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RESEARCH ARTICLE

Effect of biostimulants on soybean (*Glycine max* L. Merr.) tolerance to hydrothermal stress under organic farming in Ukraine

Chaika T[1*], Korotkova I[2], Liashenko V[3], Shevnikov M[3], Pospielova G[4] & Rybalchenko A[5]

[1]Department for Ecological and Economic Development of Rural Areas, Poltava Branch of the Ukrainian Academy of Technological Cybernetics, Poltava 36003, Ukraine
[2]Department of Crop Production and Agrochemistry, Poltava State Agrarian University, Poltava 36003, Ukraine
[3]Department of Plant Growing, Poltava State Agrarian University, Poltava 36003, Ukraine
[4]Department of Plant Protection, Poltava State Agrarian University, Poltava 36003, Ukraine
[5]Department of Breeding, Seed Production and Genetics, Poltava State Agrarian University, Poltava 36003, Ukraine

*Correspondence email - chayka_ta@ukr.net

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Abstract

Climate change-induced drought and extreme temperatures pose major challenges to global soybean (*Glycine max* L. Merr.) production. Therefore, sustainable strategies to mitigate hydrothermal stress are crucial, particularly in organic farming systems. A field experiment (2022–2024) was conducted in Ukraine to assess the effects of biostimulants on soybean yield under contrasting hydrothermal conditions. The tested biostimulants contained arbuscular mycorrhizal fungi (AMF) *Glomus intraradices* (MycoFix), nitrogen-fixing rhizobia *Bradyrhizobium japonicum* (Anderis) and phytohormonal metabolites from *Streptomyces violaceus* (Violar). Biostimulant effectiveness was assessed by abscisic acid (ABA) and relative water content (RWC), stomatal resistance (Rs) and yield of organic soybean cv. Adamos. The experimental design included 5 treatments from an untreated control to a combination of 3 biostimulants. Integrated application of MycoFix + Anderis + Violar reduced stress-induced ABA accumulation by 35.8–40.0 % vs. the control. This effect was associated with improved plant water status, reflected in higher RWC (94.8–97.2 % vs. 73.2–77.6 %) and lower Rs (1.87–2.39 vs. 3.04–4.53 $s \times m^2/mol$), resulting in a 40.6–58.9 % increase in seed yield (3.21–3.82 vs. 2.02–2.63 t/ha). Correlation analysis showed strong relationships between ABA and Rs (r=0.970), ABA and RWC (r=-0.871) and RWC and yield (r=0.992), confirming the integration of hormonal, physiological and agronomic responses. Principal component analysis (PCA) showed that biostimulant treatments explained 93.6 % of total variance, far exceeding temporal climatic variation (4.4 %) and confirming their role in plant adaptation. These findings highlight the potential of multi-component biostimulant systems to improve soybean stress tolerance in organic farming under climate change.

Keywords: abscisic acid; arbuscular mycorrhizal fungi; *Bradyrhizobium japonicum*; climate adaptation; drought; organic farming; phytohormonal metabolites; relative water content; rhizobia; stomatal resistance

Introduction

Global climate change is increasingly accompanied by a higher frequency and intensity of drought, which significantly affects crop production, with potential yield losses reaching up to 50 % (1). Soybean (*Glycine max* (L.) Merr.), one of the most important protein and oilseed crops worldwide, is particularly vulnerable to drought stress, with yield reductions under extreme water deficit conditions reaching up to 100 % (1). In Ukraine, soybeans rank first in Europe in production and are among the top global exporters of organic soybeans, making adaptation strategies to climate stress critically important (2).

In this regard, understanding the physiological and biochemical processes behind plant adaptation to hydrothermal stress is crucial. Abscisic acid (ABA), a key phytohormone, plays a crucial role in how plants react to various abiotic stresses, such as drought, high temperatures and salinity (3). Underwater deficit, ABA production is initially increased in the roots and subsequently transported to the leaves via the xylem (4). Elevated ABA levels in leaf tissues initiate a signalling cascade within guard cells, leading to stomatal closure (which is reflected in stomatal resistance (Rs) and reduced transpiration (5, 6). This process is essential for regulating plant water balance and ensuring survival during stressful conditions (7). The relative water content (RWC) is a key measure of a plants' water balance and is strongly linked to ABA-induced stress responses (8).

Abscisic acid also interacts with other plant hormones to regulate stress-related physiological responses (9) and activates a range of biochemical defence mechanisms, including the synthesis of proline, antioxidants, reactive oxygen species-detoxifying enzymes, heat shock proteins and unsaturated fatty acids. Additionally, ABA stimulates the production of cuticular waxes, enhancing plant tolerance to abiotic stress (10, 11). On a molecular level, ABA influences stress-related gene expression and enhances the functionality of the antioxidant system (12, 13). Notably, the effectiveness of ABA signalling pathways can be substantially influenced by microorganisms in the rhizosphere and endosphere used in biostimulants (14, 15).

