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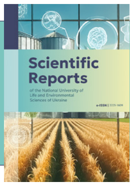

Abscisic acid and biopreparations as markers of organic soybean adaptation to hydrothermal stress

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Abstract. Given the growing demand for organic soybeans and the need to mitigate the adverse effects of climate change, the study of biopreparations of various origins and their influence on plant adaptive mechanisms under stress conditions has become increasingly relevant. This study aimed to assess changes in abscisic acid (ABA) content under the influence of biopreparations in a multi-year field experiment and to analyse the relationship between ABA and the biochemical, physiological, and agronomic indicators of soybean adaptation to hydrothermal stress under organic farming conditions. The study involved the use of biological preparations based on mycorrhizal fungi, rhizosphere and nitrogen-fixing bacteria, as well as phytohormones. A range of field, laboratory (physiological and biochemical), and statistical methods was employed. The findings revealed the integration of molecular, biochemical, physiological, and agronomic stress resistance markers as a result of the enhanced adaptive potential of soybean following the application of biopreparations. Their effectiveness was evident through a comprehensive influence on key adaptive mechanisms, including: a 9.2%-36.3% reduction in ABA concentration, indicating alleviation of stress pressure; a 10.0%-27.6% increase in relative water content, contributing to the maintenance of optimal cellular water balance; a 19.2%-65.4% improvement in stomatal conductance, attributed to the optimisation of stomatal function; a 15.6%-41.8% reduction in lipid peroxidation levels (malondialdehyde), indicating effective membrane protection against oxidative stress; a 9.1%-46.6% increase in proline content, suggesting the activation of natural defence mechanisms and the maintenance of osmotic balance. Correlation analysis confirmed that the biopreparations facilitated a complex modulation of the plant stress response, optimising the balance between defence reactions and productivity, which led to a 12.3%-45.5% increase in yield even under adverse weather conditions. It was established that under water stress, the biological preparations modulated ABA biosynthesis and its regulatory role, contributing to coordinated changes in osmoprotective mechanisms, antioxidant defence, and the plant water regime. The most effective approach proved to be the triple combination of biopreparations, which ensured an optimal balance between phytohormonal regulation, physiological processes, and soybean productivity under hydrothermal stress. The results obtained may be recommended for the cultivation of organic soybeans to improve yield under climate-related challenges

Keywords: organic farming; relative water content; stomatal conductance; proline; malondialdehyde; yield

Introduction

An integrative study of stress resistance in agricultural crops – encompassing molecular-biochemical processes, physiology, and productivity – is crucial for developing effective adaptation strategies to climate-related risks, particularly drought and temperature extremes. This is especially relevant for soybean (*Glycine max* L. Merr.), which holds strategic importance for Ukraine's economy and export potential (Bobos et al., 2025). Investigating the relationships between the application of biopreparations, phytohormonal regulation (particularly abscisic acid (ABA)), and the physiological mechanisms of plant adaptation to hydrothermal stress offers a pathway toward the development of environmentally safe technologies and enhances the competitiveness of organic agricultural production under climate change conditions.

According to K. Bogati & M. Walczak (2022), global climate change is transforming soil environments and adversely affecting the functioning of the soil biota, including microorganisms and plants, which significantly complicates crop cultivation. Under such conditions, potential yield losses may reach up to 50%, as reported by S. Poudel *et al.* (2023). Soybean is one of the most important protein-oil crops in global agriculture, with yield losses under severe water deficiency potentially reaching 100%.

According to the findings of H.M. Zabolotnyi *et al.* (2020), soybean holds a leading position on both the global and Ukrainian agricultural markets, serving not only as an export crop but also as a strategically important raw material for the food and feed industries. According to the European Commission (EU Imports of Organic Agri-food Products, 2025), Ukraine ranks second among suppliers of organic soybeans to the EU, making this crop critically important for national food and economic security. However, as demonstrated by A. Abhilasha & S.R. Choudhury (2021), increasing hydrothermal stress necessitates a deeper understanding of the mechanisms underlying plant adaptation to extreme growing conditions. A key role in regulating adaptive responses is played by phytohormones, particularly ABA, which coordinates complex signalling pathways aimed at maintaining water balance in plants. The study by S. Das *et al.* (2025) confirmed that ABA synthesis is activated in the roots under water deficit conditions, after which the hormone is transported to the aerial organs, triggering a cascade of adaptive responses. These include the regulation of stomatal conductance, accumulation of osmoprotectants, reinforcement of the cuticular wax layer, and enhancement of the antioxidant defence system.

Considerable attention has been devoted to investigating the relationship between rhizosphere microbiota and the functioning of ABA-dependent signalling pathways. As shown in the study by B. Wang *et al.* (2025), microorganisms contained in biopreparations are capable of influencing endogenous ABA levels, modulating its signalling pathways, and activating the physiological and biochemical mechanisms of stress tolerance. In the study by M. del C. Orozco-Mosqueda *et al.* (2023), the potential of biopreparations for targeted regulation of the plant phytohormonal status was emphasised. However, the relationship between ABA levels, the activity of physiological stress markers, and soybean productivity under contrasting hydrothermal conditions remains insufficiently explored.

Research by I. Yaghoubian *et al.* (2022) demonstrated that the application of plant growth-promoting microorganisms (PGPM) helps to mitigate the adverse effects of drought on soybean yield. As confirmed by the findings of J. Naamala *et al.* (2022), plant growth-promoting rhizobacteria (PGPR) can activate phytohormone synthesis – particularly ABA – increase the activity of antioxidant enzymes, and regulate the expression of stress-responsive genes. According to G.A. dos Reis *et al.* (2024), microbial inoculants are playing an increasingly important role in agriculture by reducing reliance on chemical fertilisers, enhancing crop productivity, and restoring soil biota. Thus, recent research highlights the need for further investigation into the interaction between biological preparations and ABA as key markers of organic soybean adaptation to hydrothermal stress in the context of climate change.

This study aimed to evaluate the patterns of ABA content variation in soybean plants under the influence of biopreparations of various origins in the context of a multi-year field experiment, and to analyse the relationships between ABA levels and a set of biochemical, physiological, and agronomic indicators of soybean adaptation to hydrothermal stress within an organic farming system. This objective is directed towards identifying the mechanisms through which biopreparations modulate the hormonal status of soybean to enhance its resilience to adverse climatic conditions.

