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ADAPTABILITY OF SOYBEAN UNDER COMPLEX TREATMENT WITH BIOPREPARATIONS OF DIFFERENT NATURE IN CONDITIONS OF ORGANIC FARMING AND HYDROTHERMAL STRESS

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Background. In organic farming conditions and increasing hydrothermal stress, the use of biological preparations as effective tools for improving the adaptability of soybeans is becoming particularly relevant. The integrated application of arbuscular mycorrhizal fungi, nitrogen-fixing bacteria, and phytohormonal regulators allows activating the plant physiological and biochemical processes. This biotechnological approach provides mitigation of the negative impact of hydrothermal stresses and maintains crop productivity even under unfavorable growing conditions.

Materials and Methods. The object of the study is the total chlorophyll content, leaf surface area, proline, and malondialdehyde (MDA) concentration, as well as the productivity of soybeans Khorol variety under unstable moisture conditions and influenced by rhizosphere microorganisms and various phytohormones in the composition of the biopreparations. Field, laboratory (physiological, biochemical), and statistical methods were used.

Results. The use of biopreparations improved biochemical, physiological, and morphological properties, as well as soybean productivity, indicating an improved plant status. The highest levels of total Chl (*a*+*b*) content were recorded in 2023 under the combined application of Mycofriend, Profix, and Violar, showing an average increase of 42.3 % and 26.7 % compared to the control, respectively. The leaf area reached 29.24 thousand m²/ha, while the yield amounted to 3.23 t/ha, exceeding the control

by 28.9 % and 47.5 %, respectively. The elevated proline content (7.27 mg/g) and a lower MDA level (7.14 mg/g) during stress reflect reduced oxidative damage and better osmoregulation due to the synergistic action of mycorrhiza, rhizobia, and phytohormones.

Conclusion. The complex application of biopreparations (Mycofriend + Profix + Violar) in the organic cultivation system of the soybean Khorol variety promotes the activation of physiological and biochemical processes, increasing productivity and plant stress resistance under hydrothermal stress conditions.

Keywords: arbuscular mycorrhizal fungi, phytohormonal regulators, chlorophylls, leaf area, yield, proline, malondialdehyde

INTRODUCTION

The growing global demand for environmentally safe products, transformation of climatic conditions, progressive loss of biodiversity, and soil degradation prioritize the development of organic farming as an effective strategy to minimize anthropogenic pressure on agroecosystems (Didora & Kluchevych, 2021; Sanders *et al.*, 2025). Thereby, the implementation of biological preparations to enhance crop productivity and stress resistance becomes particularly significant.

Soybean (*Glycine max* (L.) Merr.), as a leading protein-oil crop occupies key positions in the structure of organic production in Ukraine, providing 24.7 % of the European Union's needs and ranking second among countries exporting organic soybeans to the European market (Chaika *et al.*, 2023). At the same time, global climate change and the increasing frequency of periods of unstable moisture during vegetation are critical risk factors, creating an ever-growing threat to yield stability and overall sustainability of agricultural production (Gouli *et al.*, 2024). The search for effective adaptation strategies to maintain stable productivity of organic soybeans under hydrothermal stress has become increasingly relevant, as drought conditions can lead to crop losses of up to 100 % (Poudel *et al.*, 2023).

A promising approach to addressing this problem is the use of microbial biopreparations based on rhizobia, arbuscular mycorrhizal (AMF), and plant growth-promoting bacteria (PGPB) – microorganisms that stimulate plant growth and form various types of symbiotic and associative interactions with them (Ahmad *et al.*, 2022). Not only can these microorganisms optimize plant mineral nutrition but also modulate their physiological-biochemical response to abiotic stresses, including water deficit (Bouremani *et al.*, 2023). Symbiotic interactions of soybeans with AMF significantly improve the absorption of phosphorus and other mineral elements (Basiru *et al*., 2020), while associations with rhizobia, primarily *Bradyrhizobium japonicum*, increase the efficiency of biological fixation of atmospheric nitrogen, stimulate root system development, and improve the overall physiological status of plants (Inbaraj, 2021). Importantly, PGPB also demonstrate the ability to enhance mycorrhizal colonization of the root system, induce spore germination of AMF, synthesize a wide range of phytohormones (auxins, cytokinins, gibberellins, abscisic acid), and activate plant antioxidant defense systems (Umashankar *et al.*, 2023). This creates prerequisites for increasing the crops' adaptability to adverse environmental factors (Sun & Shahrajabian, 2023).

Products of microbial origin with phytohormonal activity, including biopreparations with complexes of low-molecular biologically active compounds synthesized by PGPB

strains without the participation of viable cells, are of great importance (Liao *et al.,* 2025). These compounds function as signaling molecules, regulating the expression of stress resistance genes, osmotic balance, morphogenetic processes, and biosynthesis of protective metabolites, which collectively provide plant adaptation to water deficit conditions (Shaukat *et al.*, 2024). Integration of such metabolites with AMF or rhizobia promotes synergistic effects on key physiological and biochemical processes, contributing to increased soybean productivity, stress resistance, and formation of a balanced biological nutrition system (Ferreira *et al.*, 2020).

Thus, the ecologization of agricultural production through the implementation of multicomponent biological preparations that combine the functions of growth stimulators, stress resistance inducers, and phytopathogen antagonists opens fundamentally new possibilities for increasing the productivity of agricultural crops, particularly soybeans. At the same time, comprehensive studies of the synergistic interaction of AMF, rhizobia, and bioactive metabolites in soybean agrocenoses within organic farming systems remain fragmentary. This determines the high relevance of the proposed research, the aim of which is to study the synergistic effect of complex biological preparations based on AMF, rhizobia, and bioactive metabolites for enhancing the soybean adaptability under organic farming conditions and hydrothermal stress.

MATERIALS AND METHODS

Experimental site and conditions. The field experiments were conducted in 2022–2024 under the agroecological conditions of the Left-Bank Forest-Steppe of Ukraine (Kremenchuk District, Poltava Region), using the early-maturing soybean Khorol variety, which has been included in the Register of Plant Varieties of Ukraine since 2011.

