Agronomy Research **20**(4), 730–750, 2022
https://doi.org/10.15159/AR.22.075

The efficiency of combined application of mineral fertilizers, inoculants in soybean growing technology, and functioning of nitrogen-fixing symbiosis under increasing nitrogen rates

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Received: May 16th, 2022; Accepted: October 13th, 2022; Published: November 18th, 2022

Abstract. Soy is a valuable leguminous crop, whose productivity directly depends on many factors, among which nitrogen fertilizers are one of the most important. Nitrogen fertilizing of this crop is often given insufficient attention because the yield increase can be quite insignificant. **The purpose of the research** is to study the combined effect of biofertilizers and increasing rates of nitrogen mineral fertilizers on the growth, development, and yield of soybean varieties. The experiment is three-factorial: factor A - early ripening soybean varieties Annushka and Ustya, factor B – seeds inoculation by biofertilizer, and factor C - rates of mineral fertilizers application. **Results of research shows** the efficiency of nitrogen-fixing symbiosis during seed inoculation was established for nitrogen rates from N_0 to N_{60} against the background of $P_{60}K_{60}$ - the biomass of nodule bacteria is 418–675 mg plant^{-1}. The application of $N_{90}P_{60}K_{60}$ significantly reduces the number and mass of nodule bacteria - 207–241; $N_{120}P_{60}K_{60}$ - 32.0–42.0 mg plant^{-1}, and with the introduction of $P_{60}K_{60} + N_{150–180}$ nodule bacteria are not formed and the effectiveness of microbiological drugs is not recorded. Nitrogenase activity with $N_{0–90}$ application on the background of $P_{60}K_{60}$ and seed inoculation ranged from 3.25 to 7.76 µmol C_2H_4 per plant ha^{-1}. With a further increase in nitrogen levels, nitrogenase activity was not recorded. On typical chernozems of the Forest-Steppe of Ukraine, higher yields of early-ripening soybean varieties are formed by applying $N_{60}P_{60}K_{60}$ and pre-sowing seeds treatment with rhizohumin or rhizohumin/hetomics combination. $P_{60}K_{60}N_{150–180}$ showed a partial decrease in soybean yield due to the distress effect.

Key words: hetomic, mineral fertilizers, nodules, rhizohumin, soybean, variety, yield.

INTRODUCTION

Soybeans are a valuable legume that accumulates nitrogen in the soil, forming root nodules and accumulating protein in the seeds. Growing soybeans improves nitrogen circulation by approximately 3.6 times, which is accompanied by an increase in nitrogen-fixing bacteria in the soil (Matsumiya et al., 2013).

Soybean yield directly depends on many factors, among which one of the most important is nitrogen fertilizers in research at the Mississippi Agriculture and Forestry Experiment Station in Starkville, MS. (Adeli et al., 2005). Nitrogen is one of the most important nutrients affecting soybean yields (Liu et al., 2008; Dong et al., 2010; Wan, 2013). With fertilizer applying, the rate of phosphorus-potassium fertilizers to create a deficit-free balance in the soil can be calculated, but this can't be done for nitrogen, which has a much more complex cycle of transformations in nature. The nitrogen cycle is not limited to the plant-soil system but also covers the atmosphere due to the nitrogen-fixing and denitrifying ability of microorganisms. Therefore, the use of nitrogen fertilizers should be based on specific knowledge of the natural nitrogen cycle and on knowledge of the real use scale of biological nitrogen due to nitrogen fixation by microorganisms (Temrienko, 2018; Novytska et al., 2020).

Effective use of nitrogen fertilizers is crucial for soybean cultivation, in particular, optimization of chemical form, rate, time, and application of fertilizers for crop growing in different conditions. Muchow & Sinclair (1986) in studies of yield formation of early-maturing and late-maturing soybean varieties (*Glycine max* (L.) *Merr*.), which were grown on two different types of soil both under irrigation conditions and under conditions of limited water supply in a semi-arid tropical environment noted that the introduction of high levels of nitrogen was the 'main obstacle' to obtaining a high yield of soybeans. The researchers obtained the maximum grain yield at an average level of nitrogen application. Excessive or insufficient nitrogen fertilizers did not increase soybean yields, while intermediate levels of starting nitrogen fertilizers (N_{50}) increased crop yields. Similarly, an increase in soybean yields in response to moderate nitrogen levels was observed by Tian (2007). Studies conducted in the Sanjiang River Plain in China have also shown that nitrogen fertilizers at a rate of 50 kg ha^{-1} at sowing promote activation of the soybean root system, rate of leaf photosynthesis, and maximum grain yield (Gai et al., 2017).

However, Maw et al. (2011) at the National Corn and Sorghum Research Center, Nakhon Ratchasima, Thailand reported that four rates of nitrogen fertilizer application (0, 25, 50, and 75 kg N ha^{-1}) were applied as starting nitrogen, and the highest grain yield in both seasons was observed at N_{75}. Some researchers believe that a small amount of starting nitrogen fertilizer placed near the seeds can have a positive effect on the early growth and yield of soybean grain, especially in situations where soil fertility is low or there are adverse environmental conditions (Sij et al., 1979). However, the need to apply increased rates of nitrogen when growing soybeans is due to the high removal of nitrogen from the soil, which is 3–4 times greater than phosphorus.

Takahashi et al. (Takuji et al., 2017) investigated the effect of deep placement of coated urea (CU) on soybean seed yield in a rotating rice field in Niigata, Japan. They found that deep (20 cm) application of lime nitrogen or coated urea did not increase nitrate or ammonium concentrations in the upper layer, where nodules are primarily formed and promoted soybean growth and seed yield by promoting nitrogen fixation after the initial flowering stage. This method of feeding does not inhibit the formation of nodules and nitrogen fixation. The constant supply of nitrogen from the lower part of the soil contributes to the photosynthetic activity of the leaves until the seed ripening stage. Thus, a large amount of photosynthate can be transported to the nodules as well as to the seeds. A constant supply of N and C for seed growth increases seed yield and improves seed quality. The stimulating effect on the formation of nodules by the deep application of lime nitrogen was confirmed by an experiment with a rhizobox.

The application of nitrogen fertilizers at the beginning of soybean flowering helped to increase crop yields by increasing nitrogen fixation (Takuji et al., 2017). Other studies conducted in 23 northern parts of the Midwestern United States have shown that the application of mineral nitrogen fertilizers in the full flowering phase of soybean plants reduced the efficiency of biological nitrogen fixation by 16% and yield by 13 kg ha^{-1} (Tamagno et al., 2018).

Soybean yield depends on both the mineral nitrogen of the soil and its biological fixation. The interaction of these two nitrogen sources is biologically interesting and agronomically important (Kalenska et al., 2021). Studies in Brazil show, that seed inoculation increases nitrogen fixation efficiency by up to 85%, grain yield by up to 5%, and protein content in grain by up to 7%. Application of mineral nitrogen fertilizers at the rate of 30 kg ha^{-1} at sowing did not significantly affect the number and mass of nodules, while the application of 200 kg ha^{-1} of nitrogen dramatically reduced the number of nodules and up to 44% nitrogen fixation efficiency (Hungria et al., 2006). The results of the study by Caliskan et al. (2008), conducted in Turkey, demonstrated the positive effect of inoculation and nitrogen fertilizers on plant growth parameters and soybean productivity at nitrogen application rates up to 80 kg ha^{-1}. Further increases in nitrogen levels led to negative interactions and reduced crop yields.

The introduction of nitrogen fertilizers under legumes can be excluded only under optimal environmental conditions for the symbiotic activity of nodule bacteria. The high intensity of this process is achieved with optimal humidity, the reaction of the environment, the presence of a sufficient amount of phosphorus and potassium, the presence of active strains of nodule bacteria in the soil, or when plants are infected with virulent strains of specific races of nodule bacteria. If any of the factors are in suboptimal sizes, nitrogen fixation is weak or it is not assimilated at all. Unfortunately, in real agricultural production, the agrochemical properties of the soil (pH, the content of macro- and microelements), water and temperature regimes, or other environmental factors do not always contribute to symbiotic nitrogen fixation. Leguminous plants in this case experience nitrogen starvation, and switch to heterotrophic nitrogen nutrition, just like non-leguminous crops, and with mineral nitrogen deficiency, they give low yields. Therefore, under unfavorable conditions for nitrogen fixation, it is possible to increase the productivity of legumes only by using nitrogen fertilizers. At the same time, it is important to consider that the introduction of nitrogen fertilizers under leguminous crops increases the yield only in the absence of nitrogen fixation or when the symbiosis is weakened. The high efficiency of nitrogen fertilizers in leguminous crops indicates their low symbiotic activity (Kalenskiy et al., 2016; Novytska et al., 2020).

The presented research results show that when developing a soybean fertilization system and creating optimal crop nutrition conditions, the soil and climatic conditions of the growing region and the features of the symbiotic nitrogen fixation process should first of all be taken into account. The yield and quality of soybean seeds largely depend on the optimal combination of symbiotic and mineral nitrogen nutrition in the crop fertilization system. Therefore, the purpose of the research was to establish the effectiveness of the nitrogen-fixing symbiosis, the formation of yield, seed quality, structure, and yield index with the introduction of increasing nitrogen rates in combination with biofertilizers in soybean cultivation technology in the Right Bank Forest Steppe of Ukraine.

