.3	40.5	72.0
Fixed residue, g/l	14.9	9.3	21.4
Organic matter, g/l	51.4	16.2	62.1
Humidity, %	93.4	92.1	97.0
Total nitrogen, %	0.28	0.21	0.33
Total phosphorus, %	0.12	0.06	0.19
Total potassium, %	0.11	0.09	0.13
$N-NH_4$, mg/l	143.0	71.0	224.0
$N-NO_3$, mg/l	5.8	2.9	11.0
Ca^{+2}, mg/l	97	60	100
Mg^{+2}, mg/l	234	183	224
Na^{+}, mg/l	450	185	550
K^{+}, mg/l	783	649	850
Cl^{+}, mg/l	299	138	321
SO_4^{-2}, mg/l	357	188	533

From above, we conclude that the dregs from ethyl alcohol production must be included in the agricultural circuit and used as fertilizer.

Change in the main indicators of soil from applying waste from the alcoholic beverages production. Application of these waste on the cambic chernozem positively influenced the main agrochemical properties. Statistically significant increases in the content of organic matter and nutrients were recorded (Table 5).

Table 5. Influence of waste from the alcoholic beverages production on the organic matter and nutrients content in the arable layer of cambic chernozem, 2011-2022

Variant	Organic matter, %			P_2O_5, mg/100 g soil			K_2O, mg/100 g soil		
	Content	Increase over control		Content	Increase over control		Content	Increase over control	
		%	kg/ha		mg/100 g	kg/ha		mg/100 g	kg/ha
The experience with waste from wineries									
Control - unfertilized	3.90	-	-	2.11	-	-	28	-	-
Wine yeast, 13 t/ha	4.06	0.16	4200	2.81	0.70	18.5	39	11	252
Wine yeast, 26 t/ha	4.31	0.41	11000	3.23	1.12	29.7	43	15	344
Vinasse, 300 m^3/ha	4.01	0.11	3000	2.52	0.41	10.8	41	13	275
Vinasse, 600 m^3/ha	4.51	0.31	8000	2.69	0.58	15.4	45	17	389
Dl $_{0,5}$, %	0.11	0.15	5200	0.13	0.13	3.2	6.5	6.5	42
Sx, %	7.00	5.50	5.5	6.40	6.40	6.4	9.5	9.5	9.5
The experience with cereal waste									
Control - unfertilized	3.00	-	-	2.14	-	-	25	-	-
Grain mash, 47 m^3/ha	3.12	0.12	3000	2.45	0.31	6.09	29	4	78
Grain mash, 94 m^3/ha	3.26	0.21	6500	2.68	0.54	10.61	34	9	180
Dl $_{0,5}$, %	0.11	0.09	2041	0.12	0.19	0.38	2.8	3.8	62
Sx, %	8.10	8.10	8.1	7.20	7.20	7.20	10.4	10.4	10.4

Crop productivity from waste applying. The application of waste from the production of alcoholic beverages positively influenced the yields obtained. The application of wine yeast at a dose of 13-26 t/ha annually ensured a significant increase in grape yield on average over 12 years of research of 1.4-2.4 t/ha, which is 15-25% more, compared to the unfertilized control (9.5 t/ha).

Significant actions on the productivity of grapevine plants had a vinasse incorporated at a dose of 300 and 600 m^3/ha annually. The average yield increase was 1.0-1.3 t/ha or 11-14% more than the control. Starting from 2012, sunflowers were grown on the experimental plot, then winter wheat, sunflowers, corn, winter wheat, and in 2017 winter wheat again.

Cereal porridge applied at a dose of 47 and 94 m^3/ha (equivalent to N_{120} and N_{240}) resulted in average production increases in the years studied - 1.19-1.71 t/ha of cereal units or 23-31% more compared to the unfertilized variant.

Significant increases in grape yields (Table 6) and crop plants (Table 7) were presented.

Table 6. Influence of wine waste on the yield of Sauvignon grapes obtained on cambic chernozem, t/ha

Variant	Average for 2011-2022		
	Harvest, t/ha	Yield increase	
		tons	%
Control - unfertilized	9.5	-	-
Wine yeast (N_{100}), 13 t\ha annual	10.9	1.4	15
Wine yeast (N_{200}), 26 t\ha annual	11.9	2.4	25
Vinasse (K_{450}), 300 m^3\ha annual	10.5	1.0	11
Vinasse (K_{900}), 600 m^3\ha annual	10.8	1.3	14

Table 7. Influence of cereal waste fertilization on crop productivity, cereal units

Variant	Average for 2012-2017		
	Harvest, t/ha	Yield increase	
		tons	%
Control - unfertilized	3.62	-	
Grain mash (N_{120}), 47 m^3/ha annual	4.71	1.19	23
Grain mash (N_{240}), 94 m^3/ha annual	5.33	1.71	32
Dl $_{0,5}$, %	0.53	-	-
Sx, %	10.4	0	-

Based on the research carried out, technological models for the application of these wastes were developed (Siuris, 2017a; 2017b; Siuris & Ciolacu, 2019).

