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# INFLUENCE OF RHIZOBIA AND ENDOPHYTIC BACTERIA ON ADAPTATION AND PRODUCTIVITY OF *GLYCINE MAX* (L.) MERR. UNDER HYPERTHERMIA AND DROUGHT CONDITIONS

*In the context of global adverse climate change, the use of microorganisms to increase plant stress resistance is becoming increasingly important. In this aspect, the use of rhizobia and endophytic bacteria for legume protection to abiotic stresses is still poorly understood. The **aim** of the study was to investigate the modulating effect of pre-sowing endophyte-rhizobial seed inoculation on the level of antioxidant activity of leaves and roots, water regime, and productivity of soybeans under conditions of hyperthermia and drought, as well as the preservation of this beneficial effect on the physiological homeostasis in the next generation of plants. **Methods.** The following methods were used in the study: microbiological (cultivation of microorganisms to obtain microbial bioformulations), biochemical (determination of total antioxidant and catalase activities), physiological (determination of plant water balance), field (soybean plant cultivation, determination of their productivity), and statistical (the significance of the differences determination). The endophytic bacteria Bacillus sp.4 in composition with Bradyrhizobium japonicum UCM B-6018, B-6023 and B-6035, soybean plants of the Sculptor variety were used in the study. **Results.** The most negative impact of hydrothermal growing conditions was experienced by non-inoculated plants of the control variant, which was confirmed by high values of antioxidant activity — 32% for leaves and 55% for soybean roots, while in the case of endophytic-rhizobial inoculation, these values were lower by 12.5 and 5.5%, respectively. The level of catalase activity was the highest in the inoculated plants, which may indicate an active functioning of the enzyme antioxidant system. Inoculation improved the water regime of soybean plants. The values of water supply, humidity, and water-holding capacity of the leaves of inoculated plants were higher than those of control plants, which directly contributed to better adaptation to hydrothermal conditions. The positive effect of pre-sowing endophytic-rhizobial inoculation of seeds on plant stress resistance was confirmed by an increase in*

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*their productivity, in particular, an increase in yield per plant.* ***Conclusions.*** *The use of complex inoculants of soybean based on rhizobia and endophytic bacteria is promising for the creation of new adaptive biotechnologies capable of maintaining the physiological homeostasis of the phytopartner under adverse conditions, which will help prevent yield losses of this crop from abiotic stresses.*

***Keywords:*** *endophytes, rhizobia, soybean, stress resistance, antioxidant activity, water regime, productivity.*

Microbial preparations are an integral part of environmentally friendly and effective biotechnologies, which are increasingly required in modern agricultural production. Such preparations stimulate plant growth and development, improve nitrogen and phosphorus nutrition, suppress phytopathogens, and increase plant resistance to environmental stressors (Karnwal, 2017). In contrast to agrochemicals, biological products are environmentally friendly, do not pollute the environment, and their production is less energy-intensive.

Abiotic stresses resulting from current climate change can offset the benefits of existing agricultural technologies and reduce crop yield. Global warming and drought cause oxidative stress in plants, which occurs due to excessive production of reactive oxygen species and insufficient $CO_2$, which disrupts the functioning of the plant photosynthetic system (Choudhury et al., 2016).

The development and implementation of the modern biotechnologies for crop production based on soil plant growth-promoting hizobacteria (PGPR) is extremely relevant today. Such biotechnologies can be used as an inexpensive and environmentally friendly approach to increase crop yields under abiotic stress, preserve soil fertility, and stabilize the functioning of agrophytocenoses. When developing biological products, special attention is paid to the ability of bacteria to exhibit their positive properties under extreme factors of local climatic conditions (high and low temperatures, insufficient moisture), as well as the ability to form productive symbiotic systems with plant varieties of domestic and foreign selection.

Soybean (*Glycine max* (L.) Merr.) is an economically important legume crop grown worldwide as a major source of vegetable oil and protein (Wu et al., 2024). Ensuring high soybean yields is crucial for agriculture (Liu et al., 2025). The productivity of this crop is influenced by various biotic and abiotic factors, most of which cannot be controlled by agronomic methods alone (dos Santos et al., 2022).

