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EFFECT OF DIFFERENT TYPES OF MINERAL FERTILIZATION ON YIELDS AND SOME PHYSIOLOGICAL CHARACTERISTICS IN FIELD BEANS

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Abstract

The study was conducted within the Longstanding Stationary Fertilizer Experiment (LSFE) in IASS "Obraztsov chiflik", Rousse with the aim of establishing the influence of different options of mineral fertilization on yield and some physiological characteristics, including resistance to environmental stress in field beans. It was found that the highest yield for the period - 1,070 kg ha^{-1} - was obtained in the experimental plot with combined N_5K_7 fertilization, represents nearly 70% increase compared to the average yield of the control. The content of photosynthetic pigments in the leaves of field beans varies significantly in different fertilization options, with the highest levels of chlorophyll a+b being found in the variant with nitrogen fertilization (N_5) - 1.520 mg g^{-1}. The variants with combined N_5P_{12} fertilization stand out as the most resistant to atmospheric drought during the four-year research period, with the reported values - 98.28 µS cm^{-1}, being 9,8% lower, compared to the control. The highest resistance to soil drought was established again in the variants with combined N_5P_{12} fertilization (500.00 µS cm^{-1}) and the lowest - within the control (711.58 µS cm^{-1}).

Key words: *chlorophyll, field beans, mineral fertilizers, physiological characteristics, yield.*

INTRODUCTION

The efficient use of resources to improve agricultural production is of utmost importance in view of the continuous growth of the world population. It is expected to reach 9.9 billion by 2050, which requires an increase in food production by over 70% (Sarfraz et al., 2023). The extreme events of abiotic stresses – high temperatures and prolonged periods without precipitation, affects the balance of natural and anthropogenic ecosystems and reduces crop production worldwide (Hossain et al., 2021). This place supporting plant growth and development in conditions of abiotic and biological stress and obtaining higher yields among the main challenges facing agriculture (Reynolds et al., 2011).

Leguminous crops are known for their unique nitrogen fixing ability. Legumes introduced into a cropping system can not only add to the fertility of the soil by their nitrogen fixing ability but also gives additional benefit of soil organic matter improvement, nutrient recycling, improving soil porosity and soil structure, etc. Because the growing season of legumes is typically short, they fit well into cropping systems, providing flexibility in crop planning (Praharaj & Maitra, 2020).

Fertilization is among the most significant factors influencing the productivity of agricultural crops, making it the subject of numerous scientific studies (Nenova, 2008; Naskova et al, 2019). Particularly valuable information can be obtained in studies conducted within longstanding stationary fertilizer experiments (Samarlieva & Nikolova, 1996; Panayotova, 2007).

Fertilizers improve soil fertility and plant nutrition, making them essential components in agriculture and forestry (Boteva & Cholakov, 2009). Soil fertilization increases nutrient reserves in the root zone of crops, increases pH values and the amount of organic carbon, thus improving the structure of the root zone of the soil (Karcauskiene & Repsiene, 2009).

Macronutrients (N, P and K) are of particular importance for the normal course of physiological processes in plants, for their growth and development. Nitrogen (N) significantly improves and increases the yield and its quality

due to its critical role for the normal course of biochemical and physiological processes (Amancio & Stulen, 2005). It is a key component of enzymes and structural proteins, nucleic acids, pigments and a wide range of secondary metabolites in plants (Leghari et al., 2016). Phosphorus (P) is part of the structure of many enzymes and proteins, DNA and RNA, and plays a significant role in assimilating solar energy and turning it into useful organic compounds. It is a vital component of adenosine triphosphate (ATP), which is formed during photosynthesis, and is actively involved in energy metabolism. Phosphorus increases plant resistance and regulates the physiological response to abiotic stress – high temperatures, salinity, drought, waterlogging, high CO_2 levels and the toxic effects of heavy metals (Hawkesford et al., 2012; Lambers, 2022; Khan et al., 2023). Potassium (K) is among the elements of particular importance for the normal course of a large number of physiological processes, for the growth of agricultural crops, for the quality and quantity of yields, as well as for the resistance of crops to stress (Zörb et al., 2014). It has an important role in limiting water loss from plants at high ambient air temperatures and in drought conditions. In plants under drought conditions, the intensity of photosynthesis is directly related to potassium levels (Chaerle et al, 2007).

Changes in cell membrane organization occur during seed development as seeds reach physiological maturity, as a result of moisture loss prior to harvest and water uptake prior to germination The electrical conductivity test is based on the measurement of electrolytes released from seeds as a result of changes in cell membrane permeability (Matthews & Powell, 2006). It allows for a rapid and reliable determination of seed quality and viability, in contrast to the standard method for determining germination (Standard Germination Test), which is more species specific and time-consuming (Montenegro et al., 2003).

