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EFFECTS OF INOCULATION AND PHOSPHORUS NUTRITION ON YIELD AND SEED QUALITY OF SOYBEAN VARIETIES IN THE CENTRAL FOREST-STEPPE ZONE

Yaroslav Chabaniuk ^{1,2}, Iryna Brovko ,
Andrii Kovtun , Myroslava Milova ¹

¹ National University of Life and Environmental Sciences of Ukraine
15, Heroiv Oborony St., Kyiv 03041, Ukraine

² LLC “Institute of Agrobiology”, 4 Vatslav Havel Blvd., Building 45, Kyiv 03067, Ukraine

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Background. The relationship between the seed yield of soybean (*Glycine max* (L.) Merrill) and nitrogen (N) has been well studied, whereas the effect of other elements, particularly phosphorus (P), remains insufficiently explored. Phosphorus, the second most important element after nitrogen, is crucial for obtaining high-quality soybean yields. At the same time, despite numerous studies on the effects of phosphate-mobilizing and nitrogen-fixing microorganisms on sustainable agricultural development, the effectiveness of these scientific efforts is insignificant.

Materials and Methods. General scientific and specialized methods were used, including field, laboratory, chemical and statistical methods. The research was conducted in the Forest-Steppe Zone from 2019 to 2021 at the experimental field of the LLC “Institute of Agrobiology” (Velykyi Mytnyk village, Khmilnyk district, Vinnytsia region). In this experiment, three foreign-bred soybean varieties were used: Mentor (early-maturing), Cordoba, and Moravia (mid-early varieties), using mineral phosphorus fertilization (P_{30}) and biofertilization based on a ternary composition of phosphate-mobilizing strains – *Bacillus megaterium* de Bary, 1884 (eko/207), *B. amyloliquefaciens* (ex Fukomoto, 1943) Priest, Goodfellow, Shute & Berkeley, 1987 (eko/205), and *Trichoderma harzianum* Rifai, 1969 (eko/101). These treatments were applied both without inoculation and with seed inoculation with three strains of *Bradyrhizobium japonicum* (Kirchner, 1896) Jordan, 1982 from the LLC “Institute of Agrobiology” (active ingredients of the applied formulations).



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Results and Discussion. All studied soybean varieties responded positively to the application of phosphorus fertilization and biofertilization based on phosphate-mobilizing strains, both without inoculation and with seed inoculation using nitrogen-fixing bacterial strains. Overall, the grain yield increase (%) in the experimental variants ranged from +7.70 % to +18.28 %. The best yield performance (compared to the control) was recorded in the variant combining inoculation with bacterial-micromycete (BM) biofertilization, resulting in a grain yield increase of +0.31 t/ha or 11.27 % (Mentor), +0.31 t/ha or 13.58 % (Cordoba), and +0.48 t/ha or 18.28 % (Moravia). The protein content (%) in soybean seeds ranged from 38.75 % to 42.75 %, depending on the variety, treatment variant, and fertilization background. The mid-early Moravia variety was the only one that showed an increase in protein percentage across all experimental variants (P_{30} and BM), both without inoculation and with seed inoculation. In contrast, the Mentor and Cordoba varieties exhibited a quantitative reduction in protein content in the P_{30} and BM variants (compared to the control) only in the absence of seed inoculation.

Conclusion. In the Central Forest-Steppe Zone of Ukraine, the combination of seed inoculation with phosphorus fertilization and biofertilizers based on phosphate-mobilizing microorganisms significantly improved soybean yield and seed protein content. In the overall variance, the factorial share of influence (%) on yield was lower than on protein content, amounting to 34 % and 67 %, respectively. The greatest impact on protein content was from inoculation (29 %), while yield was most affected by inoculation (17 %) and phosphorus supply (14 %). Variety-related factors were less influential for yield but contributed 17 % to protein variation. Factor interactions were mostly insignificant for yield but notable for protein.

Keywords: cultivated soybean, *Glycine max* (L.) Merrill, phosphorus nutrition, phosphate-mobilizing microorganisms, yield, protein content

INTRODUCTION

At the current stage of agricultural development, a substantial increase and stabilization of legume crop production, particularly soybean seeds, is a major challenge faced by the agrarian sector of Ukraine. Soybeans are a source of balanced and nearly “ideal” protein in terms of amino acid composition, containing 40 % of protein and 20 % of oil, with an environmentally friendly profile (Sydiakina & Ivaniv, 2023). No other plant in the world can produce as much protein and oil within 4–5 months as soybeans. Additionally, soybeans play a significant role in biological farming, as they fix nitrogen from the air, meeting 50–70 % of their own nitrogen requirements while also enriching the soil with nitrogen through plant residues after harvest (Zimmer *et al.*, 2016; Fedoruk *et al.*, 2022). For instance, the yield increase of grain crops grown after soybeans reaches 3–4 c/ha.

Depending on demand, the dynamics of soybean production and cultivated areas follow specific patterns. In 2019, the global soybean cultivation area amounted to 122 million hectares. The top ten soybean-producing countries include the United States, Brazil, Argentina, China, India, Paraguay, Canada, Russia, Ukraine, and Bolivia. The five leading countries in soybean acreage – Brazil, the USA, Argentina, India, and China – account for 106 million hectares, or over 85 % of the world’s soybean fields (Sedibe *et al.*, 2023). It is projected that this crop will occupy 6 % of the world’s arable land (Hamza *et al.*, 2024). In Ukraine, soybean acreage continues to expand, which highlights

its crucial nature. As mentioned earlier, Ukraine ranks ninth globally in soybean cultivation, with a total sown area of 1.55 million hectares, representing 1 % of the global figures (Stryzhak, 2018; Korobko, 2021).

A similar trend is observed in global soybean yield levels. In 2019, Brazil led in yield with 3.3 t/ha, followed closely by the United States (3.2 t/ha) and Argentina (3.0 t/ha). The EU and Paraguay ranked fourth and fifth with yields of 2.9 t/ha and 2.8 t/ha, respectively. Italy emerged as the leading soybean producer in Europe. In Ukraine, the average soybean yield in 2019 was 2.40 t/ha, dropping to 1.77 t/ha in 2020, and recovering slightly to 2.23 t/ha in 2021 (Korobko, 2021).

It is well known that soybean yield is highly sensitive to moisture availability. Therefore, the main soybean cultivation areas in Ukraine are located in regions with sufficient water supply, forming the so-called “soybean belt” (or “soybean-maize belt”). This belt includes the Forest-Steppe Zone, which comprises nine oblasts: Vinnytsia, Kyiv, Poltava, Sumy, Ternopil, Kharkiv, Khmelnytskyi, Cherkasy, and Chernivtsi. It also extends to Steppe regions with “forest-steppe” conditions, covering parts of Kirovohrad, Dnipropetrovsk, Odesa, and Mykolaiv oblasts, as well as the southern districts of Polissia with “forest-steppe” conditions in Zhytomyr, Chernihiv, Rivne, and Volyn oblasts. This vast territory is well-suited for soybean cultivation due to its soil properties, thermal and light resources, water availability, and the duration of the growing season (Stryzhak, 2018).

