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EFFECT OF DIFFERENT TYPES OF MINERAL FERTILIZATION ON YIELDS AND RESISTANCE TO PHYTOPATHOGENS AND ENVIRONMENTAL STRESS IN WHEAT

Gergana IVANOVA-KOVACHEVA, Iliana IVANOVA, Lyubomir IVANOV, Galin GINCHEV

Agricultural Academy - Sofia, IASS "Obraztsov Chiflik", 1 Prof. Ivan Ivanov, Rousse, Bulgaria

Corresponding author email: gi_kovacheva@abv.bg

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 resistance to environmental stress and the development of phytopathogens in common wheat.
It was found that the highest yield for the period - 6,080 kg ha^{-1}, was obtained in the experimental plot with full mineral fertilization ($N_{15}P_{12}K_7$), which represents more than a two-fold increase compared to the average yield obtained from the control. Phytopathological analysis shows that the seeds obtained from the variant with full mineral fertilization have the lowest percentage of phytopathogens (0.75-2.00%) while 22% of the seeds in the control was damaged by Tilletia. The variants with potassium fertilization (K_7) stand out as the most resistant to atmospheric drought during the four-year research period, with the reported values - 58.61µS cm^{-1}, being 12% lower, compared to the control. The highest resistance to soil drought was established for the variants with potassium (K_7) and phosphorus (P_{12}) fertilization, respectively 83.02 µS cm^{-1} and 83.05 µS cm^{-1}.

Key words: *drought resistance, mineral fertilizers, phytopathogens, wheat, yield.*

INTRODUCTION

In recent years, there has been an increase in the frequency of extreme weather events, which cause damages of varying nature and intensity in all aspects of agriculture. Extremely high temperatures and prolonged periods without precipitation are becoming an increasingly common phenomenon in North-Eastern Bulgaria, which affects the balance of natural and anthropogenic ecosystems, including, yields from field crops. According to the latest climate models, many regions of the world will experience droughts of varying duration or sharp increases in temperatures. 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). After soil and climatic conditions, fertilization is among the most significant factors influencing the productivity of agricultural crops (Nenova, 2008), making it the subject of numerous scientific studies. Particularly valuable information can be obtained in studies conducted within longstanding stationary fertilizer experiments (Samalieva & Nikolova, 1996; Panayotova, 2007).

Nitrogen (N) is the fourth most common chemical element in plant tissues. It is a key component of enzymes and structural proteins, nucleic acids, pigments and a wide range of secondary metabolites. All plants use nitrogen in the form of $NO3^−$ and $NH4^+$. It is the element of greatest importance for the growth and development of plants, which 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). If a plant's access to N is cut off, its lifespan will not be directly affected, but within just a few days growth will become impossible (Leghari et al., 2016).

Phosphorus (P) is another macronutrient of key importance for plant growth and development. It 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. Phosphorus 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. According to literature data, in plants under drought conditions, the intensity of photosynthesis is directly related to potassium levels (Chaerle et al., 2007).

Seeds are a good substrate for the development of parasitic microorganisms, as they are rich in nutrients (Pellegrino et al., 2010; Thines, 2014; Bever et al., 2015). Seed-borne pathogens reduce seed germination and vigor, cause rotting, necrosis, systemic or local infections, changes in plant morphology and yield reductions of 15 to 90% (Wiese, 1984; Bateman & Kwasna, 1999; Khanzada et al., 2002).

Many factors influence the formation of yields in common wheat (Kuneva & Stoyanova, 2015), the most important of which are genetic potential, ecological plasticity, disease resistance, etc. Wheat is attacked by a number of pathogens from the genera *Fusarium*, *Tilletia*, *Alternaria*, *Aspergillus*, *Penicillium*, etc. (Nirenberg et al., 1994; Strausbaugh et al., 2004; Yanashkov et al., 2016; 2017; Yanashkov & Vatchev, 2018). They are widespread and common causes of damage and rotting of sprouts before emergence, which leads to lower yields.

As stated above, macronutrients (N, P and K) are of particular importance for the normal course of physiological processes in plants, for their growth and development. The use of healthy seeds with high vigor is one of the key factors in obtaining high yields from field crops (Rajput et al., 2005). Time often is a critical factor in determining seed viability and quality, while managing big quantities of different types of seeds. Determination of seed germination using the Standard Germination Test is time consuming which has necessitated the development of other, more rapid, methods of analysis such as quick aging test, cold test, electrical conductivity test, etc. (Montenegro et al., 2003).