The soil of the experimental site was classified as residual-solonetzic chernozem on loess deposits, characterized by a medium supply of nitrogen and phosphorus and a high content of potassium. Agrochemical properties of the arable layer (0–20 cm), determined with a multiparametric photometer Palintest SK500 (Palintest Ltd., UK), were as follows: pH_{KCl} – 6.3; humus content – 5.2 %; total nitrogen – 58.6 mg/kg; available phosphorus – 78.3 mg/kg; exchangeable potassium – 138.4 mg/kg.

Field trials were laid out in a randomized block design with three replications. The total experimental area was 0.3 ha, with an accounting area of 0.1 ha. Standard agrotechnical practices for the region were employed, adapted to comply with organic farming principles. Soybean was sown following spring barley as the preceding crop. Sowing was carried out at the optimal time for each year, at a depth of 5 cm, with row spacing of 38 cm and a seeding rate of 700,000 viable seeds per hectare.

Meteorological conditions varied markedly throughout the cultivation period (2022–2024), which had a substantial impact on the growth, development, and physiological responses of the soybean Khorol variety. The growing season of 2022 was marked by a relatively even distribution of precipitation during May–July (46–57 mm), followed by a moderate decline in August (38 mm). Average daily air temperatures ranged from 15 to 21 °C. These hydrothermal parameters closely matched the long-term climatic norms and were generally favorable for the realization of the crop's productive potential. In 2023, both precipitation (48–67 mm) and air temperature (16–22 °C) increased compared to the previous year, particularly during June–July. These conditions can be regarded as optimal for soybean growth and development, promoting the activity of biopreparations and facilitating the establishment of efficient symbiotic interactions.

In contrast, the 2024 season was characterized by anomalous hydrothermal conditions. Precipitation levels dropped sharply to just 9 mm in May and 3 mm in both July and August, indicating the onset of severe drought. Combined with elevated average daily temperatures (15–24 °C), which peaked in July, these factors induced pronounced stress during critical stages of soybean ontogenesis (bud formation, bloom, and grain filling). Such unfavorable conditions limited the crop's ability to fully realize its genetic yield potential.

Design and treatments. The study involved a selection of microbial biopreparations representative of current-generation inoculants with diverse modes of action and compositions: Mycofriend® and Profix® as a microbial consortium, and Violar® as a metabolic bioproduct based on biologically active compounds of microbial origin.

Profix® (Certis Belchim, Belgium) comprises a consortium of nitrogen-fixing bacteria, *Bradyrhizobium japonicum* strain USDA442 (532 C) and *B. diazoefficiens* strains SEIMA 5079 and SEIMA 5080, with a total concentration of $5 \cdot 10^9$ CFU/g. Sterile ground peat serves as the carrier matrix, maintaining microbial viability and promoting the development of effective symbiosis.

Mycofriend® (solid form; BTU-Center, Ukraine) is a microbial complex including AMF (*Glomus* sp., *Trichoderma harzianum*) and rhizosphere bacteria from various functional groups (*Pseudomonas fluorescens*, *Streptomyces* sp., *Bacillus subtilis*, *B. megaterium* var. *phosphaticum*, *B. mucilaginous*, *Enterobacter* sp.), with a minimum total concentration of $1.0 \cdot 10^8$ CFU/g.

Mycofriend® (liquid form; BTU-Center, Ukraine) contains the same microbial composition as the solid form, supplemented with biologically active substances including phytohormones, vitamins, fungicidal metabolites, and amino acids. Viable cell concentration ranges from 1.0 to $1.5 \cdot 10^8$ CFU/mL.

Violar® (LLC "IC Bioinvest-Agro", Ukraine) is a cell-free metabolic preparation containing a complex of bioactive metabolites produced by *Streptomyces violaceus* IMV Ac-5027. Its multifunctional activity is attributed to the presence of phytohormones such as auxin (3.6 mg/L), cytokinin (1.9 mg/L), and gibberellin (1.6 mg/L) classes, along with free amino acids (2.2 mg/L), lipids (5.5 mg/L), sterols (1.71 mg/L), abscisic acid (0.02 mg/L), and a range of unsaturated fatty acids.

The scheme of the experiment included five variants for treatment: 1) Without inoculation – seed and plant treatment with an equivalent volume of water (control); 2) Mycofriend – seed treatment with Mycofriend® (liquid form), diluted 1:10, at a dose of 1.5 L/t, applied 1 hour before sowing; 3) Mycofriend + Profix – complex seed inoculation with Mycofriend® (solid form, 1.5 kg/t, dry method) followed, after 30 minutes, by inoculation with Profix® (1.25 kg per 500 kg of seed), both applied 30 minutes prior to sowing; 4) Mycofriend + Violar – seed inoculation with Mycofriend® (solid form, 1.5 kg/t, dry method) followed by foliar spraying at the BBCH 61 stage with Violar® (100 mL/ha, diluted in 200 L of water); 5) Mycofriend + Profix + Violar – combined treatment involving seed inoculation with Mycofriend® (solid form, 1.5 kg/t, dry method), followed by Profix® inoculation (1.25 kg per 500 kg of seed, 30 minutes later), and foliar application of Violar® (100 mL/ha, diluted in 200 L of water) at the BBCH 61 stage.

Pre-sowing soybean seed treatment with the biological preparation Mycofriend in dry form was performed using a stationary batch-type seed treater AL-50 (AGRALEX, Poland). Seed treatment with solutions of biopreparations involved seed soaking according to the specified doses and exposure time, ensuring uniform coverage of the seed material. Foliar spraying of soybean crops at the BBCH 61 stage was conducted

using a Solo backpack sprayer (Germany), which ensures uniform application of the working solution to the leaf surface.

Physiological and biochemical measurements. Plant material for the determination of chlorophyll, proline, and MDA content was collected during the BBCH 61 stage. For the quantitative analysis of photosynthetic pigments, freshly harvested plant material was used, with pigment extraction performed in 96 % ethanol. The determination of chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*) was carried out by direct spectrophotometric analysis of ethanol extracts without prior chromatographic separation. Optical density was measured at wavelengths of 665 nm (Chl *a*) and 649 nm (Chl *b*) using a ULAB 108 UV spectrophotometer (ULAB, China). Pigment content was calculated according to the equations proposed by A. R. Wellburn (1994).