Soybean productivity is largely affected by many abiotic factors, including drought, salinity, cold, heat, and fluctuations in soil pH, which prevent optimal crop yields (Singh et al., 2021). Under current global warming, droughts are projected to become more frequent and aridity more severe in most semi-arid and arid areas (Vicente-Serrano et al., 2020). During severe drought, the amount of available water in the soil decreases and the salt content increases, causing osmotic stress, and higher salinity concentrations lead to ionic toxicity and osmotic stress in roots (Ma et al., 2020; Munns et al., 2019). Drought is one of the most severe abiotic stressors that affects root physiology, leaf structure, nutrient uptake, photosynthetic activity, and seedling germination, resulting in crop growth inhibition (Zhang et al., 2018; Gupta et al., 2020).

Abiotic stresses usually cause oxidative damage to plants (Choudhury et al., 2016). Under stress conditions, plants respond in many ways in terms of signal transduction, stress-sensitive genes, activation or inactivation of functional proteins and reactions in chloroplasts, mitochondria, and peroxisomes to develop stress resistance. Plant systems also improve their molecular behavior under stress by secreting stress hormones and reactive oxygen species (ROS), which regulate cellular physiology to maintain normal plant functioning (Huang et al., 2019).

ROS can be considered endogenous signalling molecules or regulators produced by several plant organelles, such as mitochondria, chloroplasts, or peroxisomes, during stresses (Demidchik, 2015). ROS are by-products of aerobic metabolic processes in living organisms and include superoxide anions ($O_2^-$), hydrogen peroxide ($H_2O_2$), and hydroxyl radicals ($OH^-$) (Mittler et al., 2022).

ROS are produced in plants as part of normal metabolism (Foyer et al., 2015; Mignolet-Spruyt et al., 2016). Under normal conditions, ROS act as signalling molecules and serve to maintain symbiosis with rhizobial bacteria (Gupta et al., 2019; White et al., 2017). However, plants maintain homeostasis conditions with the help of ROS scavengers, including amino acids, enzymes, and other antioxidant systems (Rehman et al., 2018). Excessive accumulation of ROS in cells can cause oxidative stress, which damages DNA, lipids, and proteins, disrupting normal metabolism and leading to cell death (Juan et al., 2021). ROS are strong oxidants that can damage important macromolecules, such as membrane lipids, pigments, nucleic acids, carbohydrates, and proteins, and therefore contribute to premature leaf senescence (Fortunato et al., 2015).

In the plant system, excessive concentrations of ROS activate enzymatic or non-enzymatic antioxidant systems (Hasanuzzaman et al., 2020; Kumari et al., 2020). They effectively neutralize ROS and significantly minimize their potential harm under stressful conditions (Bamary & Einali, 2021). Enzymatic antioxidants include catalase, peroxidase, and superoxide dismutase, and non-enzymatic antioxidants include ascorbic acid, carotenoids, glutathione, and proline (Mittler et al., 2022; Hasanuzzaman et al., 2020; Kumari et al., 2020; Kapoor et al., 2019).

Certain types of endophytic microorganisms are particularly effective in maintaining the health of the host plant while receiving nutrients and protection from the phytopartner. They participate in resistance mechanisms, providing tolerance to various stresses, thus increasing the competitiveness of the host plant (Samreen et al., 2021).

In increasing plant resistance to adverse factors, the priming effect is important, which is expressed in advance preparation for enhancing plant defence responses by exposing them to various exogenous factors. Priming contributes to a stronger defence response and prolonged survival against stress. Seed priming involves physical methods of activating seed germination (magnetic field, UV, microwave and X-ray radiation, ultrasound, etc.), temperature treatment (low or high temperatures), chemical methods of exposure (Hydro-, Halo-, Nutri-, Osmo- and PGR- or Hormo-priming, respectively, treatment with water, solutions of mineral salts, trace elements, osmotics, plant growth regulators), and treatment with biological agents. One variant of Seed Priming involves treating seeds with water or solutions of various substances for a certain period of time, followed by drying. In this case, the supply of sufficient moisture to the seeds and their swelling triggers a germination process, while drying stops biochemical germination processes. After sowing, soil moisture restores the initiated physiological processes, which accelerates germination and improves the germination of already prepared seeds (Jatana et al., 2024).

The ability of endophytic microorganisms to spread 'vertically', i.e. to be transmitted to subsequent generations through seeds and restore the complementary microbiome selected by the parent plant, can be considered as a variant of Bio-priming or Bioagent-priming (Geisen et al., 2017). The existence of vertical transmission allows a plant with an established endophytic community to transfer bacteria with beneficial characteristics to its offspring (Truyens et al., 2014).