Materials and Methods

The experimental research was conducted over a three-year period (2022-2024) under the agroecological conditions of the Left-Bank Forest-Steppe of Ukraine, within the territory of Kremenchuk District, Poltava Region. The subject of the study was the early-maturing soybean variety Khorol, developed by breeders at the Soybean Research Institute Limited Liability Company (LLC). The soil cover of the experimental plots was represented by residual-solonetzic chernozem on loess deposits. According to the agrochemical analysis of the arable layer (0-20 cm), carried out using a multiparameter photometer Palintest SK500 (Palintest Ltd., United Kingdom), the following characteristics were recorded: slightly acidic soil solution (pHKCl – 6.3); high humus content (5.2%); moderate availability of nitrogen compounds (total nitrogen – 58.6 mg/kg) and phosphorus (available phosphorus – 78.3 mg/kg); and a high level of exchangeable potassium (138.4 mg/kg). Field experiments were laid out using a randomised block design with three replications. The total area of the experimental site was 0.3 ha, with an accounting (measured) plot area of 0.1 ha. The cultivation technology was consistent with the typical practices for the relevant agroclimatic zone, adapted to meet the requirements of organic farming. In the crop rotation structure, the preceding crop for soybean was spring barley.

As soybean is a thermophilic crop, the sowing date in each year of the study was determined based on soil temperature reaching 10°C-12°C at a depth of 5 cm (from 20 April to 5 May). The spatial arrangement of the plants was defined by a row spacing of 38 cm and a seeding rate of 700,000 viable seeds per hectare, which ensured the formation of an optimal plant density for varieties of this maturity group under conditions of variable water availability. An analysis of the temperature regime during the growing season (May-August) showed relative stability in 2022 and 2023, with average monthly temperatures ranging from 14.5°C-21.3°C and 15.5°C-22.0°C, respectively. In contrast, 2024 was characterised by elevated temperatures, particularly in July and August, when average monthly values reached 24.1°C and 22.4°C, respectively – exceeding those of previous years. The research complied with ethical standards as outlined in the Convention on Biological Diversity (1992).

Regarding the moisture regime, the following dynamics were observed: in 2022, rainfall was evenly distributed, with monthly totals ranging from 38 to 57 mm. The year 2023 was marked by a slightly higher amount of precipitation, ranging from 47 to 67 mm per month. In stark contrast, the situation in 2024 was characterised by unprecedentedly low rainfall during critical stages of the growing season: 9 mm in May, 3 mm in July, and 3 mm in August. These values fall within the classification of extreme drought. The hydrothermal coefficient for the 2024 growing season was significantly below optimal levels, resulting in stressful conditions for soybean plants. Particularly detrimental was the combination of critically low atmospheric precipitation and elevated air temperatures during the formation of generative organs and seed filling – stages that are among the most sensitive in the ontogenesis of this crop. The prolonged lack of adequate moisture supply led to pronounced hydrothermal stress, which had a negative impact on soybean yield.

To obtain representative data on the effectiveness of biopreparation application in soybean cultivation under organic farming systems, preparations with different mechanisms of action on plant physiological processes and various forms of symbiotic interactions were selected. Particular attention was given to the potential for synergistic effects between components:

1. In the preparations Mycofriend-wp® and Mycofriend-sc® (BTU-Centre, Ukraine), synergy is achieved through the mutually enhancing action of mycorrhizal fungi (*Glomus* sp., *Trichoderma harzianum*) and rhizosphere bacteria (*Pseudomonas fluorescens*, *Streptomyces* sp., *Bacillus subtilis*, *B. megaterium* var. *phosphaticum*, *B. muciloginosus*, *Enterobacter* sp.). For instance, *Pseudomonas fluorescens* secretes metabolites that stimulate the development of *Glomus* sp. hyphae, while the mycorrhizal fungi improve phosphorus availability for *Bacillus* species.

2. Strains of *Bradyrhizobium* (*japonicum* strain USDA442 (532 C) and *diazoefficiens*

strains SEIMA 5079 and SEIMA 5080), included in the Profix® preparation (Certis Belchim, Belgium), demonstrate functional complementarity due to differences in root colonisation dynamics and varying temperature optima for nitrogenase activity. This ensures stable nitrogen fixation across a wide range of agroecological conditions.

3. In the Violar® preparation (IC Bioinvest-Agro LLC, Ukraine), synergy is realised through a balanced composition of phytohormones from different classes (auxin, cytokinin, and gibberellin types), as well as free amino acids, lipids, sterols, and abscisic acid. This combination enables the integrated regulation of growth and adaptive processes within the plant organism.

The experimental design included eight treatment variants involving seed treatment and foliar application of bioproducts during the BBCH 61 growth stage (Table 1).

Table 1. *Experimental design involving biopreparation application*

No.	Variants	Doses	Treatment
1	Control (water)	–	seed treatment 30 minutes before sowing
2	Profix (Prof)	1.25 kg/500 kg	seed inoculation 30 minutes before sowing
3	Violar (Vio)	0.5 L/t + 100 mL/ha	seed treatment 1 hour before sowing + foliar spraying
4	Mycofriend (Myc)	1.5 L/t +	seed treatment 1 hour before sowing
5	Profix + Violar (Prof + Vio)	1.25 kg/500 kg + 100 mL/ha	seed inoculation 30 minutes before sowing + foliar spraying
6	Mycofriend + Profix (Myc + Prof)	1.5 kg/t + 1.25 kg/500 kg	seed treatment for 30 minutes + inoculation 30 minutes before sowing
7	Mycofriend + Violar (Myc + Vio)	1.5 kg/t + 100 mL/ha	seed treatment + foliar spraying
8	Mycofriend + Profix + Violar (Myc + Prof + Vio)	1.5 kg/t + 1.25 kg/500 kg + 100 mL/ha	seed treatment for 30 minutes + inoculation 30 minutes before sowing + foliar spraying

Source: developed by the authors

The relative water content (RWC) in soybean leaves was measured weekly over a seven-week period, starting from the second half of May. For the analysis, one leaf was collected from ten representative plants in each experimental plot. Samples were immediately weighed to determine their fresh weight, after which they were immersed in distilled water for 24 hours at room temperature to achieve full turgor. The following day, the leaves were carefully blotted to remove excess moisture and reweighed to determine their turgid weight. The samples were then dried to a constant weight at 70°C to determine their dry weight. The RWC was calculated as a percentage using the following formula:

$$RWC = \frac{(FW - DW)}{(TW - DW)} \cdot 100, \quad (1)$$

where *FW* (g) is the fresh weight of the leaf immediately after sampling, *DW* (g) is the dry weight of the leaf after drying at 70°C, and *TW* (g) is the turgid weight of the leaf. The analytical determination of ABA content was carried out using fluorescence spectroscopy with a PerkinElmer LS-45 fluorescence spectrometer (PerkinElmer, Inc., USA). The slit widths for both emission and excitation were set at 5 nm. The spectral range covered 200-700 nm. The measurement error in determining peak positions was ± 2 nm, with a spectral band half-width of 5 nm. Fluorescence intensity measurements of ABA solutions were performed using thin quartz cuvettes (spatula-type) with a path length of 1.00 cm. The procedure for preparing ABA leaf extracts for fluorimetric measurements followed the method described by Y.-N. Li *et al.* (2009).