Weather conditions significantly impact soybean yield, as droughts in key soybean-producing regions worsen yield prospects. The Institute of Fodder and Agriculture of Podillia, NAAS has scientifically substantiated the concept of Ukraine’s “soybean belt,” identifying zones of stable and unstable soybean production on non-irrigated lands, as well as a zone of guaranteed soybean production on irrigated lands (Didora *et al.*, 2017). Given this, soybean cultivation areas should be expanded primarily in these regions.

A scientifically grounded selection of varieties for cultivation is essential for increasing yield and improving the quality of soybean production. As stated by the authors (Korobko, 2021; Sydiakina & Ivaniv, 2023), the variety is one of the resource-saving elements of the cultivation technology, and it accounts for 30–35 % of yield increase. In extreme weather years, the variety plays a decisive role in determining the productivity of the crop.

Ukraine has a large variety of soybean cultivars across different maturity groups: ultra-early, early, mid-early, mid, and mid-late. The State Register of Plant Varieties of Ukraine lists 246 different soybean varieties. The main criteria for variety selection are productivity, duration of the growing season, resistance to shattering and lodging, resistance to diseases and pests, and, in the case of irrigated areas, resistance to temporary waterlogging, or, in dry areas, drought tolerance. In intensive agriculture under extreme weather conditions, it is essential to grow several varieties of different maturity groups. In general, for the conditions of the Ukrainian Steppe, medium-late and late varieties are most suitable; for the Forest Steppe, these are early and medium-early varieties; and for Polissia – ultra-early and early varieties (Zabolotnyi *et al.*, 2022; Tkachuk *et al.*, 2022). Furthermore, attention must be paid to the nutritional properties, such as protein content, which directly affects the value of the harvested crop. For instance, Canadian breeding focuses on creating ultra-early soybean varieties with minimal response to the duration of the light period, capable of producing 3.0–3.5 tons per hectare in regions north of 53–54° N. European breeders, particularly in Sweden, Germany, the Czech Republic, Austria, and others, are also working in this direction (Zabolotnyi *et al.*, 2020).

Among the factors determining soybean yield, plant nutrition plays a crucial role in promoting better growth, development, and productivity. Recently, the use of biopreparations with various modes of action in agricultural crop cultivation technologies, including soybean, has become increasingly relevant (Hadzovskyi *et al.*, 2020; Shepilova *et al.*, 2023).

The use of inoculants is a scientifically proven natural method for increasing the availability of nitrogen for plants, enhancing and unlocking their yield potential. Inoculants provide for the effective incorporation of beneficial rhizobial bacteria (*Bradyrhizobium* genus) into the seed, improving the ability of leguminous plants to fix atmospheric nitrogen and increase yield (Zimmer *et al.*, 2016; Fedoruk *et al.*, 2022; Mirriam *et al.*, 2023).

The relationship between soybean seed yield and nitrogen (N) has been widely studied in scientific literature, but the relationship with other nutrients, such as phosphorus (P), potassium (K), and sulfur (S), has received less attention (Salvagiotti *et al.*, 2021). However, it has been proven that phosphorus, which ranks second after nitrogen, is crucial for obtaining high-quality soybean grain yield. It increases the rate of photosynthesis, energy transfer, enzymatic activity, root development, movement and absorption of other nutrients, nodule formation, and, consequently, nitrogen fixation, seed growth, and maturation, including seed size and germination. It is also worth noting that phosphorus, together with potassium, has properties that reduce plant susceptibility to diseases (Fedoruk *et al.*, 2022; Mirriam *et al.*, 2023). Traditionally, the problem of phosphorus deficiency in the soil is addressed by applying phosphorus fertilizers. However, most of the phosphorus in fertilizers is not available to plants (Alori *et al.*, 2017).

Although extensive research on phosphate mobilizers and their impact on sustainable agriculture has been conducted over the past few decades, the effectiveness of these scientific efforts is still in its early stages (Alori *et al.*, 2017; Raymond *et al.*, 2021). The use of effective phosphate-transforming microorganisms opens new opportunities for improving crop yields and ensuring soil health (Cheng *et al.*, 2023). As noted by the authors (Zabolotnyi *et al.*, 2020), preparations based on phosphate-mobilizing bacteria allow for the efficient mobilization of 30 % or more of the phosphorus fixed in the soil. However, the mechanisms of interaction between phosphate-mobilizing microorganisms, the mineral base of the soil, and plants, particularly legumes, have not yet been fully uncovered, which could lead to improvements in both phosphorus and nitrogen nutrition.

The LLC "Institute of Agrobiology" houses a collection of agronomically beneficial soil microorganisms aimed at increasing agricultural crop yields, with strains under investigation, including *Bacillus megaterium* de Bary, 1884 (eko/207), *Bacillus amyloliquefaciens* (ex Fukomoto, 1943) Priest, Goodfellow, Shute & Berkeley, 1987 (eko/205), *Trichoderma harzianum* Rifai, 1969 (eko/101), and *Bradyrhizobium japonicum* (Kirchner, 1896) Jordan, 1982 (eko/001, eko/002, and eko/003).

The effectiveness of the ternary association of phosphate-mobilizing organisms is ensured by the complex action of the soil-spore bacteria strains *Bacillus megaterium* (eko/207), *Bacillus amyloliquefaciens* (eko/205), and the micromycete *Trichoderma harzianum* (eko/101). The microorganisms *B. megaterium* and *T. harzianum* mobilize inorganic phosphorus through the synthesis of a complex of organic and inorganic acids, while *B. amyloliquefaciens* mobilizes organic phosphate compounds by producing enzymes – phosphatases. Due to the different directions of action of these bioagents, all possible sources of phosphorus nutrition become available to the plant (Chabaniuk *et al.*, 2019).

The application of new selection strains of rhizobial bacteria in combination with a composition of phosphate-mobilizing organisms (bacteria and fungi) in the conditions of the Central Forest-Steppe Zone of Ukraine has been insufficiently studied, which significantly impacts various performance indicators of soybean plants in this region. As a result of the agroecological justification of the role of soybean in addressing the problem of plant protein in Ukraine, the authors believe that the prospects for further research lie in improving the general cultivation technologies of early and mid-early varieties of soybean under the soil and climatic conditions of the Central Forest-Steppe Zone of Ukraine. This includes the application of mineral phosphorus fertilizers and the use of a composition of phosphate-mobilizing organisms combined with seed inoculation with nitrogen-fixing bacteria. In this context, the aim of this study was to evaluate the effect of seed inoculation with *Bradyrhizobium japonicum* and/or phosphorus nutrition (mineral fertilizers and a biofertilizer based on phosphate-mobilizing bacteria and micromycetes) on soybean yield and seed protein content under the conditions of the Central Forest-Steppe Zone of Ukraine.

