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# Enhancing soybean adaptive potential and productivity with endophyte-rhizobial inoculation

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**Abstract.** The research aim was to investigate the effect of pre-sowing complex inoculation with endophytic and nodule bacteria on the rhizosphere microbiocenosis, soil macronutrient content, adaptive potential and productivity of soybean under the influence of stressful weather and climatic factors. A split-plots design was used to conduct a two-factor field experiment with soybean varieties of different maturity groups: the ultra-early maturing 'Diona' and the medium-early maturing 'Sviatohor'. Microbiological methods were used to obtain inoculants based on nodule and endophytic bacteria (*Bradyrhizobium japonicum* as part of the complex bioformulation Ryzobin$^{K}$ and their mixtures with endophytic bacteria *Bacillus velezensis* IMV B-8134, B-8135, *Pseudomonas* sp. 6), as well as to determine the abundance of microorganisms from various ecological-functional groups by inoculating soil suspensions onto selective agar media. Agrochemical methods were used to determine soil macronutrients, and statistical methods were applied for data analysis. The stimulatory and stress-protective effects of co-inoculation of seeds with nodule and endophytic bacteria on microbial communities in the rhizosphere soil, as well as on the development and yield

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of various soybean varieties on irrigated lands in southern Ukraine, have been demonstrated. An increase in the number of microorganisms belonging to the main ecological- functional groups and in the content of available forms of nitrogen, phosphorus and potassium in the rhizosphere soil was established. The most effective treatment in terms of synergistic action on soybean was found to be the treatment of seeds with Ryzobin$^{K}$ in combination with *Bacillus velezensis* IMB B-8134. The average yield for the 'Diona' variety was 2.69 t/ha and for the 'Sviatohor' variety – 2.95 t/ha, which exceeded the control values by 38-40%. Practical value lies in effective pre-sowing endophyte-rhizobial co-inoculation of soybean seeds, improving productivity and stability of agrophytocenoses under climate change

**Keywords:** *Glycine max* (L.) Merr.; symbiotic bacteria; stressful climate factors; irrigation; rhizosphere microbiota; macronutrients; effectiveness

## INTRODUCTION

In the context of global climate change, the design of agricultural systems with enhanced resistance to stress factors has become one of the priority areas of scientific research and innovation. The use of biological preparations based on active strains of symbiotic microorganisms capable of forming mutualistic relationships with the host plant contributes to plant adaptation to extreme environmental conditions and increases crop productivity. The use of complex multi-strain formulations with a multi-vector stress-protective effect is particularly promising. As noted by L. Shangguan (2023), global warming alters the duration of the growing season and the geographical distribution of agricultural crops, resulting in some traditional cultivation regions potentially losing suitability for certain crops, while the demand for water resources increases. At the same time, W. Zhu *et al*. (2024) emphasised that changes in precipitation patterns negatively affect plant growth and productivity, which may lead to yield reductions and create risks to food security. According to M. Yanagi (2024), climate change affects the distribution and availability of water resources, potentially causing water shortages and droughts and, consequently, significantly complicating irrigated agriculture. Soybean (*Glycine max* (L.) Merr.) is an important global agricultural crop that supplies protein and oil for the food industry and feed production. However, soybean is highly vulnerable to various abiotic stresses, particularly drought and heat during the flowering stage, which may significantly reduce yield (Xia *et al*., 2026). Soybean grows best at temperatures between 20°C and 25°C, but prolonged exposure to temperatures above 29°C results in a reduction in bean number and seed weight. Temperatures above 35°C severely disrupt flowering, bean setting and grain filling (Thenveettil *et al*., 2025). With ongoing global climate change, the simultaneous occurrence of heat and drought stress is becoming increasingly frequent, especially in semi-arid and drought-prone regions.

Modern agricultural practices, as highlighted by H. Tariq *et al.* (2025), depend heavily on synthetic fertilisers and pesticides, which are major contributors to greenhouse gas emissions, groundwater contamination and disruption of agroecosystem dynamics. These issues underline the urgent need for sustainable alternatives that maintain crop productivity while minimising environmental impact. In the study by I.S. Marquez *et al.* (2025), it was shown that despite the significant influence of abiotic factors – temperature, precipitation regime and nutrient availability – on plant resilience and productivity, interactions with microorganisms constitute an important mechanism for improving physiological status and yield. According to D.M. Zeffa *et al.* (2020), co-inoculation of soybean with *Bradyrhizobium* strains and plant growth-promoting rhizobacteria provides a synergistic effect manifested in enhanced root system development, increased nodule formation, improved efficiency of symbiotic nitrogen fixation and, consequently, increased productivity. Bacterial endophytes, both rhizobial and non-rhizobial, are considered critically important for the growth and resilience of legume host plants, as they participate in atmospheric nitrogen fixation, phytohormone synthesis, nutrient mobilisation and regulation of metabolic processes, while also increasing plant tolerance to abiotic and biotic stresses, including drought, temperature fluctuations and pathogen infection (Mayhood & Mirza, 2021). According to N. Shevchuk *et al.* (2025) and Y. Zhakypbek *et al.* (2026), due to their localisation within internal plant compartments, endophytes are able to interact closely with plant tissues and influence processes such as regulation of water balance and stomatal activity, nutrient distribution and stress signalling pathways, thereby contributing to improved tolerance to water deficit. The development and implementation of new environmentally safe biological preparations based on rhizobial and non-rhizobial endophytic bacteria therefore remain highly relevant. Accordingly, the aim of the study was to determine the effect of pre-sowing complex inoculation with endophytic and nodule-forming bacteria on the rhizosphere microbiocenosis, soil macronutrient content, adaptive potential and productivity of soybean varieties differing in maturity group under the influence of stressful weather and climatic factors.