The bright green chlorophyll is the most widely distributed natural pigment in nature. The chlorophylls are omnipresent in photosynthetic tissue where they are responsible for photoreception (Formaggio et al., 2001). They play a key role in the process of photosynthesis, which allows plants to absorb energy from sunlight (Willows, 2009; Hörtensteiner & Kräutler, 2011, Filimon et al., 2016). The chlorophylls of higher plants, ferns, mosses and green algae, as well as the prokaryotic organism *Prochloron*, consist of chlorophyll *a* as the main pigment and chlorophyll *b* as an accessory pigment. These two pigments were for a time thought to be the only natural chlorophylls in higher plants, but it has since been shown that a variety of precursors and degradation products are also present in nature (Andersen & Francis, 2009).

Carotenoids are derivatives of isoprene, with a dark red to yellow color found in all higher plants and in many types of microorganisms. Carotenoids are also found in many flowers and fruits where they are valuable in terms of providing signals to other species on the availability of food sources, and in this way ensure pollination and the spread of seeds (Goodwin, 1980; Gross, 1991). Carotenoids perform two main roles in plants – they are functioning as auxiliary pigments: as such, carotenoids participate in the construction of photosystems and participate in the absorption of light in that part of the spectrum (400-500 nm) in which the efficiency of chlorophyll is lower and they protect chlorophyll molecules from photooxidation (Lichtenthaler, 1987; Young and Frank, 1995; Britton, 2008; Cuttriss and Pogson, 2009; Belous et al., 2018).

MATERIALS AND METHODS

Climatic and Soil Characteristics of the Experimental Field

The study covers the four-year period of the 16th rotation (from October 2018 to June 2022) of a Longstanding Stationary Fertilizer Experiment (LSFE), located in the Experimental Field of IASS "Obraztsov chiflik", Rousse, Bulgaria. The northern climatic region of the Danubian rolling plain, in which the Obraztsov chiflik falls (152 m above sea level, 43º48´ north latitude, 26º02´ east longitude), is characterized by the largest average annual temperature amplitude for Bulgaria – from 24.5 to 26.0ºC. In january and february the lowest minimum temperatures here may reach -25ºC, while in the hot summer mounths have been recorded maximum temperatures up to 42-43ºC. The rainfall in Obraztsov chiflik follows a continental pattern,

with a maximum in June and a minimum in February. Due to the nature of their distribution (the rainfalls in Obraztsov chiflik are most often torrential, interrupted by long periods without precipitation), dry periods occur in the summer. The drought is relatively strong and prolonged not only in August, but also during most of September and even the beginning of October (Lingova, 1965).

Kostov (1981) found that the LSFE was located on a medium sandy-clayey haplic chernozem, uneroded and slightly eroded. According to a new study conducted in 2014, the modern soil classification in Bulgaria defines these soils as leached (Luvic) or degraded (Teoharov et al., 2014). As a result of the multi-year uniform fertilization of the experimental plots, permanent changes in soil acidity and humus content occurred in the different variants. Soil acidity (pH) ranged from 5.25 in plots with nitrogen-potassium fertilization to 5.74 in the no-fertilizer control. The lowest humus content (1.67%) was found in the plots of the control, and the highest (2.65%) – in the variant with full mineral fertilization ($N_{15}P_{12}K_7$).

Setting Up the Experiment

The longstanding stationary fertilizer experiment was established in 1912 on an area of 1 ha according to the eight-plot scheme of Georg Ville, in two repetitions with an experimental plot of 100 m^2 and a harvest plot of 60 m^2. Research was interrupted in 1942 and resumed in the fall of 1958 with a four-pole crop rotation of wheat-maize-barley-field beans (Ermolaev, 1965).

Seven variants of fertilization are being tested with individual and combined application of the macro elements nitrogen, phosphorus and potassium. The eighth variant is the unfertilized control ($N_0P_0K_0$). The amount of nitrogen fertilizers applied to the individual crops is as follows: for wheat and corn – 150 kg ha^{-1} a.s. (N_{15}); for barley – 100 kg ha^{-1} a.s. (N_{10}) and for beans – 50 kg ha^{-1} a.s. (N_5). The amount of phosphorus and potassium fertilizers applied is the same for all four crops in the crop rotation – 120 kg ha^{-1} a.s. in the form of P_2O_5 and 70 kg ha^{-1} a.s. in the form of K_2O. Phosphorous (P_{12}) and potassium (K_7) fertilizers are applied once, before the main tillage, and nitrogen fertilizers – once after crop emergence.

Characteristic of the Variety

The sixteenth, after the restoration of the LSFE in 1958, rotation of the four-pole crop rotation included common wheat "Dunavia" variety, winter barley, "Ahat" variety, field beans, "Obratzov Chiflik 12" variety and maze, hybrid Ps 464.

The “Obraztsov Chiflik 12” field beans variety is semi-climbing with erected, tidy bush without lodging. The height of the plants is averagely 48.3 cm with average height of the first formation of pods is 9.9 cm. It does not form pods on stem tendrils. The seeds are semi-flat type, the mass of 1000 seeds – averagely 311 g. The plants ripen at one and the same time and have good resistance to crumbling. It is a high yielding variety with good boiling time and excellent gustatory qualities.