The study tested the hypothesis that a combined application of seed inoculation with nitrogen-fixing bacteria and phosphorus nutrition, including phosphate-mobilizing microorganisms, would have a synergistic effect, leading to a significant increase in soybean yield and seed protein content, with varying responses depending on the variety's maturity group.

MATERIALS AND METHODS

The productivity and quality of grain from various soybean (*Glycine max* (L.) Merrill) varieties were studied in field experiments conducted between 2019 and 2021 in the village of Velyky Mytnyk, Khmilnytskyi District, Vinnytsia Region, located in the Forest-Steppe Zone (Fig. 1).

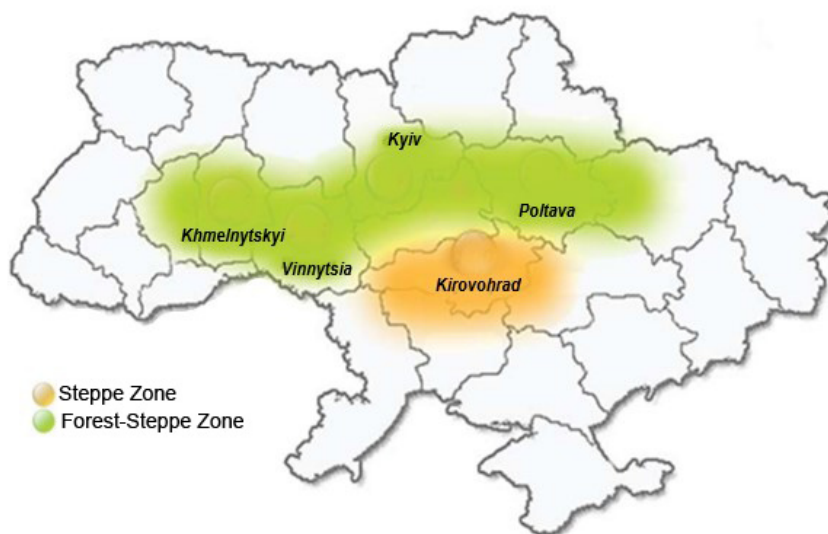


Fig. 1. Schematic map of the main soybean cultivation areas (the so-called “soybean belt”) in Ukraine. The red point represents the experiment site. Source: according to Petrychenko, 2012 (modified by the authors)

The focus was on the inoculation of seeds with a combination of active strains of rhizobial bacteria (*Bradyrhizobium japonicum*), different fertilization backgrounds – mineral (phosphorus), and bacterial-mycetous based on a ternary composition of active strains of phosphate-mobilizing organisms (*Bacillus megaterium*, *Bacillus amyloliquefaciens*, and *Trichoderma harzianum*), both in combination with inoculation and without it.

The total experimental plot area was 150 m², with a recorded area of 100 m². The experiment was repeated four times, with the predecessor crop being fallow. The experimental design followed a randomized split-plot method. The agro-technique used was typical for the growing zone, except for the nutritional elements being studied. Early and medium-early maturing soybean varieties (105–125 days) of foreign selection were grown: Mentor (originator – Euralis Semences, France), Cordoba (originator – AgReliant Genetics Inc., Canada), and Moravia (originator – Semences Prograin Inc., Canada). The land type was arable, with the soil being typical low-humus chernozem. Mineral fertilizers (at a rate of P₃₀) were applied in a split, broadcast method by hand according to the experimental scheme, prior to autumn plowing.

For the inoculation of soybean seeds, a combination of three synergistic indigenous strains of nodulating bacteria *Bradyrhizobium japonicum* (Kirchner, 1896) Jordan, 1982: eko/001, eko/002, and eko/003 (titer 10 billion per 1 mL/g) was used. These strains are stored in the microorganism museum at the Institute of Agrobiology LLC (active ingredients of the products). Strains of *B. japonicum* were cultured in yeast extract mannitol (YEM) broth at 28 ± 2 °C for 5 days under constant shaking at 150 rpm (Vincent, 1970). The inoculation of soybean seeds with the three-component combination of *B. japonicum* strains eko/001, eko/002, and eko/003 was carried out on the day of sowing. Approximately 2 mL of suspension was applied per 100 g of seeds, followed by incubation for 1 h at room temperature in the shade.

The bacterial-mycetous complex was represented by a ternary composition of active strains of phosphate-mobilizing microorganisms – *Bacillus megaterium* eko/207, *Bacillus amyloliquefaciens* eko/205, and *Trichoderma harzianum* eko/101 (titer 1 billion per 1 mL/g), also from the culture collection of the Institute of Agrobiology LLC (active ingredients of the products).

Phosphate-mobilizing microorganisms (*B. megaterium*, *B. amyloliquefaciens*, and *T. harzianum*) were applied in the form of biologically active granules containing viable spores of the respective strains. These granules were incorporated into the soil at sowing to ensure local colonization of the rhizosphere and mobilization of phosphorus compounds. *Bacillus* spp. were cultured in nutrient broth at 28 ± 2 °C for 3–5 days under static conditions, following standard microbiological procedures (Cappuccino & Sherman, 2014). *Trichoderma harzianum* was cultured in potato dextrose broth (PDB) at 28 ± 2 °C for 4–5 days (Aneja, 2003).

The field experiment design included the following variants: 1) control – without fertilizers and inoculation with the *B. japonicum* strain combination; 2) phosphorus fertilization (P₃₀) without inoculation with the *B. japonicum* strain combination; 3) bacterial-mycetous bio-fertilization (BM) based on the association of *B. megaterium*, *B. amyloliquefaciens*, and *T. harzianum* without inoculation with the *B. japonicum* strain combination; 4) without fertilizers (control) + inoculation with the *B. japonicum* strain combination; 5) P₃₀ + inoculation with the *B. japonicum* strain combination; 6) BM + inoculation with the *B. japonicum* strain combination.

Sowing was carried out when the soil temperature at the seed placement depth was +10–12 °C, with a row spacing of 15 cm. The research was conducted using the “Methodology of agronomic research in agronomy” (Didora *et al.*, 2013). Statistical data from government sources and reference materials from scientific publications were also used (Ministry of Economic Development, Trade and Agriculture, 2021; Information and reference system “Sort”, n. d.). Throughout all years of vegetation, regular phenological observations and biometric records were conducted.