Stomatal conductance (SC) is a direct indicator of stomatal opening and closing in plants (Jiang *et al*, 2006) and is typically measured using a porometer. In this study, SC in soybean leaves ($mol/m^2 \cdot s$) was measured using an SC-1 porometer (Decagon Devices, Inc., USA), with 30-second intervals and three replications for each treatment variant. The concentrations of proline and malondialdehyde (MDA) in soybean leaf tissues were determined at the BBCH 61 growth stage, following modified methodological protocols developed by M.K. Fatema *et al.* (2023). Soybean productivity was assessed by fully harvesting plants manually from the accounting plots after cutting the above-ground phytomass. Seed yield from each experimental plot was adjusted to a standard moisture content of 15% and expressed in tonnes per hectare. To summarise the results and identify key factors influencing soybean adaptive responses under varying hydrothermal conditions, principal component analysis (PCA) was conducted.

For each experimental treatment, the arithmetic mean ($\bar{X}$) and standard error of the mean (SE) were calculated. The statistical significance of differences between treatment groups was assessed using one-way analysis of variance (ANOVA), followed by Tukey's HSD post hoc test at a significance level of $P < 0.05$. Data visualisation and statistical processing were performed using RStudio. Relationships between experimental parameters were evaluated through correlation analysis, with Pearson correlation coefficients calculated at a significance level of $P < 0.05$. Statistical analysis of the experimental data was conducted using Statistica 12.0 software.

Results and Discussion

Determining the level of ABA is of fundamental importance, as this phytohormone acts as a key regulator of stress-induced signalling cascades that trigger complex plant adaptation mechanisms under water deficit conditions. An integrated analysis of physiological markers (RWC, SC), in combination with biochemical indicators (proline, MDA), provides a comprehensive understanding of the functional state of the plant at various levels of organisation. It also enables the identification of correlation links between molecular-biochemical processes and the final agronomic performance of soybean cultivation under hydrothermal stress conditions (Table 2).

Based on the experimental data obtained, a detailed analysis was conducted to evaluate the effects of climatic factors and different biological seed treatments on the ABA content in plants of the early-maturing soybean variety Khorol. Weather conditions were found to correlate strongly with changes in ABA levels. In 2024, regardless of treatment type, a significant increase in ABA content was observed compared to 2022-2023: in the control variant, it reached 8.5 nmol/g, compared to 6.8 and 6.5 nmol/g, respectively. This increase of 25.0%-30.8% confirms the activation of plant defence mechanisms in response to water deficit, as ABA is a key regulator of drought response in plants.

Table 1. *Effect of biopreparation treatments on molecular and physiological parameters and soybean yield under hydrothermal stress conditions (20222024)*

Treatment	ABA, nmol/g DW			Proline, µg/g			MDA, µg/g			RWC, %			SC, mol/m²·s			Yield, t/ha		
	2022	2023	2024	2022	2023	2024	2022	2023	2024	2022	2023	2024	2022	2023	2024	2022	2023	2024
Control	6.8 ± 0.10^{a}	6.5 ± 0.10^{a}	8.5 ± 0.13^{a}	5.1 ± 0.07^{e}	5.4 ± 0.08^{e}	6.9 ± 0.10^{e}	9.4 ± 0.14^{a}	9.2 ± 0.14^{a}	17.9 ± 0.27^{a}	73.8 ± 1.09^{e}	75.1 ± 1.10^{e}	69.7 ± 1.02^{d}	0.28 ± 0.004^{e}	0.31 ± 0.005^{g}	0.19 ± 0.003^{g}	2.24 ± 0.033^{f}	2.46 ± 0.035^{f}	1.86 ± 0.029^{g}
Profix	6.1 ± 0.09^{b}	5.9 ± 0.09^{ab}	7.8 ± 0.11^{b}	6.2 ± 0.09^{c}	6.4 ± 0.09^{cd}	8.6 ± 0.13^{c}	7.2 ± 0.11^{c}	6.9 ± 0.10^{bcd}	13.9 ± 0.21^{c}	80.5 ± 1.18^{cd}	82.5 ± 1.21^{cd}	77.5 ± 1.14^{c}	0.32 ± 0.005^{d}	0.37 ± 0.005^{ef}	0.25 ± 0.004^{f}	2.50 ± 0.037^{e}	2.82 ± 0.041^{e}	2.11 ± 0.029^{f}
Violar	5.4 ± 0.08^{cd}	5.2 ± 0.08^{cd}	6.9 ± 0.10^{c}	6.8 ± 0.10^{b}	7.5 ± 0.11^{ab}	9.5 ± 0.14^{b}	5.9 ± 0.09^{d}	5.4 ± 0.08^{d}	11.3 ± 0.17^{e}	85.1 ± 1.25^{bc}	87.2 ± 1.28^{bcd}	84.0 ± 1.24^{b}	0.36 ± 0.005^{cd}	0.39 ± 0.006^{de}	0.28 ± 0.004^{de}	2.71 ± 0.039^{d}	2.93 ± 0.044^{de}	2.32 ± 0.035^{e}
Mycofriend	5.6 ± 0.08^{c}	5.4 ± 0.08^{c}	7.1 ± 0.10^{c}	5.7 ± 0.08^{d}	5.9 ± 0.09^{de}	7.4 ± 0.11^{e}	8.2 ± 0.12^{b}	7.9 ± 0.12^{ab}	14.8 ± 0.22^{b}	79.2 ± 1.17^{de}	81.2 ± 1.20^{de}	77.8 ± 1.14^{c}	0.31 ± 0.005^{d}	0.35 ± 0.005^{f}	0.26 ± 0.004^{ef}	2.73 ± 0.039^{cd}	3.01 ± 0.045^{de}	2.41 ± 0.036^{de}
Prof + Vio	5.1 ± 0.07^{d}	4.8 ± 0.07^{e}	6.7 ± 0.10^{c}	7.4 ± 0.11^{a}	7.8 ± 0.11^{a}	10.4 ± 0.15^{a}	6.1 ± 0.09^{d}	5.6 ± 0.08^{cd}	10.9 ± 0.16^{e}	86.3 ± 1.27^{b}	88.7 ± 1.30^{abc}	85.7 ± 1.26^{b}	0.36 ± 0.005^{b}	0.43 ± 0.006^{c}	0.31 ± 0.005^{bc}	2.91 ± 0.044^{bc}	3.10 ± 0.045^{cd}	2.53 ± 0.037^{cd}
Myc + Prof	5.3 ± 0.08^{cd}	5.0 ± 0.07^{de}	6.2 ± 0.09^{d}	6.1 ± 0.09^{cd}	6.3 ± 0.09^{cd}	8.0 ± 0.12^{d}	7.3 ± 0.11^{c}	6.9 ± 0.10^{bcd}	12.6 ± 0.19^{d}	85.4 ± 1.26^{bc}	87.8 ± 1.29^{abcd}	84.6 ± 1.25^{b}	0.35 ± 0.005^{bc}	0.41 ± 0.006^{cd}	0.29 ± 0.004^{cd}	2.93 ± 0.043^{b}	3.24 ± 0.048^{bc}	2.65 ± 0.039^{bc}
Myc + Vio	4.6 ± 0.07^{e}	4.4 ± 0.06^{f}	6.0 ± 0.09^{de}	6.3 ± 0.09^{c}	6.4 ± 0.09^{b}	8.3 ± 0.12^{cd}	6.9 ± 0.10^{c}	7.5 ± 1.14^{abc}	6.4 ± 0.09^{c}	87.1 ± 1.28^{ab}	89.5 ± 1.32^{ab}	86.7 ± 1.27^{ab}	0.37 ± 0.005^{b}	0.46 ± 0.007^{b}	0.32 ± 0.005^{b}	3.02 ± 0.045^{ab}	3.36 ± 0.050^{ab}	2.76 ± 0.041^{b}
Myc + Prof + Vio	4.2 ± 0.06^{f}	4.1 ± 0.06^{f}	5.6 ± 0.08^{e}	6.5 ± 0.10^{bc}	6.6 ± 0.10^{cd}	8.7 ± 0.13^{c}	6.3 ± 0.09^{d}	5.8 ± 0.09^{cd}	9.4 ± 0.14^{f}	92.3 ± 1.36^{a}	94.7 ± 1.39^{a}	91.9 ± 1.35^{a}	0.41 ± 0.006^{a}	0.51 ± 0.008^{a}	0.39 ± 0.006^{a}	3.15 ± 0.046^{a}	3.57 ± 0.053^{a}	2.96 ± 0.045^{a}