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. The lowest minimum temperatures here reach -25ºC in the months of January and February, and during the more intense summer warming, maximum temperatures up to 42-43ºC are recorded. 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).

In a soil survey conducted in 1980, it was found that the LSFE was located on a medium sandy-clayey haplic chernozem, uneroded and slightly eroded (Kostov, 1981). According to a new study by Teoharov et al. (2014), the modern soil classification in Bulgaria defines these soils as leached (Luvic) or degraded (Ninov, 2005; 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, beans, 'Obratzov Chiflik 12' variety and maze, hybrid Ps 464. 'Dunavia' is the newest common wheat variety, product of the breeding program of IASS "Obratzov Chiflik", obtained from the inter varietal cross Mironovskaya 33 x RS 14/98. The variety is medium early with a medium height, resistant to stem and rhizome lodging. The grain is medium large with a red color and vitreousness 50%. The mass of 1000 grains vary from 37 g to 42 g depending on the conditions of the year.

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

Phytopathological Assessment

The research was carried out in the Laboratory of Phytopathology at IASS "Obratzov Chiflik", Rousse. For phytopathological analysis, 100 seeds of common wheat from all variants of LSFE were obtained for analysis. The seeds were soaked for 2 hours in distilled water and then placed in a humid chamber to incubate pathogens for a period of 7 days. Pathogen determination was performed using standard phytopathological methods (SEV, 1988). On the 10th day, the assessment was carried out to establish the quantitative and qualitative composition of the seed mycoflora (Stancheva, 2007; Mancini et al., 2016). The number of infected seeds from individual *Fungi* genera is reported in percentage (%). The determination of the health status of the seeds is carried out on the basis of a phytopathological analysis of the set of pathological changes of the seeds and the morphological signs of the causative agent of the disease.

Sampling and Analysis

- To determine the electrical conductivity of leaf membranes, in the heading stage of wheat, 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 & 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 (2014) 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.

The aim of the present research is to study the influence of different nitrogen, phosphorus and potassium fertilization options on the yield of common wheat variety 'Dunavia' and to determine the influence of long-term mineral fertilization on its resistance to diseases and environmental stress.

RESULTS AND DISCUSSIONS

Brief Meteorological Characteristics

The beginning of the wheat vegetation period 2018-2019 is characterized by temperature sums close to the climatic norm for the region (620.7ºC), 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).

Table 1a. Temperature sum by quarter for the period October - June from 2018 to 2022

Wheat growing season	Temperature sum, ºC		
	X-XII	I-III	IV-VI
2018-2019	591.3	410.7	1540.3
2019-2020	885.8	508.6	1495.4
2020-2021	752.4	310.7	1408.3
2021-2022	618.6	314.1	1521.1
ΣºC - norm	*620.7*	*411.3*	*1541.0*

The amounts of precipitation in the last quarter of 2018 exceeded the climatic norm of 150.8 mm by 16.2 mm, while the first three months of 2019 can be defined as dry - the total amount of precipitation for the period January-March reached only 47.1 mm, which is significantly below the norm of 103.6 mm. The precipitation (255.5 mm) that fell during the last months of the wheat vegetation significantly exceeded the multi-year norm of 214.0 mm, while no deviations were noted in the temperature sum.

The months from October to December in the second and third years of the 16th LSFE rotation were unusually warm with temperature sums of 885.8ºC for 2019 and 752.4ºC for 2020, respectively. High temperatures at the start of the 2019 wheat growing season were accompanied by significant drought - the total amount of precipitation that fell in the area of Obraztsov Chiflik in the last three months of the year was only 113.1 mm, 38 mm less than the norm.

Table 1b. Distribution of precipitation by quarter for the period October - June from 2018 to 2022

Wheat growing season	Precipitation, mm		
	X-XII	I-III	IV-VI
2018-2019	167.0	47.1	255.5
2019-2020	113.1	140.0	206.7
2020-2021	148.4	243.1	240.1
2021-2022	214.5	44.4	165.9
Precipitation – norm	*150.8*	*103.6*	*214.0*