The average yield was determined by the ratio of the mass of harvested grain to the total area of fields where it was grown according to the methodology of the Ukrainian Institute for Plant Variety Examination (Tkachyk *et al.*, 2017). Soybeans were harvested in sections using direct threshing with a small-plot combine harvester during the fully ripe phase, when seed moisture content was 14–16 %. The content of “crude protein” (protein) was determined using infrared spectroscopy according to the State Standard of Ukraine DSTU 4117:2007 (State Consumer Standard of Ukraine, 2007).

The statistical processing of the obtained primary data was carried out using descriptive and variation statistics methods, with the use of IBM SPSS Statistics (v.23.0) and Microsoft Excel 2016 data processing software. To describe general quantitative patterns, the main statistical indicator – the measure of central tendency – was used, with the calculation of the arithmetic mean (\bar{x}). The trend analysis for grain yield formation and seed quality of soybeans in the respective comparison variants was performed using multifactorial analysis of variance (ANOVA). If necessary, an additional post hoc comparison (Tukey’s HSD test) was carried out. The threshold for statistical significance was set at the generally accepted 5 % level (or $p \leq 0.05$).

Experimental studies of plants (both cultivated and wild), including the collection of plant material, complied with institutional, national, or international guidelines. The authors adhered to the standards of the Convention on Biological Diversity (CBD) (Verkhovna Rada of Ukraine, 2010) and the Convention on Trade in Endangered Species of Wild Fauna and Flora (CITES) (Verkhovna Rada of Ukraine, 1999).

RESULTS

Research showed that in the first year of the study, the average soybean yields for the Mentor, Cordoba, and Moravia varieties were 2.89, 2.70, and 2.46 t/ha, respectively. In the second year, yields slightly declined to 2.67, 2.68, and 2.22 t/ha. However, in the final and most favorable year, the respective yields increased to 2.73, 2.91, and 3.32 t/ha.

It was found that the average yield of all soybean varieties (Mentor, Cordoba, and Moravia) with inoculation using the combination of *B. japonicum* strains eko/001, eko/002, and eko/003 was higher than the corresponding values without inoculation for each year of the study. Specifically, in 2019, the soybean yield (averaged across all variants) with and without inoculation ranged from 2.33 to 3.02 t/ha. In 2020, the yield of the crop decreased to 2.10 and 2.78 t/ha, respectively. However, in the final year, 2021, the yield peaked, reaching 2.58 and 3.62 t/ha, respectively.

The average yield of all soybean varieties (Mentor, Cordoba, and Moravia) in the control group without inoculation (overall) during the study period was 2.38 t/ha, while with inoculation, it was 2.68 t/ha. It was established that the highest yields in the control variants (both with and without inoculation) were achieved by the Mentor and Cordoba varieties, which exhibited almost identical values – 2.70 and 2.71 t/ha, respectively. The lowest yield was observed in the early-maturing Moravia variety, with a yield of 2.28 t/ha

(Table 1; Fig. 2). The yield analysis also showed that, overall, all soybean varieties in the experimental variants (P_{30} and BM) demonstrated increased yields compared to the control group (both with and without inoculation) (Table 1; Fig. 2).

Table 1. Yield (t/ha) of soybean varieties depending on inoculation with the combination of *B. japonicum* strains eko/001, eko/002, and eko/003 and the phosphorus fertilization background (average for 2019–2021)

Varieties (Factor C)	Pre-sowing seed treatment (Factor A)										Ave to (C)
	Without inoculation					Inoculation					
	Nutrient background (Factor B)										
	1	2	± to C	3	± to C	4	5	± to C	6	± to C	
	C	P ₃₀		BM		C	P ₃₀		BM		
Early-maturing group											
Mentor	2.45	2.73	+0.28	2.73	+0.28	2.70	2.96	+0.26	3.01	+0.31	2.75
Medium-early group											
Cordoba	2.43	2.65	+0.22	2.72	+0.29	2.71	3.01	+0.30	3.08	+0.37	2.76
Moravia	2.28	2.56	+0.28	2.62	+0.34	2.63	2.83	+0.20	3.11	+0.48	2.67
Ave to (A)	2.57					2.89					–
Ave to (B)	2.38	2.64	+0.26	2.69	+0.30	2.68	2.93	+0.25	3.06	+0.38	–
sign. F	Factor A*; Factor B*; Factor Cns; Interaction: A × Bns; A × Cns; B × Cns; A × B × Cns										

Comments: Conventional symbols: ave to (A), (B), (C) – average value for the corresponding factor; C – control; P_{30} – phosphorus nutrient element; BM – bacterial-mycetous bio-fertilization (ternary association of phosphate-mobilizing organisms); sign. F – ANOVA F-value (ns, not significant; * – $p < 0.05$). Source: authors' own elaboration

According to our observations, the highest yield increase (in %) compared to the corresponding control indicator was recorded for the Moravia variety in the BM variant, both with and without inoculation: +14.74 % and +18.28 %, respectively. The lowest yield increase (%) compared to the corresponding control in the P_{30} variant was observed for the Moravia variety, at +7.70 %, while without inoculation, the lowest increase in yield compared to the control was noted for the Cordoba variety in the P_{30} variant at +9.12 % (Table 1; Fig. 2).

Specifically, when compared to the control, the use of P_{30} without inoculation resulted in an average yield increase of +11.39 % for the early-maturing Mentor variety, and with inoculation, the increase was +9.55 %. The application of BM bio-fertilization in soybean crops of the Mentor variety also resulted in a yield increase both with and without inoculation: +11.53 % and +11.27 %, respectively (Table 1; Fig. 2).

It was found that the yield increase for the medium-early Cordoba variety when using P_{30} was +9.12 % without inoculation and +10.72 % with inoculation, compared to the control. The yield of this soybean variety under BM bio-fertilization showed an increase (compared to the corresponding control) both with and without inoculation: +12.18 % and +13.58 %, respectively (see Table 1; Fig. 2).

On average, the highest yield increase for the medium-early Moravia variety, compared to the control, was observed when applying P_{30} both without inoculation and with inoculation: +12.37 % and +7.70 %, respectively. A slightly higher increase was recorded with the use of BM both without inoculation and with inoculation: +14.74 % and +18.28 %, respectively (see **Table 1**; **Fig. 2**).