CONCLUSIONS

The research confirmed that the waste from the production of alcoholic beverages increased the content of humified organic matter in the soil by 0.2-0.4. A significant increase in mobile phosphorus was found (0.3-1.0 mg/100 g). The application of the waste did not change the content of exchangeable potassium. The wine yeasts application ensured a significant increase in grape Sauvignon production, on average for 2011-2022, of 1.4-2.4 t/ha. The harvest increase when incorporating vinasse was on average 1.0-1.3 t/ha. Cereal waste determined the achievement of average plant production increases of 1.2-1.7 t/ha cereal units or 23-32% compared to the unfertilized control.

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AGROECOLOGICAL PRACTICES THAT PROMOTE AND SUPPORT SUSTAINABLE AGRICULTURE

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Abstract

In recent decades, sustainable agriculture has become essential in the joint effort of agricultural policies and all stakeholders against the backdrop of climate changes and concerns about natural resources conservation. This agricultural system focuses on practices that increase agricultural yields, while protecting the environment and ensuring resources for the future. These practices include, among others, soil and plant health through crops diversification and rotations, the use of organic fertilizers, water and soil conservation by new tillage methods, or the implementation of innovative technologies for crops growing and animal husbandry. On the other hand, agroecology is an innovative concept that aims to ensure sustainable agricultural production and is based on the collaboration between people, science and environment to find solutions to the major problems that agriculture is currently facing, such as: diminishing soil and crop health, pollution, climate changes, pests control, or to ensure the high quality of agri-food products. The agroecological practices could be the path to success towards a sustainable agri-food system, through the involvement and effort of all key actors, farmers, decision-makers, citizens, by co-creating an environment favorable to knowledge exchange, progress, adaptation to change, and dissemination of good results to all. In this context, this paper aims to provide an overview of some examples of agroecological practices that promote and support sustainable agriculture in Romania.

***Key words**: agroecology, agroecological practices, crops health, soil health, sustainable agriculture.*

INTRODUCTION

Sustainable agriculture is an approach that seeks to minimize the negative impact of agricultural activities on the environment, while maintaining the ability to produce agrifood products in the most sustainable way possible. At the same time, according to FAO documents, agroecology involves, in an integrated approach, ecological and social concepts, principles with the aim of designing and managing agricultural and food systems in a sustainable way (FAO, 2025).

By optimizing the interactions between plants, animals, people and the environment, by including ecological, socio-cultural, technological, economic and political dimensions, from production to consumption, equitable and sustainable food systems can result that ensure food security and safety. In the realm of food security, agroecological practices play a vital role in ensuring sustainable nourishment. By bolstering soil fertility, encouraging crop diversity, and supporting local food production, agroecology acts as a buffer against the challenges posed by a changing climate (McLennon et al., 2021; Madsen et al., 2021). Consumers are increasingly interested in food products obtained through sustainable and ecological methods. This leads to an increased demand for agroecological products, stimulating the transition to more sustainable food systems (https://www.ecoruralis.ro/programe/agroecologie/).

Agroecological principles such as polyculture, crop diversity, and integrated pest control help to improve food security by increasing stability and nutrition. Agroecology encourages carbon sequestration, soil health, and greenhouse gas reductions, resulting in climate-resilient farming systems (Ranjan, 2024).

MATERIALS AND METHODS

This article presents an analysis of the specialized literature that seeks to make a connection between the importance and role of implementing agroecological practices in the approach and development of sustainable agriculture, protecting the long-term health of people, animals, landscapes and the environment, as well as the health of soil and plants.

In summary, a series of aspects related to the current global issues of the agroecological transition will be discussed through the lens of soil degradation processes and soil health management and its microbiology, emphasizing the causal relationships between factors, the dynamics of degradation processes, and the impact produced on both the soil, the environment, and human society.

Examples of agroecological practices related to improving soil fertility through nutrient management and crop rotation diversity through the use of legumes as nitrogen-fixing plants were addressed. Also, the issues of integrated pest management were addressed, which are based mainly on preventive and then curative methods.