## MATERIALS AND METHODS

Field experiments were conducted during 2024-2025 on irrigated land located in the urban-type settlement of Naddniprianske, Kherson Region, on a specially equipped irrigated field site, following generally accepted agronomic methods and methodological guidelines. The geographical coordinates of the site were 46°44'53.6" N latitude and 32°42'55.7" E longitude, at an altitude of 50 m above sea level. The soil was dark chestnut, medium loamy, slightly solonetzic, formed on carbonate loess. The humus content (according to Tyurin) in the arable layer was 2.2%, total nitrogen 0.17%, available phosphorus (according to Machigin) 30.0 mg/kg, and exchangeable potassium 300 mg/kg of soil. The lowest moisture capacity of the 0-100 cm soil layer was 21.3%, and the wilting point was 9.5% relative to the mass of absolutely dry soil. Bulk density was 1.41 t/m$^3$, and the pH of the aqueous soil extract in the arable layer ranged from 6.8 to 7.2. A two-factor field experiment was established using a split-plot design. The sowing plot area was 15 m$^2$ and the accounting plot area was 10 m$^2$, with four replications. In the experimental design, the main plots (first-order plots, factor A) consisted of soybean varieties: the ultra-early maturing ‘Diona’ and the medium-early maturing ‘Sviatohor’. The subplots (second-order plots, factor B) included microbial inoculants: Control 1 (without water spraying); Control 2 (water spraying); Ryzobin$^K$ (an association of three strains of *Bradyrhizobium japonicum*: *B. japonicum* UCM B-6018, UCM B-6023, UCM B-6035); Ryzobin$^K$ + *Bacillus velezensis* IMV B-8134; Ryzobin$^K$ + *B. velezensis* IMV B-8135; and Ryzobin$^K$ + *Pseudomonas* sp. 6. For the complex endophyte-rhizobial inoculation of soybean seeds of different maturity groups, strains of microorganisms from the culture collection of the Department of General and Soil Microbiology of the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine were used. Seed inoculation was performed according to the methods described by H. Iutynska *et al*. (2022). The titre of symbiotic bacteria in the inoculant was at least 3-5 × 10$^9$ cells/ml. Treatment was carried out by spraying the seeds, with an inoculant application rate of 1.0 l per 1 t of seed.

Weather and climatic factors influencing production processes during the yield formation period of soybean varieties differing in maturity group were determined by assessing average temperature, relative air humidity and atmospheric precipitation during interphase periods, i.e. the main indicators significantly affecting evaporation rate, moisture deficit and the moisture coefficient (Romanchuk, 2020). These indicators, taking weather conditions into account, were determined according to generally accepted methods described by V.O. Ushkarenko *et al.* (2019). The overall characterisation of the main weather parameters during the interphase periods of both soybean varieties was based on observations from the meteorological station in the settlement of Naddniprianske. Soybean varieties were sown during the first ten days of May with a row spacing of 45 cm and a sowing depth of 2-3 cm. The sowing rate for the ‘Diona’ variety was 800,000 viable seeds per hectare and for ‘Sviatohor’ 600,000 viable seeds per hectare. During the flowering to early bean formation stage, the abundance of microorganisms belonging to the main ecological-functional groups in the soybean rhizosphere was determined. Enumeration was performed using the surface plating method of soil suspensions with appropriate tenfold dilutions onto selective agar media. In particular, phosphate-mobilising microorganisms were cultured on mineral medium with sodium phenolphthalein phosphate, pedotrophic microorganisms on soil agar, oligotrophic microorganisms on starvation (water) agar, and oligonitrotrophic and nitrogen-fixing microorganisms on Ashby medium. Incubation was carried out at 28°C for 5-7 days. The number of microorganisms was expressed as colony-forming units per 1 g of absolutely dry soil, taking soil moisture into account (CFU $g^{-1}$ soil), which was determined gravimetrically. At the bean maturity stage, yield assessment was conducted with adjustment to standard seed moisture content. Harvesting was carried out using a Sampo-130 combine harvester (Sampo Rosenlew Ltd, Finland). Soybean yield was evaluated according to the generally accepted methods of field experimentation described by V.O. Ushkarenko *et al.* (2019) and expressed in t/ha. The results of all laboratory and field experiments with four replications were analysed using Microsoft Excel 2010 software. Multifactor analysis of variance (ANOVA) was applied for statistical analysis. Differences were considered statistically significant at $p < 0.05$. Field studies were conducted in accordance with the principles of biodiversity conservation under the provisions of the Convention on Biological Diversity (1992), as well as bioethical principles and environmental safety standards. Sampling was performed with minimal impact on plants and their habitats.

## RESULTS AND DISCUSSION

The agrometeorological conditions of the growing season in the southern part of the Steppe zone (Kherson Region) were characterised by a moderately hot and extremely arid climate. Evaporation and moisture deficit during the growing season varied considerably and depended on average daily temperature, relative air humidity and the amount of productive atmospheric precipitation. The total duration of the interphase period "emergence – beginning of branching" during the cultivation of both soybean varieties in 2024-2025 did not exceed 18 days, during which 21.1 mm of precipitation fell, accounting for 12.3-20.9% of the total precipitation received throughout the crop growing season. The duration of the interphase period "beginning of branching – beginning of flowering" for the soybean variety 'Diona' was 22 days, during which 24.6 mm of atmospheric precipitation fell, representing 24.5% of the total precipitation amount (Table 1).