Note: data are presented as mean ± SE (n = 10). Values followed by different letters (a, b, c, d, e, f, g) for each variable indicate statistically significant differences at the $p < 0.05$ level according to Tukey's HSD post hoc test

Source: compiled by the authors

The findings showed that the use of biopreparations with different modes of action contributed to a reduction in ABA levels compared to the control in all years of the study, indicating their beneficial role in enhancing plant stress resistance. The greatest reduction in ABA content relative to the control was observed under the combined treatment Myc + Prof + Vio, which led to a 36.9%-38.2% decrease in 2022-2023 and 34.1% in the drought-stricken year 2024. The Myc + Vio combination also demonstrated high effectiveness, reducing ABA levels by 32.3% in favourable years and by 29.4% in 2024. Among single treatments, Violar proved the most effective, resulting in a 20.0%-20.6% reduction in ABA content in favourable years and an 18.8% reduction in 2024.

Analysis of the results from 2022-2024 confirmed a significant influence of climatic factors on the biochemical indicators of soybean adaptive responses, with the most pronounced changes observed in 2024, a year marked by extreme weather conditions (Table 2). In particular, in response to the 2024 drought, a substantial increase in proline content was recorded across all treatment variants compared to 2022-2023. In the control, the proline level reached 6.9 μg/g, which was 26.7%-34.7% higher than in previous years. This indicates the activation of natural defence mechanisms, as proline is a key osmoprotectant that accumulates in plant cells under water deficit conditions, helping to maintain osmotic balance.

The highest proline accumulation was recorded under the combined Prof + Vio treatment: in 2022-2023, proline levels ranged from 7.4 to 7.8 μg/g (an increase of 43.9%-45.1% compared to the control), and in 2024 reached 10.4 μg/g (51.4% higher). This effect can be attributed to the synergistic action of nitrogen-fixing bacteria and the phytohormonal complex in regulating metabolic processes. The integrated application of all three biopreparations (Myc + Prof + Vio) also led to a significant increase in proline content – up to 6.5-6.6 μg/g in 2022-2023 and 8.7 μg/g in 2024 (21.0%-27.6% above the control), confirming the effectiveness of a combined approach in stimulating plant adaptive mechanisms.

The changes in MDA content proved particularly significant. In 2024, the control treatment recorded an MDA level of 17.9 μg/g, nearly double that of 2022-2023 (9.2-9.4 μg/g). This sharp increase indicates an intensification of lipid peroxidation processes in plant cell membranes under drought conditions, which negatively affects their structural integrity and function. The most effective treatment in reducing MDA content was the combined application of three biopreparations (Myc + Prof + Vio), which resulted in a 33.2%-37.2% decrease in 2022-2023 and a 47.7% reduction in 2024. These findings suggest a strong antioxidant effect and the development of a comprehensive cellular defence system against oxidative stress. The Myc + Vio combination also demonstrated high efficacy, reducing MDA content by 26.4%29.8% in 2022-2023 and by 37.8% in 2024, confirming the important role of mycorrhizal fungi and phytohormones in maintaining membrane integrity under abiotic stress.

Relative water content (RWC) and stomatal conductance (SC) are key physiological indicators that reflect a plant's water status and its capacity to withstand drought. The relationship between these parameters highlights various aspects of water exchange, the adaptive potential of plants under adverse environmental conditions, and overall physiological activity. Analysing the dynamics of these indicators concerning climatic conditions and the influence of biopreparations provides a valuable foundation for developing effective agronomic strategies for soybean cultivation in the context of climate change and increased aridity.

In the control treatments, RWC was recorded at 73.8% in 2022, 75.1% in the wetter year of 2023, and dropped to a critical 69.7% during the drought conditions of 2024. This 7.2% decrease in 2024 compared to 2023 indicates a significant disruption of the plant's water balance under severe water deficit. It is well established that a decline in RWC to 70%-80% in plant cells suppresses key metabolic processes, including photosynthesis and respiration, signalling severe physiological stress and potentially irreversible damage to the structure of cell membranes (Filipović, 2020). The SC in the control plots decreased by 38.7% in 2024 compared to the wetter 2023 season, reflecting the activation of plant defence responses aimed at reducing water loss through stomatal closure.

The application of biopreparations significantly improved the water status of plants in all study years, although the most pronounced effect was observed under drought conditions. In the favourable year of 2023, Profix application increased RWC to 82.5% (+ 9.9% compared to control), Violar to 87.2% (+ 16.1%), and Mycofrend to 81.2% (+ 8.1%). The combination of biopreparations produced an even more pronounced effect: Prof + Vio increased RWC to 88.7% (+ 18.1%), Myc + Prof to 87.8% (+ 16.9%), and Myc + Vio to 89.5% (+ 19.2%). The most effective treatment was the comprehensive application of Myc + Prof + Vio, which raised RWC by 26.1% compared to the control, indicating a strong synergistic effect of biological agents with different mechanisms of action.