Proline and malondialdehyde (MDA) concentrations in soybean leaf tissues were analyzed following modified protocols developed by M. K. Fatema *et al.* (2023).

The assimilation surface area of leaves was precisely calculated using the Easy Leaf Area software (Easlon & Bloom, 2014). The integral index of the leaf area per unit crop area (m^2/ha) was obtained by multiplying the average leaf area per plant by the number of plants per hectare, taking into account actual plant density.

Soybean productivity was assessed by complete manual harvesting of plants from designated plots following preliminary cutting of the aboveground biomass.

Statistical analysis. For each treatment variant, the arithmetic mean ($\bar{X}$) and standard error of the mean (SE) were calculated. Statistical significance of differences among treatments was determined using one-way analysis of variance (ANOVA), followed by Tukey's HSD post hoc test at a significance level of $P < 0.05$. Data processing and visualization were performed in the R software environment (R Core Team, New Zealand, version 4.4.3, 2025-02-28).

RESULTS AND DISCUSSION

For a comprehensive assessment of the soybean adaptability under hydrothermal stress, key physiological-biochemical and morphological indicators reflecting the functional state of plants at different levels of organization were analyzed. The total chlorophyll *a* and *b* content (Chl (*a*+*b*)) served as a marker of the photosynthetic apparatus condition. Leaf surface area was used as an integral morphological indicator of photosynthetic activity. Proline content was measured to assess the level of osmotic adaptation. Malondialdehyde (MDA) concentration indicated the degree of oxidative damage to cell membranes. Yield was considered an integral productivity indicator reflecting the effectiveness of adaptive mechanisms in response to stress factors.

Chlorophyll content in soybean leaves. Three-year studies of the biopreparations' impact on the Chl (*a*+*b*) content in soybean Khorol variety plants revealed significant differences both between treatment variants and between vegetation seasons (**Fig. 1**). A clear relationship between hydrothermal conditions and pigment complex formation was established. Inter-annual dynamics of Chl (*a*+*b*) demonstrates a pronounced connection with weather conditions: maximum pigment content was recorded in 2023 (4.12 mg/g of fresh weight), which exceeded the 2022 indicators by 11.7 % (3.69 mg/g). In contrast, in 2024, which was characterized by critical moisture deficiency, a sharp decrease in chlorophyll content to 2.94 mg/g was observed, which is 28.6 % less compared to the previous year, confirming the crucial role of water supply in the biosynthesis of photosynthetic pigments.

Application of biopreparations promoted chlorophyll accumulation, with the extent of stimulation varying depending on the treatment. Mono-treatment with Mycofriend solution increased Chl (*a*+*b*) content by 10.3 % relative to the control. Binary compositions proved more effective: combining Mycofriend with Profix provided an increase of 23.3 %, and the combination of Mycofriend with Violar – by 26.3 %. The highest efficiency was demonstrated by the three-component complex (Mycofriend + Profix + Violar), which increased chlorophyll content by 37.3 % relative to the control and by 24.5 % compared to mono-treatment with Mycofriend.

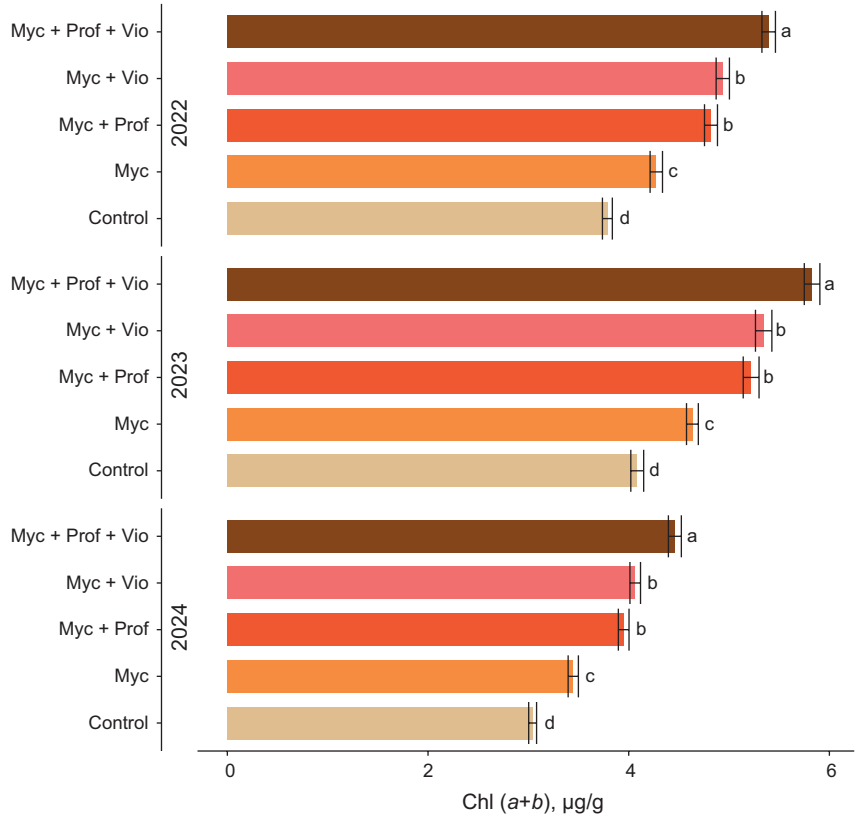

Fig. 1. Chl (*a*+*b*) content in soybean plants at the beginning of the blooming phase
Note: x ± SE, n = 10; 2022, 2023, 2024 – research years. Different letters a, b, c, d indicate statistically significant differences in Chl (*a*+*b*) content between treatments, determined using Tukey's test (P <0.05)

Notably, the relative effectiveness of complex treatment was maintained throughout all years of research, independent of hydrothermal conditions. In the favorable 2023, the three-component combination resulted in an increase in Chl (*a*+*b*) content of 37.1 % (4.73 vs. 3.45 mg/g in control), and in the dry 2024, the relative increase was 37.8 % (3.39 vs. 2.46 mg/g), demonstrating the effectiveness of the biopreparation complex in sustaining the photosynthetic apparatus of soybean plants under different hydrothermal conditions.