**Table 1.** Hydrothermal conditions during the interphase periods of soybean varieties differing in maturity group, average for 2024-2025

| Calendar dates | Average air temperature, °C | Precipitation, mm | Relative air humidity, % | Evaporation, mm | Moisture deficit, mm | Moisture coefficient (MC) |
|---|---|---|---|---|---|---|
| **'Diona' variety** | | | | | | |
| Emergence – beginning of branching (18 days) | | | | | | |
| 18.05-04.06 | 19.3 | 21.1 | 57.0 | 151.9 | 130.8 | 0.14 |
| Beginning of branching – beginning of flowering (22 days) | | | | | | |
| 05.06-26.06 | 22.9 | 24.6 | 46.0 | 223.0 | 198.4 | 0.11 |
| Beginning of flowering – beginning of bean formation (15 days) | | | | | | |
| 27.06-11.07 | 26.5 | 19.0 | 40.0 | 286.4 | 267.4 | 0.07 |
| Beginning of bean formation – beginning of seed filling (16 days) | | | | | | |
| 12.07-27.07 | 28.3 | 9.3 | 41.0 | 301.7 | 292.4 | 0.03 |
| Beginning of seed filling – beginning of seed ripening (16 days) | | | | | | |
| 28.07-12.08 | 25.8 | 6.4 | 44.0 | 260.1 | 253.7 | 0.02 |
| Beginning of seed ripening – full seed maturity (14 days) | | | | | | |
| 13.08-26.08 | 24.2 | 19.9 | 40.0 | 261.4 | 241.5 | 0.08 |
| Total | | | | | | |
| 101 days | 24.5 | 100.3 | 45.0 | 1,484.5 | 1,384.2 | 0.07 |
| **'Sviatohor' variety** | | | | | | |
| Emergence – beginning of branching (18 days) | | | | | | |
| 19.05-05.06 | 19.8 | 21.1 | 56.0 | 158.9 | 137.8 | 0.13 |
| Beginning of branching – beginning of flowering (26 days) | | | | | | |
| 06.06-01.07 | 23.2 | 24.6 | 46.0 | 225.8 | 201.2 | 0.11 |

*Table 1. Continued*

| Calendar dates | Average air temperature, °C | Precipitation, mm | Relative air humidity, % | Evaporation, mm | Moisture deficit, mm | Moisture coefficient (MC) |
|---|---|---|---|---|---|---|
| Beginning of flowering – beginning of bean formation (20 days) | | | | | | |
| 02.07-21.07 | 28.1 | 28.3 | 40.0 | 304.5 | 276.2 | 0.09 |
| Beginning of bean formation – beginning of seed filling (22 days) | | | | | | |
| 22.07-12.08 | 26.3 | 6.4 | 43.0 | 270.0 | 263.6 | 0.02 |
| Beginning of seed filling – beginning of seed ripening (22 days) | | | | | | |
| 13.08 -03.09 | 24.2 | 28.6 | 41.0 | 257.1 | 228.5 | 0.11 |
| Beginning of seed ripening – full seed maturity (16 days) | | | | | | |
| 04.09-19.09 | 20.7 | 61.5 | 49.0 | 191.7 | 130.2 | 0.32 |
| Total | | | | | | |
| 124 days | 24.1 | 171.0 | 46.0 | 1,408.5 | 1,237.5 | 0.12 |

**Source:** compiled by the authors

The duration of this interphase period for the variety ‘Sviatohor’ was 26 days, during which 24.6 mm of precipitation fell, accounting for 14.4% of the total precipitation during the crop growing season. At an average daily temperature of 22.9°C and relative air humidity of 46.0%, evaporation during this interphase period under cultivation of the variety ‘Diona’ reached 223.0 mm, while the moisture deficit amounted to 198.4 mm. During cultivation of the variety ‘Sviatohor’ in the interphase period “beginning of branching – beginning of flowering”, 24.6 mm of atmospheric precipitation fell and evaporation amounted to 225.8 mm, resulting in a moisture deficit of 201.2 mm. The moisture coefficient during cultivation of the variety ‘Diona’ reached 0.11 and, correspondingly, 0.11 for the variety ‘Sviatohor’, indicating conditions characteristic of desert environments in the southern part of the Steppe zone. During the interphase period “beginning of flowering – beginning of bean formation”, the total duration of which for the variety ‘Diona’ was 14 days, at an average temperature of 26.5°C and relative air humidity of 40.0%, evaporation increased to 286.4 mm and the moisture deficit reached 267.4 mm. The amount of precipitation during cultivation of the variety ‘Diona’ in this interphase period was 19.0 mm, or 18.9% of the total precipitation received during the crop growing season. Under these weather conditions, the moisture coefficient for the variety ‘Diona’ was 0.07, which is characteristic of desert conditions, while for the variety ‘Sviatohor’ it was 0.09. During the interphase period “beginning of bean formation – beginning of seed filling”, the amount of atmospheric precipitation during cultivation of the variety ‘Diona’ was 9.3 mm, or 9.2% of the total precipitation received during the variety’s growing season. At an average temperature of 28.3°C and relative air humidity of 41.0%, evaporation during this interphase period under cultivation of the variety ‘Diona’ increased to 301.7 mm, while the moisture deficit reached 292.4 mm. During cultivation of the medium-early variety ‘Sviatohor’ in the interphase period “beginning of bean formation – beginning of seed filling”, the total duration of which was 22 days, adverse weather conditions negatively affected crop yield formation. At an average daily temperature of 26.3°C and relative air humidity of 43.0%, evaporation amounted to 270.0 mm and the moisture deficit reached 263.6 mm. Under these weather conditions, the moisture coefficient was 0.03, which is characteristic of desert conditions. During the interphase period “beginning of seed filling – full seed maturity”, the moisture coefficient during cultivation of the variety ‘Diona’ over 16 days did not exceed 0.02, which is characteristic of desert conditions, while for the variety ‘Sviatohor’ it amounted to 0.11. The moisture coefficient during cultivation of the variety ‘Diona’ was 0.08 (desert conditions) and, correspondingly, 0.32 for the variety ‘Sviatohor’ (semi-arid zone). The total duration of both interphase periods during cultivation of the variety ‘Diona’ was 30 days, while for the variety ‘Sviatohor’ it was 38 days.