Under the acute drought conditions of 2024, the impact of biopreparations on plant water status was even more evident. The combined application of Myc + Prof + Vio increased RWC by 31.9% relative to the control, demonstrating the biopreparations' effectiveness in enhancing cellular water retention. Notably, even under extreme drought, plants treated with the full combination reached an RWC of 91.9%, significantly exceeding the control value under favourable 2023 conditions (75.1%). This confirms the high efficacy of biopreparations in improving stress tolerance and mitigating the adverse effects of water deficit.

The application of biopreparations also significantly influenced SC, ensuring optimal levels in line with environmental conditions. In 2023, Profix seed treatment increased SC by 19.4% compared to the control, Violar by 25.8%, and Mycofriend by 12.9%. The most pronounced effects were observed with combined treatments: Prof + Vio enhanced SC by 38.7%, Myc + Prof by 32.3%, and Myc + Vio by 48.4%. The highest stomatal conductance was recorded with the full combination Myc + Prof + Vio, reaching 0.51 mol/m^2 · s (+ 64.5% compared to control), which ensured a high level of photosynthetic activity.

Under the drought conditions of 2024, the action of the biopreparations was not aimed at maximising stomatal opening but rather at its optimal regulation. The combined treatment with Myc + Prof + Vio increased SC to 0.39 mol/m^2 · s, which was 2.1 times higher than the control. This ensured a sufficient level of photosynthesis while simultaneously limiting water loss. The unfavourable climatic conditions of 2024 led to a marked reduction in soybean yield across all experimental treatments (Table 2). In the control variant, the yield was 1.9 t/ha, which was 17.0%-24.4% lower compared to 2022-2023. A decrease in RWC below 70% confirms that water deficit is a critical factor disrupting water exchange, photosynthesis, respiration, and assimilate transport, all of which directly affect yield formation.

The highest yield was achieved through the combined application of the three biological preparations, which increased yield by 40.6%-45.1% in 2022-2023 and by 59.1% in 2024 relative to the control. These results are supported by statistical analysis and confirm the high effectiveness of an integrated approach to enhancing drought tolerance and soybean productivity. High yield levels were also ensured by the Myc + Vio combination (+ 34.8%-36.6% in 2022-2023 and + 48.4% in 2024), highlighting the key role of mycorrhizal symbiosis and hormonal regulation in shaping soybean performance.

To systematise the results and facilitate interpretation of the effects of biological preparations on plant adaptive traits, a heat map was constructed. This approach enables a clear assessment of the effectiveness of individual and combined biotreatment variants under varying hydrothermal conditions (Fig. 1).

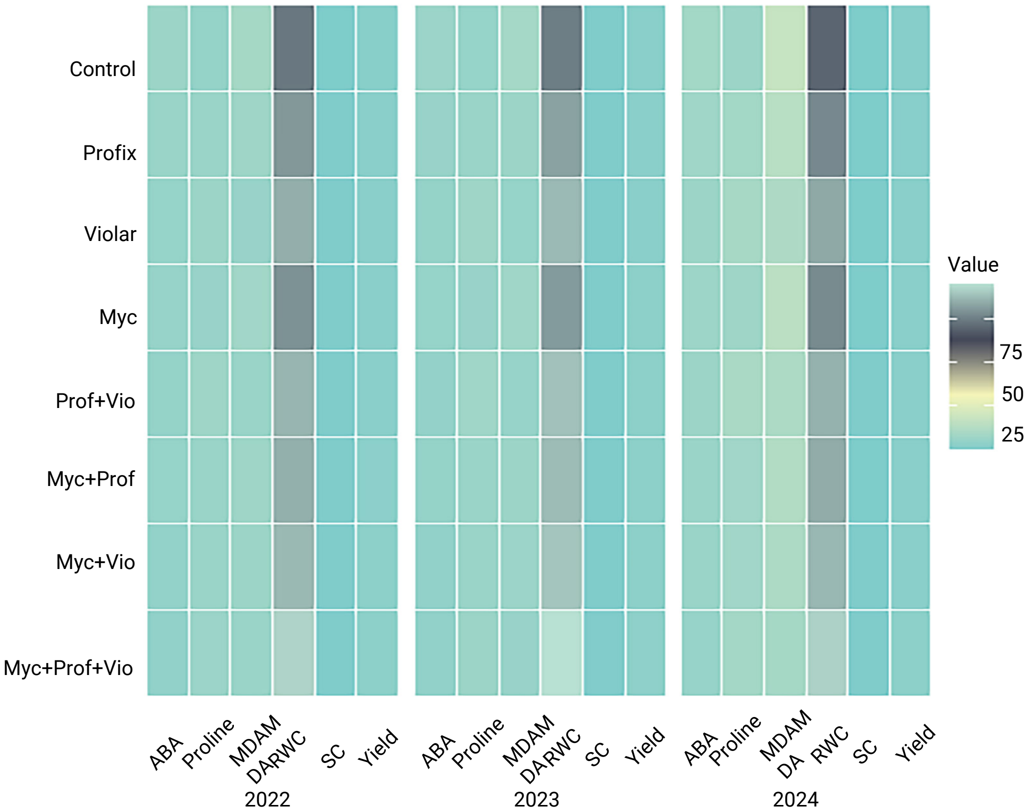

***Figure 1.** Heat map of comprehensive experimental indicators of soybean under different biotreatment variants in contrasting hydrothermal conditions (2022-2024)*

Source: compiled by the authors

The heat map results reveal a clear dependence of ABA levels on hydrothermal conditions and the influence of biopreparations. In 2022, all variants exhibited moderate concentrations of this stress-related phytohormone. In contrast, the extreme weather conditions of 2024 led to a peak accumulation of ABA in the control treatment, indicating the activation of stress-induced mechanisms in response to water deficit. The lowest ABA values were observed in the treatments with combined application of Myc + Prof + Vio and Myc + Vio, even under drought conditions in 2024, confirming their effectiveness in reducing stress levels.

The accumulation dynamics of proline correlated with the intensity of abiotic stress. The highest concentrations of this osmoprotectant were recorded in 2024 across all variants; however, the combined biopreparations resulted in the smallest increase, demonstrating a stabilising effect on metabolic processes. Throughout all study years, the Myc + Prof + Vio treatment ensured the most consistent proline concentrations. The MDA content, as a marker of the intensity of lipid peroxidation processes, increased sharply in 2024 in the control variant, indicating the activation of oxidative stress. The most effective reduction in MDA levels was achieved through the use of biopreparations containing mycorrhizal components, particularly Mycofriend, confirming their antioxidant properties.