When carrying out the experiment, the agrotechnics adopted for North-Eastern Bulgaria for growing field beans are followed. The obtained data were statistically processed with Microsoft Corporation's *Excel* and TIBCO Software's *Statistica 13* programs.

Sampling and Analysis

- *To determine the electrical conductivity of leaf membranes*, in the flowering stage of field beans a sample of healthy, with normal turgor and without signs of damage, leaves of 10 randomly selected plants in each of the experimental plots was taken. The leaves are placed in clean containers and taken to a laboratory for analysis. Fresh leaves were washed well, rinsed with deionized water and after drying, an average sample was taken from them to measure the electrical conductivity of leaf membranes (Pavlova et al., 2005; Pavlova & Panayotova, 2005; Pavlova & Dochev, 2010). The measurement of the electrical conductivity of leaf membranes is performed with a COND 51 laboratory conductometer after a two-hour incubation of the samples in a thermostat at a temperature of 42°C.

- *The measurement of the electrical conductivity of the seed membranes* makes it possible to quickly and reliably determine the viability of the seeds. The electrical conductivity test is based on measuring electrolytes that are released from the seeds as a result of changes in the permeability of cell membranes (Matthews & Powell, 2006). In our study to determine

electrical conductivity of seed membranes, the methodology described by Avramiuc (2016) was used with some modifications. A month after harvesting 50 seeds of each variant of the LSFE were selected, placed in a suitable container and poured with 50 mL of deionized water. The samples thus prepared were kept in a thermostat at a temperature of 20°C for 24 hours, after which the electrical conductivity of the water was measured using a COND 51 laboratory conductometer.

- *Extraction and determination of the content of photosynthetic pigments*

The photosynthetic pigments contained in the leaves of the plants in flowering stage were extracted under vacuum, using an 80% acetone solution as a solvent. A sample of the obtained extract was diluted with pure 80% acetone, then photometrically measured. The quantitative determination of the pigments was carried out using a DR 3900 spectrophotometer from HACH LANGE, Germany. The light absorption in the extract was measured at three wavelengths: 663 nm and 644 nm, respectively for chlorophyll *a* and *b*, and 440 nm for carotenoids with a blank sample of pure, 80% acetone solution as a control. The pigments content was calculated according to Berova et al. (2007) in mg g^{-1} fresh mass, taking into account the concentration of the pigments, the volume of the total extract, the mass of the leaf sample and the proportion of dilution.

The aim of the present research is to study the influence of different nitrogen, phosphorus and potassium fertilization options on the yield of field beans variety "Obraztsov chiflik 12" and to determine the influence of long-term mineral fertilization on its photosynthetic pigments content and resistance to environmental stress.

RESULTS AND DISCUSSIONS

Brief meteorological characteristics

The spring of 2019 is characterized by temperature sums and precipitation close to the climatic norm for the region formed on the basis of the multi-year data obtained from a meteorological cell located in Obraztsov chiflik at the beginning of the 20th century (Table 1a and 1b). The temperature and amounts of precipitation in April and May are close to normal, while June can be defined as hot and humid with a temperature sum of 672.6ºC, significantly exceeding the norm of 604.0ºC, and 129.1 mm of total precipitation at a norm of 82.1 mm. The precipitation that fell in July is unevenly distributed over 8 days, with their total amount reaching only 19.5 mm, or slightly over 1/3 of the norm for the period, amounting to 65.9 mm. No significant deviations are observed in terms of the temperature sum.

Table 1a. Temperature sum for the period April-July from 2019 to 2022

Year	Temperature sum, ºC			
	IV	V	VI	VII
2019	340.6	527.1	672.6	711.7
2020	366.9	511.6	616.9	746.0
2021	287.4	521.5	599.4	763.5
2022	339.7	542.0	639.4	766.8
Climate norm	***341.9***	***509.5***	***604.0***	***699.1***

Table 1b. Distribution of precipitation for the period April – July from 2019 to 2022

Year	Precipitation, mm			
	IV	V	VI	VII
2019	56.0	70.4	129.1	19.5
2020	13.6	67.1	126.0	1.1
2021	45.6	73.5	121.0	1.0
2022	74.9	18.0	73.0	11.6
Climate norm	***50.6***	***66.5***	***82.1***	***65.9***

The temperature sum for the period April – June in 2020 and 2021 is close to normal for the Obraztsov Chiflik region, with the exception of April 2021, which can be defined as cool, with a temperature sum of 287.4ºC compared to a norm of 341.9ºC. The precipitation in May 2020 and April-May 2021 is close to normal for the period with a significant drought in the early spring of the second year of the 16th LSFE rotation. The total amount of precipitation in April is only 13.6 mm compared to a norm of 50.6 mm. In these meteorological conditions the amount of precipitation in June – distributed mainly in the second ten days of the month – significantly exceeds the norm of 82.1 mm, respectively by 43.9 mm in 2020 and 38.9 mm in 2021. The last month of the crop's vegetation is characterized by serious drought, with notable increase in average daily temperatures. The temperature sum recorded in 2020 exceeds the norm of

699.1ºC for the month of July by 46.9ºC, in 2021 – by 66.4ºC and in 2022 – by 67.7ºC.