The reduction of the negative effects of extreme weather conditions observed during the cultivation of both soybean varieties in the interphase periods “emergence – beginning of branching”, “beginning of seed filling – beginning of seed ripening” and “beginning of seed ripening – full seed maturity” was achieved through irrigation during the growing season using drip irrigation. During cultivation of the ultra-early variety ‘Diona’, 18 irrigations were carried out during the growing season with an irrigation rate of 5,315 $m^3$/ha, while for the medium-early variety ‘Sviatohor’, 20 irrigations were applied with an irrigation rate of 5,548 $m^3$/ha. The abundance of microorganisms and their metabolic activity are regarded as indicators of soil biological activity (Jezierska-Tys *et al.*, 2020). Determination of the abundance of microorganisms belonging to different ecological-functional groups in the soybean rhizosphere, depending on inoculation with rhizobia and endophytic bacteria, was carried out during the “flowering – beginning of bean formation” stage. It was established that soybean seed inoculation influenced the abundance of microorganisms belonging to different ecological-functional groups in the rhizosphere soil of both soybean varieties under investigation (Table 2).

**Table 2.** Abundance of microorganisms in rhizosphere soil (CFU $g^{-1}$ soil), average for 2024-2025

| Treatment | Phosphate-mobilising microorganisms | Pedotrophic microorganisms | Oligotrophic microorganisms | Nitrogen-fixing microorganisms |
|---|---|---|---|---|
| **‘Diona’ variety** | | | | |
| 1 | 9.9 ± 1.1 | 47.7 ± 5.7 | 46.6 ± 9.3 | 16.1 ± 2.9 |
| 2 | 30.8 ± 2.4 | 56.4 ± 2.2 | 88.9 ± 18.5 | 17.1 ± 1.8 |
| 3 | 13.9 ± 3.2 | 49.1 ± 1.1 | 110.7 ± 17.9 | 22.9 ± 3.7 |
| 4 | 16.3 ± 2.1 | 57.7 ± 9.7 | 28.2 ± 4.7 | 22.5 ± 3.3 |
| 5 | 13.7 ± 1.0 | 25.5 ± 3,9 | 64.7 ± 6.2 | 17.2 ± 2.8 |
| **‘Sviatohor’ variety** | | | | |
| 1 | 26.1 ± 1.2 | 42.7 ± 7.7 | 67.7 ± 11.1 | 73.9 ± 16.8 |
| 2 | 33.2 ± 2.4 | 44.8 ± 6.1 | 72.7 ± 11.7 | 75.4 ± 11.0 |
| 3 | 32.7 ± 6.7 | 41.1 ± 6.7 | 58.0 ± 6.7 | 40.9 ± 4.5 |
| 4 | 31.6 ± 6.3 | 50.2 ± 7.5 | 57.3 ± 11.8 | 72.1 ± 13.7 |
| 5 | 25.1 ± 2.5 | 45.2 ± 8.1 | 78.4 ± 6.9 | 68.9 ± 7.5 |

**Note:** 1 – Control (without treatment); 2 – Ryzobin$^K$; 3 – Ryzobin$^K$ + *B. velezensis* IMV B-8134; 4 – Ryzobin$^K$ + *B. velezensis* IMV B-8135; 5 – RyzobinK + *Pseudomonas* sp. 6
**Source:** compiled by the authors

Soils frequently contain large amounts of insoluble mineral and organic phosphorus compounds, although phosphorus is essential for the growth of most plants and microorganisms. One approach to improving phosphorus nutrition of plants is the use of microorganisms capable of increasing the mobility of phosphorus compounds (Malynovska, 2021). The highest abundance of phosphate-mobilising microorganisms was recorded following the application of Ryzobin$^K$ alone and in combination with the endophytic strains *B. velezensis* IMV B-8134 and *B. velezensis* IMV B-8135. In these treatments, their abundance reached 13.9-33.2 million CFU/g of soil. At the same time, the best values exceeded the control by 3.1 times for the variety ‘Diona’ and by 1.3 times for the variety ‘Sviatohor’. Pedotrophic microorganisms participate in the transformation of organic matter and humus formation, while an increase in their abundance indicates intensification of these processes (Maslovska *et al.*, 2025). The greatest abundance of pedotrophic microorganisms in the rhizosphere soil of both studied varieties was promoted by inoculation with the complex Ryzobin$^K$ + *B. velezensis* IMV B-8135. In these treatments, the abundance of pedotrophs was higher than in the non-inoculated control by 21.0% for the variety ‘Diona’ and 17.6% for the variety ‘Sviatohor’. Oligotrophic microorganisms are adapted to ecological niches with low substrate concentrations and limited energy flow, and an increase in their abundance in soil, according to N.B. Dragone *et al.* (2024), may indicate more active utilisation of residual available nutrients. The highest abundance of oligotrophic microorganisms in the rhizosphere soil of soybean variety ‘Diona’ was promoted by inoculation with the complex Ryzobin$^K$ + *B. velezensis* IMV B-8134, which exceeded the control value by 2.4 times. In the rhizosphere soil of soybean variety 'Sviatohor', the highest abundance of oligotrophic microorganisms was observed under inoculation with Ryzobin$^K$ + *Pseudomonas* sp. 6, which was 1.2 times higher than the control.