MATERIALS AND METHODS

Field research was conducted in 2006–2010 in a stationary field experiment at the 'Agronomic Research Station' of NULES of Ukraine in a 10-field field crop rotation and on the basis of educational and scientific laboratory 'Demonstration collection field of agricultural crops of the Plant Science Department of NULES of Ukraine. The soil is typical low-humus chernozem, medium-loamy in mechanical composition. The thickness of the humus horizon is 25–30 cm. The content of humus (Table 1) in the arable layer of the soil (0–30 cm) according to Tyurin is 5.3%, the pH of the salt extract is 7.8, the content of mobile phosphorus and exchangeable potassium according to Chirikov, respectively 35.7 and 90.19 mg kg^{-1} of soil, nitrogen - 213.2 mg kg^{-1} of soil, the reaction of the soil solution is close to neutral.

Table 1. Agrochemical indicators of the experimental field

INDEX	Test results	Accuracy
pH of salt extract, pH units	7.8	± 0.30
Humus substance (organic matter), %	5.3	± 0.53
Nitrogen (alkalin-hydrolized), mg kg^{-1}	213.2	± 26.65
Mass content of Potassium mg kg^{-1}	90.19	± 0.24
Mass content of Potassium %	0.00902	± 0.00002
Labile Phosphorus, mg kg^{-1}	35.7	± 5.35
Exchange Calcium, mmol per 100 g	8.7	± 0.65
Exchange Magnesium, mmol per 100 g	1.5	± 0.15
Carbonates, mmol per 100 g	0.1	± 0.001
Bicarbonates, mmol per 100 g	0.59	± 0.059
Mass content of Iron, mg kg^{-1}	0.067	± 0.09
Mass content of Iron, %	0.00007	± 0.00001
Mass content of Manganese, mg kg^{-1}	12.88	± 0.20
Mass content of Manganese, %	0.00129	± 0.00002
Mass content of Copper, mg kg^{-1}	0.141	± 0.001
Mass content of Copper, %	0.0000141	± 0.0000001
Mass content of Zink, mg kg^{-1}	0.38	± 0.01
Mass content of Zink, %	0.000038	± 0.000001
Particle size classification	Medium clay-loam soil, rough-dusty-muddy	
Sand 1–0.05 mm, %	9.0	
Rough dust 0.05–0.01 mm, %	49.85	
Dust 0.01–0.001 mm, %	18.0	
Silt < 0.001 mm, %	23.10	
Clay < 0.01 mm, %	34.80	

The soil of the experimental field has high natural fertility, is characterized by optimal parameters of agronomic properties, and has a slightly alkaline or neutral reaction of the soil solution. Soils of this type are well humus-rich, as a result of which they have a dark color and considerable depth, and are well structured. Such soils are rich in nutrients, and the physical and mechanical qualities of the soil are quite favorable for the cultivation of cultivated plants.

Climate conditions

The research area (Kyiv-Sviatoshynskyi district of Kyiv region) is a zone of sufficient humidity (HTC-1.2), with a warm, moderately humid climate. Winters are mild, cloudy with frequent thaws and only in some years with severe frosts, and summers are mostly warm, and moderately humid. Transitional periods (spring, autumn) are mostly long, and unstable, but on average warm springs prevail with sufficient (160–180 mm) reserves of productive moisture in a meter layer of soil.

Starting from 2006, the average temperature for the period April-October exceeded the average long-term indicator, and the dynamics of a gradual increase of this indicator were observed (Table 2).

Table 2. Weather conditions during the years of research

Year	Month							
	IV	V	VI	VII	VIII	IX	X	IV–X
	Monthly precipitation indicators, mm							
2006	30.2	120.4	44.0	25.3	130.4	43.0	15.4	408.7
2007	4.1	57.8	53.2	43.7	51.3	7.9	0.6	218.6
2008	53.6	9.8	14.7	43.9	38.2	132.4	22.0	314.6
2009	0.0	27.8	56.2	96.8	8.1	10.8	26.4	226.1
2010	42.1	46.8	53.9	110.2	33.6	29.7	26.7	343
Average multi-year	38.4	43.3	73.9	72.9	57.8	46.3	31	363.6
	Average monthly air temperature, °C							
2006	11.1	15.6	17.5	19.8	19.4	15.3	9.3	15.4
2007	9.8	18.6	21.3	21.9	21.8	15.0	8.9	16.8
2008	10.1	13.9	18.2	19.6	20.8	12.9	9.8	15.0
2009	9.4	14.2	20.0	21.3	18.6	16.4	8.8	15.5
2010	9.5	17.0	20.7	23.8	23.1	14.4	5.6	16.3
Average multi-year	8.9	15.3	18.4	20.1	19.3	14.0	8.2	15.5
	Hydrothermal coefficient (HTC)*							
2006	1.3	2.5	0.8	0.4	2.2	0.9	0.9	1.3
2007	0.2	1.1	0.8	0.6	0.8	0.2	0.0	0.7
2008	2.8	0.2	0.3	0.7	0.6	5.2	1.4	1.1
2009	0.0	0.7	0.9	1.5	0.1	0.2	1.7	0.7
2010	2.7	0.9	0.9	1.5	0.5	0.7	0.0	1.1
Average multi-year	1.8	1.0	1.1	1.4	1.0	0.8	1.6	1.2

*Note. HTC > 1.6 – excessive moisture; HTC 1.3–1.6 – wet conditions; HTC 1.0–1.3 – slightly arid conditions; HTC 0.7–1.0 – arid conditions; HTC 0.4–0.7 – very arid; HTC < 0.4 – dry conditions.

The average daily air temperature during the growing season of spring crops from April to October was on average 1.1 °C higher, compared to long-term data - 14.9 °C. In recent years, excessively high temperatures are increasingly observed in April, August, and September. In addition, the years of research significantly differed in humidity. Thus, the conditions of 2006 were generally typical, but the meteorological spring began earlier than usual, so April was atypical in terms of temperature as the average air temperature was 11.1 °C, which exceeded the long-term by 2, 2 °C. Accordingly, the sum of active temperatures above +5 and +10 °C was 80–82 °C higher than the long-term value, which accelerated the emergence of seedlings and the development of plants in the initial phases of ontogenesis. During the April-October period, 408.7 mm of precipitation fell, which is 45.1 mm more than the long-term value. The distribution during the growing season was uneven - the most precipitation fell in May (120.4 mm) and August (130.4 mm), which to some extent compensated for their lower amount in the following months. In particular, June of this year was characterized by dry conditions (Hydrothermal coefficient = 0.8), and July – severe drought (HTC = 0.5). In general, in terms of moisture supply and temperature, the year was typical, and dry periods had an insignificant effect on the productivity of most crops.

According to the coefficient of deviations significance of the temperature regime from the long-term value for the period April-October, the conditions of 2007 were rare with a significant excess. During this period, the average value of the air temperature was 16.8 °C, which is 1.9 °C higher than the long-term indicator. May, June, July, and August were the months with an atypical temperature regime (C_s 1–2), that is, during the period of active vegetation of spring crops, the sum of active temperatures also increased, which affected the duration of individual phenological phases. Regarding the wetting regime of the territory, only 218.6 mm of precipitation fell during the period from April to October, which is 145 mm lower than the long-term indicator. The main reason for this phenomenon was the low amount of precipitation in April, September, and October (0.6–7.9 mm per month). It should be noted that in months with atypically high air temperature, the amount of precipitation was within the range of the multi-year value, however, due to the inflow of thermal energy, the HTC decreased compared to typical years. In general, the year was characterized by dry conditions (HTC = 0.7), and very severe drought conditions were observed in April, September, and October. At the same time, June and August were dry (HTC = 0.8), and July was very dry (HTC = 0.5), which limited the productivity of spring crops, the critical periods of which fell on these months.

The temperature regime in 2008 corresponded to typical conditions, but in August it was atypically warmer (C_s = 1.1). The total amount of precipitation for the April-October period was 314.6 mm, which is 49 mm lower than the long-term norm, but its distribution during the growing season was uneven, which manifested itself in droughts at certain stages of crop development. In particular, after a wet April (HTC > 1.6) with 53.6 mm of precipitation, May and September experienced very severe drought (HTC = 0.2–0.3), and very dry conditions in July and August (HTC 0.6–0.7). The excessive amount of precipitation in September (132.4 mm) did not affect the formation of crops productivity, although in general the year was characterized by sufficient moisture (HTC 1.1), in critical periods there were severe droughts that limited productivity.

The year 2009 was typical in terms of temperature, although atypically hot conditions were observed in July and September (C_s 1–2), which negatively affected the productivity of spring crops. During this period, 226.1 mm of precipitation fell, which is by137.5 mm less than the long-term value, and its distribution was anomalous, since in June and July a total of 153 mm fell, and the remaining 73.1 mm was distributed over other months, while that there was no precipitation at all in April. According to the HTC, conditions in April, August, and September were characterized by very severe drought, while May and June were dry. The productivity of spring crops depended mostly on the reserves of productive moisture, given that the year was generally dry, especially in critical periods for moisture supply.