Leaf surface area and soybean yield. Figure 2 shows the leaf surface area of soybean plants under different treatments. Mono-treatment with Mycofriend provided an

increase in leaf surface area by an average of 12.6 % compared to the control. Binary combinations demonstrated increased efficiency: combining Mycofriend with Profix and Violar caused an additional increase of 7.1 % and 8.4 % respectively, relative to mono-treatment. Maximum development of assimilation surface (29.24 thousand m²/ha) was achieved with the complex application of three biopreparations, which exceeded the variant with Mycofriend by 14.5 %, indicating synergistic interaction between components.

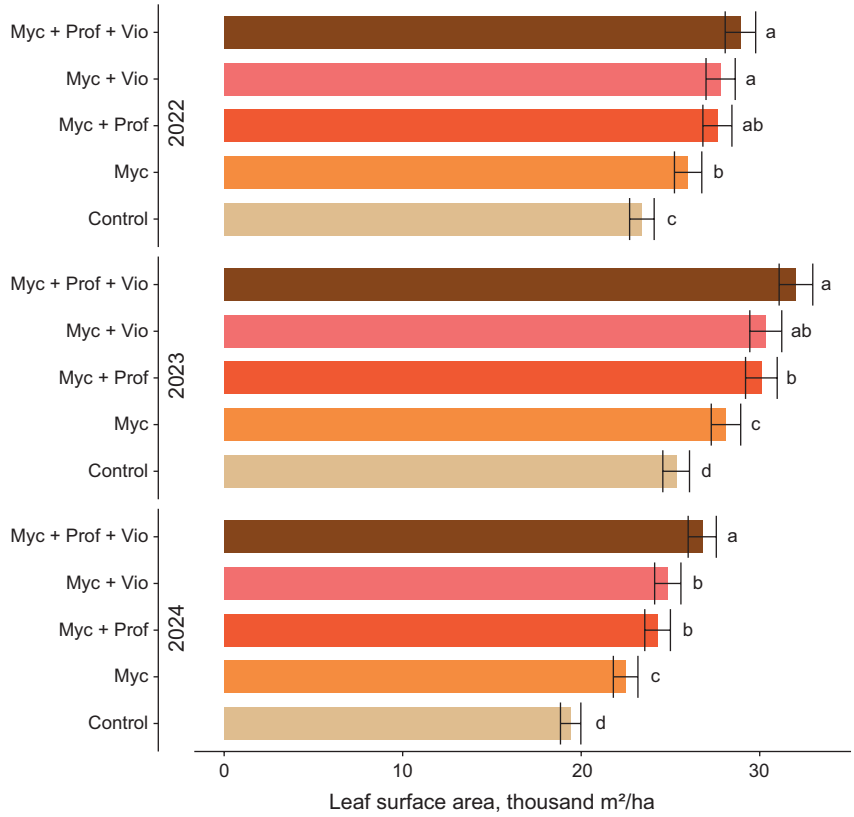

Fig. 2. Leaf surface area of soybean plants at the beginning of the blooming phase
Note: x ± SE, n = 10; 2022, 2023, 2024 – research years. Different letters a, b, c, d indicate statistically significant differences in leaf surface area between treatments, determined using Tukey's test (P <0.05)

A similar trend was observed regarding yield (**Fig. 3**). The use of Mycofriend increased productivity by 24.2 % compared to the control. Combinations of Mycofriend + Profix and Mycofriend + Violar provided additional yield growth of 8.1 % and 12.1 % respectively. The three-component treatment demonstrated the highest efficiency – 3.27 t/ha on average over three years, which exceeded the variant with Mycofriend mono-treatment by 20.2 %, confirming the synergism of biopreparations of different nature.

A direct correlation was established between the development of assimilation surface and yield formation depending on hydrothermal conditions during vegetation (**Fig. 4**). In 2022, leaf surface area varied from 23.4 to 28.92 thousand m²/ha, in 2023 – from 25.10 to 32.03 thousand m²/ha, and in the dry 2024 – from 19.55 to 26.39 thousand m²/ha.

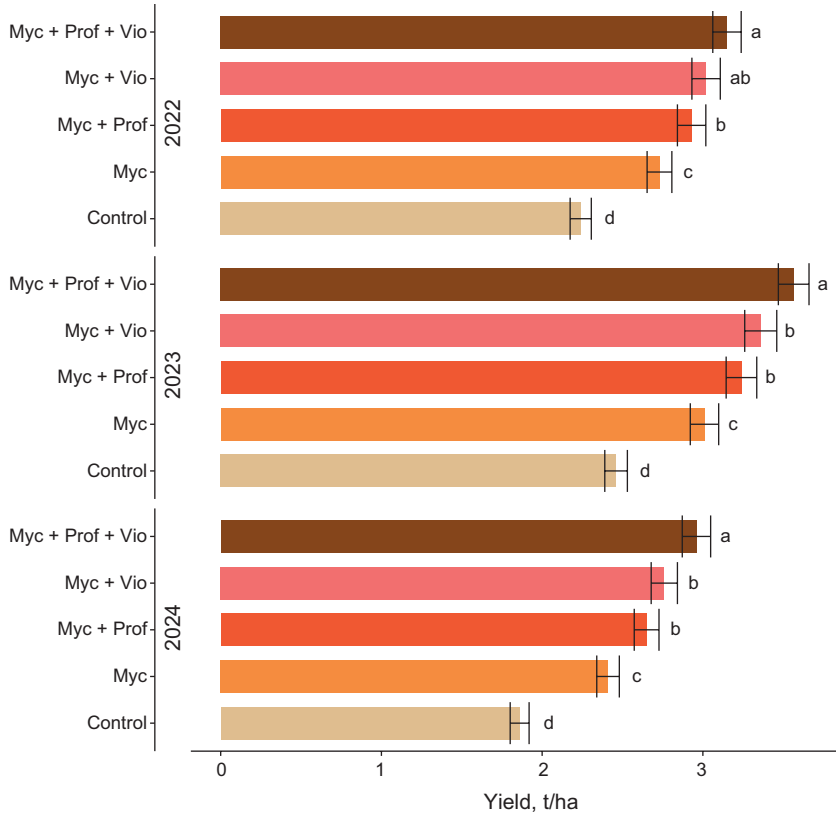

Fig. 3. Soybean yield depending on treatment variants
Note: x ± SE, n = 10; 2022, 2023, 2024 – research years. Different letters a, b, c, d indicate statistically significant differences in yield between treatments, determined using Tukey's test (P <0.05)