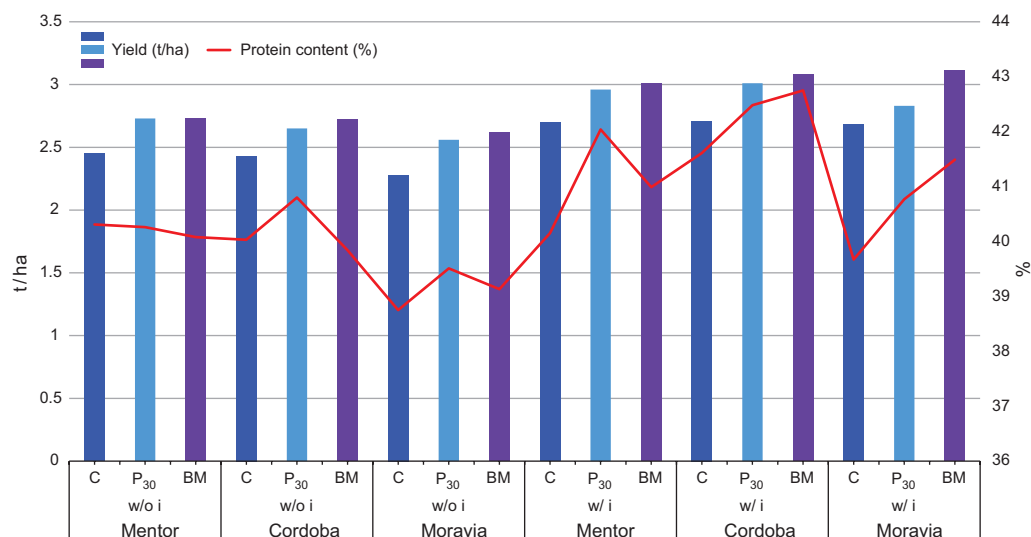


Fig. 2. Yield (t/ha) and protein content (%) of soybean varieties depending on phosphorus nutrition backgrounds (Control (C), P_{30} , BM) and in combination with inoculation (with inoculation, labeled as "w/ i") or without inoculation (labeled as "w/o i") of *B. japonicum* strains eko/001, eko/002, and eko/003 (average for 2019–2021). Source: authors' own elaboration

Based on the results of the analysis of variance, it was shown that the most significant factors were: the seed treatment variant with the combination of *B. japonicum* strains eko/001, eko/002, and eko/003 (Factor A) – 17.0 % ($F(3.888) = 49.620$, $p < 0.05$), phosphorus nutrient background (Factor B) – 14.0 % ($F(2.650) = 20.480$, $p < 0.05$), and variety by maturity group (Factor C) – 1.2 % ($F(2.650) = 1.838$, $p > 0.05$). The interaction of the main factors (AB, AC, BC, ABC) was not significant (Factual < Ftheoretical, p-value > 0.05), and their influence was almost absent or minimal – up to 1 % (0.2–0.4 %). The remaining "random" factors accounted for 66.5 % of the total variance (**Fig. 3**).

Post-hoc pairwise comparison of the main effects on yield indicators between groups using Tukey's test revealed that for Factor A, all levels showed significant differences ($p < 0.05$). For Factor B, the control group significantly differed from the P_{30} group ($p < 0.05$) and the BM group ($p < 0.05$), but the P_{30} group did not significantly differ from the BM group ($p > 0.05$). Factor C did not require further investigation, as its main effect was insignificant.

According to the biochemical analysis, the protein content in soybean seeds ranged from 38.75 % to 42.75 %, depending on the maturity group of the variety, seed treatment variant, and phosphorus nutrition background (see **Table 2**). The protein content in the seeds of the investigated soybean varieties remained relatively consistent throughout the study period, with a tendency towards higher values in the inoculation treatment using *B. japonicum* strains eko/001, eko/002, and eko/003. In the seeds of

the varieties (Mentor, Cordoba, and Moravia), the average protein content was 40.6 %, 41.2 %, and 40.5 %, respectively (see **Table 2**; **Fig. 2**). The protein content in the seeds of the soybean varieties did not show significant variation across the years of the study.

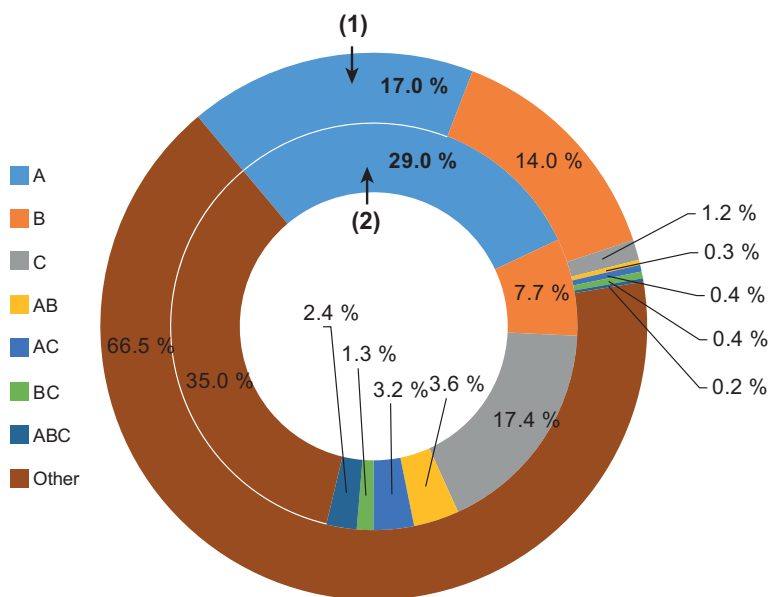


Fig. 3. Contribution (%) of factors to the variability of soybean yield (1) and protein content (2) (2019–2021): Factor A – seed treatment variant with *B. japonicum* strains eko/001, eko/002, and eko/003; Factor B – phosphorus nutrition background; Factor C – variety by maturity group; AB, AC, BC, ABC – interaction of main factors; Other – residual portion. Source: authors' own elaboration

The results revealed an increase in protein content among all investigated soybean varieties in all experimental variants (P_{30} and BM) with inoculation by the combination of *B. japonicum* strains eko/001, eko/002, and eko/003, compared to the control. The protein content increase ranged from +2.09 % to +4.71 % (see **Table 2**; **Fig. 2**). A trend toward higher protein content in the soybean seeds was also observed in both experimental variants (P_{30} and BM) compared to the control in the absence of inoculation by the combination of *B. japonicum* strains eko/001, eko/002, and eko/003 in the Moravia variety, and in only one variant (P_{30}) for the Cordoba variety (P_{30} variant: +1.98 % and +1.92 %; BM variant: +0.99 % and -0.47 %). The Mentor variety was the only one (except for the Cordoba variety in the BM variant) that showed a decrease in protein content in the absence of inoculation in all experimental variants (P_{30} and BM), with decreases of -0.12 % and -0.58 %, respectively, compared to the control (see **Table 2**; **Fig. 2**).