According to the temperature regime, the year 2010 belonged to the category of atypically warm (C_s 1.6), since the average temperature for the April-October period was 16.3 °C, which is by1.4 °C higher than the long-term value. It should be noted that some months were abnormally hot, while others were unusually cool. In particular, June of this year was atypically hot, and July and August were abnormally hot (C_s 3.0 and 2.9, respectively), which respectively affected the productivity of crops due to stressful conditions. At the same time, it was unusually cool in October, which indicated an earlier end of the growing season. The total amount of precipitation in April-October this year was 343 mm, which was only 20 mm less than the long-term value, and their distribution was relatively even. This is what made it possible to attribute the year to slightly arid conditions (HTC = 1.1), although in some months there was a moisture deficit. In particular, May, June, and October were dry, and a severe drought was in August. Due to the peculiarities of the October temperature regime, HTC was not calculated (the sum of active temperatures > 10 °C was 0 °C). In general, the year was hot, but the moisture regime allowed crops to form a competitive yield level.

Sampling and methods

The purpose of the research is to study the combined effect of biofertilizers and increasing rates of nitrogen fertilizers on the growth, development, and yield of soybean varieties. The experiment is three-factor (Table 3). Agricultural technology for growing soybeans is generally accepted for the Northern Forest Steppe. The seeds were sown at a soil temperature of 10–12 °C with a SON-4.2 vegetable planter. The total area of the elementary plot is 84 m^2, and the area of the accounting plot is 50 m^2 with four repetitions. The location of the plots is systematic (Ermantraut et al., 2014). Seeding sowing rate - 600 thousand similar seeds ha^{-1}.

Table 3. The scheme of the experiment

Factor A: variety	Factor B: seed inoculation	Factor C: fertilizing, kg ha^{-1} a.s.
Annushka	1. Without inoculation (control)	1. Without fertilizers (control)
Ustya	2. Rhizohumin	2. $P_{60}K_{60}$
	3. Hetomic	3. $N_{30}P_{60}K_{60}$
	4. Rhizohumin + Hetomic	4. $N_{60}P_{60}K_{60}$
		5. $N_{90}P_{60}K_{60}$
		6. $N_{120}P_{60}K_{60}$
		7. $N_{150}P_{60}K_{60}$
		8. $N_{180}P_{60}K_{60}$

Field research was carried out during 2006–2010 in the stationary and temporary experiments of the Plant Science Department at the PF of the National University of Life and Environmental Sciences of Ukraine ‘Agronomic Research Station’ (Pshenichne village, Vasylkiv district, Kyiv region) in the Right Bank Forest Steppe of Ukraine. Laboratory studies were carried out in the educational and scientific laboratories of the Plant Science Department ‘Analytical research in plant science’, ‘Quality of seeds and planting material’ of the National University of Life and Environmental Sciences of Ukraine and in the analytical biochemical scientific research laboratory ‘Physiological bases of plant productivity’ of the NSC ‘Institute of Biology and Medicine’ Taras Shevchenko Kyiv National University.

The studies used recommended soybean varieties for the Forest-Steppe zone: ultra-early Annushka (PE Scientific Breeding and Seed Company 'Soevyi vik', Kropyvnytskyi) and early-ripening Ustya (NSC 'Institute of Agriculture NAAS', Chabany); biofertilizers of the Institute of Agricultural Microbiology of NAAS (Melnyk et al., 2007): inoculant Rhizohumin (strain *Bradirizobium Japonicum* M-8), microbial biofungicide Hetomic (*Chaetomium cochliodes* 3250), application rate - 200 g $hectare^{-1}$ seeding rate.

In soybean varieties Annushka and Ustya, at 1–2 variants of factor B and 1–8 variants of factor C in the main phases of plant development - flowering and bean swelling, physiological and biochemical indicators of plant condition were determined: the content of photosynthetic pigments in leaves (chlorophyll a, b, carotenoids) and the amount of malondialdehyde (MDA) and grain quality (protein and fat content).The content of chlorophyll *'a'* and *'b'* in the leaves of plants was determined by biochemical analysis using a spectrophotometer, followed by calculation of pigments concentration according to the equation of Wetstein and Holm (Hrytsaenko et al., 2003). Nitrogenase activity of nodules of the soybean root system was determined by the acetylene-ethylene method (Makrushin et al., 2006). The intensity of lipid peroxidation processes was studied in soybean plant leaves for the content of TBA-active products (Platonova & Kostyshyn, 2000). Harvest accounting was performed separately by direct combining and 'test sheaf' methods. Grain quality was determined by infrared spectrometry on a NIP Scanner 4250 infrared analyzer with ADI DM 3114 computer software.

The productivity index was calculated as the coefficient of reduction of the level of average productivity of a separate agricultural crop relative to the level of average productivity in specific soil and climatic conditions of research.

Statistical data processing was performed using the Microsoft Excel program, the SAS 9.4 software package, and the 'Statistica 6' software package.

Fisher *LSD* was conducted for establishing significant difference between variants in yield structure (pods and seeds per plant, seed weight, and thousand seed weight, seed yield, MDA content, protein, and fat content). Analysis of variance was conducted by Statistica 13.3 for average seed yield. Means of yield structure were presented with standard error (*SE*).

RESULTS AND DISCUSSION

Adaptation and stability of plants largely depend on the functioning of their photosynthetic apparatus (Halliwel & Guteridge, 1984). Photosynthetic pigments change their quantitative indicators in response to the influence of various factors. It is known that lipid peroxidation (LPO) is one of the first consequences of oxidative damage to membrane systems, and the amount of malonic dialdehyde (MDA) - a stable product of this process, indicates the depth of this process and is used as an indicator of stress (Halliwel & Guteridge, 1984; Tuanjie et al., 2017). Intensification of lipid peroxidation (LPO) processes is the primary nonspecific response to a stress factor manifested in an increase in malonic aldehyde (MDA) in photosynthetic soybean tissues with nitrogen application (Platonova & Kostyshyn, 2000; Batsmanova et al., 2020). Therefore, the possible stressors of different rates of nitrogen fertilizers on soybean varieties were evaluated by the accumulation of MDA. In our research it was found that the processes of LPO were more actively developed in the phase of flowering and maximum growth - 55% in terms of control without fertilizers was achieved with the introduction of N_{180}; with pre-sowing seeds treatment by Rhizohumin and with the introduction of N_{30} the content of MDA in the leaves increased by only 12.5% (Table 4).

Table 4. The content of malonic dialdehyde in soybean plants leaves of different varieties depending on fertilizer (μm g^{-1} of raw material)

Fertilizing	Annushka		Ustya	
	flowering	beans swelling	flowering	beans swelling
	Control			
Control	50.86	55.44	53.95	88.20
$P_{60}K_{60}$ – background	48.73	56.70	54.77	88.83
background + N_{30}	48.40	65.37	73.59	112.68
background + N_{60}	49.88	76.69	79.80	108.78
background + N_{90}	54.03	87.63	81.71	107.42
background + N_{120}	60.09	97.67	92.80	109.01
background + N_{150}	58.93	97.09	92.02	110.51
background + N_{180}	61.60	101.07	94.88	109.97
	Rhizohumin			
Control	66.89	66.89	65.40	99.65
$P_{60}K_{60}$ – background	60.18	68.15	66.22	100.28
background + N_{30}	59.85	76.82	85.04	124.13
background + N_{60}	61.33	88.14	91.25	120.23
background + N_{90}	65.48	99.08	93.16	118.87
background + N_{120}	71.54	109.12	104.25	120.45
background + N_{150}	70.38	108.54	103.47	121.96
background + N_{180}	73.05	112.52	106.33	121.42
$LSD_{0.05}$	0.24	2.18	0.67	1.12

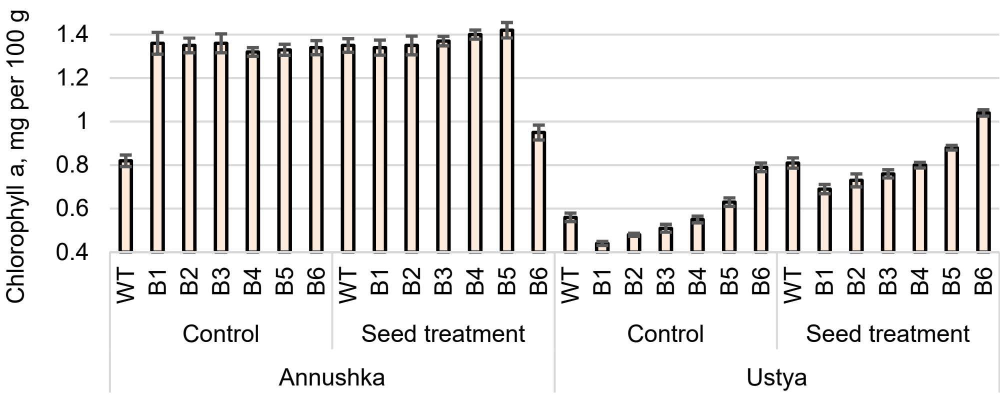
The biggest increase in the MDA content was observed in the phase of bean swelling with the use of high rates of nitrogen (180 kg ha^{-1}): in the Ustya variety the MDA content increased by 25% compared to the control; in the Annushka variety, the MDA content increased by 69% in the flowering phase. In the phase of bean swelling with pre-sowing seeds treatment by Rhizohumine and application of N_{30} and without its addition, the MDA content decreased by 16 and 18%, respectively, and with the application of N180 - increased by 17% relative to control.