The principal component analysis (PCA) biplot visually demonstrates the correlation between leaf area and soybean yield during the 2022–2024 period. The first two principal components explain 100% of the data variation (Dim1 = 98.8 %, Dim2 = 1.2 %), confirming the established direct correlation between assimilation surface and yield formation.

Vector analysis of the biplot confirms a strong positive correlation between leaf area and soybean yield, indicated by the acute angle between the corresponding vectors. Analysis of point distribution in the principal component space clearly identifies three clusters corresponding to different hydrothermal conditions of the research years.

In the favorable year of 2023, the most compact grouping of all variants was observed in the right part of the biplot, corresponding to positive values of both leaf area and yield. This is consistent with optimal hydrothermal conditions (48–67 mm precipitation per month), which ensured the maximum realization of the correlation between assimilation surface and productivity.

The distribution for 2024 presents a contrast with extremely dry conditions (3 mm precipitation in July–August), where points are located in the lower part of the biplot. This demonstrates the disruption of normal correlation due to water stress, which critically affected assimilation surface formation.

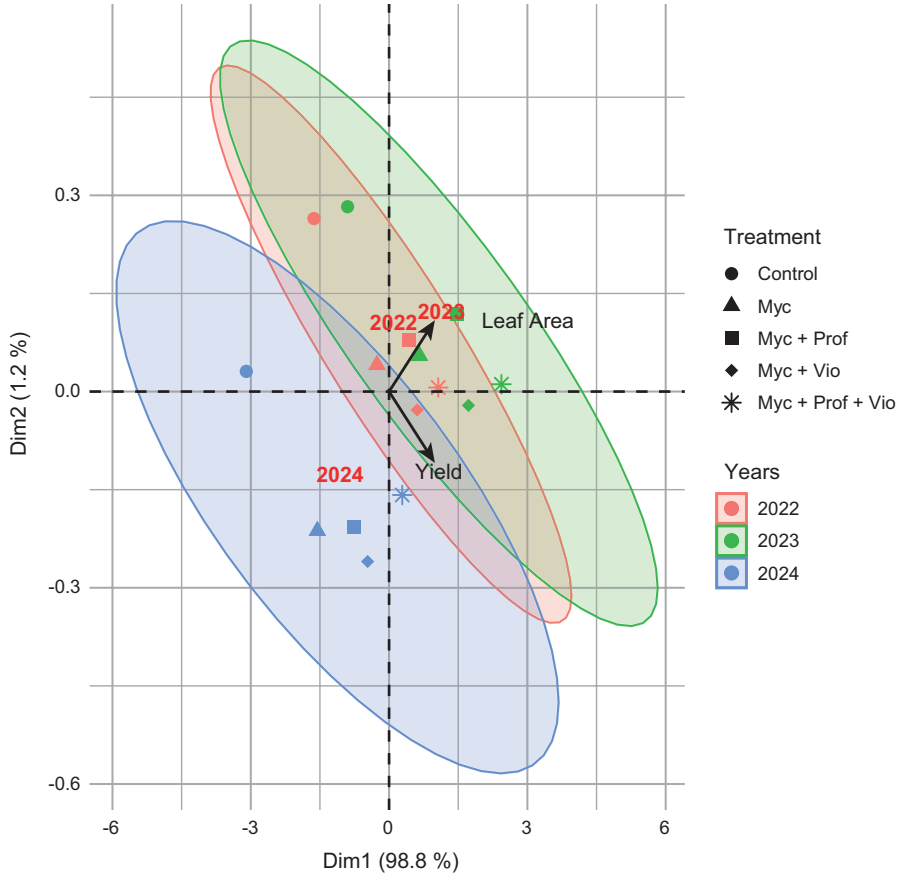

Fig. 4. Principal component biplot showing relationships between soybean leaf area and yield under different treatments and hydrothermal conditions (2022–2024)

Note: each point represents the average mean of a specific treatment in a given year. The PCA was based on standardized values of leaf surface area and yield. Colors indicate the year of observation. Ellipses represent groupings by year. The proximity of points and arrows indicates the strength and direction of correlation between variables. The plot illustrates that both leaf area and yield were positively associated and influenced by seasonal hydrothermal conditions

Data from 2022 occupy an intermediate position, demonstrating moderate correlation strength under average hydrothermal conditions.

The vector arrangement demonstrates that the first principal component (98.8 % of variation) reflects the general nature of the correlation between the studied parameters, confirming the statistical significance of the established relationship between assimilation surface development and yield formation depending on hydrothermal conditions during vegetation.

Favorable conditions in 2023 contributed to an increase in leaf surface by an average of 8.7 % compared to 2022, with treatment efficiency proportionally increasing with the biopreparations' composition: in the control, the increase was 7.3 %, with Mycofriend mono-treatment – 8.2 %, in binary combinations – 8.9–9.1 %, and with the three-component scheme – 10.8 %. This confirms that biopreparations with different action optimize the functioning of the photosynthetic apparatus and enhance crop productivity.

Particularly indicative is the differentiated reaction of plants to hydrothermal stress in 2024. The decrease in leaf surface area compared to 2023 was: in the control – 22.1 %, with Mycofriend mono-treatment – 20.0 %, in binary combination variants – 19.3 % and 18.1 %, while with complex treatment – only 16.4 %. This indicates the ability of biopreparations, especially in combination, to support the development of the photosynthetic apparatus even under unfavorable growing conditions.