Nitrogen is an essential macronutrient for plants; however, most of it is present in the atmosphere in the inaccessible form of $N_2$. Thus, replenishment of nitrogen reserves occurs, among other mechanisms, through biological fixation by nitrogen-fixing microorganisms, which convert it into compounds available to plants (Zulfarina *et al.*, 2017). An increase in their abundance in rhizosphere soil contributes to the supply of available nitrogen to plants, improvement of plant growth and maintenance of soil fertility. In the rhizosphere soil of soybean variety 'Diona', the highest abundance of nitrogen-fixing microorganisms (22.9 million CFU/g of soil) was recorded in the treatment Ryzobin$^K$ + *B. velezensis* IMV B-8134, which was 42.2% higher than in the control treatment. In the rhizosphere of soybean variety 'Sviatohor', the differences between treatments averaged over the years of study were not statistically significant. Thus, the rhizosphere soil of both soybean varieties under complex endophyte-rhizobial inoculation was characterised by increased biological activity, as evidenced by the high abundance of microorganisms belonging to the main ecological-functional groups. This contributed to increased plant resistance to arid weather and climatic conditions and to higher productivity.

Determination of nutrient content in the 0-20 cm layer of dark chestnut soil in the experimental field showed that the complex microbial preparations had a positive residual effect on the soil, enriching it with nutrients available to plants. In the soil of treatments where inoculation with nodule-forming and endophytic bacteria was applied, the content of mineral nitrogen increased. During cultivation of soybean variety 'Diona' in the interphase period "beginning of branching – beginning of flowering", the mineral nitrogen content under bacterial inoculation increased by 4.75-5.72 mg/kg compared with the control treatments. It was also established that during cultivation of soybean varieties under irrigation with complex endophyte-rhizobial seed inoculation, the content of available phosphorus changed during the interphase period "beginning of branching – beginning of flowering". In the 0-20 cm soil layer, its maximum content was recorded in the treatment Ryzobin$^K$ + *B. velezensis* IMV B-8134 at 38.4 mg/kg, which was higher than in the soil of the control treatments (Table 3).

**Table 3.** Agrochemical composition of dark chestnut soil under soybean plants in the 0-20 cm layer during the "beginning of branching – beginning of flowering" stage, mg/kg

| Inoculants | Mineral nitrogen content ($N\text{-}NO_3$ + $N\text{-}NH_4$) | Available phosphorus content ($P_2O_5$) | Exchangeable potassium content ($K_2O$) |
|---|---|---|---|
| **'Diona' variety** | | | |
| Control 1 (without seed treatment) | 23.67 | 23.4 | 155 |
| Control 2 (seed treatment with water) | 22.69 | 26.3 | 162 |
| Ryzobin$^K$ | 28.82 | 38.0 | 200 |
| Ryzobin$^K$ + *B. velezensis* IMV B-8134 | 27.62 | 38.4 | 242 |
| Ryzobin$^K$ + *B. velezensis* IMV B-8135 | 28.42 | 37.6 | 230 |
| Ryzobin$^K$ + *Pseudomonas* sp. 6 | 26.00 | 37.3 | 216 |
| $LSD_{0.05}$ | 2.27 | 5.58 | 28.4 |
| **'Sviatohor' variety** | | | |
| Control 1 (without seed treatment) | 15.77 | 26.4 | 225 |
| Control 2 (seed treatment with water) | 17.58 | 246 | 202 |
| Ryzobin$^K$ | 27.75 | 37.8 | 228 |
| Ryzobin$^K$ + *B. velezensis* IMV B-8134 | 30.22 | 38.2 | 235 |
| Ryzobin$^K$ + *B. velezensis* IMV B-8135 | 32.06 | 37.3 | 207 |
| Ryzobin$^K$ + *Pseudomonas* sp. 6 | 24.80 | 37.4 | 218 |
| $LSD_{0.05}$ | 5.83 | 5.08 | 10.1 |

**Source:** compiled by the authors

In the other treatments, the values of this parameter ranged within 37.6-38.3 and 37.3-38.0 mg/kg of soil. The content of exchangeable potassium during the interphase period "beginning of branching – beginning of flowering" in the 0-20 cm soil layer under the treatment Ryzobin$^K$ + *B. velezensis* IMV B-8134 was the highest for the 'Diona' variety, reaching 242.75 mg/kg; correspondingly, for the 'Sviatohor' variety it amounted to 235 mg/kg, whereas in the control treatments its content was the lowest, amounting to 202 and 225 mg/kg of soil. During the interphase period "beginning of bean formation – beginning of bean filling", the content of mineral nitrogen increased in the soil under plants inoculated with nodule-forming and endophytic bacteria. During cultivation of the soybean variety 'Diona', the mineral nitrogen content under bacterial inoculation increased by 18.0-22.4 mg/kg compared with the control treatments. In the cultivation of the soybean variety 'Sviatohor', the mineral nitrogen content in the soil of inoculated treatments increased by 1.8-3.4 mg/kg compared with the control values (Table 4).