RESULTS AND DISCUSSIONS

From the point of view of the importance of agroecology on sustainable agricultural systems, some benefits are discussed based on some general principles that refer to: soil conservation, nutrients management, crop rotation, biodiversity, energy balance of farm and environmental impact (Figure 1). The importance of each will be briefly presented below.

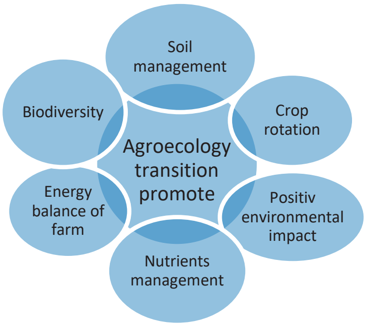

Figure 1. Agroecology practices to support sustainable agriculture

Soil health management. Broad sustainable soil development is only available it if includes forms of conservation and sustainability within the processes of soil management. Of the 4.40 billion ha of agricultural land on worldwide, 33% of the Earth's soils are already degraded and over 90% could become degraded by 2050 (FAO and ITPS, 2015; IPBES, 2023). Soil erosion and land degradation pose a major threat to global food security and to the achievement of the Sustainable Development Goals (SDGs) – compromising the well-being of at least 3.2 billion people around the world (FAO, 2025).

The loss of fertile arable land is accelerating annually with a rate of around 24 million ha and 23 ha per minute and economic losses amounting to 10% of the global GDP annually (Gebremedhin et al., 2022). Because 95% of the food comes from the soil, soil erosion is critical for protecting the soil while ensuring a sustainable and food security in the world (https://www.weforum.org/stories/2023/02/soil-degradation-biodiversity-planet/).

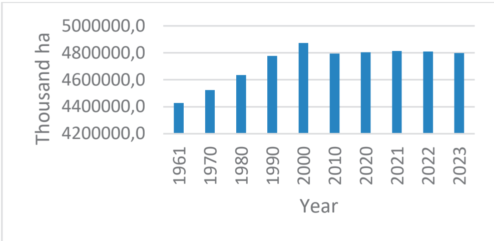

Figure 2. Evolution of agricultural land in the world in the period 1961-2023 (FAOSTAT, 2023)

According to Alderman et al. (1990), on the global basis, the soil degradation is caused primarily by overgrazing (35%), agricultural activities (28%), deforestation (30%), over exploitation of land to produce fuelwood (7%), and industrialization (4%). In Europe, according to the same authors, the soil degradation is caused primarily by agricultural activities (38%), deforestation (29%), overgrazing (44%) and industrialization (9%). Romania's agricultural area impacted by critical levels of singular processes, highlighting the degradative pathways of soil compaction (40%), aridity (24%), tillage erosion (22%), soil nutrient imbalances (22%) and water erosion (20%) as the greatest threats to

agricultural landscapes countrywide (Prăvălie et al., 2025).
Under no-till conditions the earthworm populations are much more abundant than in those where plowing is applied (Amuza and Ilie, 2024).

Table 1. Soil organism functions and agronomic consequences

Soil organisms	Functions	Agronomic consequences
Earthworm burrows	Consume dead plant material (roots, residues) make galleries, redistribute organic matter and nutrients from the topsoil layer, secrete a plant growth stimulant	Soil aeration, water infiltration, root penetration, nutrient recycling, plant growth stimulation
Arthropods	Consume plant and animal residue	Nutrient recycling
Bacteria	Descompose organic matter wastes, realease plant growth hormones, fix atmosferic nitrogen, transform nitrogen to ammonium to nitrites and viceversa, secretae the polysaccharides	Provision of available nutrients (N, P, Al), plant grow stimulation, improvement of the soil structure, root diseases resistance
Fungi	Decompose organic matter, release plant hormones and antibiotics	Nutrient recycling, plant stimulation
Mychorhizae (fungi)	Extend the reach of roothairs, release plant hormons and antibiontics	Increase in water and nutrient (p) intake, plant diseases resistance, root growth stimulant
Actinomicetes	Descopose organic matter, release antibiotics	Nutrient recycling, root disease resistance
Algae	Photosynthesize, secrete organic substance	Improvement in soil structure
Protozoa	Consume bacteria and other microbes	Increase in the rate of nutriet cycling
Nematodes	Eat decaying organic matter and microorganisms (bacteria, fungi, algae, protozoa, nematodes	Increase in the rate of nutrient cycling