Biostimulants provide promising strategies to enhance stress tolerance in soybeans. Rhizobial inoculants containing *Bradyrhizobium japonicum* improve nitrogen fixation, arbuscular mycorrhizal fungi (AMF) such as *Glomus* species enhance phosphorus uptake and drought tolerance and phytohormonal preparations also promote stress tolerance (16–18). Biostimulants, including microbial components such as AMF and plant growth-promoting rhizobacteria (PGPR) and non-microbial substances (seaweed extracts, protein hydrolysates), have become key tools for sustainable agriculture. They enhance plant growth, nutrient uptake and stress tolerance while reducing reliance on synthetic fertilisers (19–21). These compounds modulate plant hormonal balance, activate antioxidant responses and improve water and nutrient absorption, making them especially valuable for organic farming systems (22).

Rhizophagus intraradices and *Glomus intraradices* are well-studied AMF species, has been shown to improve plant drought tolerance by enhancing water and nutrient uptake, increasing photosynthetic efficiency and activating antioxidant protection mechanisms (23). The effectiveness of AMF-based biostimulants can be further enhanced when combined with seaweed extracts, which provide additional osmoregulatory compounds and growth stimulants (18). Co-inoculation strategies combining rhizobia with other microorganisms have shown yield increases of 10–15 % (16, 24). However, the integrated use of AMF, rhizobia and phytohormonal stimulants in organic systems remains largely unexplored, particularly regarding their effects on ABA-mediated responses under field conditions. This study aimed to evaluate ABA content variation in soybean under the influence of biostimulants (mycorrhizal, rhizobial and phytohormonal) in a 3 year field experiment under organic farming in Ukraine and to analyse relationships between ABA levels, plant water status (RWC), Rs and yield performance under contrasting hydrothermal conditions. Understanding these integrative mechanisms is essential for developing sustainable adaptation strategies for organic soybean production under climate change.

Materials and Methods

Description of the study site

A 3 year research trial was conducted during the soybean growing seasons of 2022–2024 in the experimental field of Poltava State Agrarian University. The early-maturing soybean variety Adamos, developed by the Yuriev Plant Production Institute of the National Academy of Agrarian Sciences of Ukraine, was used in the experiment. The soil of the experimental plots was classified as residual saline chernozem. Five soil samples from every plot were taken with an auger from depths of 0–20 cm for analysis. A composite sample was prepared by thoroughly mixing the collected subsamples. The sample was air-dried, crushed and passed through a 2 mm sieve. The soil properties in the experimental plots were analysed at the Soil Testing Laboratory, Poltava State Agrarian University, using a Palintest SK500 multiparametric photometer (Palintest House, United Kingdom, 2020) in four replicates. Soil humus content was determined using the Tyurin wet oxidation method (modified by Simakov) according to the Ukrainian national standard (DSTU) 4289:2004. The method is based on oxidation of organic carbon with potassium dichromate in concentrated sulfuric acid, followed by back titration of the excess oxidant with ferrous ammonium sulfate (Mohrs' salt), consistent with the Anne-type wet oxidation procedure (25). Available phosphorus (P) and exchangeable potassium (K) were determined according to the Chirikov acid extraction method in accordance with DSTU 4114:2002, followed by photometric measurement using a Palintest SK500 multiparametric photometer (Palintest Limited, UK). Nitrate and nitrite nitrogen (NO_3^-–N + NO_2^-–N) were determined after extraction with 1 M ammonium chloride (NH_4Cl), followed by reduction of nitrate to nitrite and colourimetric determination using the Palintest Nitratest™ method with the same photometer (26). The obtained results showed that the humus content was 4.8 %, available P–75.6 mg/kg, exchangeable K–136.7 mg/kg, nitrate–nitrite nitrogen of 60.2 mg/kg and pH 6.9. The soil cultivation technology corresponded to standard organic soybean production practices. Spring barley was used as the preceding crop for soybeans. The field experiment was carried out using a randomised block design (RBD) with three replications. For physiological measurements (ABA, RWC, Rs), 10 fully expanded trifoliate leaves were sampled per plot (30 leaves per treatment). Stomatal resistance was measured on the same leaves before sampling. Yield was recorded from the entire plot area of each replication. As soybeans are a thermophilic crop, the sowing date each year was determined based on soil temperatures reaching 10–12 °C at a depth of 5 cm, generally between April 20 and May 5. Soybean seeds were sown, maintaining 38 cm from line to line with a seeding rate of 700.000 seeds/ha. The crop was harvested in mid-September each year.