At these unusually high temperatures, the amount of precipitation is insignificant, with precipitation for the entire month of July in 2020 and 2021 being only 1.1 mm and 1.0 mm, respectively. In conclusion, it can be said that the years from 2019 to 2022 are characterized by a warm spring and hot summer with unevenly distributed precipitation and prolonged periods of drought during the summer months.

Yield

Only the results for the productivity of the crops included in the crop rotation in the 9th, 10th and 12th rotations of LSFE after its restoration in 1952 were analyzed and published (Dimitrov & Beykov, 1995; Stoyanov, 2001; Nenova, 2008; Nenova & Stoyanova, 2011). So far there is just one published study on the influence of different fertilization options on the resistance of some of the crops in crop rotations of LSFE to environmental stress (Ivanova-Kovacheva et al., 2024).

The yields obtained during the fertilization of field beans variety "Obraztsov Chiflik 12" with the macro elements nitrogen (N_5), phosphorus (P_{12}) and potassium (K_7), applied alone and in combination between them, for the period of the 16th rotation of LSFE are presented on Table 2.

Table 2. Yields of field beans variety "Obraztsov Chiflik 12" obtained from LSFE for the period from 2019 to 2022

Variants	Yield, kg ha^{-1}				Average yield, kg ha^{-1}	Differences +,- kg ha^{-1}
	2019	2020	2021	2022		
Control ($N_0P_0K_0$)	540	780	320	890	632.50	0.0
N_5	980	1,140	780	1,090	997.50	365 +++
P_{12}	730	820	490	800	710.00	77.5 n.s.
K_7	720	850	310	740	655.00	22.5 n.s.
N_5P_{12}	1,130	1,260	830	1,050	1,067.50	435 +++
N_5K_7	1,090	1,160	880	930	1,015.00	382.5 +++
$P_{12}K_7$	600	1,070	420	800	722.50	90 n.s.
$N_5P_{12}K_7$	960	1,100	910	1,050	1,005.00	372.5 +++
Average	843.75	1,022.50	617.50	918.75		
LSD 0.05						150
LSD 0.01						200
LSD 0.001						270

The most favorable year for field beans was 2020, with an average yield of 1,022.5 kg ha^{-1}. The highest yield was reached in the same year – 1,260 kg ha^{-1}, obtained with the two-component fertilization combination (N_5P_{12}), followed by 1,160 kg ha^{-1} with the N_5K_7 combination.

The data also shows that the highest average yield for the rotation in the variants with combinations of the studied macroelements was achieved in the one with the two macroelements N_5P_{12} – 1,067.5 kg ha^{-1}, with the increase compared to the unfertilized control being 435 kg ha^{-1}. In the other fertilization combinations in which the macroelement nitrogen participates, a proven increase in yield was also observed, with the N_5K_7 variant being 1,015 kg ha^{-1} or 382.5 kg ha^{-1} higher than the unfertilized control. In the case of phosphorus and potassium fertilization alone and in the combination between them, the differences in yield obtained are unproven.

Electrical Conductivity of Leaf Membranes

The measurement of the electrical conductivity of the cell membranes of the leaves during the active vegetation of plants can be used as an indicator of the resistance to atmospheric drought (Pavlova, 2005; 2010). Figure 1 presents the reported values of electrical conductivity of leaf membranes in flowering stage of the field beans for the entire period of the 16th rotation of LSFE.

It can be seen that the lowest measured values vary within very narrow limits – from 70.10 µS cm^{-1} in the variant with only nitrogen fertilization (N_5) in 2020 to 77.73 µS cm^{-1} in the variant with combined nitrogen-phosphorus fertilization (N_5P_{12}) in 2021.

The maximum electrical conductivity of leaf membranes during the four years of the study were found in the non-fertilized variant ($N_0P_0K_0$), with the values falling within significantly wider

margins – from 98.53 μS cm^{-1} in 2020 to 124.60 μS cm^{-1} in 2021.

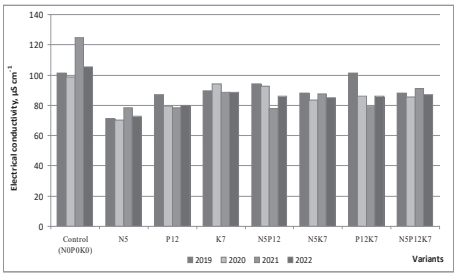

Figure 1. Electrical conductivity of leaf membranes in flowering stage of field beans by year, 16th LSFE rotation

The summarized results show that the leaching of electrolytes through the cell membranes of the leaves in the variants with only N_5 and P_{12} fertilization is lower, compared to the variants with only K_7 fertilization, as well as the variants with combined fertilization (Table 3).