Soybean yield also reflected the influence of climatic factors: maximum indicators were recorded in 2023 (2.46–3.57 t/ha), while minimum – in 2024 (1.86–2.96 t/ha), which is on average 19.2 % less compared to the previous year and 10.0 % less compared to 2022. Under favorable conditions in 2023, the increase in yield relative to 2022 was differentiated depending on the treatment variant: from 9.8 % in the control to 13.3 % with the three-component scheme.

Under conditions of hydrothermal deficit in 2024, the decrease in yield compared to 2023 was the greatest in the control (24.4 %) and the lowest with the application of the three-component composition (17.1 %), demonstrating the protective effect of complex biopreparation treatment. Intermediate positions were occupied by variants with Mycofriend mono-treatment (22.5 %), as well as combinations of Mycofriend + Profix (21.3 %) and Mycofriend + Violar (20.2 %).

The obtained results confirm the ability of biological preparations to mitigate the impact of abiotic stressors, particularly under conditions of hydrothermal deficit, maintaining the morphophysiological state of plants and preserving their productive potential. The combination of Mycofriend + Profix + Violar proved to be most effective in ensuring a stable yield of soybean Khorol variety even under unfavorable growing conditions, confirming the expediency of using complex biopreparation schemes in organic farming.

The revealed differentiated adaptive response of soybeans to hydrothermal stress demonstrates the potential of combined biopreparations as an effective tool for increasing stress resistance of cultivated plants under conditions of climatic factor instability.

Proline and MDA as stress indicators. Analysis of proline and MDA content in soybean leaves revealed regular changes in these biochemical stress markers depending on hydrothermal conditions and the biopreparations applied (**Fig. 5, 6**). Both indicators serve as reliable indicators of plant adaptive reactions to abiotic stressors, primarily soil moisture deficit.

Proline content as an osmoprotector. During the three-year study, a clear trend toward an increasing proline concentration was observed, which correlated with deteriorating water supply to crops and differentiated depending on the treatment variants (**Fig. 5**). In the control plots, the average proline level was 5.80 mg/g fresh weight, while Mycofriend mono-treatment increased this indicator by 9.1 % (6.33 mg/g). Binary combinations revealed a more powerful stimulatory effect: combining Mycofriend with Profix and Violar contributed to an increase in proline content by 16.9 % and 20.2 % respectively, compared to mono-treatment. Maximum concentration (7.27 mg/g) was recorded with the three-component scheme, which exceeded the variant with Mycofriend by 14.8 %.

Inter-annual differences in proline accumulation demonstrate a pronounced dependence on hydrothermal conditions. With a favorable moisture supply in 2023, the increase in proline concentration was minimal: in control – 6.3 %; with Mycofriend mono-treatment – 2.8 %; in variants with binary combinations – 2.1 % and 1.1 %; with the three-component scheme – 1.1 %. In contrast, in the dry 2024, a sharp increase

in proline content was recorded in all experimental variants (6.87–8.71 mg/g), on average by 28.6 %, however, with different intensities: in control – 26.8 %; with Mycofriend mono-treatment – 25.7 %; in variants Mycofriend + Profix – 27.7 %; Mycofriend + Violar – 30.2 %; with three-component treatment – 32.4 %.

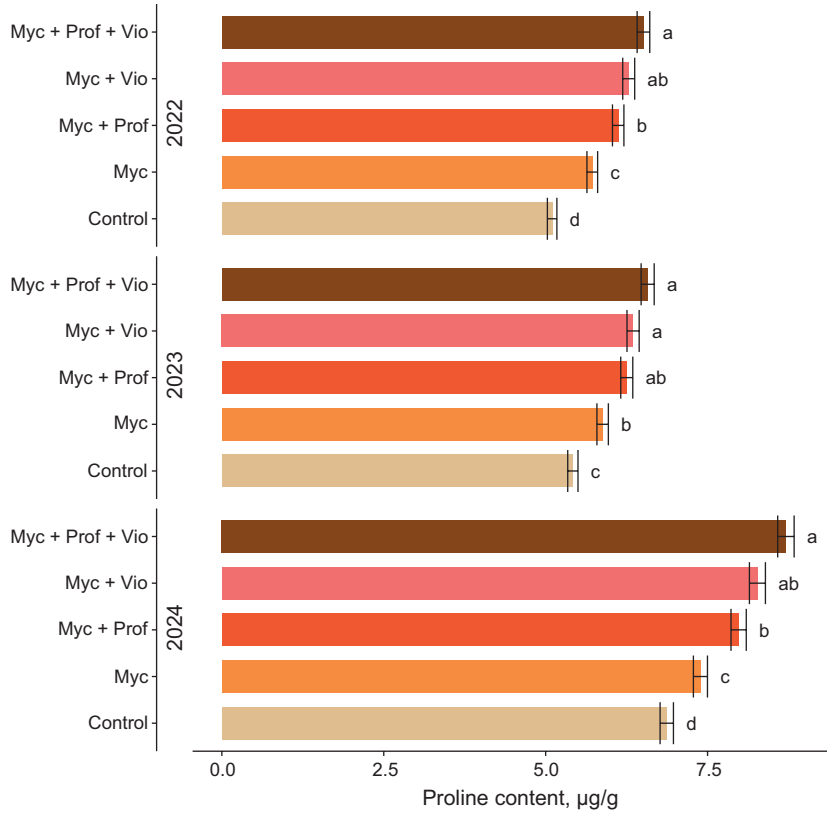

Fig. 5. Proline content in soybean leaf tissues at the BBCH 61 stage depending on treatments
Note: x ± SE, n = 10; 2022, 2023, 2024 – research years. Different letters a, b, c, d indicate statistically significant differences in proline content between treatments, determined using Tukey's test (P <0.05)

Malondialdehyde as a marker of oxidative stress. An inverse relationship was established regarding the MDA content, – a decrease in its concentration with the application of biopreparations (**Fig. 6**). In the control, an average MDA level was 12.17 mg/g fresh weight, while in treatment variants, its gradual decrease was observed: Mycofriend – 10.30 mg/g; Mycofriend + Profix – 8.89 mg/g; Mycofriend + Violar – 8.17 mg/g; Mycofriend + Profix + Violar – 7.14 mg/g.