Meteorological conditions were collected from the Ukrainian Hydrometeorological Institute of the National Academy of Sciences of Ukraine, as given in Fig. 1. The climatic conditions during the soybean growing season in 2022–2023 were characterised by a moderate average summer temperature of approximately 21 °C and a relatively uniform monthly precipitation ranging from 48 to 58 mm. In contrast, the 2024 growing season was marked by episodes of extreme heat, with temperatures in July reaching 24 °C and occasionally peaking at 30–32 °C, accompanied by critically low and unevenly distributed rainfall (3–53 mm), creating significant stress conditions for the plants.

Experimental procedures

The treatments aimed to study the impact of biostimulants on enhancing the soybean tolerance to hydrothermal stress: MycoFix (Legume Technology Limited, UK) contains the mycorrhizal fungus *G. intraradices* (1 %, minimum 2000 spores/g) and seaweed extract of *Ascophyllum nodosum* (99 %). Anderis (BTU-Centre, Ukraine) contains nitrogen-fixing bacteria (*B. japonicum*, *Rhizobium leguminosarum* bv. *viceae*, *R. leguminosarum* bv. *phaseoli*, *Mesorhizobium ciceri*, *R. pusense* (*Sinorhizobium* sp.) at 1.0–3.0 × 10^9 CFU/cm and the phosphate-mobilising fungus *Penicillium bilaiei* (*P. bilaiae*). Violar (Bioinvest-Agro LLC, Ukraine) - supernatant of the culture liquid + ethanol extract of the biomass (4:1) of the producer *Streptomyces violaceus* IMV Ac-5027) - contains natural phytohormones, including auxins (3.6 mg/L), cytokinins (1.9 mg/Lgibberellins (1.6 mg/L), ABA (0.02 mg/L), free amino acids (2.2 mg/L), sterols (1.71 mg/L) and lipids (5.5 mg/L).

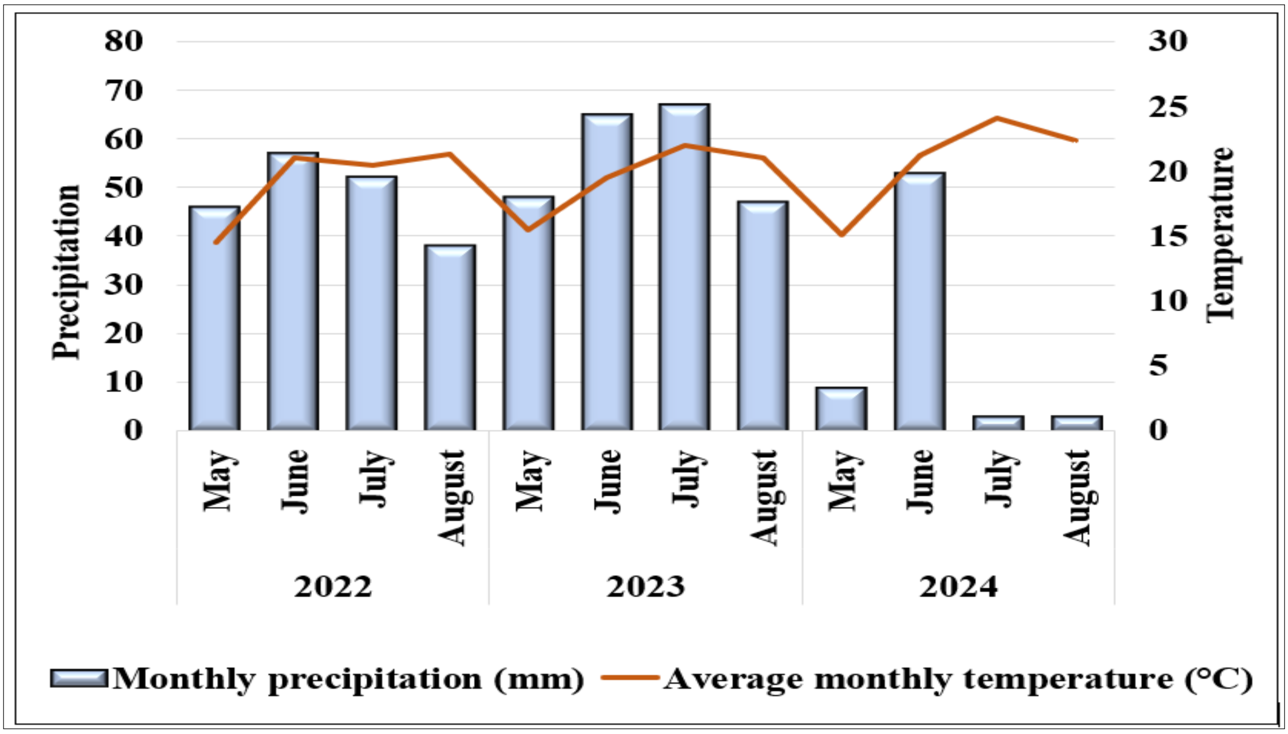

Fig. 1. Monthly average temperature and cumulative precipitation during the soybean growing seasons (2022–2024).