The influence of endophytic microorganisms on stress resistance and plant productivity under adverse abiotic factors, as well as their ability to bio-priming, has not been studied sufficiently to date.

The **aim** of the study was to investigate the modulating effect of pre-sowing endophyte-rhizobial seed inoculation on the level of antioxidant activity of leaves and roots, water regime and productivity of soybeans under conditions of hyperthermia and drought, as well as the preservation of this beneficial effect on the physiological homeostasis in the next generation of plants.

**Materials and Methods.** A small-scale field experiment on the cultivation of the medium-early soybean variety Sculptor was conducted at the experimental field of the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine. The research design included the following options: 1 — treatment of seeds with sterile water (control); 2 — inoculation of seeds with the complex bioformulation Ryzobin$^K$ (R$^K$); 3 — inoculation with the composition of the complex inoculant Ryzobin$^K$ together with endophytic strain *Bacillus* sp. 4 (R$^K$+4); 4 — cultivation of the second generation of soybeans inoculated with Ryzobin$^K$ in the previous year (R$^K$ priming); 5 — cultivation of the second generation of soybeans inoculated with the composition Ryzobin$^K$+*Bacillus* sp. 4 in the previous year (R$^K$+4 priming). The field experiment was performed in 3 replicates.

Ryzobin$^K$ is a complex bioformulation that contains three strains of highly effective nodule bacteria *Bradyrhizobium japonicum* UCM B-6018, B-6023 and B-6035, which differ in the spectra of synthesized phytohormones and complement each other. The complex inoculant Ryzobin$^K$+*Bacillus* sp. 4 were prepared as described in (Iutynska et al., 2022). Soybean rhizobia strains *Bradyrhizobium japonicum* UCM B-6018, B-6023 and B-6035 were obtained from the Ukrainian Collection of Microorganisms. Endophytic strain *Bacillus* sp. 4 were isolated from soybean nodules.

The soil of the experimental field, according to the analysis by the laboratory of the National Scientific Centre «Institute of Agriculture of NAAS of Ukraine», is dark grey podzolized. The particle size distribution (according to Kachinsky) is medium loam. The 0—25 cm layer of soil contains: humus (by Tiurin's method in Simakov's modification) — 1.15%; mobile phosphorus by Chirikov — 72.5 mg/kg of soil; mobile potassium by Chirikov — 113.8 mg/kg, and easily hydrolyzable nitrogen — 41.3 mg/kg of soil; exchangeable acidity (pH) — 6.4.

The hydrothermal conditions of the growing season of soybean cultivation are given in Table 1. In general, in May-September, the average daily air temperature exceeded the long-term average by 4.0° C, and the moisture deficit was 22.7%. The driest months were July and August, when the average daily air temperature was 24.4° C and rainfall was 19.6 mm. The hydrothermal coefficient for the growing season was 0.8, which indicates an insufficient level of moisture. Under such air temperature and rainfall conditions, plant transpiration was rapid.

The antioxidant activity of ethanolic extracts of leaves and roots of the soybean variety Sculptor in the flowering phase was determined using DPPH (2,2-diphenyl-1-picrylhydrazyl) as a source of stable free radical, according to the method described by Arabshahi-Deloue, Urooj (2007). The method is based on the ability of DPPH to be reduced with a change in color (loss of purple color) when interacting with antioxidants that donate a hydrogen atom. Visual monitoring of the DPPH neutralisation reaction

*Table 1.* **Meteorological conditions of the growing season 2024 (Kyiv)** (Meteopost 2010)

| Month | Average daily air temperature, °C | Total rainfall, mm | Hydrothermal coefficient |
|---|---|---|---|
| May | 16.2 | 15.4 | 0.3 |
| June | 21.5 | 137.0 | 2.1 |
| July | 24.4 | 51.9 | 0.7 |
| August | 23.1 | 25.2 | 0.4 |
| September | 20.5 | 33.0 | 0.5 |
| Total for the growing season | 21.1 | 309.2 | 0.8 |
| Long-term average for the period May-September | 17.1 | 339.0 | — |