The temperature sum at the beginning of 2020 exceeded the norm by 97.3ºC, and the precipitation - by 36.4 mm, while during the period April - June the reported values for temperature and precipitation did not deviate from the usual for the region. The reported temperatures from January to June 2021 are lower than the multi-year norm, respectively by 100.6ºC for the period January - March and by 132.7ºC for the period April - June. The cold weather is accompanied by significant amounts of precipitation - 243.1 mm for the first and 240.1 mm for the second quarter of the year, with values of the climatic norm of 103.6 mm and 214.0 mm, respectively. The last economic year of the four-year rotation of the LSFE was characterized by a near-normal temperature sum for the first and third quarters of the wheat growing season and cooler weather in the second quarter (97.2ºC below the norm for the area). The total amount of precipitation (424.8 mm) is unevenly distributed throughout the year with a peak in the month of October, when their amounts exceed the norm by more than 70 mm. The least precipitation was reported in the

months of January - 6.7 mm (with a climatic norm of 30.6 mm) and May - 18.0 mm (climatic norm of 80.5 mm).
In conclusion, it can be said that the meteorological conditions during wheat growing season from 2018 to 2022 are characterized by mild winters and unevenly distributed rainfall throughout the year.

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 are no published studies on the influence of different fertilization options on the resistance of crops in crop rotations of LSFE to phytopathogens and environmental stress.

The yields obtained during the fertilization of common wheat variety 'Dunavia' with the macro elements nitrogen (N_{15}), 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. The first year (2019) stands out as the most favourable for wheat with an average yield of 4,590 kg ha^{-1}. The data shows that the highest yield was reached in 2021 - 6,590 kg ha^{-1}, followed by 6,500 kg ha^{-1} in 2019, both were obtained in the full mineral fertilization ($N_{15}P_{12}K_7$).
It is also evident from the data that the highest average yield for the rotation among the variants with combinations of these macro elements was obtained in the one with the three macro elements ($N_{15}P_{12}K_7$) - 6,085 kg ha^{-1}, and the increase of yield compared to the unfertilized control was 3,342.5 kg ha^{-1}.

Table 2. Yields of common wheat 'Dunavia' variety 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 ($N_0P_0K_0$)	3,320	2,170	2,540	2,940	2,742.5	0.0
N_{15}	5,580	5,590	5,430	5,240	5,460.0	2,717.5 +++
P_{12}	2,940	2,030	2,470	2,640	2,520.0	-222.5 n.s.
K_7	3,000	1,970	2,330	2,840	2,542.5	-200.0 n.s.
$N_{15}P_{12}$	6,340	5,750	6,290	5,630	6,002.5	3,260.0 +++
$N_{15}K_7$	6,100	5,420	6,020	5,550	5,772.5	3,030.0 +++
$P_{12}K_7$	2,950	2,580	3,110	2,970	2,902.5	160.0 n.s.
$N_{15}P_{12}K_7$	6,500	5,630	6,590	5,580	6,085.0	3,342.5 +++
Average	4,590	3,890	4,350	4,170		
LSD 0.05						410
LSD 0.01						560
LSD 0.001						750

In the rest of the fertilization combinations in which the macro element nitrogen is involved, a proven increase in yield is also observed, with the $N_{15}P_{12}$ variant surpassing the unfertilized control by 3,260 kg ha^{-1}, and the $N_{15}K_7$ variant - by 3,030 kg ha^{-1}. With separate fertilization with the three macro elements, a proven increase in yield compared to the unfertilized control was observed only with the nitrogen (N_{15}) by 2,717.5 kg ha^{-1}. In the separate phosphorus and potassium fertilization and in the combination between these elements, the obtained differences in yield are unproven. The highest efficiency of wheat fertilization, expressed by the obtained additional yield, was achieved, expectedly, with full mineral fertilization with $N_{15}P_{12}K_7$ and amounted to 3,342.5 kg ha^{-1}.

Phytopathological Assessment

Our results confirm those obtained by other authors (Porras-Alfaro & Bayman, 2011; Malfanova et al., 2013; Truyens et al., 2015). The group of seed-borne phytopathogens includes both non-specialized species (of the genus *Fusarium*, *Alternaria*, *Aspergillus*, *Penicillium*, etc.) and closely adapted species (of the genus *Tilletia*, *Ustilago*). From the analyzed samples, 4 fungal species were found, which belong to 4 genera (Table 3). The averaged results for the period 2019-2022 show that for pathogens belonging to the genus *Tilletia*, the highest percentage of infection was observed in the sample taken from the variant without fertilization (22%) and the lowest – in the sample from the variant with combined $N_{15}P_{12}K_7$ fertilization (1.75%), followed by the variant with only phosphorus (P_{12}) fertilization.