The analysis of variance revealed that the following factors had the most significant impact on protein content: seed treatment with a combination of *B. japonicum* strains eko/001, eko/002, and eko/003 (Factor A) – 29.0 % ($F(3.888) = 21.353$, $p < 0.05$), phosphorus nutrition background (Factor B) – 7.7 % ($F(3.041) = 21.353$, $p < 0.05$), and variety according to maturity group (Factor C) – 17.4 % ($F(3.041) = 48.471$, $p < 0.05$). The interaction of the main factors (AB; AC; BC; ABC) was significant for (AB) and (AC), recorded at nearly the same level, respectively 3.6 % ($F(3.041) = 10.036$, $p < 0.05$) and 3.2 % ($F(3.041) = 6.343$, $p < 0.05$). In the other cases, interactions (BC and ABC) had

an F-value lower than the theoretical F-value ($F_{\text{fact.}} < F_{\text{theory}}$, $p > 0.05$), and their effect was minor, ranging from 1.3 % to 2.4 %. The residual portion in the total variance of protein content accounted for 35 % (**Fig. 3**).

Table 2. The protein content in soybean seeds depending on the cultivation variant, % (average for 2019–2021)

Varieties (Factor C)	Pre-sowing seed treatment (Factor A)										Ave to (C)
	Without inoculation					Inoculation					
	Nutrient background (Factor B)										
	1	2	± to C	3	± to C	4	5	± to C	6	± to C	
	C	P ₃₀		BM		C	P ₃₀		BM		
Early-maturing group											
Mentor	40.31	40.26	-0.05	40.08	-0.23	40.15	42.04	+1.89	40.99	+0.84	40.63
Medium-early group											
Cordoba	40.03	40.80	+0.77	39.84	-0.19	41.61	42.48	+0.87	42.75	+0.27	41.21
Moravia	38.75	39.51	+0.76	39.13	+0.38	39.67	40.77	+1.10	41.49	+0.72	39.88
Ave to (A)	39.79					41.26					–
Ave to (B)	39.60	40.19	–	39.60	–	40.40	41.70	–	41.70	–	–
sign. F	Factor A*; Factor B*; Factor C*; Interaction: A × B*; A × C*; B × Cns; A × B × Cns										

Comments: Conventional symbols: ave to (A), (B), (C) – average value for the corresponding factor; C – control; P₃₀ – phosphorus nutrient element; BM – bacterial-mycetous bio-fertilization (ternary association of phosphate-mobilizing organisms); *sign. F* – ANOVA F-value (ns, not significant; *, $p < 0.05$). Source: Authors' own elaboration

A posterior comparison of the main effects and some of their interactions regarding protein content between groups using Tukey's test showed that for Factor A, all levels exhibited significant differences ($p < 0.05$). For Factor B, the control group significantly differed from both the P₃₀ ($p < 0.05$) and BM groups ($p < 0.05$), but the P₃₀ group did not significantly differ from the BM group ($p > 0.05$). For Factor C, all levels showed significant differences ($p < 0.05$). Pairwise comparison for the factor AB revealed that the group (AB₁) "no inoculation/control" significantly differed from the groups "inoculation/control," "inoculation/ P₃₀," and "inoculation/BM" ($p < 0.05$), but did not differ from the groups "no inoculation/ P₃₀" and "no inoculation/BM" ($p > 0.05$). The group (AB₂) "inoculation/control" significantly differed from the groups "inoculation/P₃₀" "no inoculation/BM," and "inoculation/BM" ($p < 0.05$), but did not differ from the "no inoculation/ P₃₀" group ($p > 0.05$). The group (AB₃) "no inoculation/P₃₀" significantly differed from the groups "inoculation/P₃₀" and "inoculation/BM" ($p < 0.05$), but did not differ from the "no inoculation/BM" group ($p > 0.05$). The group (AB₄) "inoculation/P₃₀" significantly differed from the "no inoculation/BM" group ($p < 0.05$), but did not differ from the "no inoculation/ BM" group ($p > 0.05$). The group (AB₅) "no inoculation/BM" significantly differed from the "inoculation/BM" group ($p < 0.05$). Other interaction factors (AC, BC, and ABC) did not require further investigation as their main effect was insignificant.

DISCUSSION

Yield is a key integral indicator of the effectiveness of agronomic strategies for crop production, particularly for soybeans. It reflects the influence of soil-climatic conditions and cultivation practices on crop productivity and harvest outcomes. At the same time, the quality of the harvested product is equally important. For soybeans, seed quality is largely determined by the variety's biological characteristics, environmental conditions, and applied agronomic technologies, with crude protein content serving as the main quality parameter (Hubenko *et al.*, 2019). Thus, an integrated assessment of both yield and seed quality is required to evaluate the effectiveness of strategies for the improvement of soybean cultivation.

An analysis of the available data in the State Register of Varieties Eligible for Distribution in Ukraine as of 2021 (Ministry of Economic Development, Trade and Agriculture, 2021) and official descriptions of plant varieties and agronomic suitability indicators presented in the "Protection of Plant Variety Rights" Bulletins, available in the Information and Reference System "Sort" (Information and reference system "Sort", n. d.), showed that the yield of the early-maturing soybean variety Mentor in Ukraine is: Polissya – 2.49 t/ha, Forest-Steppe – 2.25 t/ha, and Steppe – 1.97 t/ha, with protein content in the seeds ranging from 39.6 % to 40.7 %. For the medium-early soybean varieties, Cordoba and Moravia, the average yield for the Cordoba variety is: Polissya – 2.07 t/ha, Forest-Steppe – 2.37 t/ha, and Steppe (irrigation) – 1.76 t/ha, with protein content ranging from 38.0 % to 39.2 %; for the Moravia variety, these indicators are on average 2.00 t/ha for yield and 41 % for protein content.

The obtained results demonstrate the importance of an integrated approach to soybean cultivation that combines mineral phosphorus fertilization, phosphate-mobilizing microorganisms (PMOs), and inoculation with nitrogen-fixing rhizobia. Each of these factors individually contributes to the improvement of plant nutrition and symbiotic efficiency, but their combined application ensures a synergistic effect that optimizes yield and seed quality.

Numerous studies have confirmed the general positive effect of phosphorus fertilization on soybean productivity. For example, M. R. Kabiru *et al.* (2024) reported yield improvements of up to 0.42 t/ha when phosphorus was applied in moderate rates. Similarly, S. Adjei-Nsiah *et al.* (2022) found that phosphorus fertilization and inoculation significantly increased soybean grain yield by 88 % and 108 %, respectively, compared to the control in the semi-deciduous forest agro-ecological zone of Ghana. *Notably*, the combined application of phosphorus fertilizer and *Bradyrhizobium* inoculant resulted in a threefold increase in grain yield compared to the control.