The RWC index exhibited an inverse trend in relation to the intensity of hydrothermal stress. The lowest values were recorded in 2024 under the control conditions, reflecting a significant disruption of water exchange. Biopreparations effectively supported water balance, particularly due to mycorrhizal symbioses, which enhanced the water-absorbing capacity of the root system. The highest RWC values were recorded in the combined treatment variants, notably Myc + Prof + Vio, even under the extreme water deficit conditions of 2024. The dynamics of SC reflected the complex nature of plant adaptive responses to water stress. In 2024, a critical reduction in SC was observed in the control, associated with stomatal closure aimed at reducing water loss. Biopreparations contributed to the optimisation of stomatal functioning, maintaining a balance between adequate photosynthetic activity and water conservation. The most stable SC values were demonstrated by the combined biopreparation treatments.

Soybean yield showed a direct correlation with the effectiveness of adaptive mechanisms. The highest productivity levels were achieved in the combined treatments incorporating mycorrhizal fungi, nitrogen-fixing bacteria, and phytohormonal formulations. Under the conditions of severe water deficit in 2024, biopreparations significantly mitigated the negative effects of stress, whereas a marked decline in yield was observed in the control treatment. The use of individual biopreparations (Profix, Violar, Mycofriend) resulted in moderate improvements in adaptive parameters; however, their effectiveness was insufficient under extreme conditions. Combinations of two biopreparations (Prof + Vio, Myc + Prof, Myc + Vio) exhibited a synergistic effect, particularly in stabilising water balance and reducing oxidative stress indicators. The highest overall efficacy across all experimental parameters was achieved with the integrated application of Myc + Prof + Vio, which led to a reduction in stress markers (ABA, MDA), maximal preservation of RWC, SC, balanced proline accumulation, and consistently high yields.

The combined action of the biopreparations was driven by several interacting factors: improved nitrogen nutrition provided by *Bradyrhizobium japonicum* and *B. diazoefficiens* (Profix); the formation of mycorrhizal symbioses involving *Glomus* sp. and *Trichoderma harzianum* (Mycofriend); hormonal balance regulation via naturally derived phytohormones (Violar); and the antagonistic activity of *Bacillus subtilis* and *Pseudomonas fluorescens* against pathogenic microorganisms.

To validate the observed patterns, a correlation analysis was conducted between the physiological and biochemical indicators of soybean adaptation to hydrothermal stress. Most notably, a strong negative correlation was observed between ABA content and yield (r = −0.909), confirming the central role of this phytohormone in reducing soybean productivity under stress conditions. A strong positive correlation was also identified between ABA and MDA levels (r = 0.882), indicating a direct relationship between the activation of ABA synthesis and the onset of oxidative stress in plant tissues. ABA content showed a strong negative correlation with SC (r = -0.885), confirming the physiological role of ABA as a key regulator of stomatal closure in response to water deficit. A negative correlation was also observed between ABA and relative water content (RWC) (r = −0.723), indicating a deterioration of the plant's water status as the concentration of this stress hormone increased. A weak positive correlation between ABA and proline content (r = 0.306) suggests their parallel yet partially independent involvement in stress-adaptive mechanisms.

A significant negative correlation was found between MDA content and SC (r = −0.799), reflecting the disruption of gas exchange processes under oxidative stress. Furthermore, the correlation between MDA and RWC (r = −0.574) confirms the adverse impact of enhanced lipid peroxidation on the water balance of plants. A strong negative correlation between MDA and yield (r = −0.754) highlights the direct inhibitory effect of oxidative stress on soybean productivity. Conversely, a strong positive correlation between RWC and SC (r = 0.832) reflects the close relationship between the plant's water regime and stomatal function. A similarly high positive correlation between RWC and yield (r = 0.847) underscores the critical role of maintaining optimal water status in achieving high productivity. The strongest positive correlation was recorded between SC and yield (r = 0.955), emphasising the decisive importance of stable stomatal function in ensuring maximum soybean productivity.

The results obtained clearly confirmed that the application of biopreparations contributes to the optimisation of the water regime, the reduction of oxidative stress intensity, and the maintenance of crop productivity under hydrothermal stress, through a complex influence on key physiological and biochemical mechanisms of plant adaptation. Figure 2 presents the findings of a three-year study (2022-2024) on the effects of biopreparations with different functional properties on the molecular and physiological-biochemical indicators, as well as the yield, of the soybean cultivar Khorol under organic farming conditions.

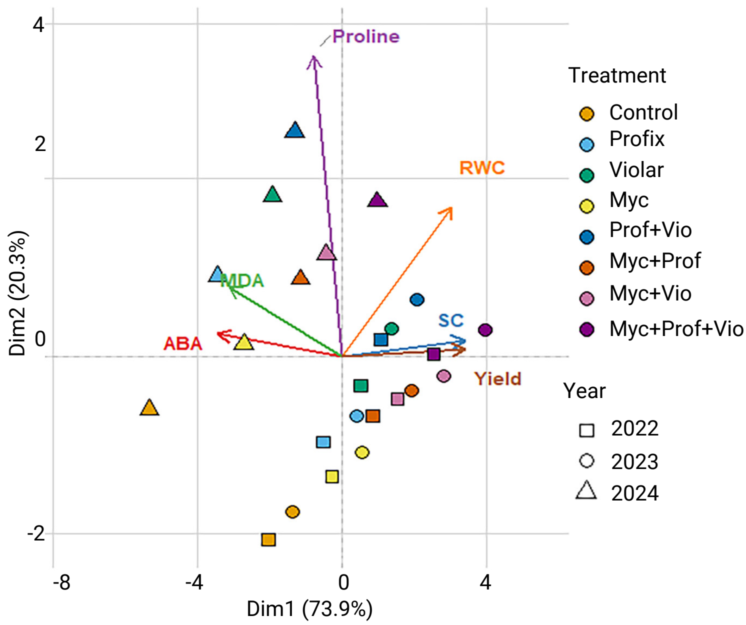

***Figure 2.** Principal component analysis – relationship between the action of biopreparations and the set of experimental indicators (2022-2024)*

Source: compiled by the authors

The total explained variance accounted for 91.8% (Dim1 – 63.3%, Dim2 – 28.5%), indicating the high informativeness of the data obtained and the validity of the applied approach. The biplot analysis clearly illustrates the differentiation of experimental data by year, reflecting the decisive influence of the hydrothermal regime on the development of adaptive responses in plants. For instance, the year 2022 was characterised by moderate moisture availability and optimal temperature conditions, as evidenced by the clustering of relevant points predominantly in the lower section of the biplot. The 2023 season, marked by evenly distributed rainfall throughout the growing period, resulted in the highest recorded yields, which is confirmed by the grouping of "Yield-2023" data points in the right-hand section of the biplot.