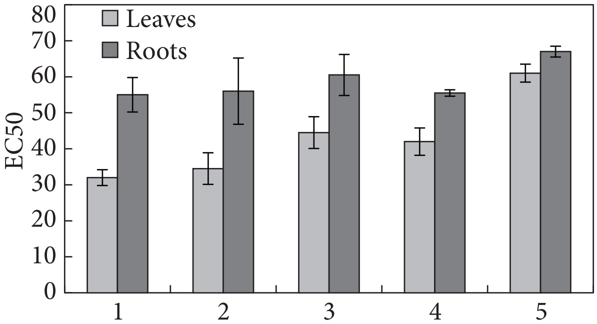


***Fig. 1.*** Antioxidant activity in soybean leaves and roots: 1 — control; 2 — inoculation of seeds with the complex bioformulation Ryzobin$^K$; 3 — inoculation with the composition of the Ryzobin$^K$ + *Bacillus* sp. 4; 4 — seeds of the next generation of soybeans treated with Ryzobin$^K$ in the previous year (Ryzobin$^K$ priming); 5 — seeds of the next generation of soybeans treated with Ryzobin$^K$+*Bacillus* sp. 4 in the previous year (Ryzobin$^K$+*Bacillus* sp. 4 priming)

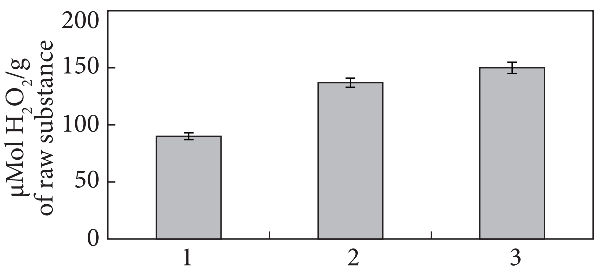


***Fig. 2.*** Catalase activity in soybean leaves: 1 — control; 2 — inoculation with the composition of complex inoculant Ryzobin$^K$ + *Bacillus* sp. 4; 3 — seeds of the next generation of soybeans treated with Ryzobin$^K$ + *Bacillus* sp. 4 composition in the previous year (Ryzobin$^K$ + *Bacillus* sp. 4 priming)

allows the antioxidant activity of the sample to be assessed by the EC50 value (the 'effective concentration' that causes a 50% loss of DPPH activity and color). The optical density of the solution was measured spectrophotometrically at 520 nm using an FLUOstar Omega microplate reader (BMG Labtech, Germany) in four replicates.

Catalase activity was determined by the iodometric method (Francis & Alexander, 1972). The enzyme activity was calculated using special formulas and expressed in micromoles of decomposed hydrogen peroxide.

To study the water regime, soybean plants of the Sculptor variety were grown in a greenhouse, 4 plants per pot, at an air temperature of 20—22 °C and soil moisture of 45%. Soybean seeds were pre-inoculated with soybean-specific rhizobia together with endophytic bacteria *Bacillus* sp. 4. In the phase of the first leaves, they were selected and water regime parameters were determined, namely moisture, dry matter content, water deficit, water supply, transpiration intensity, and transpiration coefficient (TC) (Perepelytsia et al., 2022).

The laboratory experiments were performed in 3 replicates.

Data analysis of laboratory and field experiments was performed using Microsoft Excel 2017 software. Numerical data are presented as an arithmetic mean (M) and standard error (± m). Differences between groups were considered statistically significant at $p < 0.05$.

**Results**. When studying the antioxidant activity of plants, the highest values were observed in the control variant, which may indicate a significant effect of drought on untreated plants (Fig. 1). The DPPH method assesses non-enzymatic antioxidant activity, which means the ability of compounds in an extract to neutralize free radicals by donating an electron or a hydrogen atom to them (Haida & Hakiman, 2019). The values of EC50 were 32% for leaf extracts and 55% for roots, indicating a high content of ROS in plants. The use of complex endophytic-rhizobial inoculation contributed to a better adaptive potential of plants, which was reflected in the level of antioxidant activity, which was lower than in the control and the variant with Ryzobin$^K$ treatment alone. This indicated that less ROS was formed in the plants; as a result, the antioxidant activity was lower, and the EC50 values were higher, respectively. Plants obtained from primed seeds retained high EC50 values in the variant using Ryzobin$^K$, and the use of the complex Ryzobin$^K$ + *Bacillus* sp.4 for seed priming contributed to an increase in EC50 values by

1.1 and 1.3 times for the extracts from roots and leaves, respectively. The results emphasize the role of endophytes in increasing soybean thermotolerance and resistance to arid growing conditions suggesting that this effect may be maintained in subsequent generations.