However, phosphorus efficiency in soils often remains limited due to fixation in unavailable forms. In this context, PMOs, such as *Bacillus megaterium*, *B. amyloliquefaciens*, and *Trichoderma harzianum*, are gaining attention for their ability to solubilize bound phosphorus and promote root development. M. Satyaprakash *et al.* (2017) demonstrated that co-application of PMOs with mineral phosphorus increased soybean yield by 12–18 % and improved nutrient use efficiency. S. K. Singh *et al.* (2025) also found a 15–20 % increase in nodulation and protein content in soybean when *Bacillus* spp. were applied in phosphorus-deficient soils.

The role of *Bradyrhizobium japonicum* inoculation remains central to sustainable soybean cultivation due to its ability to biologically fix nitrogen. In our study, seed inoculation with indigenous strains of *B. japonicum* (eko/001–003) consistently improved

yield in all experimental years. This confirms earlier work by M. Hungria *et al.* (2005), who reported that inoculation increased soybean yield by 0.4–0.6 t/ha and raised seed protein content by 1.5–2.3 %, depending on soil fertility and weather conditions.

Importantly, the combined use of P_{30} fertilization, PMOs, and rhizobial inoculation had the greatest positive effect on yield and seed quality. This synergistic interaction likely enhances both phosphorus and nitrogen uptake, as suggested by H. B. Houngnandan *et al.* (2020), who observed that this tripartite approach improved nutrient acquisition, nodulation, and productivity in legume crops.

At the same time, an analysis of the available literature data dedicated to specific aspects of soybean cultivation technology for these varieties in the Forest-Steppe Zone of Ukraine revealed higher yields and better product quality. As is well known, varieties of different genotypic origins do not realize their potential productivity in the same way. Some varieties significantly reduce their yield in the absence of optimal nutrition and plant protection measures, while others can provide high productivity under any, even unfavorable, growing conditions (Sydiakina & Ivaniv, 2023).

According to H. L. Hadzovskyi and N. V. Novytska (2018), when inoculating soybean seeds with *Bradyrhizobium japonicum* 532c bacteria (Legum Fix preparation) and treating crops with Vuks Oil Seed (a fertilizer suspension containing boron, manganese, and molybdenum), the early-maturing Mentor variety in the conditions of the Western Forest-Steppe (Volyn region) formed a maximum yield of 3.45 t/ha.

Research by I. V. Fedoruk (2019) in the Forest-Steppe Zone of Khmelnytskyi Oblast on the impact of soybean seed inoculation with different maturity groups, including the medium-maturing Cordoba variety, showed positive results regarding yield indicators from the use of the inoculants HiCot Super, HiCot Super Extender (which contain the highly effective strain 532C of the nodulating bacterium *B. japonicum*), as well as from the use of the preparations Standak Top and Vuksal Boron (suspension with high boron, nitrogen, and phosphorus content). The average yield of the Cordoba variety ranged from 2.53 to 3.49 t/ha, depending on the experimental variant.

Other studies (Baida, 2021) on the effectiveness of cultivating the Cordoba soybean variety under the influence of various technological factors, including micronutrients with molybdenum and phosphorus content (Yara Vita Molitrak during the budding stage + during flowering) and growth regulators with compositions of biologically active compounds – phytohormone analogs, amino acids, and fatty acids (Biosil, Radostim) – showed that the best yield for the Cordoba variety was achieved with the application of Yara Vita Molitrak during the budding stage + Radostim, reaching 3.03 t/ha. Additionally, when applying Yara Vita Molitrak twice in combination with Biosil or Radostim, the yield was 3.03 t/ha and 3.07 t/ha, respectively. The protein content (%) in the Cordoba variety, according to the author's data (Baida, 2021), was 43.5 % when treated with Yara Vita Molitrak (during the budding stage + Radostim), and 43.3 % and 46.6 % when using Yara Vita Molitrak during the budding stage + flowering phase, combined with Biosil or Radostim.

Summarizing the above, it can be noted that the results of our research align in some aspects and differ in others compared to other studies regarding the potential yield and quality (protein content) of the seeds of these soybean varieties under the influence of various agronomic practices in the Forest-Steppe Zone of Ukraine.

Overall, according to the literature data for the Forest-Steppe Zone, these varieties can be ranked in terms of yield (t/ha) in the following descending order: Cordoba > Mentor > Moravia. As for protein content (%), the varieties rank as follows: Moravia > Mentor > Cordoba.

Our three-year studies have shown that in terms of yield, these varieties, under all feeding backgrounds and without inoculation, are arranged similarly. However, a completely different pattern is observed when seeds are inoculated with a combination of *B. japonicum* strains eko/001, eko/002, and eko/003 using phosphorus biofertilizer (BM), where the ranking changes to the following (in descending order): Moravia > Cordoba > Mentor. As for the protein content in the experimental soybean varieties, their ranking (in descending order) both with and without inoculation across all feeding backgrounds differs from the aforementioned general literature data, and is as follows: Cordoba > Mentor > Moravia.

Our findings revealed a significant main effect of Factor (A) in the overall variance, suggesting that the process of seed treatment with a synergistic combination of three strains (eko/001, eko/002, and eko/003) of *Bradyrhizobium japonicum* bacteria significantly affects both the yield and protein content of soybeans, thus emphasizing the importance of using inoculation with nitrogen-fixing bacteria. The significant main effect of Factor (B) indicates that the type of phosphorus fertilization background plays a crucial role in soybean yield. However, its impact on protein content in the seeds was lower, highlighting the need for a rational approach to the selection of phosphorus fertilizers and the use of biofertilization based on phosphate-mobilizing bioagents. Although the effect of factor (C) related to the maturity group of the variety was not significant for yield, the small share of its influence suggests that it may be worth investigating in future studies, as this could have practical implications under specific soil-climatic conditions. Regarding protein content, the effect of Factor (C) was significant, with its influence increasing by 14.5 times compared to yield.

The interaction of these factors was less pronounced than their individual effects, both in terms of yield and protein content. However, in the latter case, the combined effect was 9.5 times higher and statistically significant. Therefore, the authors recommend that future studies more thoroughly examine the interaction between seed treatment with rhizobial bacteria, the nutritional background (particularly biological preparations with phosphate-mobilizing microorganisms and mineral fertilizers in combinations), and varietal characteristics, to better understand which factors (and their combined effects) influence the variability of yield and the improvement of product quality. The high residual share in the total variance regarding soybean yield (66.5 %) requires consideration of other characteristics during the crop's growth aimed at forming seed productivity indicators. Regarding protein content, the share of random factors was almost halved (35 %), but still remained at a significant level.