**Table 4.** Agrochemical composition of dark chestnut soil under soybean plants in the 0-20 cm layer during the "beginning of bean formation – beginning of bean filling" stage, mg/kg

| Inoculants | Mineral nitrogen content ($N\text{-}NO_3 + N\text{-}NH_4$) | Available phosphorus content ($P_2O_5$) | Exchangeable potassium content ($K_2O$) |
|---|---|---|---|
| **'Diona' variety** | | | |
| Control 1 (without seed treatment) | 12.27 | 62.5 | 163 |
| Control 2 (seed treatment with water) | 12.75 | 62.7 | 163 |
| Ryzobin$^K$ | 19.72 | 64.3 | 197 |
| Ryzobin$^K$ + *B. velezensis* IMV B-8134 | 35.11 | 64.8 | 201 |
| Ryzobin$^K$ + *B. velezensis* IMV B-8135 | 30.30 | 69.2 | 246 |
| Ryzobin$^K$ + *Pseudomonas* sp. 6 | 29.10 | 60.6 | 176 |
| $LSD_{0.05}$ | 7.77 | 2.34 | 25.0 |
| **'Sviatohor' variety** | | | |
| Control 1 (without seed treatment) | 38.8 | 61.8 | 227 |
| Control 2 (seed treatment with water) | 38.9 | 61.9 | 227 |
| Ryzobin$^K$ | 42.20 | 63.5 | 154 |
| Ryzobin$^K$ + *B. velezensis* IMV B-8134 | 40.60 | 62.2 | 231 |
| Ryzobin$^K$ + *B. velezensis* IMV B-8135 | 40.00 | 68.4 | 208 |
| Ryzobin$^K$ + *Pseudomonas* sp. 6 | 41.90 | 69.3 | 187 |
| $LSD_{0.05}$ | 1.16 | 4.22 | 28.0 |

**Source:** compiled by the authors

During the cultivation of soybean varieties under irrigation conditions with complex endophyte-rhizobial seed inoculation, the content of available phosphorus changed during the interphase period "beginning of bean formation – beginning of bean filling". In the 0-20 cm soil layer, its maximum content was recorded in the treatment Ryzobin$^K$ + *B. velezensis* IMV B-8135, reaching 69.2 mg/kg, which was higher than in the control treatments. In the remaining treatments, the values of this parameter ranged within 63.2-64.8 and 54.6-68.4 mg/kg of soil. The content of exchangeable potassium compounds in the 0-20 cm layer under the treatment Ryzobin$^K$ + *B. velezensis* IMV B-8135 during cultivation of the soybean variety 'Diona' was the highest, amounting to 246 mg/kg, whereas in the control treatments its content was the lowest at 163 mg/kg of soil; correspondingly, for the variety 'Sviatohor' it amounted to 231 mg/kg. The obtained data regarding the increase in mineral nitrogen and available phosphorus content in the soil under complex inoculation are consistent with the findings of P. Xing *et al.* (2022), who demonstrated that co-inoculation of *B. japonicum* 5038 with *Bacillus aryabhattai* MV35-5 promoted an increase in soil nitrogen content and had a significant effect on its accumulation in soybean plants, while inoculation with *B. japonicum* 5038 and *Paenibacillus mucilaginosus* 3016 contributed to an increase in the content of available phosphorus in the soybean rhizosphere soil. Thus, the effectiveness of bacterial preparations is determined by the ability of the bacteria on which they are based to fix atmospheric nitrogen and promote mineralisation of soil organic matter, thereby improving the mineral nutrition of plants. Modern bacterial preparations contribute to the formation of a powerful nitrogen-fixing apparatus (nodules) on roots, intensive development of leguminous plants and protection against diseases, which helps to obtain high seed yields with superior sowing qualities. For obtaining consistently high soybean seed yields, optimum plant density is of considerable importance, as it promotes intensive crop growth and development. In the present study, the sowing rate for the variety 'Diona' was 800 thousand seeds/ha and, correspondingly, for the variety 'Sviatohor' 600 thousand viable seeds/ha (Table 5).

**Table 5.** Plant density, field germination and survival of soybean plants depending on variety and application of bacterial preparations

| Inoculants | Seed sowing rate, thousand/ha | Emerged plants, thousand/ha | Field seed germination, % | Plants at full maturity stage, thousand/ha | Plant survival, % |
|---|---|---|---|---|---|
| **'Diona' variety** | | | | | |
| Control 1 (without seed treatment) | 800,000 | 400,000 | 50.0 | 340,000 | 85.0 |
| Control 2 (seed treatment with water) | 800,000 | 400,000 | 50.0 | 340,000 | 85.0 |
| Ryzobin$^K$ | 800,000 | 480,000 | 60.0 | 470,000 | 97.9 |
| Ryzobin$^K$ + *B. velezensis* IMV B-8134 | 800,000 | 570,000 | 71.3 | 560,000 | 98.2 |
| Ryzobin$^K$ + *B. velezensis* IMV B-8135 | 800,000 | 560,000 | 70.0 | 540,000 | 96.4 |
| Ryzobin$^K$ + *Pseudomonas* sp. 6 | 800,000 | 520,000 | 65.0 | 510,000 | 98.1 |
| | $LSD_{0.05}$ | 64,1 | 8.02 | 82.7 | 5.42 |
| **'Sviatohor' variety** | | | | | |
| Control 1 (without seed treatment) | 600,000 | 310,000 | 51.7 | 290,000 | 93.5 |
| Control 2 (seed treatment with water) | 600,000 | 310,000 | 51.7 | 290,000 | 93.5 |
| Ryzobin$^K$ | 600,000 | 360,000 | 60.0 | 350,000 | 97.2 |
| Ryzobin$^K$ + *B. velezensis* IMV B-8134 | 600,000 | 380,000 | 63.3 | 370,000 | 97.4 |
| Ryzobin$^K$ + *B. velezensis* IMV B-8135 | 600,000 | 380,000 | 63.3 | 370,000 | 97.4 |
| Ryzobin$^K$ + *Pseudomonas* sp. 6 | 600,000 | 360,000 | 60.0 | 350,000 | 97.2 |
| | $LSD_{0.05}$ | 26.3 | 4.33 | 29.7 | 1.62 |