Combined use of Rhizohumin + $N_{180}P_{60}K_{60}$ stimulated the doubling of MDA content compared to the control (without fertilizers + Rhizohumin) and higher by 35% compared to the absolute control, which may be due to the negative impact of high rates of mineral fertilizers on legume-rhizobial symbiosis formation.

According to the results of research, a rate-dependent increase in MDA content in the leaves of experimental varieties was established. In particular, the biggest increase in the MDA content was observed in the phase of swelling beans with the use of high rates of nitrogen (180 kg ha^{-1}): in the Ustya variety, the MDA content increased by 25% compared to the control. For the Annushka variety, the MDA content increased by 69% in the flowering phase, and already in the bean swelling phase, its values approached the control. The effect of the rate of 180 kg ha^{-1} was most negative for the Annushka variety in the flowering phase - the largest accumulation of its content was recorded compared to other experimental varieties. An increase in the amount of MDA in experimental soybean varieties may indicate a shift in the pro/antioxidant balance in the direction of enhanced ROS (reactive oxygen species) generation and their involvement in the oxidation of membrane lipids, which ultimately leads to pathological changes and growth inhibition.

Chlorophyll is vital for photosynthesis, allowing soybeans to absorb light energy (Tucker, 1979; Wan, 2013). The chlorophyll content is one of the most pronounced characteristics of the photosynthetic apparatus of plants' adaptation to environmental conditions (Zhang et al., 2001). The rate of photosynthesis of soybeans is negatively affected by stressful environmental conditions, in particular, during drought, respiratory conductivity decreases, significant over moistening causes the accumulation of starch in the leaves (Raymond et al., 2015). In the critical phase of plant development (flowering) there is a tendency for a rate-dependent increase in the content of photosynthetic pigments in plants without inoculation by Rhizohumine. Seed inoculation, depending on nitrogen rates, showed an increase in chlorophyll by 64% compared to control (without fertilizers), and a decrease of 30% with N_{180} and without seed inoculation - a manifestation of inhibitory action of excess nitrogen for effective nitrogen-fixing symbiosis as also noted by Shen et al. (2012). With applying N_{30} kg ha^{-1}, the quantitative content of pigment did not change. The content of chlorophyll *'b'* and carotenoids was at the control level.

In the phase of bean swelling there is a redistribution of assimilates with more intensive outflow to new attracting seed centers, which affected the reduction of photosynthetic pigments. In addition, the use of Rhizohumin showed a tendency to reduce the content of photosynthetic pigments, compared with the control without inoculation (Fig. 1).



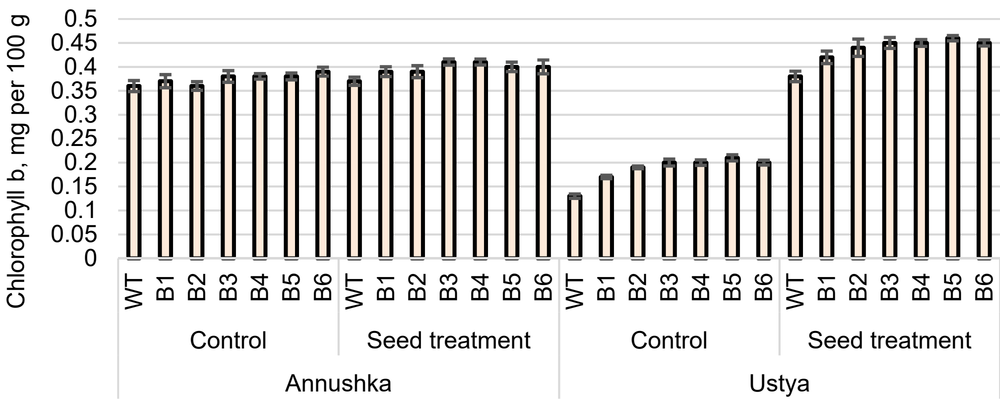


Figure 1 (continued)

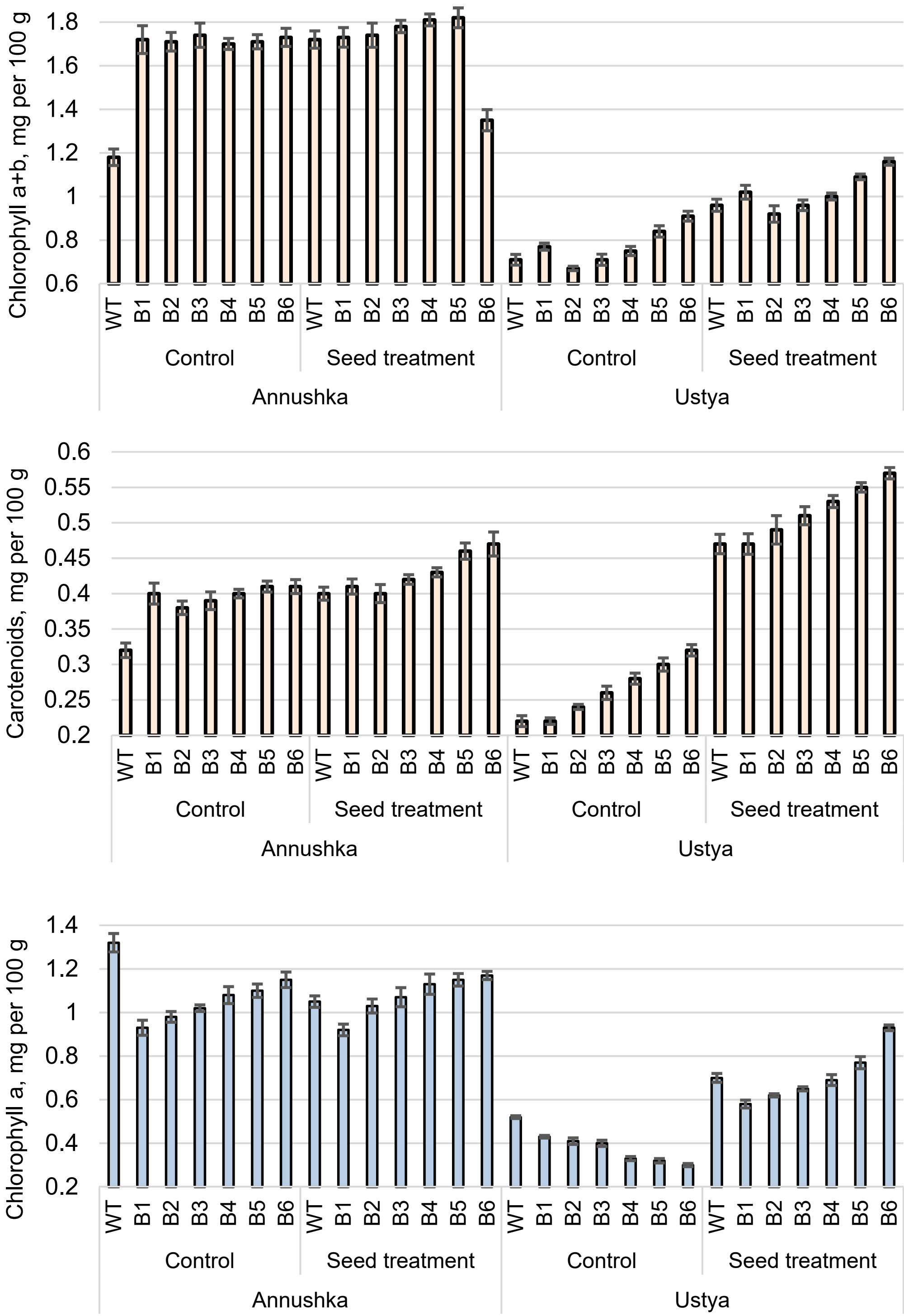


Figure 1 (continued)