The decrease in the total antioxidant activity of ethanol extracts of leaves and roots in the variants with bacterial inoculation, compared to the control, is a probable indication of a decrease in the level of oxidative stress in the treated plants. Drought conditions during the growing season caused a significant accumulation of ROS in untreated plants, which stimulated the increased production of low molecular mass antioxidants and, accordingly, led to lower EC50 values in the DPPH test. The obtained data, in our opinion, can be explained by the fact that the control plants were under a higher oxidative stress caused by unfavorable drought and hyperthermic conditions. At the same time, endophytic-rhizobial inoculation contributed to a better adaptation of soybean plants, which was confirmed by a lower amount of antioxidant substances formed in the extracts of experimental plants.

Catalase activity is an important enzymatic component of plant tolerance mechanisms to abiotic stresses. This parameter in soybean leaves in the variants with pre-sowing complex seed treatment with Ryzobin$^K$ + *Bacillus* sp.4 and with seed priming with this complex was higher than in control plants by 52.2% and 66.6%, respectively (Fig. 2).

The detected increase in the catalase activity in plants treated with the composition Ryzobin$^K$ + *Bacillus* sp. 4 indicates the activation of the enzymatic link of antioxidant defense. Catalase, which is responsible for the cleavage of $H_2O_2$, is a key enzyme in the neutralization of excess ROS, which ensures the stability of metabolic processes under abiotic stress. At the same time, an increased level of catalase activity in these plants does not necessarily indicate a state of acute stress. On the contrary, it may indicate that the antioxidant enzyme system is prepared for potential danger. This «on the alert» status of catalase is probably part of an early adaptive response induced by endophytes, which are known for their ability to modulate the expression of defense genes in plants.

The results of the study of the water regime of juvenile soybean plants in the vegetation experiment showed that the moisture content in leaves in all variants of the experiment was at the level of 77.5—81.2% (Table 2).

The water deficit, which is the lack of water to full saturation, expressed as a percentage of the total content at full saturation of tissues with water, was the lowest in the experimental variants with complex endophytic-rhizobial inoculation — 13.5 and 13.8%, while in the control variant it was 16.3%. Water availability expresses the water content in the native tissue relative to the water content in the water-saturated leaf. It is an essentially relative urgency. This indicator ranged from 67.4% in the control to 69.4% in the variant with complex inoculation, and the highest water content in the leaf tissue (82.0 %) was observed in the variant with primed seeds. Thus, the plants of the experimental variants were the most supplied with water compared to the control. The water-holding capacity of leaves, i.e., the ability to lose water before

*Table 2.* **Indices of water regime of soybean leaves at the initial stage of ontogeny**

| Variants | Moisture content, % | Water deficit, % | Water availability, % | Water holding capacity, % (water loss after 2 hours) |
|---|---|---|---|---|
| Control | 77.5 | 16.3 | 67.4 | 33.4 |
| Inoculation with Ryzobin$^K$ + *Bacillus* sp. 4 | 81.2 | 13.8 | 69.4 | 25.0 |
| Prime by inoculation Ryzobin$^K$ + *Bacillus* sp. 4 | 80.6 | 13.5 | 82.0 | 25.6 |

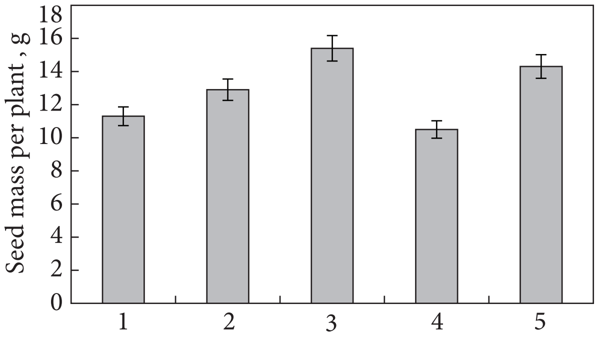


***Fig. 3.*** Yield of soybean variety Sculptor under drought conditions in the Kyiv region. 1 — control; 2 — inoculation of seeds with the complex bioformulation Ryzobin$^{K}$; 3 — inoculation with the composition Ryzobin$^{K}$ + *Bacillus* sp. 4; 4 — seeds of the next generation of soybeans treated with Ryzobin$^{K}$ in the previous year (Ryzobin$^{K}$ priming); 5 — seeds of the next generation of soybeans treated with Ryzobin$^{K}$+*Bacillus* sp. 4 in the previous year (Ryzobin$^{K}$+*Bacillus* sp. 4 priming)