**Source:** compiled by the authors

During the growing season of both soybean varieties under study, part of the plants died off, resulting in a reduction in crop density within the field experiment plots. Complex endophyte-rhizobial seed inoculation had a significant effect on plant preservation. The highest survival rate of plants of the variety 'Diona' was observed under seed inoculation with Ryzobin$^K$ + *B. velezensis* IMV B-8134 at 98.2% and Ryzobin$^K$ + *B. velezensis* IMV B-8135 at 96.4%, while for the variety 'Sviatohor' the highest survival rate was recorded under inoculation with the composition Ryzobin$^K$ + *B. velezensis* IMV B-8134 at 97.4%. The lowest plant survival was observed in the control treatments, ranging from 85.0 to 93.5%. Soybean inoculation with rhizobia promotes improved nitrogen nutrition of plants, their growth and yield formation. During the experiments, the application of inoculation with the preparation Ryzobin$^K$, containing three strains of *B. japonicum*, resulted in a yield increase compared with the non-inoculated treatment of 9.23% (0.18 t/ha) for the soybean variety 'Diona' and 14.2% (0.30 t/ha) for the variety 'Sviatohor'. Considerably better results were achieved under complex endophyte-rhizobial inoculation. During the years of research, the maximum seed yield of both studied varieties was obtained in the treatment with complex seed inoculation Ryzobin$^K$ + *B. velezensis* IMV B-8134, averaging 2.69 t/ha for the ultra-early maturing variety 'Diona' and 2.95 t/ha for the medium-early maturing variety 'Sviatohor', which exceeded the control values by 38-40% (Table 6).

**Table 6.** Seed yield of soybean varieties differing in maturity group under endophyte-rhizobial inoculation on irrigated lands of the Southern Steppe of Ukraine, t/ha, 2024-2025

| Inoculants (B) | Yield, t/ha | | | Average increase relative to Control 1 | |
|---|---|---|---|---|---|
| | 2024 | 2025 | average | t/ha | % |
| **'Diona' variety ($A_1$)** | | | | | |
| Control 1 (without seed treatment) | 2.77 | 1.13 | 1.95 | – | – |
| Control 2 (seed treatment with water) | 2.80 | 1.15 | 1.97 | – | – |
| Ryzobin$^K$ | 3.02 | 1.25 | 2.13 | 0.18 | 9.23 |
| Ryzobin$^K$ + *B. velezensis* IMV B-8134 | 3.57 | 1.82 | 2.69 | 0.74 | 37.9 |
| Ryzobin$^K$ + *B. velezensis* IMV B-8135 | 3.26 | 1.77 | 2.51 | 0.56 | 28.7 |
| Ryzobin$^K$ + *Pseudomonas* sp. 6 | 3.01 | 1.66 | 2.33 | 0.38 | 19.4 |
| **'Sviatohor' variety ($A_2$)** | | | | | |
| Control 1 (without seed treatment) | 2.84 | 1.37 | 2.10 | – | – |
| Control 2 (seed treatment with water) | 2.85 | 1.38 | 2.11 | – | – |
| Ryzobin$^K$ | 3.11 | 1.69 | 2.40 | 0.30 | 14.2 |
| Ryzobin$^K$ + *B. velezensis* IMV B-8134 | 3.77 | 2.14 | 2.95 | 0.85 | 40.4 |
| Ryzobin$^K$ + *B. velezensis* IMV B-8135 | 3.39 | 1.98 | 2.68 | 0.58 | 27.6 |
| Ryzobin$^K$ + *Pseudomonas* sp. 6 | 3.11 | 1.98 | 2.54 | 0.44 | 20.9 |

**Note:** $LSD_{0.05}$ for partial effects: factor A – 0.154 t/ha, factor B – 0.091 t/ha; for mean (main) effects: factor A – 0.058 t/ha, factor B – 0.065 t/ha
**Source:** compiled by the authors

High yields were also obtained in the treatment Ryzobin$^K$ + *B. velezensis* IMV B-8135, amounting to 2.51 t/ha for the variety 'Diona' and 2.68 t/ha for the variety 'Sviatohor'. The yield increase of the variety 'Diona' under complex seed inoculation in the different experimental treatments, compared with Control 1, was as follows: Ryzobin$^K$ – 0.18 t/ha; Ryzobin$^K$ + *B. velezensis* IMV B-8134 – 0.74 t/ha; Ryzobin$^K$ + *B. velezensis* IMV B-8135 – 0.56 t/ha; and Ryzobin$^K$ + *Pseudomonas sp.* 6 – 0.38 t/ha. During cultivation of the medium-early variety 'Sviatohor', the yield increase was also sufficiently high and, in the respective experimental treatments, reached: Ryzobin$^K$ – 0.30 t/ha; Ryzobin$^K$ + *B. velezensis* IMV B-8134 – 0.85 t/ha; Ryzobin$^K$ + *B. velezensis* IMV B-8135 – 0.58 t/ha; and Ryzobin$^K$ + *Pseudomonas sp.* 6 – 0.44 t/ha. The lowest yield of conditioned seed for both soybean varieties was obtained in the control treatments (Control 1 and Control 2), amounting to 1.95-1.97 t/ha for the variety 'Diona' and 2.10-2.11 t/ha for the variety 'Sviatohor'. During the years of research, the highest seed yield for both soybean varieties was obtained in the treatment with complex seed inoculation Ryzobin$^K$ + *B. velezensis* IMV B-8134, averaging 2.69 t/ha for the ultra-early maturing variety 'Diona' and 2.95 t/ha for the medium-early variety 'Sviatohor', which exceeded the control values by 38-40%.