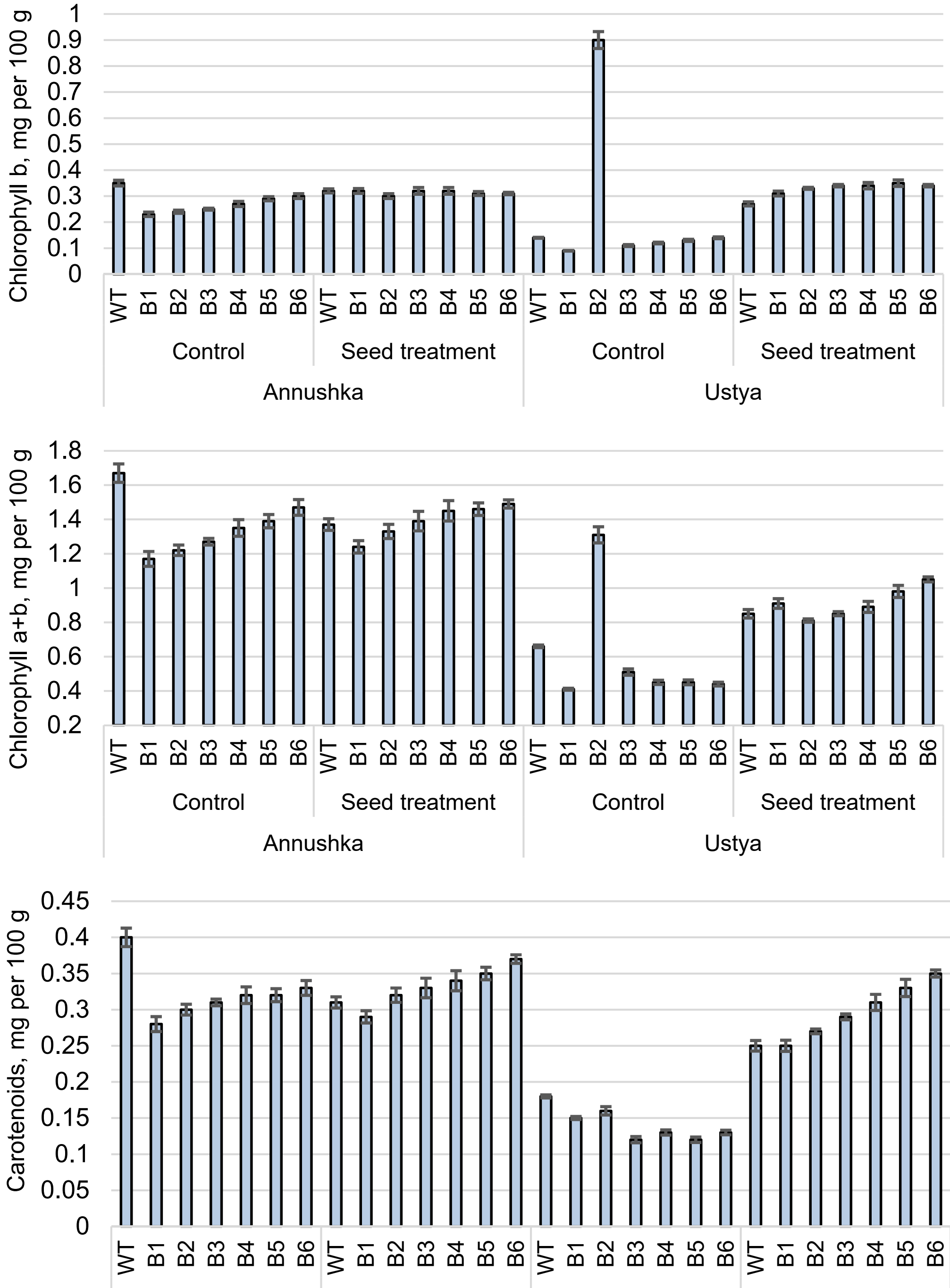


Figure 1. The content of photosynthetic pigments in the leaves of soybean plants depending on the rate of nitrogen fertilizers.

Note: yellow bars are the flowering phase, and blue bars are the iswelling of beans. Error bars describe the standard error of the mean (*SE*).

The results of our research are consistent with the data obtained by Zhang et al. (2001) at the Soybean Institute of Jilin Academy of Agricultural Sciences, Honchar et al. (2021) at the National University of Life and Environmental Sciences of Ukraine and Mutava et al. (2015) in the greenhouses of the Department of Plant Science, University of Missouri, Columbia show that the increase in the ratio of chlorophyll *'a'* and *'b'* with the introduction of nitrogen on the background of inoculation indicates the development of stress and reduced stability of the plant. In the flowering phase, there is an increase in this ratio with the combined use of nitrogen fertilizers with Rhizohumin and without it. The same trend persists in the bean swelling phase for almost all combinations of nitrogen and inoculation.

With the introduction of N_{30} and inoculation by Rhizohumin, in the phase of swelling beans, the ratio decreased by 30% compared to the control. This, in turn, indicates an adaptation aimed at improving the efficiency of photosynthesis. In soybean plants, this figure differed significantly in the experimental phases of development - flowering and swelling of beans. Thus, in the flowering phase in all experimental varieties, there was a tendency for a rate-dependent increase in chlorophyll *'a'*, while the content of other photosynthetic pigments (chlorophyll *'b'* and carotenoids) did not differ significantly from control values. In the bean swelling phase, which is characterized by an increase in the intensity of nitrogen use by non-photosynthetic tissues for seed formation, the expected decrease in pigment content in the studied soybean varieties was observed, depending on the rate of fertilizers. In the experimental varieties Annushka and Ustya, a significant decrease in the content of all pigments was recorded. In particular, the content of chlorophyll *'b'*, which is more sensitive to various anthropogenic influences, in the variety Annushka decreased by 33.5%, and in the variety Ustya by 27%.

The symbiotic activity of soybean varieties differed in such indicators as: the number (Table 5) and weight of nodules per plant, nitrogenase activity (Fig. 2). The increase in the number and weight of nodules occurred from the absolute control with seed inoculation and drugs to the introduction of $N_{60}P_{60}K_{60}$ and seed inoculation; from $N_{90}P_{60}K_{60}$ there is a sharp decrease in symbiotic activity; against the background of $N_{120}P_{60}K_{60}$ only traces of symbiotic activity are recorded, and when $N_{150}P_{60}K_{60}$ and $N_{180}P_{60}K_{60}$ are applied, symbiotic nitrogen fixation is absent.

Table 5. Number of active nodules, psc per plant

Marking	Rate of fertilizer, *factor C*	Variety, *factor A*							
		Annushka, A1				Annushka, A1			
		Seed treatment[1], *factor B*							
		C	H	R	R+H	C	H	R	R+H
		B1	B2	B3	B4	B1	B2	B3	B4
C1	Control	12.4	22.1	24.1	32.2	13.4	19.5	22.3	28.5
C2	$P_{60}K_{60}$ – background	17.2	40.6	42.4	50.4	22.1	36.4	38.8	42.8
C3	background + N_{30}	21.2	64.4	69.5	68.6	20.4	49.9	58.2	62.4
C4	background + N_{60}	26.6	84.9	85.1	88.3	28.6	64.4	76.1	78.2
C5	background + N_{90}	17.1	35.2	36.5	36.6	19.2	33.3	35.6	38.6
C6	background + N_{120}	9.1	11.2	12.4	16.6	10.8	9.2	10.6	12.1
C7	background + N_{150}	0	0	0	0	0	0	0	0
C8	background + N_{180}	0	0	0	0	0	0	0	0
$LSD_{0.05}$		2.1	8.8	11.4	9.5	1.7	12.1	10.6	7.6

The study by Osborne & Riedell (2006) in the unique environment of the northern Great Plains, USA and Wang & Han (2009) in China show that small amounts of nitrogen should be applied under soybeans, assuming that plants will be provided with it due to natural nitrogen fixation. However, under stress, not all plants or even crops form a nitrogen-fixing symbiosis, which significantly reduces yields. Our studies are consistent with the results of Matsumiya et al. (2013) conducted at Department of Biotechnology, College of Lifesciences, Ritsumeikan University, Japan, Gai et al. (2016, 2017), on the plains of the Sanjiang River in China, which confirm that the use of pre-sowing treatment of soybean seeds also had a positive effect on grain yield formation. Thus, in the variants with inoculation of soybean seeds by Rhizohumin and Hetomic, the yield of the crop was higher by 0.12–0.25 t ha^{-1} compared to the control. Among the varieties, the ultra-early variety Annushka turned out to be more productive when grown on typical chernozems.

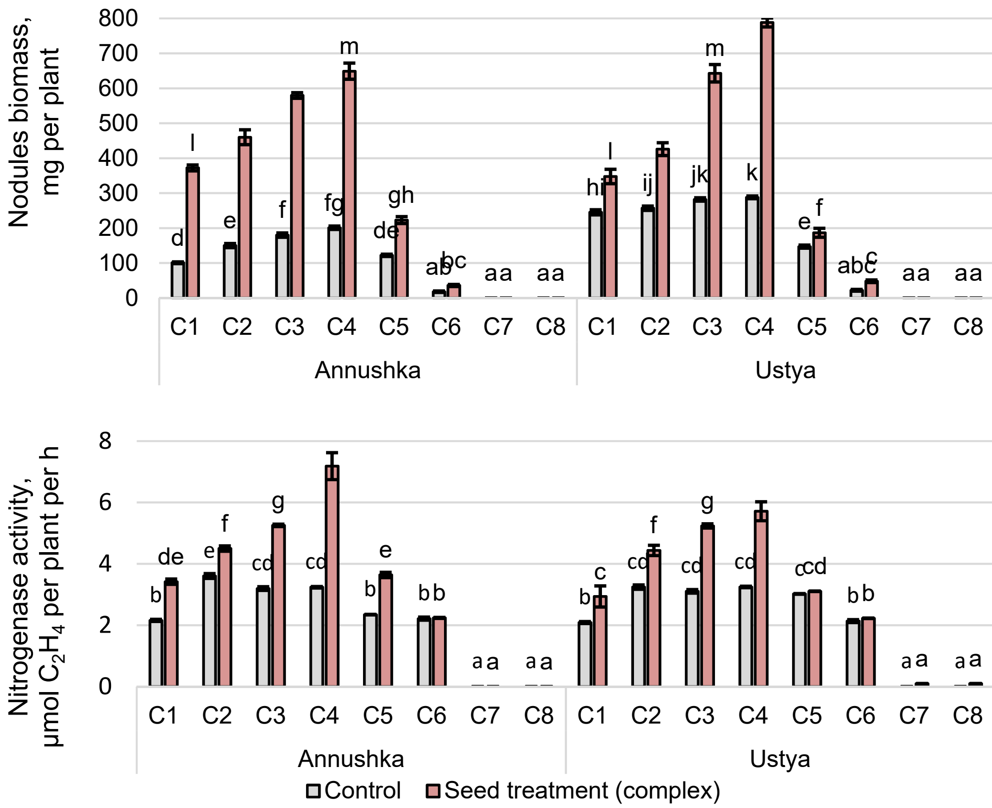


Figure 2. Nodules mass and soybean nitrogenase activity depending on the fertilizer and inoculation.