Agroecological practices that support technological progress, as well as better tillage systems, supporting biodiversity through crop rotation, balanced fertilization plans, and controlling weeds, pests, and diseases, can offset the negative effects of soil degradation (Al-Musavi et al., 2025).
Organic matter is anessential component of soil. Among its several properties, it supplies carbon, energy and nutrients, stabilizes soil aggregates, improves storage characteristics and water flow in soil, acts as a sourse of nutrients, has a great capacity of cationic exchange, reduces the soil apparent density while minimizing the effect of compression, makes soil much more friable an less adherent, thus easier to till, reduces negative effects of pesticides, heavy metals and other polluters. The quantity of organic material in soil at a given moment is determined by the equilibrum between the addition of material and its loss and decomposition.
Nutrients management. Balanced fertilization refers to the optimal supply of nutrients necessary for plants for healthy and vigorous growth, without excesses or deficiencies. Such a balance involves the adequate supply of nitrogen (N), phosphorus (P) and potassium (K), as well as other minor elements, depending on the specific needs of the plant and the soil. The Code of Good Agricultural Practices (CBPA) formulates several principles to ensure efficient and non-polluting fertilization. Rational fertilization and the preservation of soil quality are interdependent. The principles it is based on include application of fertilizers only during the active vegetation period, because during periods of vegetative rest, significant losses of nutrients occur in surface waters and groundwater and implicitly pollution with nitrates.
The mechanisms of involvement and participation of nutrients in physiological processes in plants are the same, regardless of their origin (from natural sources or from chemical fertilizers). At the same time, the quantitative requirements of nutrients vary with the crop, the expected harvest and the climatic conditions. The fertility level of a soil can be degraded if the cultivation technologies are incorrect or, on the contrary, it can increase if it is cultivated in a manner that improves its chemical, physical and biological properties (Furey and Tilman, 2021).

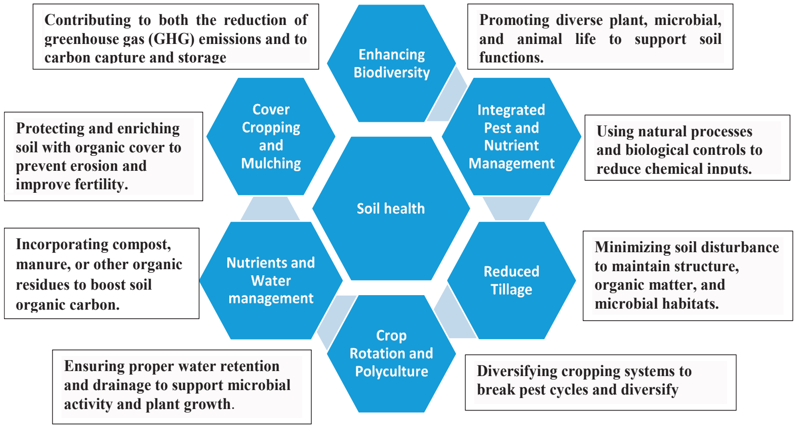

Figure 3. Benefits of soil health to agroecology transition

A soil with good natural fertility can become productive by correcting the limiting factors that prevent the normal growth and development of plants (acidity, excess or deficiency of nutrients) or it can depreciate by impoverishing in one or more nutrients, by degrading some properties or it can be completely destroyed by erosion phenomena (Karlen, 2004).

In recent decades, sustainable agriculture has become essential in the joint effort of agricultural policies and all stakeholders against the backdrop of climate change and concerns about resource conservation. This agricultural system focuses on practices that increase agricultural yields, while protecting the environment and ensuring resources for the future. These practices include soil and plant health through crop diversification and rotation, the use of organic fertilizers, water and soil conservation, or the implementation of innovative technologies in plant cultivation and animal husbandry. On the other hand, agroecology is an innovative concept that aims to ensure sustainable agricultural production and is based on the collaboration between man, science and the environment to find solutions to the major problems that agriculture is currently facing, such as: diminishing soil and crop health, pollution, climate change, or the need to ensure the quality of agri-food products (Chable et al., 2020). The agroecological transition could be the path to success towards a sustainable agrifood system, through the involvement and effort of all key actors, farmers, decision-makers, citizens, by co-creating an environment favorable to knowledge exchange, progress, adaptation to change, which would help to popularize, accelerate the transition and implementation of agroecological practices.

In this context, as examples of agroecological practices that promote and support sustainable agriculture are the cultivating of plants that can be grown for green manuring are those that yield relatively much green mass under the farms climatic and soil conditions. In addition, nitrogen-fixing plants are advantageous. The effect of green manure depends not only on the crop ploughed in but also on the soil. In sandy soil poor in nitrogen, the bacteria within root nodules fix more nitrogen. In spite of this, the yield increasing effect of green manuring is higher in latter because, together with decomposition of ploughed in green manure, the decomposition of the soils organic substances accelerates too, so that more nutrients available to the plant are produced.