complete wilting, was the highest in the control and amounted to 33.4% after 2 hours of wilting, and in the experimental variants, it was at the level of 25.0 and 25.6%. The plants of the experimental variants lost the least water.

According to the results of small-scale field studies, the use of biological products based on a complex of nodule and endophytic bacteria had a stress-protective effect in the extreme drought conditions of the Kyiv region and contributed to an increased yield of the soybean variety Sculptor.

The highest increase in grain mass per plant was observed in the treatment with the Ryzobin$^{K}$ + *Bacillus* sp.4 complex — 36.2% more than the control. In the variant with primed seeds, a tendency to increase the yield was also noted, by 26.5%. In the variant with priming with Ryzobin$^{K}$ alone, no such effect was observed, which may indicate the ability of the endophytic strain of *Bacillus* sp.4 to be transmitted to subsequent generations of soybean plants (Fig. 3).

**Discussion.** The impacts of climate changes on plants, soil microorganisms, and their interactions have gained considerable relevance in recent years (Dubey, 2021). Endophytes are essential internal partners; they mitigate abiotic stresses by modulating local or systemic mechanisms and producing antioxidants to counteract ROS in plants (Verma et al., 2021). The mitigation of oxidative damage by endophytes may be related to their ability to provide plants with antioxidants, promote their production, or stimulate the activity of existing ones (Li et al., 2020; Sadeghi et al., 2020; Chakrabarti et al., 2022). Endophytes contribute to the adaptation of cultivated plants under abiotic stress, stimulate growth, and help activate plant stress resistance genes (Verma et al., 2021). Our data are in line with the results of other researchers who have shown that inoculation of soybeans with endophytic and rhizobial microorganisms significantly mitigates the damage from oxidative stress caused by unfavorable hydrothermal conditions. Both our studies and the works of other authors have shown that colonization by endophytic microorganisms increases enzymatic activity and the content of non-enzymatic antioxidant molecules (Lata et al., 2018). Plants have other defense mechanisms, such as enzymatic antioxidants — catalase and superoxide dismutase. Catalase is involved in the decomposition of hydrogen peroxide, one of the main types of ROS. A high level of catalase activity may indicate that the plant is actively degrading ROS enzymatically, and a low level may indicate either a low stress or the realization of protection through non-enzymatic antioxidants (Haida & Hakiman, 2019). It is known that in stressful situations, endophytes simultaneously reduce oxidative damage and increase antioxidant content in plants, and the accumulation of antioxidants is a consequence of the endophytes' ability to regulate the expression of antioxidant genes in plants (Nie et al., 2024). Both enzymatic and non-enzymatic pathways are often involved in antioxidant defence. It has been established that endophyte inoculation of tomato plant with *Bacillus safensis* BTL5, *Bacillus haynesii* GTR8, *Bacillus paralicheniformis* GTR11, and *Bacillus altitudinis* GTS16 activates the antioxidant enzyme system *via* the modulation peroxidase, ascorbate peroxidase, superoxide dismutase, and catalase, simultaneously contributing to increasing the content in plants proline and total phenolics (Sahu et al., 2021). Similar results were obtained in the experiments with faba bean (*Vicia faba* L.) inoculated with *Rhizobium leguminosarum* biovar *viciae* (USDA 2435) in combination with *Pseudomonas putida* (RA MTCC5279). It was noted that under drought conditions in all experimental plants, the activities of catalase, peroxidase, and superoxide dismutase significantly increased with increasing levels of water stress. However, the activities of the enzymes were significantly increased in plants infected with *R. leguminosarum* and/or *P. putida* under drought conditions compared to those in non-inoculated plants (Mansour et al., 2021).