Note. Seed treatment: B1 – without inoculant (control); B2 – H, B3 – R, B4 – H + R; fertilizer option: 1 – control; 2 – $P_{60}K_{60}$ (Background); 3 – background + N_{30}; 4 – background + N_{60}; 5 – background + N_{90}; 6 – background + N_{120}; 7 – background + N_{150}; 8 – background + N_{180}.

Error bars describe the standard error of the mean (*SE*). The same letters indicate belonging to the same similarity group (no significant difference according to Fisher's LSD_{05}) within the factor. Columns without a letter are significantly different for Fisher's LSD_{05} from other variants.

Table 6. Soybean yield depending on fertilizer rates and pre-sowing seed treatment, t ha^{-1}

Marking	Rate of fertilizer, *factor C*	Variety, *factor A*									
		Annushka, A1					Ustya, A2				
		Seed treatment[1], *factor B*									
		C	H	R	R+H	+from	C	H	R	R+H	+from
		B1	B2	B3	B4	$B^{2–4}$	B1	B2	B3	B4	$B^{2–4}$
C1	Control	1.96	2.08	2.12	2.20	0.16	1.78	1.87	1.93	2.00	0.15
C2	$P_{60}K_{60}$ - background	2.10	2.24	2.28	2.36	0.19	1.94	2.03	2.09	2.16	0.20
C3	background + N_{30}	2.64	2.80	2.95	3.16	0.32	2.40	2.52	2.68	2.88	0.34
C4	background + N_{60}	3.84	4.07	4.15	4.18	0.29	3.49	3.66	3.76	3.80	0.25
C5	background + N_{90}	3.69	3.80	4.01	4.06	0.27	3.36	3.42	3.65	3.67	0.22
C6	background + N_{120}	3.61	3.63	3.64	3.63	0.16	3.27	3.23	3.24	3.28	0.03
C7	background + N_{150}	3.43	3.45	3.47	3.47	0.03	3.29	3.33	3.31	3.30	0.02
C8	background + N_{180}	3.29	3.33	3.33	3.36	0.05	2.71	2.72	2.71	2.72	0.05
$LSD_{0.05}$		0.13	0.14	0.15	0.14	-	0.13	0.14	0.13	0.12	-

Note: 1C – control; R – rhizohumin; R + H – rhizohumin + hetomic; H – hetomic; + from $B^{2–4}$ – average yield increase from seed treatment by inoculants.

The highest yield is formed by the application of $N_{60}P_{60}K_{60}$ and pre-sowing treatment of seeds with Rhizohumin or a combination of Rhizohumin and Hetomic (Table 6, 7).

With a further increase in nitrogen fertilizers to 180 kg ha^{-1} a.s. on background $P_{60}K_{60}$, there was a partial decrease in yield due to a significant increase in vegetative mass, which confirms the results of studies by Zhang et al. (2013). The leaf area of soybean crops Annushka variety at the end of flowering with the application of $N_{150}P_{60}K_{60}$ and $N_{180}P_{60}K_{60}$, regardless of seed treatment, exceeded 50.0 thousand m^2 ha^{-1}, Ustya variety - 49.1 thousand m^2 ha^{-1}.

The effectiveness of biofertilizer was manifested from N_0 to N_{90}, and the application of nitrogen fertilizers in the rate of N_{150} and N_{180} showed the absence of nodules on the root system of soybeans. Similar results were obtained by Tamagno et al (2018) and Mehmet (2008), who proved that soybean yields did not change depending on biofertilizer at high rates of nitrogen fertilizer application. Yield formation occurred only through the application of mineral fertilizers.

Table 7. ANOVA of soybean yield data

Effect	SS	*df*	MS	Partial eta-square
Variety (V)	23.672	1	23.672	0.984916
Fertilizing (F)	404.257	7	57.751	0.999104
Seed treatment (ST)	6.074	3	2.025	0.943676
Year (Y)	95.209	4	23.802	0.996207
V*F	4.732	7	0.676	0.928836
V*ST	0.047	3	0.016	0.114789
F*ST	4.143	21	0.197	0.919531
V*Y	10.219	4	2.555	0.965737
F*Y	157.956	28	5.641	0.997710
ST*Y	0.710	12	0.059	0.661860
V*F*ST	0.043	21	0.002	0.106712
V*F*Y	11.146	28	0.398	0.968497
V*ST*Y	0.120	12	0.010	0.248153
F*ST*Y	2.415	84	0.029	0.869458
V*F*ST*Y	0.694	84	0.008	0.656868
Error	0.363	640	0.001	

Note: All effects are significant at $p < 0.001$.

Note: Error bars describe the standard error of the mean (*SE*). The same letters indicate belonging to the same similarity group (without a significant difference according to Fisher's $LSD_{0.5}$) within the factor. Columns without a letter are significantly different for Fisher's $LSD_{0.5}$ from other variants.

The studied factors differently affect the variation in yield of soybean varieties Ustya and Annushka (Fig. 3).

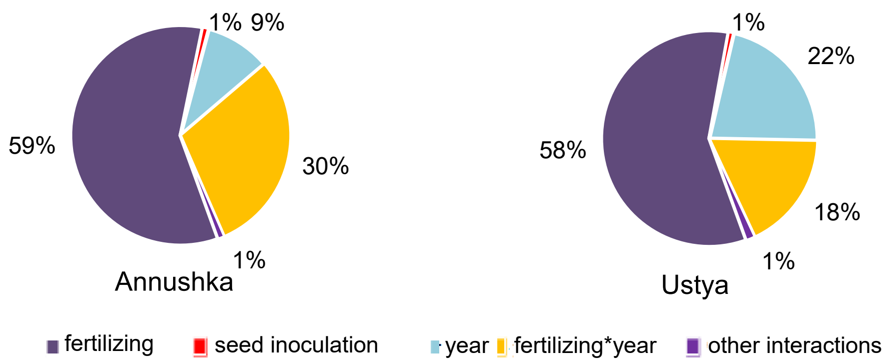


Figure 3. The share of factors in the yield formation of soybean varieties Annushka and Ustya.

In particular, what is common is that the studied fertilizer system accounted for 58–59% of the total yield variation, and seed inoculation only 1%. At the same time, the ratio of the influence of weather conditions (year) and the interaction of weather conditions with the fertilizer system differed. The influence of weather conditions on the yield of Annushka variety was 9 vs. 30% in the interaction of weather conditions with the fertilizer system, which indicates the sensitivity of this variety to nutrients and their utilization in the crop depending on weather conditions. At the same time, the influence of weather conditions on the yield of Ustya variety was 22%, and their interaction - 18%, which indicates greater inertia to the assimilation of nutrients under different conditions, but higher sensitivity to the various conditions of the year.

The yield index is parabolic depending on the rate of fertilizer within a particular inoculant - if in the control version the yield index was low due to lack of nutrients and differentiation of a small number of generative organs, then the introduction of $N_{150}P_{60}K_{60}$ and $N_{180}P_{60}K_{60}$ decreased by accumulating significant by-products (Fig. 4).

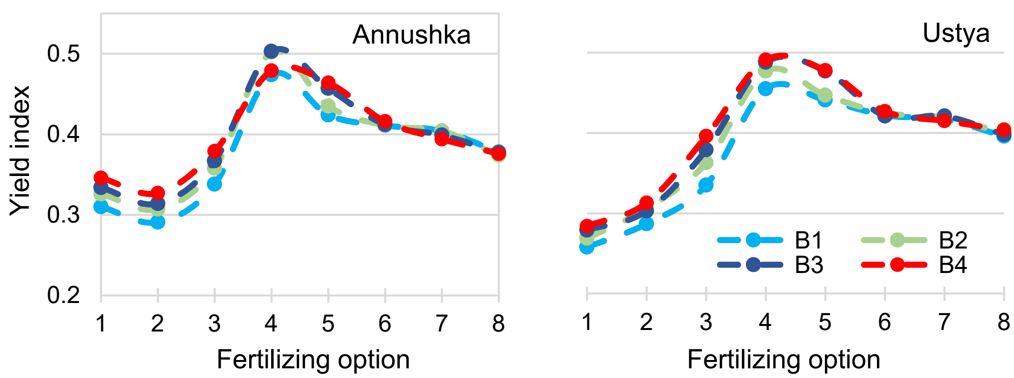


Figure 4. The soybean yield index depending on the fertilizer system and seed inoculation.