Table 3. Phytopathological analysis of 'Dunavia' wheat seeds obtained during the 16th rotation of the LSFE for the period from 2019 to 2022

Variants	**Infected seeds in sample, %**				
	Fusarium	*Tilletia*	*Alternaria*	*Aspergillus*	*Penicillium*
2019					
Control ($N_0P_0K_0$)	-	10	20	6	4
N_{15}	-	10	20	4	1
P_{12}	-	5	4	1	4
K_7	-	5	4	3	4
$N_{15}P_{12}$	-	5	10	-	-
$N_{15}K_7$	-	-	-	6	-
$P_{12}K_7$	-	-	-	5	4
$N_{15}P_{12}K_7$	-	5	4	1	1
2020					
Control ($N_0P_0K_0$)	-	37	10	10	15
N_{15}		-	-	-	-
P_{12}	-	-	4	-	-
K_7	-	20	1	-	-
$N_{15}P_{12}$	-	28	1	-	-
$N_{15}K_7$	-	4	1	4	-
$P_{12}K_7$	-	28	5	8	7
$N_{15}P_{12}K_7$	-	-	1	1	7
2021					
Control ($N_0P_0K_0$)	-	31	33	20	20
N_{15}	-	8	5	-	1
P_{12}	-	2	5	-	-
K_7	-	2	1	1	1
$N_{15}P_{12}$	-	10	10	5	7
$N_{15}K_7$	-	25	31	10	18
$P_{12}K_7$	-	2	6	4	1
$N_{15}P_{12}K_7$	-	1	-	-	-
2022					
Control ($N_0P_0K_0$)	-	10	6	4	10
N_{15}	-	5	4	1	5
P_{12}	-	1	1	1	4
K_7	-	5	3	3	6
$N_{15}P_{12}$	-	8	-	-	5
$N_{15}K_7$	-	2	6	-	6
$P_{12}K_7$	-	3	5	4	9
$N_{15}P_{12}K_7$	-	1	1	1	-
Average.					
Control ($N_0P_0K_0$)	**-**	**22**	**17.25**	**10**	**12.25**
N_{15}	**-**	**5.75**	**7.25**	**1.25**	**0.75**
P_{12}	**-**	**2**	**3.5**	**0.5**	**2.00**
K_7	**-**	**8**	**2.25**	**1.75**	**2.75**
$N_{15}P_{12}$	**-**	**12.75**	**5.25**	**1.25**	**3.00**
$N_{15}K_7$	**-**	**7.75**	**9.25**	**5**	**6.00**
$P_{12}K_7$	**-**	**8.25**	**4.0**	**5.25**	**5.25**
$N_{15}P_{12}K_7$	**-**	**1.75**	**1.5**	**0.75**	**2.00**

The same trend was observed in the pathogens of the *Alternaria* genus, as in the variant without fertilization the percentage of infected seeds was 17.25%, and in those with combined $N_{15}P_{12}K_7$ fertilization it was 1.5%, followed by the variant with only potassium (K_7) fertilization. The results show that pathogens of the genus *Aspergillus* appeared from 0.5% in the variant with only phosphorus fertilization (P_{12}) and 0.75% in combined $N_{15}P_{12}K_7$ fertilization to 10% in the control. The development of pathogens from the genus *Penicillium* is the weakest – 0.75% in the variant with only nitrogen (N_{15}) fertilization and the strongest in the control – 12.25%.

Electrical Conductivity of Leaf Membranes

According to Pavlova (2005; 2010), 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. Figure 1 presents the reported values of electrical conductivity of leaf membranes in heading stage of the wheat for the entire period of the 16th rotation. It can be seen that the lowest measured values vary within relatively narrow limits - from 54.49 µS cm^{-1} in the variant with only potassium fertilization (K_7) in 2020 to 60.61 µS cm^{-1} in those with combined nitrogen-phosphorus fertilization ($N_{15}P_{12}$) in 2021.

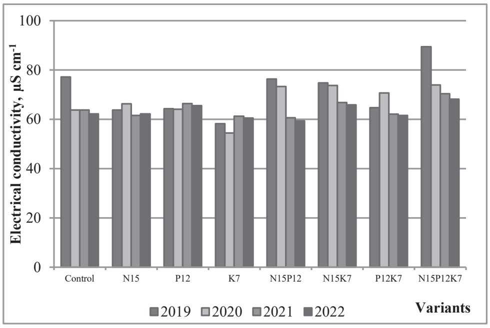

Figure 1. Electrical conductivity of leaf membranes in heading stage of wheat by year, 16th LSFE rotation

The maximum electrical conductivity of leaf membranes during the four years of the study were found in the variant with combined $N_{15}P_{12}K_7$ fertilization, with the values falling within significantly wider limits - from 68.17 µS cm^{-1} in 2022 to 89.4 µS cm^{-1} in 2019.