Thus, the data available in the literature indicate a positive effect of both rhizosphere and endophytic bacteria on the antioxidant protection of plants, which was expressed both through the synthesis of ROS deactivating substances and enzymatically. Our data showed that endophytes induced both antioxidant defense pathways synthesizing antioxidants to balance the set of free radicals that support normal cell functioning. The results of studies revealed the presence of changes in the functioning of the antioxidant system of *Glycine max* (L.) Merr. plants under the influence of pre-sowing inoculation with a complex endophytic-rhizobial bioformulation. The decrease in the total antioxidant activity of ethanolic extracts of leaves and roots in the variants with bacterial inoculation, compared to the control, is a probable evidence of a decrease in the level of oxidative stress in the treated plants. The control plants had a higher level of antioxidant activity compared to those inoculated with the complex endophytic-rhizobial bioformulation. This can be explained by the fact that in arid conditions, antioxidant activity often increases naturally, as the plant experiences oxidative stress due to water shortage, high temperatures, etc. In response, it begins to synthesize more antioxidant compounds (e.g., phenolic acids, flavonoids, etc.) to protect the cells. The control uninoculated plants were probably under more stress, and therefore synthesized antioxidants more actively. At the same time, the endophytic bacteria in the experimental plants could reduce the level of stress in them. This is typical of endophytes and rhizobia, as they improve nutrition (especially nitrogen), can synthesize phytohormones (auxins, cytokinins), and enhance adaptation to drought. Due to the lower level of stress, plants did not need to produce antioxidants as intensively, and therefore the detected activity was lower (Pokluda et al., 2021).

Water regime processes change during the vegetation period. This is mainly due to the influence of dry, hot weather, which results in a decrease in leaf moisture and an increase in transpiration, leading to inhibition of plant development. Water deficit shortens the vegetation period and accelerates plant aging (Yang et al., 2021). If the process of water evaporation by the plant outweighs its supply from the soil, the plant loses its turgor and wilts. Such a plant reduces the intensity of photosynthesis, intensifies the processes of hydrolysis and decomposition of organic substances, and disrupts the action of enzymes (Lykholat et al., 2021; Chornyi, 2020; Meychik et al., 2021).

The drought resistance provided by endophytes in crops is the result of the activation of a whole range of physiological and biochemical mechanisms in the host plant. Endophytic microorganisms isolated from drought-prone regions, where they are adapted to extreme conditions, have the potential to transfer these adaptive properties to less resistant species or varieties of cultivated plants (Byregowda et al., 2022). The drought tolerance phenomenon may be explained by enhanced accumulation of solutes in tissues of endophyte-infected plants as compared to non-infected plants, or by reduced leaf conductance and a slowdown of the transpiration stream, or due to a thicker cuticle formation. Thus, many studies have emphasized the phenomenon of increasing plant's drought tolerance by inoculation with endophytic microorganisms. In most cases, the focus is on endophytic fungi, such as *Tricho-*

*derma* sp. colonizing *Theobroma cacao* (Lata et al., 2018). As for prokaryotic endophytes, the adaptive role of the endophyte *Gluconacetobacter diazotrophicus* PALS in increasing drought tolerance of *Saccharum officinarum* cv. SP70-1143 has been reported; it describes the initial molecular events that may trigger the increased drought tolerance in the host plant and activate ABA-dependent signaling genes (Vargas et al., 2014). The role of prokaryotic inoculants in the drought tolerance of legumes has been studied to a lesser extent. The role of complex inoculation of legumes with PGP bacteria isolated from the soil was studied. Strain *Pseudomonas fluorescens* DR397 was isolated from the rhizosphere of soybean grown under arid conditions. The inoculation of the legumes *Pisum sativum* and *Phaseolus vulgaris* with this strain under drought conditions stimulated shoot and root growth by 149.1% compared to control uninoculated plants. A study of the genome of this strain showed that it contains several genes associated with the synthesis of substances that help reduce plant stress from drought: choline and glycine-betaine, exopolysaccharides (alginate and cellulose), and genes associated with plant growth stimulation (indole-3-acetic acid, transketolase, and thiamine phosphate) (Nishu & Lee, 2022).