Note. Seed treatment: B1 – without inoculant (control); B2 – H; B3 – R; B4 – H + R; fertilizer option: 1 – control; 2 – $P_{60}K_{60}$ (Background); 3 – background + N_{30}; 4 – background + N_{60}; 5 – background + N_{90}; 6 – background + N_{120}; 7 – background + N_{150}; 8 – background + N_{180}.

Nitrogen is an inducer of the productive properties of plants, determining the yield and nutritional value of soybeans in terms of protein and fat content. The results of our studies confirm the data of Boroomandan et al. (2009) that the total protein content in control seeds without treatment by Rhizohumin increased depending on the rate of nitrogen fertilizers, and conversely, in soybean plants with pre-sowing treatment decreased significantly relative to control with Rhizohumin and without its use (Fig. 5).

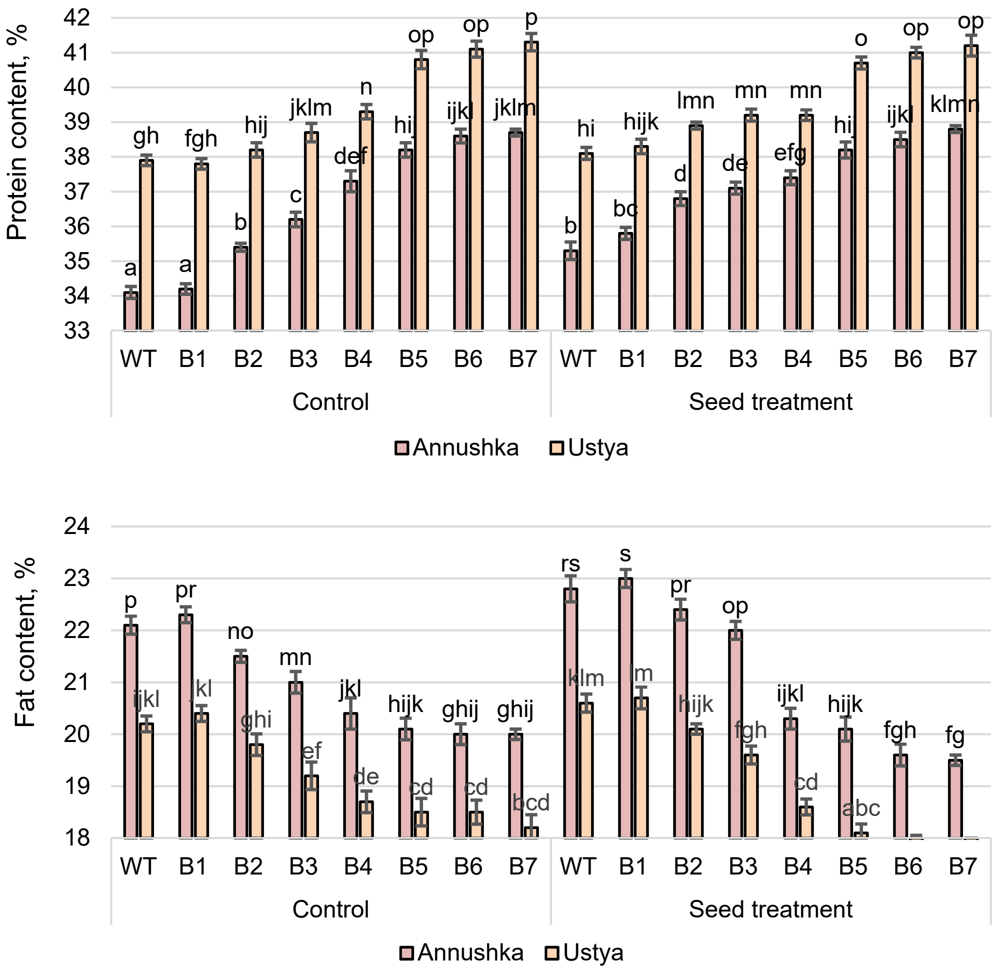


Figure 5. The protein and fat content in soybean seeds depending on the rate of nitrogen fertilizers and inoculation by Rhizohumine.

Note: Error bars describe the standard error of the mean (*SE*). The same letters indicate belonging to the same similarity group (no significant difference according to Fisher's LSD_{05}) within the factor. Columns without a letter are significantly different for Fisher's LSD_{05} from other variants.

This is due to the more efficient use of nitrogen in the metabolic reactions of plants formed from inoculated seeds (Dong et al. (2010)). Nitrogen fertilizers increased by 60%, and a significant increase in fat content of 60% was observed only with the application of N_{30}, compared with the absolute control.

CONCLUSIONS

Growing soybeans on typical low-humus chernozems of Ukraine, the balanced use of increasing rates of nitrogen fertilizers on the background of seed inoculation, in general, contributes to increasing yields, improving grain quality, and significantly reduces the chemical load on the plant due to its specific physiological response. The highest yield of soybeans on typical chernozems of the forest-steppe of Ukraine is formed by applying $N_{60}P_{60}K_{60}$ and pre-sowing seed treatment by rhizohumin or a combination of rhizohumin and hetomics. With a further increase in nitrogen rates and seed inoculation, a partial decrease in productivity is observed. The application of pre-sowing processing had a positive effect on grain yield formation. When soybean seeds were inoculated with rhizohumin and hetomik, the crop yield was higher by 0.12–0.25 t ha^{-1} compared to the control. The use of nitrogen fertilizers in the amount of 30 and 180 kg ha^{-1} is impractical for soybeans. This is evidenced by the adaptive changes in this variety to the application of these rates of nitrogen fertilizers, which are manifested in the increase in the amount of MDA and the parallel decrease in the content of photosynthetic pigments during the growing season, and as a result, the decrease in crop yield. The effectiveness of biofertilizer is manifested from N_0 to N_{90}, with the application of nitrogen fertilizers at the rate of N_{150} and N_{180}, the absence of nodules on the soybean root system was observed. At high rates of nitrogen fertilizer application, soybean productivity is formed only due to the application of mineral fertilizers, as the influence of biofertilizer is neutralized.

However, many issues of mineral nutrition have not yet been resolved. This is related to the biology of soybean plants, different requirements for nutrients during ontogenesis, and the presence of the process of biological assimilation of nitrogen. In particular, to investigate to what extent the excess mineral forms of nitrogen in the soil creates a precedent for nitrate pollution of the environment. In further research, it is also worth finding out how soil moisture deficiency affects both the level of symbiosis and the photosynthetic activity of soybean crops. It is extremely important to get strains of nodule bacteria resistant to drought. Scientific laboratories in the USA, China, and many European countries are working on this problem.

REFERENCES

Adeli, A., Sistani, R.K. & Tewlde, H. 2005. Effects of broiler litter on soybean production and soil nitrogen and phosphorus concentrations. *Agron J.* **97**(1), 314–321. https://doi.org/10.2134/agronj2005.0314

Batsmanova, L., Taran, N., Konotop, Y., Kalenska, S. & Novytska, N. 2020. Use of a colloidal solution of metal and metal oxide-containing nanoparticles as fertilizer for increasing soybean productivity. *Journal of Central European Agriculture* **21**(2), 311–319. https://doi.org/10.5513/JCEA01/21.2.2414

Boroomandan, P., Khoramivafa, M., Haghi, Y. & Ebrahimi, A. 2009. The effects of nitrogen starter fertilizer and plant density on yield, yield components and oil and protein content of soybean. *Pak J Biol Sci.* **12**(4), 378–382. 10.3923/pjbs.2009.378.382

Caliskan, S., Ozkaya, I., Caliskan, M.E. & Arslan, M. 2008. The effects of nitrogen and iron fertilization on growth, yield and fertilizer use efficiency of soybean in a Mediterranean-type soil. *Field Crops Research* **108**(2), 126–132. https://doi.org/10.1016/j.fcr.2008.04.005

Dong, S.K., Gong, Z.P. & Zu, W. 2010. Effects of nitrogen nutrition levels on N-accumulation and yields of soybean. *Plant Nutr Fert Sci*. 16(1), 65–70. https://doi.org/10.1371/journal.pone.0174841

Ermantraut, E.R., Gopcij, T.I. & Kalenska, S.M., Kryvoruchenko, R.V., Turchynova, N.P. & Prysyajnyuk, O.I. 2014. *Method of selection experiment (in plant growing).* Harkiv. 229. (in Ukrainian)

Gai, Z., Zhang, J. & Li, C. 2017. Effects of starter nitrogen fertilizer on soybean root activity, leaf photosynthesis and grain yield. *PLoS ONE*. **12**(4). https://doi.org/10.1371/journal.pone.0174841

Gai, Z.J., Zhang, W., Jiang, F.F. & Cai, LJ. 2016. Response of soybean root nodule and yield to biological-sugar nitrogen fertilizer and urea. *J Nuclear Agric Sci*. **30**(4), 124–128. https://doi.org/10.11869/j.issn.100-8551.2016.04.0822

Halliwel, B. & Guteridge, J.M.C. 1984. Oxygen toxicity, oxygen radicals, transition metals and desease. *Biochem. J.* **219**. 1–14.