Table 3. Statistically processed data on electrical conductivity of leaf membranes in flowering stage of field beans, 16th LSFE rotation

Variants	Electrical conductivity of leaf membranes, μS cm^{-1}		
	Mean	CV, %	SE, %
Control ($N_0P_0K_0$)	107.44	10.97	5.89
N_5	73.07	5.04	1.84
P_{12}	81.22	4.68	1.90
K_7	90.23	3.17	1.43
N_5P_{12}	87.72	8.56	3.75
N_5K_7	86.12	2.60	1.12
$P_{12}K_7$	88.16	10.59	4.67
$N_5P_{12}K_7$	88.05	2.80	1.23

The highest values of electrical conductivity of the leaf membranes, averaged over the period of the study, were recorded in the control variant ($N_0P_0K_0$) – 107.44μS cm^{-1} against 73.07μS cm^{-1} for the variant with nitrogen fertilization (N_5). The variants with phosphorus (P_{12}) and two-component nitrogen-potasium (N_5K_7) fertilization are also proven resistant to atmospheric drought, in which the electrical conductivity of the leaf membranes was recorded as 81.22 μS cm^{-1} and 86.12 μS cm^{-1}, respectively.

The statistical processing of the data obtained in the four-year period of the study, found that the values of the coefficient of variation (CV, %) for the different variants of field beans fertilization lie within relatively wide range – from 2.60% in the variant with combined N_5K_7 fertilization to 10.97% in the control variant ($N_0P_0K_0$). The low values of the coefficient of variation verify the higher accuracy of the results reported during the four years of the study. Low values of the statistical error (SE, %) were established in the variant with combined N_5K_7 and full mineral fertilization ($N_5P_{12}K_7$) as well as those whit applying just one of the three macro elements. The calculated values vary from 1.12% to 1.90%. The largest deviation from the average values of the parameter was reported for the variants without mineral fertilization ($N_0P_0K_0$) and the combined phosphorus-potassium fertilization ($P_{12}K_7$), where the SE reached 5.89% and 4.67%, respectively.

Electrical Conductivity of Seed Membranes

It is been established that the higher rate of restoration of the integrity of the cell membrane system during seed moistening will result in weaker extraction of electrolytes in the external environment and therefore the electrical conductivity of the water, which indicates a higher vitality (Baalbaki et al., 2009; Szemruch et al, 2019; Araújo et al., 2022). The relationship between electrical conductivity and field emergence is useful in practical seed tehnology (Mattews and Powel, 2006). Consequently, it could be assumed that it is possible to use the electrical conductivity of seed membranes as a criterion for determining the resistance of seeds to water stress, including soil drought.

The values of the electrical conductivity of field beans seed membranes varied vastly over the years and between different fertilization options (Figure 2). The seeds with the highest vitality in the individual years were obtained from experimental plots with different variants of fertilization. In the first and third years of the 16th rotation of the LSFE, the lowest extraction of electrolytes was recorded in the variant with phosphorus fertilization (P_{12}), 601.3 μS cm^{-1} and 457.5 μS cm^{-1}, respectively, and in the second and fourth years – in the one with full mineral fertilization – 370.3 μS cm^{-1} and 484.0 μS cm^{-1}. The analysis of the obtained results shows that the control and the variants with potassium and combined potassium-phosphorus fertilization had the lowest vitality, respectively, the highest amount of extracted electrolytes.

The values of seeds membrane conductivity reported in the first year of the 16th LSFE rotation are significantly higher and vary within wider range. The highest vitality was recorded in the variants with only P_{12} (601.3 μS cm^{-1}) and combined N_5P_{12} (609.3 μS cm^{-1}) fertilization, and the lowest – in the seeds obtained from plants in the plots without fertilization (1045.0 μS cm^{-1}) and with only K_7 fertilization. The conductivity in the variants with only phosphorus or combined nitrogen-phosphorus fertilization was higher than that of the control variant by 42.5-41.7%. The greater leaching of electrolytes was found in only one of the four years of the rotation, therefore the data obtained in 2019 cannot claim statistical reliability. The high heterogeneity of the results obtained necessitates the need of further studies to be conducted on the correlation between seed vitality and the different types of fertilization of field beans.

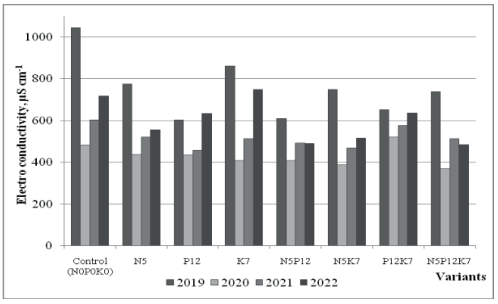

Figure 2. Electrical conductivity of field beans seeds by year, 16th LSFE rotation

The statistical processing of the data (Table 4) confirms the heterogeneity of the results, obtained during the 4-year study.