Crops rotation and legumes crops. Crop rotation is a sustainable agricultural practice that involves systematically changing the types of crops grown in a particular area across different seasons or years. This method helps improve soil health, breaking pest and disease cycles, and decrease the reliance on chemical inputs. Key benefits of crop rotation in organic farming include: enhancing soil fertility by alternating crops with different nutrient needs; breaking pest and disease cycles by disrupting their habitats; reducing weed problems through diverse planting patterns; promoting beneficial soil organisms. Common crop rotation strategies often include the use of legumes (such as beans or peas) to fix nitrogen in the soil, followed by crops that consume more nutrients (like grains or vegetables), and then cover crops or green manure to restore soil organic matter. Legumes have a positive impact on soil fertility due to their ability to fix atmospheric nitrogen in the soil through symbiotic bacteria such as those of the genus *Rhizobium*. This process helps increase the level of nitrogen available to other plants, reducing the need for chemical fertilization.

The benefits of these plants include: improving the nutrient content of the soil, especially nitrogen, improving soil structure and water retention capacity; reducing soil erosion; increasing microbiological biodiversity in the soil. In addition, legume crop rotation helps prevent nutrient depletion and reduce soil diseases, thus helping to maintain and improve soil fertility in the long term (Țopa at al., 2024).

Plant protection. Plant protection according to agroecology principles focuses on maintaining healthy crops by minimizing the impact of pests, diseases, and weeds using natural and sustainable methods. This approach emphasizes preventing problems through proactive measures and fostering a balanced ecosystem rather than relying on synthetic pesticides (Zhou et al., 2024). Unlike conventional agriculture, these methods rely on ecological principles and preventive techniques, such as crop rotation and biodiversity management, to control pests, diseases, and weeds, rather than chemical inputs. Specific techniques include the use of organic substances like compost and manure for fertilization, mechanical methods such as barriers and water sprays for pest removal, and biological controls utilizing natural predators or biopesticides. Also often incorporates IPM principles, which involve monitoring pest populations, identifying thresholds for intervention, and using a combination of preventative and direct control methods. Deguine et al. (2022) defined Agroecological Crop Protection (ACP) as the reduction of pest impacts through the reorganization of cropping practices and the improvement of agroecosystem sustainability by harnessing its ecological functions. The stimulation of ecological processes such as natural pest regulation through improved soil health and improved interactions between plant and animal communities, is a rich source of innovative crop protection models (Brévault and Clouvel, 2019). Key principles are presented in the next table (Table 2).

Table 2. Key principles and practices of plant protection according to agroecology principles (own processing)

Key principles	Practices
Biodiversity	• Promoting a diverse ecosystem helps to naturally control of pests and diseases; • Including crop rotation, intercropping, and maintaining habitats for beneficial organisms.
Soil Health	• Healthy soil is fundamental for plant health; • Practices like composting, cover cropping, and reduced tillage improve soil structure, fertility, and microbiome diversity, which in turn enhances plant resistance to pests.
Cultural Practices	• Techniques such as intercropping, crop rotation, and timed planting can reduce pest pressures and improve crop resilience; • Planting pest-resistant varieties also plays a significant role.
Biological Control	• Utilizing natural predators and parasitoids to manage pests is a standard practice in ecological farming; • Introducing or conserving beneficial insects (like ladybugs or parasitoid wasps).
Mechanical and Physical Controls	• Practices such as mulching, row covers, and traps can physically restrict pests and weed growth without chemicals.
Monitoring and Thresholds	• Regularly monitoring pest populations and understanding economic thresholds helps farmers make informed decisions about when intervention is necessary, reducing unnecessary pesticide use.
Natural Inputs	• Using of natural substances (such as neem oil, insecticidal soaps, or diatomaceous earth) that are less harmful to the environment compared to synthetic options.
Education and Knowledge Sharing	• Farmer education and participation in cooperative learning can enhance knowledge of sustainable practices and pest management strategies.

Plant protection seeks to create a balance between maintaining productive agriculture systems and conserving biodiversity.

By implementing a combination of biological, cultural, physical, and, when necessary, natural control measures, farmers can effectively manage pests while promoting environmental sustainability.

CONCLUSIONS

Agroecology applies ecological principles to design sustainable food systems that work with nature, to reduce environmental impact and promote ecological renewal.

By emphasizing biodiversity, closed nutrient cycles, and natural processes, it minimizes reliance on synthetic inputs, enhances ecosystem services like soil fertility and natural pest control, and contributes to more resilient, equitable, and sustainable food production systems.

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