Experiments with the inoculation of five commercially cultivated faba bean (*Vicia faba* L.) cultivars, i.e., Giza-716, Giza-843, Nubaria-3, Sakha-4, and Wadi-1, with the nodule bacteria *Rhizobium leguminosarum* biovar *viciae* (USDA 2435) in combination with *Pseudomonas putida* (RA MTCC5279) were conducted. However, the authors do not mention that this strain was endophytic. It has been shown that under complex inoculation, the efficiency of photosynthesis, plant-water interactions, osmotic regulation, and enzymatic antioxidant activity increased. This had a positive effect on plant productivity (Mansour et al., 2021).

The effect of inoculation with endophytic bacteria together with rhizobia is almost not described in the literature. Our research results show that this complex endophytic-rhizobial inoculation improves the water regime of soybean in the early stages of ontogeny. As a result of seed treatment and priming, the plants were better supplied with water and gained the ability to lose less of it. The use of endophytic bacteria in soybean cultivation technologies will allow for increasing the adaptability of plants to water deficit and water supply reduction, which has been observed in recent years due to global climate change, as well as to increase yields under stressful environmental conditions.

**Conclusions.** Complex endophyte-rhizobial inoculation of soybean seeds contributed to the formation of a high level of antioxidant protection, due to the synthesis of both reactive oxygen species scavengers and enzymatic (catalase) compounds, optimizing a plant water regime and ensuring an increase in yield under arid conditions. The use of complex inoculants of soybean based on rhizobia and endophytic bacteria is promising for the creation of new adaptive biotechnologies capable of maintaining the physiological homeostasis of the phytopartner under adverse conditions, which will help prevent yield losses of this crop from abiotic stresses.

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**Conflict of Interest.** The authors declare no conflict of interest.

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## ВПЛИВ РИЗОБІЙ ТА ЕНДОФІТНИХ БАКТЕРІЙ НА АДАПТАЦІЮ І ПРОДУКТИВНІСТЬ *GLYCINE MAX* (L.) MERR. В УМОВАХ ГІПЕРТЕРМІЇ ТА ПОСУХИ

В умовах глобальних негативних змін клімату використання мікроорганізмів для підвищення стресостійкості рослин набуває все більшої актуальності. У цьому аспекті використання ризобій та ендофітних бактерій для підвищення стійкості бобових до абіотичних стресів залишається маловивченим. **Метою** роботи було дослідити модуляційний вплив передпосівної ендофітно-ризобіальної інокуляції насіння на рівень антиоксидантної активності листя і коренів, водний режим і продуктивність сої за умов гіпертермії і посухи, а також збереження цього сприятливого ефекту на фізіологічний гомеостаз у рослин наступного покоління. **Методи.** В роботі використовували наступні методи: мікробіологічні (культивування мікроорганізмів для отримання мікробних препаратів), біохімічні (визначення загальної антиоксидантної та каталазної активностей), фізіологічні (визначення водного балансу рослин), польові (вирощування рослин сої, визначення продуктивності рослин). Використано ендофітні бактерії *Bacillus* sp.4 в композиції з *Bradyrhizobium japonicum* УКМ В-6018, В-6023 та В-6035, рослини сої сорту Скульптор. **Результати.** Найбільшого негативного впливу гідротермічних умов вирощування зазнали не інокульовані рослини контрольного варіанту, що підтверджується високими значеннями антиоксидантної активності — ЕС 50 сягало 32% для листків та 55% для коріння сої, тоді як за застосування ендофітно-ризобіальної інокуляції ці показники були меншими, відповідно 12.5 та 5.5%. Рівень каталазної активності був найвищим у інокульованих рослин, що може свідчити про активне функціонування ферментної антиоксидантної системи. За інокуляції покращувався водний режим рослин сої. Значення водозабезпечення, вологості та водоутримувальної здатності листків інокульованих рослин були вищими, ніж контрольних, що безпосередньо сприяло кращій адаптації до гідротермічних умов. Позитивний вплив передпосівної ендофітно-ризобіальної інокуляції насіння на стресостійкість рослин підтверджено підвищенням їх продуктивності, зокрема на збільшення врожаю з однієї рослини. **Висновки.** Використання комплексних інокулянтів сої на основі ризобій і ендофітних бактерій є перспективним для створення новітніх адаптивних біотехнологій, здатних підтримувати фізіологічний гомеостаз фітопартнера за несприятливих умов, що сприятиме запобіганню втрат урожаю цієї культури від абіотичних стресів.

***Ключові слова:*** ендофіти, ризобії, соя, стресостійкість, *антиоксидантна* активність, водний режим, продуктивність.