Table 4. Statistically processed data on electrical conductivity of field beans, 16th LSFE rotation

Variants	Electrical conductivity, μS cm^{-1}		
	Mean	CV, %	SE, %
Control ($N_0P_0K_0$)	711.58	34.02	121.03
N_5	572.75	25.18	72.12
P_{12}	531.83	18.76	49.89
K_7	633.03	33.05	104.60
N_5P_{12}	500.00	16.57	41.42
N_5K_7	530.87	29.32	77.83
$P_{12}K_7$	597.01	10.04	29.98
$N_5P_{12}K_7$	526.53	29.26	77.04

The seeds harvested from the variants with combined nitrogen-phosphorus (N_5P_{12}) and full mineral ($N_5P_{12}K_7$) fertilization have the highest vitality, on average for the period, 500.0 μS cm^{-1} and 526.53μS cm^{-1}, respectively. High vitality was also found in the variant with combined N_5K_7 (530.87 μS cm^{-1}) and with P_{12} (531.83 μS cm^{-1}) fertilization.

The seeds from the control ($N_0P_0K_0$) have the lowest vitality. The highest electrical conductivity of seed membranes (711.58 μS cm^{-1}) was measured in this variant.

Statistical processing of the data obtained during the four-year study period found that the values of the coefficient of variation (CV, %) for the different options for fertilizing field beans fall within very wide limits, but shows satisfactory uniformity of the data by year, regardless. Sampling heterogeneity, i.e., values of CV above 30%, was found in the control and in the variant with potassium fertilization ($N_0P_0K_0$ – 34.02% and K_7 – 33.05%). The lowest values of the coefficient of variation were established for the variant with combined $P_{12}K_7$ fertilization (10.04%) with N_5P_{12} close second (16.57%).

In the variant without fertilization ($N_0P_0K_0$), a significantly high level of statistical error (121.03%) was observed. According to the formula for calculating the error, in view of the small sample size due to the specific conditions of deriving the LSFE, and the significant coefficient of variation, obtaining such a value is possible. Smaller errors, but again significant in value, were obtained in the variant with potassium (K_7) and nitrogen (N_5) fertilization, 104.6% and 72.12%, respectively. In the variant with two-component fertilization with phosphorus and potassium ($P_{12}K_7$), the lowest level of statistical error was calculated – 29.98%.

Photosynthetic pigment content

The content of photosynthetic pigments in plant leaves provides important information about their physiological state and gives important information about their resistance to stress (Sims & Gamon, 2002).

The quantitative and qualitative composition of pigments and changes in their ratio in leaves are important characteristics of the physiological state of plants in general and of the photosynthetic apparatus in particular, including

adaptive responses to stress conditions. The total amount of photosynthetic pigments varies significantly depending on the dynamics of their accumulation and the location of plants (Belous et al., 2018). Similar conclusions were reached by Ilieva & Vasileva (2016), whose studies confirm the thesis that the content of plastid pigments changes under the influence of various factors.

Data representing the content of photosynthetic pigments (chlorophylls and carotenoids) during the four-year period covering the 16th rotation of LSFE are presented in Figure 3 and Figure 4.

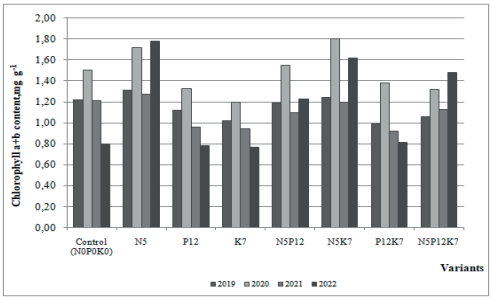

Figure 3. Chlorophyll *a+b* content of field beans in flowering stage, 16th LSFE rotation

It is apparent that the content of chlorophylls (Chl. *a+b*) and carotenoids in the leaves of the studied plants varies significantly between the different fertilization options and by year.

The smallest deviations in the values of the parameters within the studied period are in the variants with full mineral ($N_5P_{12}K_7$) and two-component fertilization with nitrogen and potassium (N_5K_7).

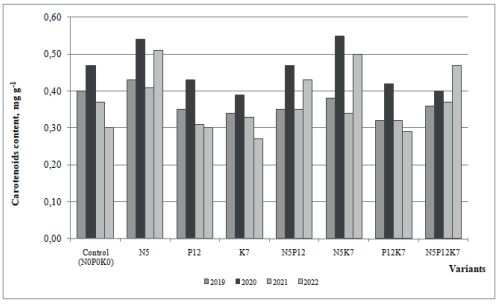

Figure 4. Carotenoid content of field beans in flowering stage, 16th LSFE rotation

The lowest content of chlorophylls (Chl. *a+b*) in the leaves of field beans in 2019 – 0.990 mg g^{-1}, was found when applying the two-component phosphorus-potassium ($P_{12}K_7$) fertilization, followed by those with only potassium (K_7) and full mineral ($N_5P_{12}K_7$) fertilization with 1.020 mg g^{-1} and 1.060 mg g^{-1}, respectively. The amount of chlorophylls in plants with fertilization including nitrogen is significantly higher. Maximum values were recorded in the variant with only nitrogen fertilization (1.310 mg g^{-1}), followed by those with combined nitrogen-potassium (N_5K_7) fertilization and the control, 1.240 mg g^{-1} and 1.220 mg g^{-1}, respectively.