Of particular interest are the results from 2024, when extreme drought conditions were observed, especially during the critical stages of reproductive organ development (July August). The corresponding data points are concentrated in the upper section of the biplot and exhibit a strong correlation with indicators of stress-induced metabolites and physiologicalbiochemical adaptive mechanisms. In particular, a positive correlation was established between drought conditions and the accumulation of proline, a typical osmoprotectant whose synthesis intensifies in response to water deficit. The elevated levels of MDA in plants grown in 2024 reflect the activation of lipid peroxidation processes in membrane structures triggered by oxidative stress.

The most significant outcome of the PCA was the clear differentiation in plant responses to various biopreparation treatments depending on the level of hydrothermal supply. Complex treatment schemes, especially Myc + Prof + Vio, demonstrated the highest efficacy under drought conditions in 2024, reflected in increased RWC and SC values. This finding is of fundamental importance for understanding the mechanisms underlying the development of drought tolerance in soybean agroecosystems under the influence of biological preparations. The synergistic action of arbuscular mycorrhizal fungi, nitrogen-fixing bacteria, and phytohormonal regulators facilitates the formation of a multi-level adaptive response to water deficit. It was shown that single treatments produced intermediate effectiveness, whereas control variants consistently exhibited the poorest performance, particularly under stress conditions. This confirms the rationale for using complex biopreparation schemes to ensure multi-component protection of the plant organism against abiotic stress factors.

The analysis of the contrasting hydrothermal conditions in 2023 and 2024 revealed that, under optimal moisture availability, the effectiveness of biopreparations was primarily expressed through increased yield and the optimisation of the water regime. In contrast, under drought conditions, their action was directed towards the activation of defence responses through the accumulation of the osmolyte proline and the reduction of oxidative stress levels. Of particular note is the consistent effectiveness of the Myc + Prof + Vio treatment across years with contrasting weather conditions, which is of critical importance for developing adaptive technologies for organic soybean cultivation in the context of climate change.

The findings are consistent with current scientific evidence regarding the influence of biopreparations of various origins on the hormonal status of plants, particularly regarding abscisic acid levels. ABA is one of the key signalling molecules that regulates numerous aspects of plant growth, development and physiological condition, playing a particularly important role in shaping adaptive responses to abiotic stresses, as noted in the study by Y. Osakabe *et al.* (2014). Hormone-mediated signalling pathways, particularly those involving ABA and auxin, are considered by V. Verma *et al.* (2016) to be essential for maintaining the balance between growth processes and the activation of defence responses under stress conditions.

The physiological role of ABA lies in its ability to initiate structural and functional changes in plant cells aimed at preserving the organism's viability under adverse conditions. The strong negative correlation identified in this study between ABA content and stomatal conductance ($r = -0.885$) confirms the regulatory function of this phytohormone in controlling stomatal activity. According to S. Munemasa *et al.* (2015), an increase in ABA concentration in response to hydrothermal stress induces the closure of stomata – microscopic pores on the leaf surface formed by guard cells, which regulate gas exchange and water vapour loss. This mechanism serves as an effective adaptive response, allowing plants to reduce transpirational losses and maintain water balance during drought. The observed relationship between ABA concentration and stomatal activity confirms the crucial role of this phytohormone in regulating the water regime of soybean under hydrothermal stress. The established correlation indicates the high activity of ABA-dependent signalling cascades in managing water exchange processes in soybean plants exposed to hydrothermal stress.

It is important to emphasise the role of rhizospheric microbiota – PGPR and arbuscular mycorrhizal fungi – in modulating the phytohormonal status of plants, particularly the level of ABA. PGPR strains present in the biopreparations

Mycofriend and Profix, as well as in the metabolite-based preparation Violar, are known to influence the hormonal balance of plants both directly and indirectly, enhancing productivity and stress resilience. Research by R. Porcel *et al.* (2014) confirms that these effects involve regulation of ABA synthesis and modulation of ethylene levels.

According to D. Barnawal *et al.* (2017), certain rhizobacterial strains are capable of metabolising ABA, thereby reducing its concentration in plant tissues, which positively influences growth processes. Similar properties are characteristic of mycorrhizal fungi. For example, H. Esch *et al.* (1994) identified the presence of ABA in the spores and hyphae of *Glomus* species, confirming their ability to synthesise or accumulate this hormone. Research by A. Martinez-Medina *et al.* (2013) showed that root colonisation by *Trichoderma harzianum* enhances the expression of ABA-responsive genes, thereby increasing the adaptive capacity of plants. These findings are supported by later studies (e.g. Rawal *et al.*, 2022), which demonstrated the ability of *T. harzianum* to improve plant tolerance to abiotic stresses, including drought, through the regulation of phytohormonal signalling pathways.

The role of rhizobacteria in regulating ABA levels is further supported by the findings of M. Saakre *et al.* (2017), who described the ability of *Pseudomonas fluorescens* to enhance plant drought tolerance through modulation of ABA levels. The positive impact of PGPR on soybean growth has also been confirmed in recent studies by S. Gouli *et al.* (2024). Certain *Streptomyces* strains, according to F.M. Rashad *et al.* (2015), demonstrate the capacity to synthesise ABA *in vitro*, reinforcing their potential role in the hormonal regulation of growth processes. The interaction of *Bacillus megaterium* var. *phosphaticum* with plant hormonal balance highlights the complex nature of phytohormonal signalling in microbe-plant associations, as discussed by H. Etesami *et al.* (2023). Particularly noteworthy are the findings of R. Hidri *et al.* (2024), which showed that dual inoculation with *Bacillus subtilis* and arbuscular mycorrhizal fungi effectively protects plants from salt stress by modulating ABA levels and activating antioxidant mechanisms.

The findings of the present study underscore the complexity and variability of plant phytohormonal responses depending on the combination of environmental conditions and type of biological treatment. As shown by E.A. Kazerooni *et al.* (2021), PGPR treatment can reduce ABA levels in plants exposed to stress factors by 45.2%-86.5%, which is consistent with the present results. Specifically, under hydrothermal stress, an increase in ABA content by 31.5%32.2% was recorded in soybean plants inoculated with mycorrhizal fungi (Myc) or rhizobacteria (Profix), compared to plants grown under favourable conditions. Comparison of the treated variants with the stress control revealed a tendency towards a reduction in ABA levels by 8.2%-16.5%, depending on the treatment scheme. This indicates the effectiveness of biopreparations in reducing the need for activation of ABA-dependent defence mechanisms by improving the physiological state of the plants.