The summarized results show that the leaching of electrolytes through the cell membranes of the leaves in the variants with combined $N_{15}P_{12}K_7$ and $N_{15}K_7$ fertilization is greater, compared to the non-fertilized variant (control), as well as to the variant with only nitrogen fertilization (Table 4).

The highest values of electrical conductivity of the leaf membranes, averaged over the period of the study, were recorded in the variant with full mineral fertilization ($N_{15}P_{12}K_7$) - 75.46 µS cm^{-1} against 66.71 µS cm^{-1} for the control.

The lowest leaching of electrolytes was found in the variant with only potassium fertilization - 58.61 µS cm^{-1}. The variants with single nitrogen (N_{15}) and combined $P_{12}K_7$ fertilization are also proven to be resistant, in which the electrical conductivity of the leaf membranes was recorded as 63.45 µS cm^{-1} and 64.75 µS cm^{-1}, respectively.

Table 4. Statistically processed data on electrical conductivity of leaf membranes in heading stage of wheat, 16th LSFE rotation

Variants	Electrical conductivity of leaf membranes, µS cm^{-1}		
	Mean	VC, %	SE, %
Control ($N_0P_0K_0$)	66.71	10.47	3.49
N_{15}	63.45	3.36	1.07
P_{12}	65.06	1.68	0.55
K_7	58.61	5.18	1.52
$N_{15}P_{12}$	67.41	12.82	4.32
$N_{15}K_7$	70.29	6.58	2.31
$P_{12}K_7$	64.75	6.43	2.08
$N_{15}P_{12}K_7$	75.46	12.71	4.79

The statistical processing of the data obtained in the four-year period of the study, found that the values of the coefficient of variation (VC %) for the different variants of wheat fertilization lie within wide range - from 1.68% in the case of phosphorus fertilization (P_{12}) to 12.82% in the variant of combined $N_{15}P_{12}$ fertilization and 12.71% when using full mineral fertilization. The low values of the coefficient of variation verify to 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 variants with applying just one of the three elements of mineral fertilization and in the combined $N_{15}K_7$ and $P_{12}K_7$ fertilization - from 0.55% in P_{12} to 2.31 in $N_{15}K_7$. The largest deviation from the average values of the parameter was reported for the options with full mineral fertilization ($N_{15}P_{12}K_7$) and the combination of nitrogen and phosphorus fertilizer ($N_{15}P_{12}$), where the SE reached 4.79% and 4.32%.

Electrical Conductivity of Seed Membranes

A number of researchers have found that the higher the rate of restoration of the integrity of the cell membrane system during seed moistening, the weaker will be the extraction of electrolytes in the external environment and therefore the electrical conductivity of the water, which indicates a higher vitality (Baalbaki et al., 2009; De Oliveira Araújo et al., 2022). 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 and soil drought. The values of the electrical conductivity of wheat seed membranes varied over the years and between different fertilization options within relatively narrow range during the first 3 years of the study - from 68.85 µS cm^{-1} in the combined $N_{15}P_{12}$ fertilization in 2021, to 84.03 µS cm^{-1} for the variant with only N_{15} fertilization, in 2020 (Figure 2). The seeds with the highest vitality in the individual years were obtained from different fertilization options, but when analyzing the obtained results, it can be seen that with potassium and phosphate fertilization alone, the loss of electrolytes through the seed membrane is smaller, in comparison with the control and variants with single or combined nitrogen fertilization.

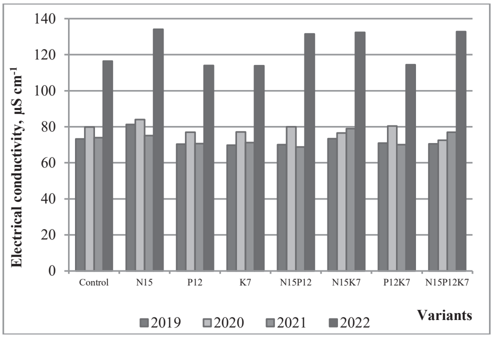

Figure 2. Electrical conductivity of wheat by year, 16th LSFE rotation

The seeds membrane conductivity values reported in the last year of the 16th LSFE rotation are significantly higher and vary within wider range. The highest vitality was recorded in the variants with only K_7 (113.9 µS cm^{-1}) and P_{12} (114.03 µS cm^{-1}) fertilization, and the lowest - in the seeds obtained from plants in the plots with full mineral fertilization (134.07 µS cm^{-1}). Viability recorded in variants with single or combined nitrogen fertilization was lower than that of the control variant by 13-15%, which can be attributed to the insufficient rainfall (48.1 mm below the climatic norm) in the period of heading and flowering, ripening and maturation of the wheat. The greater leaching of electrolytes was found in only one of the four years of the rotation, therefore the data obtained in 2022 cannot claim statistical reliability. Further studies need to be conducted on the correlation between seed vitality and rainfall during heading and ripening of wheat.