The content of carotenoids in the studied plants ranges within narrow limits – the lowest values were recorded in the variant with combined $P_{12}K_7$ (0.324 mg g^{-1}), followed by those with only potassium (0.343 mg g^{-1}) and phosphorus (0.352 mg g^{-1}) fertilization, and the highest – in the variant with only nitrogen fertilization (0.431 mg g^{-1}).

In the second year of the 16th rotation of the LSFE, the content of chlorophyll a+b varied from 1.203 mg g^{-1} and 1.324 mg g^{-1} in the variants with potassium (K_7) and full mineral ($N_5P_{12}K_7$) fertilization to 1.803 mg g^{-1} and 1.721 mg g^{-1} in the variants with combined nitrogen-potassium (N_5K_7) and nitrogen (N_5) fertilization. A similar pattern is observed in terms of carotenoid content – the lowest amount was recorded in the variants with potassium (K_7) and with full mineral fertilization ($N_5P_{12}K_7$) – 0.392 mg g^{-1} and 0.403 mg g^{-1}, respectively, and the highest – in the combination of nitrogen and potassium (0.553 mg g^{-1}) and in nitrogen (0.541 mg g^{-1}) fertilization.

The lowest chlorophyll content in 2021 – 0.922 mg g^{-1} was recorded in the variant with combined phosphorus-potassium ($P_{12}K_7$) fertilization, and the highest – in the variant with nitrogen fertilization (1.273 mg g^{-1}), followed by the control (1.210 mg g^{-1}) and the variant with combined nitrogen-potassium (N_5P_{12}) fertilization – 1.201 mg g^{-1}).

The content of the carotenoids varied from 0.311 mg g^{-1} and 0.323 mg g^{-1} in the variants with phosphorus (P_{12}) and two-component phosphorus-potassium ($P_{12}K_7$) fertilization to 0.412 mg g^{-1} and 0.371 mg g^{-1}, respectively, in nitrogen (N_5) and full mineral fertilization ($N_5P_{12}K_7$).

The variants with potassium (K_7) and phosphorus (P_{12}) fertilization had the lowest chlorophyll content in the last year of the 16th rotation of the LSFE, 0.774 mg g^{-1} and 0.783 mg g^{-1}, respectively. The variant with only nitrogen fertilization (1.782 mg g^{-1}) had the highest chlorophyll content, followed by the one with combined nitrogen-potassium fertilization (N_5K_7) with 1.623 mg g^{-1}.

In terms of carotenoid content, the variants can be divided into two groups – with low and with high carotenoid content. The first group (0.271-0.304 mg g^{-1}) includes the variants with potassium (K_7) and combined phosphorus-potassium ($P_{12}K_7$) fertilization and the control ($N_0P_0K_0$), and the second (0.433-0.514 mg g^{-1}) includes all variants with nitrogen – alone or in combination with other macronutrients.

Table 5 presents the results of the statistical processing of the obtained data, averaged over the period of the 16th rotation of LSFE. It can be seen that the content of photosynthetic pigments between the individual fertilization options of field beans varies, within wider limits in terms of chlorophylls and within significantly narrower limits – in terms of carotenoids. The lowest amounts of chlorophyll a+b (Chl. *a*+*b*) were found in the variant with potassium fertilization (0.983 mg g^{-1}) and with two-component phosphorus-potassium fertilization (1.025 mg g^{-1}). In plants with nitrogen (N_5) and nitrogen-potassium (N_5K_7) fertilization, the highest chlorophyll content was recorded, 1.520 mg g^{-1} and 1.465 mg g^{-1}, respectively.

Table 5. Content of photosynthetic pigments in field beans, 16th LSFE rotation

Variants	Chlorophyll *a*+*b*, mg g^{-1}		Carotenoids, mg g^{-1}		Chl. *a*/*b*	Chl. *a*+*b*/Car.
	$\bar{X}\pm S_{\bar{x}}$	CV, %	$\bar{X}\pm S_{\bar{x}}$	CV, %		
Control ($N_0P_0K_0$)	1.183±0.014	24.38	0.385±0.0035	18.31	3.38	3.07
N_5	1.520±0.013	17.58	0.473±0.0031	13.20	3.14	3.22
P_{12}	1.048±0.012	22.34	0.348±0.0030	17.00	3.41	3.01
K_7	0.983±0.009	18.18	0.333±0.0025	14.81	3.42	2.95
N_5P_{12}	1.268±0.010	15.47	0.400±0.0030	15.00	3.12	3.17
N_5K_7	1.465±0.015	19.98	0.443±0.0049	22.32	2.96	3.31
$P_{12}K_7$	1.025±0.012	24.19	0.338±0.0028	16.83	3.02	3.04
$N_5P_{12}K_7$	1.248±0.009	15.23	0.400±0.0025	12.42	3.09	3.12

Regarding carotenoids (Ccar.), the lowest content of this group of pigments, on average for the 16th rotation of the LSFE, was recorded in the variant with potassium fertilization (0.333 mg g^{-1}), followed by the variant with combined phosphorus-potassium (0.338 mg g^{-1}) and phosphorus fertilization (0.348 mg g^{-1}). In the variant with nitrogen fertilization (N_5), the highest content of carotenoids in the leaves of field beans was found – 0.473 mg g^{-1}.