Increasing the productivity of grain legumes through the use of the potential of symbiotic and non-symbiotic microorganisms is an economically and environmentally justified practice in modern agriculture. In the study by E. Szpunar-Krok *et al.* (2023), under rhizobial inoculation with *B. japonicum* and in the absence of nitrogen fertilisation, the soybean variety Aldana showed an average increase in seed yield of 7.1% (0.28 t/ha), while the variety Annushka showed an increase of 14.6% (0.50 t/ha). In experiments conducted by M. Leggett *et al.* (2017) in the United States of America, inoculation of soybean with *B. japonicum* resulted in an average increase in soybean grain yield of 1.67% over four years of trials, while in Argentina the increase reached 6.39% over five years of trials, equivalent to 60 and 190 kg/ha respectively. Positive effects of complex seed inoculation using several strains simultaneously have also been reported. Thus, T.C. Tu *et al*. (2021) demonstrated that co-inoculation with the *Bradyrhizobium* isolate Bra6 and various *Pseudomonas* isolates enhanced the formation of active symbiotic systems, nutrient uptake and soybean biomass accumulation. However, it should be noted that despite the general approaches, the application of different inoculants requires adjustment taking into account the characteristics of indigenous microbiota, soil conditions and especially climate change. The experiments demonstrated the positive effect of the multistrain seed inoculation applied in the present study – a consortium of microorganisms consisting of three strains of nodule-forming bacteria and one of the newly isolated strains of endophytic bacteria.

## CONCLUSIONS

It was established that the development of climate-oriented agrotechnologies is an important prerequisite for increasing the efficiency of agriculture in arid regions. The expediency of using plant growth-promoting bacteria as a promising approach to enhancing crop resilience and productivity was demonstrated. The obtained data indicated an increase in the physiological activity of microorganisms belonging to the main ecological-functional groups, as well as in the content of mineral nitrogen and available phosphorus in soybean rhizosphere soil under endophyte-rhizobial inoculation. Owing to the effectiveness of complex inoculation and the active development of agronomically beneficial groups within the rhizosphere microbiome, plant density and the intensity of crop growth and development increased. Complex endophyte-rhizobial seed inoculation promoted soybean plant survival under unfavourable climatic factors. The highest survival rate of plants of both studied varieties was observed under inoculation with the composition Ryzobin$^{K}$ + *B. velezensis* IMV B-8134. In the same treatment, the maximum yield of both soybean varieties was obtained, averaging 2.69 t/ha for the ultra-early maturing variety 'Diona' and 2.95 t/ha for the medium-early variety 'Sviatohor', which exceeded the control values by 38-40%. Thus, the effectiveness of bacterial preparations is determined by the ability of the bacteria on which they are based to fix atmospheric nitrogen, promote mineralisation of soil organic matter, improve plant mineral nutrition and stress resistance, and enhance the biological activity of the rhizosphere microbiocenosis. In summary, the present study established the stimulatory and stress-protective effects of endophyte-rhizobial inoculation on the biological activity of rhizosphere microbiota, enrichment of soil with essential nutrients, adaptive potential and productivity of soybean varieties differing in maturity group under stressful weather and climatic conditions. It was concluded that co-inoculation with nodule-forming and non-rhizobial endophytic bacteria is an environmentally safe stress-protective strategy and a powerful natural resource for modern agricultural biotechnologies. Future research perspectives include investigating the effectiveness of endophyte-rhizobial inoculation in other climatic zones of Ukraine during the cultivation of grain legumes, as well as studying the prospects for the application of endophytic bacteria in the cultivation of cereal and industrial crops.

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## CONFLICT OF INTEREST

None.

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## Підвищення адаптаційного потенціалу та врожайності сої за допомогою ендофітно-різобіальної інокуляції


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**Анотація.** Метою дослідження було вивчення впливу передпосівної комплексної інокуляції ендофітними та бульбочковими бактеріями на ризосферний мікробіоценоз, вміст макроелементів у ґрунті, адаптивний потенціал і продуктивність сої за дії стресових погодних і кліматичних чинників. Для проведення двофакторного польового досліду використано метод розщеплених ділянок із сортами сої різних груп стиглості: ультраранніим 'Діона' та середньораннім 'Святогор'. Мікробіологічні методи застосовували для отримання інокулянтів на основі бульбочкових і ендофітних бактерій (*Bradyrhizobium japonicum* у складі комплексного біопрепарату Ризобін$^{K}$ та їх сумішей з ендофітними бактеріями *Bacillus velezensis* IMB B-8134, B-8135, *Pseudomonas* sp. 6), а також для визначення чисельності мікроорганізмів різних еколого-функціональних груп шляхом висіву ґрунтових суспензій на селективні агаризовані середовища. Агрохімічні методи використовували для визначення вмісту макроелементів у ґрунті, статистичні – для обробки отриманих даних. Показано стимулювальний і стрес-протекторний ефекти коінокуляції насіння бульбочковими та ендофітними бактеріями на мікробні угруповання ризосферного ґрунту, а також на ріст і врожайність різних сортів сої на зрошуваних землях Південного Степу України. Встановлено зростання чисельності мікроорганізмів основних еколого-функціональних груп і підвищення вмісту доступних форм азоту, фосфору та калію в ризосферному ґрунті. Найефективнішим варіантом за синергічним впливом на сою виявилося оброблення насіння препаратом Ризобін$^{K}$ у поєднанні з *Bacillus velezensis* IMB B-8134. Середня врожайність для сорту 'Діона' становила 2,69 т/га, для сорту 'Святогор' – 2,95 т/га, що перевищувало контроль на 38-40 %. Практична цінність роботи полягає в ефективності передпосівної ендофітно-ризобіальної коінокуляції насіння сої, що забезпечує підвищення продуктивності та стабільності агрофітоценозів в умовах кліматичних змін

**Ключові слова:** *Glycine max* (L.) Merr.; симбіотичні бактерії; стресові кліматичні чинники; зрошення; ризосферна мікробіота; макроелементи; ефективність