Temporal differences in the MDA content also proved to be indicative. In the dry 2024, a sharp increase in its concentration was recorded in all variants (average level – 13.17 mg/g), while in 2022 and 2023, the indicators were significantly lower – 7.61 mg/g and 7.22 mg/g, respectively. In the optimal hydrothermal regime of 2023, a decrease in MDA content compared to 2022 was found in all treatment variants: in the control – by 2.8 %; with Mycofriend application – by 3.9 %; in binary combination variants – by 5.4 % and 6.9%; with the three-component scheme – by 8.3 %.

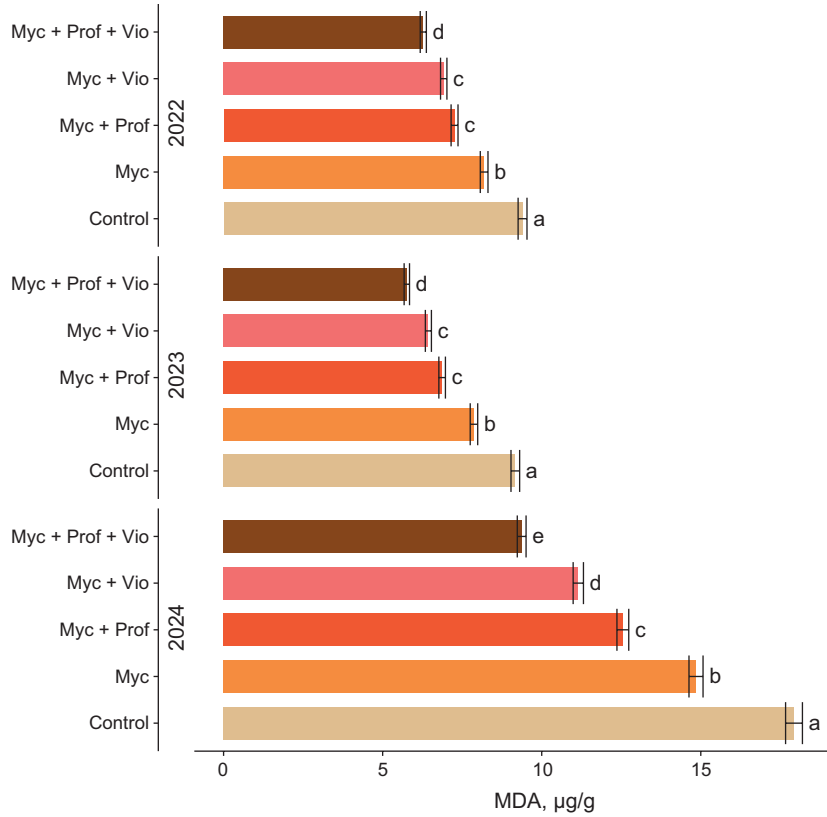

Fig. 6. MDA content in soybean leaf tissues at the BBCH 61 stage depending on treatments
Note: x ± SE, n = 10; 2022, 2023, 2024 – research years. Different letters a, b, c, d indicate statistically significant differences in the MDA content between treatments, determined using Tukey's test (P <0.05)

Stressful conditions in 2024 caused a significant increase in MDA levels compared to 2023, however, with different intensities: in control – by 96.2%; with Mycofriend mono-treatment – by 88.7 %; in variants Mycofriend + Profix – by 82.7 %; Mycofriend + Violar – by 61.8 %; with three-component treatment – only by 56.3 %. It can be assumed that the applied biopreparations exert a complex effect on the condition of cell membranes and contribute to a reduction in MDA levels under stress conditions. This is consistent with literature data indicating that MDA is a reliable marker of lipid peroxidation and oxidative stress in plants, and that its decreased content is associated with improved membrane integrity and enhanced stress tolerance (Heath & Packer, 1968; Gill & Tuteja, 2010; Hasanuzzaman *et al.,* 2020).

The obtained results align with current scientific findings regarding the role of arbuscular mycorrhizal fungi, nitrogen-fixing bacteria, and phytohormones in biopreparations in enhancing soybean adaptability under adverse growing conditions. Symbiosis of plants with AMF activates cellular energy metabolism, intensifying photosynthetic processes: chlorophyll content increases, leaf surface expands, CO_2 fixation rate increases, and assimilate transport to the root system is enhanced (Yang *et al.*, 2021; Bogati & Walczak, 2022).

Inoculation with AMF stimulates the accumulation of osmolytes (sugars, proline, glycine betaine), which play a key role in osmoregulation and provide an adaptive response of plants to water deficit, and also affect the level of photosynthetic pigments in soybean plants (Wu *et al.*, 2017; Begum *et al.*, 2023). S. A. Mona *et al*. (2017) reported that *T. harzianum* soybean seed inoculation caused an increase of 15.04 % in total chlorophylls despite the drought stress and led to a 2.0 % proline level increase. In our study, the application of the biopreparation Mycofriend, which contains arbuscular mycorrhizal fungi (AMF), contributed to a 10.3 % increase in total chlorophyll content compared to the control. AMF inoculation (Mycofriend) also caused an increase in leaf proline levels of 28.6 % on average.

Simultaneously, the activity of antioxidant enzymes increases, particularly that of catalase and superoxide dismutase, which contributes to the stabilization of cell membranes and a reduction of the MDA content by 30–50 %, indicating strengthening of the antioxidant defense system (Mirshad & Puthur, 2016; Li *et al.*, 2019).

The use of phytohormones in combination with AMF or rhizobia increases plant stress resistance, optimizes the biological nutrition system, and enhances soybean productivity (Ferreira *et al.*, 2020). Synergistic relationships between AMF and rhizobia are fundamental for ensuring effective nitrogen fixation and optimization of mineral nutrition, which forms stable productive agrocenoses resistant to hydrothermal fluctuations and extreme temperature regimes (Li *et al.*, 2019; Miljaković *et al.*, 2022). M. S. Sheteiwy *et al.* (2021) showed that the application of *B. japonicum*, AMF strains, and their combination in soybean seed inoculation contributed to an increase in chlorophyll content by 35.4 %, 41.5 %, and 39.0 %, which correlated with an increase in yield by 25.9 %, 32.5 %, and 25.0 %, respectively.