The statistical processing of the data collected for the four-year period of the study established that the values of the coefficient of variation (CV %) in the different fertilization variants lies within wide margins for both groups of plastid pigments. Nevertheless, the analysis of the obtained results shows good statistical uniformity of the sample. The coefficient of variation of the data on the chlorophyll content for different variants of fertilization of field beans ranges from 15.23% for full mineral fertilization ($N_5P_{12}K_7$) to 24.38% for the control ($N_0P_0K_0$).

The CV values of the data obtained when determining the content of yellow-red photosynthetic pigments in the leaves of field beans show a greater dispersion of the results, compared to that of chlorophylls. The lowest values were calculated for the variant with full mineral fertilization (12.42%), followed by that with nitrogen fertilization (13.20%). The largest deviations from the average values of the carotenoid content were observed for the variant with combined N_5K_7 fertilization (22.32%).

Values from 0.0025% for the determination of carotenoid content of the variants with potassium (K_7) and full mineral fertilization ($N_5P_{12}K_7$), to 0.0146% for the measurement of

chlorophyll content in the plants with nitrogen-potassium fertilization (N_5K_7), indicate a very high level of statistical reliability of the obtained results.

A number of scientific studies have reported a relationship between the ratio of chlorophyll *a* to chlorophyll *b* (Chl *a/b*) and chlorophyll *a+b* to carotenoids (Chl *a+b*/Car) and the physiological status of plants (Petkova & Poryazov, 2007). The normal ratio of relative amounts of chlorophylls *a* and *b* found in higher plants is about 3:1, although the preponderance of chlorophyll *a* is greater in those grown in bright conditions and less in those grown in shady sites (Gross, 1991; Andersen & Francis, 2009). A lower value of the green/yellow pigment ratio is associated with an increase in protection against chlorophyll photooxidation (Gilmore & Govindjee, 1999; Velinova & Glushkova, 2011).

So, it can be said that there was not observed significant deviations in the ratio of chlorophyll *a* to chlorophyll *b* in the different variants of fertilization of field beans. The lowest values (Chl.*a/b* = 2.96) were found in the variant with combined nitrogen-potassium (N_5K_7) fertilization, and the highest – in those with potassium (K_7) and phosphorus (P_{12}) fertilization, 3.42 and 3.41, respectively.

The ratio of the total amount of green to yellow plastid pigments lies in the range from 2.95 in the potassium (K_7) to 3.31 in the combined nitrogen-potassium (N_5K_7) fertilization. It is noteworthy that the variants with participation of the macroelements potassium and phosphorus had the highest ratio of chlorophyll *a/b* and the lowest of Chl.*a+b*/Car., and the combination of nitrogen and potassium (N_5K_7) had the lowest ratio of chlorophyll *a/b* and the highest of Chl.*a+b*/Car.

CONCLUSIONS

The results obtained from the research show that, on average, for the period of the 16th rotation of LSFE, fertilization with the combination of the two macroelements (N) and (P) has a decisive influence on increasing the productivity of field beans. The fertilization with only phosphorus and potassium and the combination between them has no proven effect on the yield. The highest efficiency of field beans fertilization expressed by the obtained additional yield (435 kg ha^{-1}) was achieved when fertilizing with N_5P_{12}.

The highest resistance to atmospheric drought was found in the variant with nitrogen fertilization (N_5), and the lowest – in the variant without fertilization ($N_0P_0K_0$). The highest vitality (resistance to soil drought) was found in the seeds of field beans obtained from the variants with two-component fertilization with nitrogen (N_5P_{12} and N_5K_7) and with only phosphorus (P_{12}) fertilization. The seeds of the control ($N_0P_0K_0$) has shown the lowest vitality.

The total content of chlorophylls (Chl.*a+b*) and carotenoids (Ccar.) in the leaves of the studied plants varies significantly between the different fertilization options and by year.

The lowest amounts of chlorophyll *a+b* (Chl.*a+b*) were found in the variant with potassium fertilization (K_7) – 0.983 mg g^{-1} and with combined phosphorus-potassium ($P_{12}K_7$) fertilization – 1.025 mg g^{-1}. The highest chlorophyll content was recorded in plants with nitrogen (N_5) fertilization – 1.520 mg g^{-1}.

The lowest carotenoids content (Ccar.) on average for the 16th rotation of the LSFE was found in the variant with potassium fertilization (0.333 mg g^{-1}) and the highest – in the variant with nitrogen fertilization (N_5) – 0.473 mg g^{-1}.

In the variants with applied only potassium and only phosphorus fertilization was found the highest chlorophyll *a* to chlorophyll *b* (Chl.*a/b*) ratio and the lowest chlorophyll *a+b* to carotenoids (Chl.*a+b*/Car.) ratio.

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