The statistical processing of the data (Table 5) confirms the previously commented heterogeneity of the data collected during the 4-year study.

The seeds obtained from the variants with only potassium (K_7) and phosphorus (P_{12}) fertilization have the highest vitality, on average for the period, 83.02 µS cm^{-1} and 83.05 µS cm^{-1}, respectively. High vitality was also found in the variant with combined $P_{12}K_7$ fertilization - 83.98 µS cm^{-1}.

Table 5. Statistically processed data on electrical conductivity of wheat, 16th LSFE rotation

Variants	Electrical conductivity, µS cm^{-1}		
	Mean	**VC, %**	**SE, %**
Control ($N_0P_0K_0$)	85.90	23.96	10.29
N_{15}	93.63	29.07	13.61
P_{12}	83.05	25.14	10.44
K_7	83.02	25.10	10.42
$N_{15}P_{12}$	87.63	33.88	14.85
$N_{15}K_7$	90.32	31.15	14.07
$P_{12}K_7$	83.98	24.77	10.40
$N_{15}P_{12}K_7$	88.25	33.79	14.91

The seeds obtained from the variant with only nitrogen fertilization (N_{15}) have the lowest vitality. In this variant, the highest electrical conductivity of seed membranes was measured (93.63 µS cm^{-1}). It should be noted that according measuring of electrical conductivity, seeds obtained from the variant without fertilization (85.9 µS cm^{-1}) have higher vitality than these, obtained from all of the variants with nitrogen fertilization. The values of the coefficient of variation (VC, %) for the different variants of mineral fertilization show a satisfactory uniformity of the data by year. Sampling heterogeneity, i.e., values of VC above 30%, was found in the variants with combined nitrogen fertilization ($N_{15}P_{12}$ - 33.88%, $N_{15}P_{12}K_7$ - 33.79% and $N_{15}K_7$ - 31.15%). The lowest values of the coefficient of variation were reported for the control (23.96%) and the variant with combined $P_{12}K_7$ fertilization (24.77%). The obtained values of standard error (SE, %) from 10.29% for the control to 14.91% for the variant with full mineral fertilization and 14.85% for the combined nitrogen-phosphorus fertilization fall outside the limits in which the data could be accepted as statistically reliable.

CONCLUSIONS

The results obtained from the research show that, on average, for the period of the 16th rotation of LSFE, nitrogen fertilization, applied both alone and in combination with the macro elements, phosphorus and potassium, has a decisive influence on increasing the productivity of wheat. The fertilization with only phosphorus and potassium and the combination between them has no proven effect on the yield. The highest efficiency of wheat fertilization expressed by the obtained additional yield (3,342.5 kg ha^{-1}) was achieved when fertilizing with $N_{15}P_{12}K_7$.

It was found that the phytopathogens of the genus *Tilletia* were detected with a high frequency in the samples, followed by the genus *Alternaria* and the genus *Penicillium*. No development of *Fusarium* pathogens was detected. Regarding the distribution among the different variants, the weakest development of pathogens was observed in those with combined $N_{15}P_{12}K_7$ fertilization. The strongest development of phytopathogens was observed in the control.

In wheat, the highest resistance to atmospheric drought was found in the variants with only potassium fertilization (K_7), and the lowest – in the variants with full mineral fertilization ($N_{15}P_{12}K_7$). The highest vitality (resistance to soil drought) was found in the seeds of wheat obtained from the variants with only potassium (K_7) and phosphorus (P_{12}) fertilization. The seeds of the variant with only nitrogen fertilization (N_{15}) have the lowest vitality.

The results obtained in the process of the 4-year study correspond well with the existing data on the role of phosphorus and potassium in increasing the resistance of plants to abiotic stress (high temperatures and low soil and atmospheric humidity). Further studies on the relationship between wheat resistance, yield and meteorological factors are recommended.

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