Honchar, L., Mazurenko, B., Shutyi, O., Pylypenko, V. & Rakhmetov, D. 2021. Effect of pre-seed and foliar treatment with nano-particle solutions on seedling development of tiger nut (*Cyperus Esculentus* L.) plants. *Agronomy research* **19**(S1), 767–776. https://doi.org/10.15159/ar.21.021

Hrytsaenko, Z.M., Hrytsaenko, A.O. & Karpenko V.P. 2003. Methods of biological and agrochemical research of plants and soils. K.: CJSC "Nichelava". 316 pp. (in Ukrainian).

Hungria, M., Franchini, J.C., Campo, R.J., Crispino, C.C., Moraes, J.Z., Sibaldelli, R.N.R., Mendes, I.C. & Arihara, J. 2006. Nitrogen nutrition of soybean in Brazil: Contributions of biological N2 fixation and N fertilizer to grain yield. *Can. J. Plant Sci.* **86**, 927–939. https://doi.org/10.4141/P05-098

Kalenska, S., Novytska, N., Stolyarchuk, T., Shutiy, O. & Sonko, R. 2021. Nanopreparations in technologies of plants growing. *Agronomy Research* **19**(S1), 795–808. https://doi.org/10.15159/AR.21.017

Kalenskiy, V., Novytcka, N., Kalenska, S., Pylypenko, V., Cherniy, V. & Scherbakova, E. 2016. Efficiency of symbiotic nitrogen fixation in legumes (soybean, peas, chickpeas), based on cropping technology on black soils. Die rolle der bodenmikroorganismen bei der ernahrung von kulturpflanzen. Intern. Wissenschaftliche konferenc, 17–18 november, Anhalt - Bernburg, Strenzfeld, pp. 12–13.

Liu, X.B., Jin, J., Wang, G.H. & Herbert, S.J. 2008. Soybean yield physiology and development of high-yielding practices in Northeast China. *Field Crops Res*. **105**, 157–171. https://doi.org/10.1016/j.fcr.2007.09.003

Makrushin, M.M., Peterson, N.V. & Melnykov, M.M. 2006. Physiology of plants. Vinnytsia: New book, 416 pp. (in Ukrainian).

Matsumiya, Y., Horii, S., Matsuno, T. & Kubo, M., 2013. Soybean as a Nitrogen Supplier, A Comprehensive Survey of International Soybean Research - Genetics, Physiology, Agronomy and Nitrogen Relationships. *Intech Ope*n., https://doi.org/10.5772/51017

Maw, Myint, Nakasathien, Sutkhet & Sarobol, Ed. 2011. Responses of Specific Leaf Weight, Biomass and Seed Yield of Soybean to Nitrogen Starter Rate and Plant Density. *Kasetsart Journal - Natural Science* 45.

Matsumiya, Y., Horii, S., Matsuno, T. & Kubo, M., 2013. Soybean as a Nitrogen Supplier, A Comprehensive Survey of International Soybean Research - Genetics, Physiology, Agronomy and Nitrogen Relationships. *Intech Open*. https://doi.org/10.5772/51017

Mehmet, O.Z. 2008. Nitrogen rate and plant population effects on yield and yield components in soybean. *Afr J Biotechnol*. **7**(24), 4464–4470.

Melnyk, S.I., Jylkin, V.A., Gavrylyuk, M.M., Snigovyi, V.S. & Lisovyi, M.M. 2007. *Recommendations for the effective use of microbial drugs in crop production technologies*. Ministry of Agrarian Policy of Ukraine, Ukrainian Academy of Agrarian Sciences. Kyiv, 52 pp. (in Ukrainian).

Muchow, R.C. & Sinclair, T.R. 1986. Water and nitrogen limitations in soybean grain production. II. Field and model analyses. *Field Crops Res*. **15**, 143–156. https://doi.org/10.1016/0378-4290%2886%2990083-3

Mutava, R., Prince, S., Syed, N., Song, L., Valliyodan, B., Chen, W., Nguyen, H. 2015. Understanding abiotic stress tolerance mechanisms in soybean: A comparative evaluation of soybean response to drought and flooding stress. *Plant Physiology and Biochemistry* **86**. https://doi.org/10.1016/j.plaphy.2014.11.010

Novytska, N., Gadzovskiy, G., Mazurenko, B., Svistunova, I. & Martynov, O. 2020. Effect of seed inoculation and foliar fertilizing on structure of soybean yield and yield structure in western Polissya of Ukraine. *Agronomy Research* **18**(4), 2512–2519. https://doi.org/10.15159/ar.20.203

Osborne, S.L. & Riedell, W.E. 2006. Starter nitrogen fertilizer impact on soybean yield and quality in the Northern Great Plains. *Agron J.* **98**(6), 1569–1574. http://dx.doi.org/10.2134/agronj2006.0089

Platonova, A.A. & Kostyshyn, S.S. 2000. The content of malonic dialdehyde and the activity of antioxidant enzymes in pea seedlings under the action of cadmium ions. *Physiology and biochemistry of cultivated plants* **32**(2), 146–150 (in Ukrainian).

Raymond, N.M., Silvas, J.KP., Naeem, H.S., Li, S., Babu, V., Wei, C. & Henry, T.N. 2015. Understanding abiotic stress tolerance mechanisms in soybean: a comparative evaluation of soybean response to drought and flooding stress. *Plant Physiol. Biochem*. **86**, 109–120. https://doi.org/10.1016/j.plaphy.2014.11.010

Shen, X.H., Jiang, C. & Zhang, J.T. 2012. Correlation between spectrum reflectance and chlorophyll content of soybean leaves under different nitrogen level. *Soybean Sci.* **31**(1), 73–80. https://doi.org/10.1371/journal.pone.0174841

Sij, J.W., Turner, F.T. & Craigmiles, J.P. 1979. ‘Starter nitrogen’ fertilization in soybean culture. *Commun. Soil Sci. Plan*. **10**(11), 1451–1457. https://doi.org/10.1080/00103627909366999

Takuji, O., Kaushal, T., Shinji, I., Kazuya, T., Satoshi, K., Yuki, O., Soshi, H., Norikuni, O., Kuni, S., Hideo, H., Takashi, S., Sayuri, T., Yoshifumi, N., Yoichi, F. & Yoshihiko, T. 2017. Role of Nitrogen on Growth and Seed Yield of Soybean and a New Fertilization Technique to Promote Nitrogen Fixation and Seed Yield, Soybean - The Basis of Yield, Biomass and Productivity, Minobu Kasai, *IntechOpen*. https://www.intechopen.com/chapters/53538

Tamagno, S., Sadras, V.O., Haegele, J.W., Armstrong, P. R. & Ciampitti, I.A. 2018. Interplay between nitrogen fertilizer and biological nitrogen fixation in soybean: implications on seed yield and biomass allocation. *Sci. Re.* **8**, 17502. https://doi.org/10.1038/s41598-018-35672-1

Temrienko, O.O. 2018. Formation of soybean productivity depending on agrotechnical methods of cultivation in the conditions of the right-bank Forest-Steppe. *Scientific reports of NULES of Ukraine* **3**(73). http://dx.doi.org/10.31.548/dopovidi2018.035 (in Ukrainian).

Tian, H.Y. 2007. Effects of N fertilization at different stages on nitrogenase activity and yield of soybean. Thesis, *Northeast Agric. Univ*. **12**(4), 1102–1104.

Tuanjie, Z., Muqadas, A. & Ripa, A.S. 2017. Adaptation to Water Stress in Soybean: Morphology to Genetics, Plant, Abiotic Stress and Responses to Climate Change, *IntechOpen Book Series*. https://doi.org/10.5772/intechopen.72229

Tucker, C.J. 1979. Red and photographic infrared linear combinations for monitoring vegetation. *Remote Sens Environ* **8**, 127–150. https://doi.org/10.1016/0034-4257(79)90013-0

Wan, T. 2013. Effect of nitrogen levels on photosynthesis rate and yield of soybean. Thesis, *Northeast Agric Univ*. **18**(6), 816–819.

Wang, S.Q. & Han, X.Z. 2009. Root morphology and nitrogen accumulation in soybean (Glycine max L.) under different nitrogen application levels. *Chin J Eco-Agric*. **17**(6), 1069–1073. https://doi.org/10.3724/SP.J.1011.2009.01069

Zhang, H.S., Cheng, Y.X. & Wang, D.Q. 2001. Correlation analysis of chlorophyll content and yield at pod period of soybean. *Soybean Sci*. **20**(4), 275–279.

Zhang, X., Huang, G., Bian, X. & Zhao, Q. 2013. Effects of root interaction and nitrogen fertilization on the chlorophyll content, root activity, photosynthetic characteristics of intercropped soybean and microbial quantity in the rhizosphere. *Plant Soil Environ*. **59**(2), 80–88. https://doi.org/10.17221/613/2012-PSE