The most effective result was observed with the combined treatment of Myc + Prof, which led to a 27.1% reduction in ABA levels, indicating optimised water status and mineral nutrition. The decrease in ABA under stress conditions, recorded in this study (up to 34.1% following Myc + Prof + Vio treatment), is consistent with the findings of R.A. Repke *et al.* (2022), who noted that biostimulants are capable of lowering ABA concentrations under stress, thereby helping to maintain stomatal function and photosynthetic potential.

These results are also in line with previous studies on the impact of seed priming on the physiological and biochemical parameters of emmer wheat. In particular, UV-C seed irradiation combined with treatment using the 1r Seed Treatment solution led to an increase in photosynthetic pigments and a corresponding rise in yield by 21.4%-31.0% (Korotkova *et al.*, 2023). Similar outcomes were obtained in soybean studies, where the application of the phytohormonal preparation Violar in combination with a bacterial inoculant enhanced chlorophyll content by 5.5%-17.2% and leaf surface area by 13.6%-21.2% (Chaika & Korotkova, 2025). Thus, the results of the study confirm the effectiveness of biopreparations in enhancing the molecular and physiological-biochemical resilience of plants to hydrothermal stress. This is attributed to their ability to modulate hormonal status, optimise water balance, and support crop productivity.

Conclusions

The study confirmed the key role of abscisic acid (ABA) in shaping the adaptive responses of soybean plants to hydrothermal stress through the regulation of structural and functional changes, particularly stomatal activity and water status. Under water-deficit conditions, ABA concentrations increased by 25.0%-30.8%, indicating the activation of protective mechanisms. The application of biopreparations led to a reduction in ABA levels by an average of 9.2%36.3%, confirming their positive effect on the drought tolerance of soybean plants. In response to the drought conditions of 2024, a significant increase in proline content was observed across all experimental treatments, indicating the activation of natural osmoregulatory mechanisms. The greatest accumulation of proline was recorded following the combined application of Prof + Vio, with an average increase of 47.1% compared to the control.

The drought conditions also caused a marked rise in the concentration of malondialdehyde (MDA), indicating intensified lipid peroxidation and a disruption of the structural integrity of cell membranes. The combined application of biopreparations, particularly Myc + Prof + Vio, resulted in a 15.6%-41.8% reduction in MDA content and a 9.1%-46.6% increase in proline levels compared to the control, suggesting the formation of an effective antioxidant defence system. The application of biopreparations significantly improved the water status of soybean plants throughout all years of the study – on average by 10.0%-27.6% compared to the control – confirming the development of effective cellular waterretention mechanisms and enhanced stress tolerance. The optimisation of stomatal activity under the influence of biopreparations was achieved not only through increased conductance (by 19.2%-65.4% relative to the control), but also via the formation of regulatory mechanisms that ensured the adaptive functioning of the stomatal function in accordance with hydrothermal conditions.

Correlation analysis confirmed the key mechanisms influencing productivity: a strong negative correlation was observed between yield and ABA content ($r = -0.909$) as well as MDA content ($r = -0.754$), while a strong positive correlation was found with stomatal conductance ($r = 0.955$) and relative water content ($r = 0.847$). A 17%-24% decrease in yield in the control under drought conditions confirmed the critical role of water deficit in limiting soybean productivity. At the same time, the use of biopreparations – especially the Myc + Prof + Vio complex – contributed to a 12.3%-45.5% increase in yield due to improved water status and reduced concentrations of ABA and MDA, highlighting the effectiveness of an integrated approach to enhancing crop drought tolerance.

The findings clearly demonstrate that the combined use of biopreparations is the most effective strategy for increasing the adaptive potential of organic soybeans to hydrothermal stress. The three-component combination of mycorrhizal fungi, nitrogen-fixing bacteria, and phytohormones provides systemic support for plant metabolic processes and ensures stable productivity even under extreme weather conditions. These results support the development of adaptive technologies for organic soybean cultivation in response to current climate change trends.

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Conflict of Interest

None.

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Абсцизова кислота та біопрепарати як маркери адаптації органічної сої до гідротермального стресу

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Анотація. У зв'язку зі зростанням попиту на органічну сою та необхідністю зменшення негативного впливу кліматичних змін актуальним є дослідження впливу біопрепаратів різної природи на адаптивні механізми рослин до стресових умов. Метою роботи було оцінити зміну вмісту абсцизової кислоти (ABA) під дією біопрепаратів у багаторічному польовому експерименті та проаналізувати взаємозв'язки ABA з біохімічними, фізіологічними й агрономічними показниками адаптації сої до гідротермічного стресу в умовах органічного землеробства. Дослідження проводили з використанням біопрепаратів на основі мікоризних грибів, ризосферних й азотфіксуючих бактерій і фітогормонів. Застосовано комплекс польових, лабораторних (фізіологічних, біохімічних) і статистичних методів. Встановлено інтеграцію молекулярних, біохімічних, фізіологічних і агрономічних маркерів стресостійкості завдяки підвищенню адаптивного потенціалу сої при застосуванні біопрепаратів. Їх ефективність проявлялася через комплексний вплив на ключові адаптивні механізми: зниження концентрації стресового гормону ABA на 9,2-36,3 %, що свідчило про послаблення стресового навантаження; підвищення відносного вмісту води на 10,0-27,6 %, що забезпечувало оптимальний водний статус клітин; покращення продихової провідності на 19,2-65,4 % завдяки оптимізації роботи продихів; зниження рівня перекисного окислення ліпідів (малонового діальдегіду – на 15,6-41,8 %), що вказувало на ефективний захист мембран від оксидативного стресу; підвищення вмісту проліну на 9,1-46,6 %, що свідчило про активацію природних захисних механізмів і підтримку осмотичного балансу. Кореляційний аналіз підтвердив, що біопрепарати забезпечують комплексну модуляцію стресової відповіді рослин, оптимізуючи баланс між захисними реакціями та продуктивністю, що призводило до підвищення врожайності на 12,3-45,5 % навіть за несприятливих погодних умов. Встановлено, що під час водного стресу біопрепарати модулюють біосинтез АВА та її регуляторну функцію, сприяючи координованим змінам осмопротекторних механізмів, антиоксидантного захисту та водного режиму рослин. Найбільш ефективною виявилася потрійна комбінація біопрепаратів, яка забезпечувала оптимальний баланс між фітогормональною регуляцією, фізіологічними процесами та продуктивністю сої в умовах гідротермічного стресу. Отримані результати можуть бути рекомендовані для вирощування органічної сої з метою підвищення її врожайності в умовах кліматичних викликів

Ключові слова: органічне землеробство; відносний вміст води; продихова провідність; пролін; малоновий діальдегід; урожайність