Our results confirm the synergistic effects between AMF and rhizobia, as well as between AMF and phytohormones. The addition of Profix, a preparation containing nitrogen-fixing bacteria, to Mycofriend led to a significant increase in total chlorophyll content (by 23.3 %), while the combined use of the phytohormonal preparation Violar with Mycofriend resulted in an even greater increase (by 26.3 %). These improvements in physiological parameters were reflected in soybean productivity. The use of Mycofriend alone increased yield by 24.2 % compared to the control, whereas the combinations of Mycofriend + Profix and Mycofriend + Violar further enhanced yield by 8.1 % and 12.1 %, respectively.

Under conditions of increasing climate change and growing frequency of extreme weather events, the implementation of anti-stress protection elements in crop cultivation systems becomes particularly relevant. Complex application of biopreparations of different nature – AMF, phytohormonal regulators, nitrogen-fixing bacteria – provides a synergistic effect and sustainable adaptation of plants to unfavorable conditions. Widespread implementation of such biotechnological approaches can not only stabilize yield but also become the basis for forming sustainable, resource-efficient agroecosystems under conditions of global climate change.

CONCLUSION

The conducted study demonstrates a pronounced multifaceted positive effect of biopreparations (monotreatment or in combinations) on physiological and biochemical processes, as well as on soybean productivity (cv. Khorol) under hydrothermal conditions.

The integrated application of the biopreparations Mycofriend, Profix, and Violar resulted in the maximum effect: total chlorophyll content Chl (*a*+*b*) increased by 37.3 %, leaf assimilation area grew by 28.9 %, which together led to a significant improvement in yield (up to 47.5 %). This effect can be explained by the synergistic interaction of AMF, nitrogen-fixing bacteria, and phytohormonal substances contained within the biopreparations.

The study also revealed a considerable improvement in the soybean plant's adaptability under biopreparation treatment, as reflected by a reduced intensity of oxidative stress: proline accumulation (as an osmoprotectant) increased by 25.3 %, while MDA content decreased by 41.1 %.

Considering the performance advantages observed across variable moisture years, the combined application of Mycofriend, Profix, and Violar can be recommended for organic soybean cultivation in regions facing recurrent hydrothermal stress to sustain yield and crop resilience under climate challenges.

Based on the current findings, the further studies should be conducted to evaluate (i) an expanded panel of biopreparation combinations (7 treatment variants and control) to refine dose–composition recommendations; (ii) include additional drought- and stress-responsive physiological indicators such as abscisic acid (ABA) concentration, relative water content (RWC), and stomatal conductance to better link microbial treatments with plant water status regulation. Where data permit, integrating agronomic performance with techno-economic analyses will further support on-farm decision-making and regional scaling.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: the authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Animal Rights: this article does not contain any studies with animal subjects performed by any of the authors.

AUTHOR CONTRIBUTIONS

Conceptualization, [T.C.; I.K.]; methodology, [T.C.]; validation, [T.C.; I.K.]; formal analysis, [G.P.; V.L.]; investigation, [G.P.; N.K.; V.L.]; resources, [V.L.]; data curation, [G.P.; N.K.]; writing – original draft preparation, [T.C.; I.K.]; writing – review and editing, [T.C; I.K.]; visualization, [G.P.; N.K.; V.L.] supervision, [T.C.].

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АДАПТИВНІСТЬ СОЇ ЗА КОМПЛЕКСНОЇ ОБРОБКИ БІОПРЕПАРАТАМИ РІЗНОЇ ПРИРОДИ В УМОВАХ ОРГАНІЧНОГО ЗЕМЛЕРОБСТВА ТА ГІДРОТЕРМІЧНОГО СТРЕСУ

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Обґрунтування. В умовах органічного землеробства та посилення гідротермічного стресу особливої актуальності набуває застосування біологічних препаратів як ефективних інструментів підвищення адаптивності сої. Комплексне застосування арбускулярних мікоризних грибів, азотфіксуючих бактерій і фітогормональних регуляторів дає змогу активувати фізіологічні та біохімічні процеси рослин. Такий біотехнологічний підхід забезпечує пом'якшення негативного впливу гідротермічних стресів і підтримує продуктивність культур навіть за несприятливих умов вирощування.

Матеріали та методи. Об'єктом дослідження є загальний вміст хлорофілу, площа листкової поверхні, концентрація проліну та малонового діальдегіду (МДА), а також продуктивність сої сорту Хорол за умов нестабільного зволоження під впливом ризосферних мікроорганізмів і різних фітогормонів у складі біопрепаратів. Використано польові, лабораторні (фізіологічні, біохімічні) та статистичні методи.

Результати. Використання біопрепаратів покращило біохімічні, фізіологічні та морфологічні показники, а також продуктивність сої, що свідчить про покращення стану рослин. Найвищий рівень загального хлорофілу (*a*+*b*) зареєстровано в 2023 р.

за комбінованого застосування Mycofriend, Profix і Violar, показуючи середнє збільшення відповідно на 42,3 % і 26,7 % порівняно з контролем. Площа листкової поверхні досягла 29,24 тис. м²/га, а врожайність – 3,23 т/га, перевищуючи контроль на 28,9 % та 47,5 % відповідно. Підвищений вміст проліну (7,27 мг/г) і нижчий рівень МДА (7,14 мг/г) під час стресу відображають зниження окислювального пошкодження та кращу осморегуляцію завдяки синергічній дії мікоризи, різобій і фітогормонів.

Висновки. Комплексне застосування біопрепаратів (Мікофренд + Profix + Віолар) у системі органічного вирощування сої сорту Хорол сприяє активації фізіолого-біохімічних процесів, підвищенню продуктивності та стресостійкості рослин за умов гідротермічного стресу.

Ключові слова: арбускулярно-мікоризні гриби, фітогормональні регулятори, хлорофіли, площа листкової поверхні, врожайність, пролін, малоновий діальдегід

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