CONCLUSIONS

In the Central Forest-Steppe Zone conditions, the integration of seed inoculation with *Bradyrhizobium japonicum* strains (eko/001, eko/002, and eko/003), mineral phosphorus fertilizers, and biofertilizers containing *Bacillus megaterium* (eko/207), *B. amyloliquefaciens* (eko/205), and *T. harzianum* (eko/101) significantly increased the productivity and quality of early- and mid-early-maturing soybean varieties (*G. max* (L.) Merrill).

Yield gains ranged from 8 % to 18 %, with the best results in the combined seed inoculation with a composition of *B. japonicum* strains + application of bacterial-mycromycete ternary composition of phosphate-mobilizing organisms (*B. megaterium*, *B. amyloliquefaciens*, and *T. harzianum*) variant. Protein content rose by 2–5 %, especially in mid-early varieties.

The most influential factor (in %) for yield was inoculation using nitrogen-fixing bacterial strains (17 %), followed by nutrient background (14 %). The share of the effect of the “variety by maturity group” factor was not significant and amounted to 1 %. Factor interactions were minor or insignificant, with a range of up to 1 %. For protein content, inoculation accounted for 29 %, variety – 17 %, and nutrition – 8 %. In some cases, the interaction of these factors was significant and recorded at levels ranging from 1 % to 4 %.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that the study was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

Animal rights. This article does not include animal studies.

AUTHOR CONTRIBUTIONS

Conceptualization, [Ch.Ya.; B.I.]; methodology, [Ch.Ya.; B.I.; M.M.; K.A.]; validation, [Ch.Ya.; B.I.; K.A.]; formal analysis, [Ch.Ya.; B.I.; M.M.; K.A.]; investigation, [Ch.Ya.; B.I.; K.A.]; resources, [Ch.Ya.; B.I.]; data curation, [Ch.Ya.; B.I.]; writing – review and editing, [Ch.Ya.; B.I.; K.A.]; visualization, [M.M.; K.A.]; supervision, [Ch.Ya.; B.I.]; project administration, [Ch.Ya.]; funding acquisition, [Ch.Ya.; B.I.].

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ВПЛИВ ІНОКУЛЯЦІЇ ТА ФОСФОРНОГО ЖИВЛЕННЯ НА ВРОЖАЙНІСТЬ І ЯКІСТЬ НАСІННЯ СОРТІВ СОЇ В ЦЕНТРАЛЬНОМУ ЛІСОСТЕПУ

Ярослав Чабанюк^{1,2}, Ірина Бровко², Андрій Ковтун², Мирослава Мілова¹

¹ Національний університет біоресурсів і природокористування України,
вул. Героїв Оборони, 15, Київ 03041, Україна

² ТОВ "Інститут агробіології", бульв. Вацлава Гавела, буд. 4, корп. 45, Київ 03067, Україна

Обґрунтування. Дослідження показують, що взаємозв'язок між врожайністю насіння сої *Glycine max* (L.) Merrill і азотом (N) добре вивчений, тоді як вплив інших елементів (зокрема, фосфору P), вивчено недостатньо. Фосфор, другий за важливістю після азоту, є ключовим для отримання якісного врожаю сої. Водночас, попри значну кількість досліджень дії фосфатмобілізаторів і азотофіксаторів щодо їхнього впливу на сталий розвиток сільського господарства, результати цих досліджень залишаються на початковій стадії.

Матеріали та методи. Використовували загальнонаукові та спеціальні методи, зокрема, польовий, лабораторний, хімічний і статистичний. Дослідження проводили в умовах Лісостепу впродовж 2019–2021 рр. на дослідному полі ТОВ "Інститут агробіології" (с. Великий Митник, Хмельницький район, Вінницька область). Вирощували три сорти сої іноземної селекції: Ментор (ранньостиглий), Кордоба та Моравія (середньоранні сорти) зі застосуванням мінерального фосфорного живлення (P_{30}) і біоживлення на основі тернарної композиції штамів фосфатмобілізуючих організмів – *Bacillus megaterium* de Bary, 1884 (eko/207), *B. amyloliquefaciens* (ex Fukumoto, 1943) Priest, Goodfellow, Shute & Berkeley, 1987 (eko/205) та *Trichoderma harisianum* Rifai, 1969 (eko/101) на тлі без інокуляції та з інокуляцією насіння композицією кількох штамів бактерій *Bradyrhizobium japonicum* (Kirchner, 1896) Jordan, 1982 із колекції культур ТОВ "Інститут агробіології" (діючі інгредієнти препаратів).

Результати. Усі сорти досліду позитивно відреагували на застосування фосфорного живлення, біоживлення на основі штамів фосфатмобілізуючих організмів на тлі без інокуляції та з інокуляцією насіння штамми азотфіксувальних бактерій. Загалом приріст врожаю (у %) зерна сої у дослідних варіантах коливався в межах від +7,70 до +18,28 %. Найкращі показники врожайності (порівняно з контролем) фіксували у варіанті з поєднанням процесу інокуляції та застосування бактеріально-мікроміцетного (БМ) біоживлення, що дало можливість отримати приріст врожайності зерна: +0,31 т/га, або 11,27 % (Ментор); +0,31 т/га, або 13,58 % (Кордоба); +0,48 т/га, або 18,28 % (Моравія). Вміст білка (%) в насінні сої перебував у межах від 38,75 до 42,75, залежно від сорту, варіанта обробки і тла живлення. Середньоранній сорт Моравія був єдиним, який характеризувався відсотковим приростом білка в усіх дослідних варіантах (P_{30} та БМ) на тлі без інокуляції та з інокуляцією насіння, на відміну від решти сортів (Ментор і Кордоба), у яких встановлено кількісне зменшення показників вмісту білка у дослідних варіантах P_{30} та БМ (порівняно з контролем) лише на тлі без інокуляції насіння.

Висновки. У центральному Лісостепу України поєднання інокуляції насіння з фосфорним удобренням та біодобривами на основі фосфатмобілізуючих мікроорганізмів значно покращило врожайність сої та вміст білка у насінні. У загальній

дисперсії “факторна” частка впливу (у %) на врожайність була нижчою, ніж на вміст білка, становлячи 34 % та 67 % відповідно. Найбільший вплив на вміст білка мала інокуляція (29 %), тоді як на врожайність найбільше впливали інокуляція (17 %) та забезпечення фосфором (14 %). Фактори, пов’язані зі сортом, мали менший вплив на врожайність, однак внесли 17 % до варіації вмісту білка. Взаємодія факторів була здебільшого незначною для врожайності, але помітною для вмісту білка.

Ключові слова: соя культурна, *Glycine max* (L.) Merrill, фосфорне живлення, фосфатмобілізуючі мікроорганізми, урожайність, вміст білка