Foliar treatments were applied at growth stage 61 according to the BBCH scale (Biologische Bundesanstalt, Bundessortenamt and Chemical Industry). Five treatment variants (T_1-T_5) of biostimulants application were used: T_1: control (seed treatment with water before sowing within 1 hr); T_2: MycoFix (seed inoculation before sowing within 1 hr (1 kg/2 L of water per tonne seed)); T_3: T_2 + Anderis (seed inoculation (2 L per tonne seed) after MycoFix); T_4: T_2 + foliar spray of Violar (100 mL/200 L of water per ha) at BBCH 61; T_5: T_3 + foliar spray of Violar (100 mL/200 L of water per ha) at BBCH 61. The selected application rates and timings were based on manufacturer recommendations and established agronomic practices for soybean under comparable field conditions. The BBCH 61 stage was selected because of its high physiological sensitivity to water deficit and its critical role in reproductive development.

Laboratory analysis

Abscisic acid content was measured using a PerkinElmer LS-45 fluorescence spectrometer (PerkinElmer, Inc., USA). Soybean leaf extracts for fluorescence measurement were prepared following the protocol described previously (27). Relative water content and Rs were used to evaluate plant water status. Measurements of RWC were performed using fully expanded leaves from control plants and all treatment variants. Immediately after cutting, leaves were transferred to the laboratory in plastic bags and weighed to determine their fresh weight (FW). Then, leaves are immersed in distilled water for 24 hr at room temperature to achieve full turgor. After soaking, excess moisture was gently removed from the leaf surfaces and the samples were reweighed to determine turgid weight (TW). Then, the samples were dried to a constant weight at 80 °C for 24 hr to obtain dry weight (DW) and weighed. Relative water content was calculated according to the formula (28):

$$RWC = (FW - DW)/(TW - DW) \times 100 \qquad \text{(Eqn. 1)}$$

Stomatal conductance was measured on the abaxial leaf surface using an SC-1 porometer (Decagon Devices, Inc., USA) before leaf sampling. Stomatal resistance was calculated as the inverse of stomatal conductance (29). Soybeans were harvested when the pods were completely dry. Yield was adjusted to 14.0 % moisture to ensure comparability among treatments.

Statistical analysis

Recorded data were statistically analysed and graphical visualisations were generated using packages in R (version 4.4.3). Analysis of variance (ANOVA) was performed separately for each year and parameter in accordance with the standard protocol (30). Significant treatment effects were followed by Tukeys' HSD test ($p \leq 0.05$). Different letters indicate statistically significant differences among treatments. Relationships among experimental parameters were assessed using Pearsons' correlation analysis ($p \leq 0.05$). Data are presented as mean ± standard error (SE).

Results

Abscisic acid

In the control variant, ABA concentrations were highest across all study years (Fig. 2). In 2022, under favorable conditions, ABA reached 6.5 nmol/g, slightly decreased to 6.2 nmol/g in 2023 due to optimal moisture and increased to 8.1 nmol/g in the drought-affected 2024 season, representing a 30.6 % rise compared to 2023 (Fig. 1). The application of MycoFix significantly reduced ABA concentrations. In 2022, ABA levels dropped to 5.3 nmol/g, to 5.1 nmol/g in 2023 and to 6.7 nmol/g in 2024, showing a decrease of 17.3–18.5 % compared to the control. The combined use of MycoFix with the inoculant Anderis reduced ABA concentrations to 5.0, 4.7 and 5.8 nmol/g in 2022, 2023 and 2024, respectively, a 23.1 % to 28.4 % lower than the control. Adding the phytohormonal preparation Violar to the MycoFix enhanced this effect, lowering ABA concentrations to 4.3, 4.1 and 5.6 nmol/g (30.9 % to 33.8 % decrease compared to the control). The most effective treatment was the comprehensive use of MycoFix + Anderis + Violar. In this treatment, ABA levels were 3.9, 3.8 and 5.2 nmol/g, 38.7 % to 40.0 % lower in favourable years and 35.8 % lower in the drought-affected 2024.

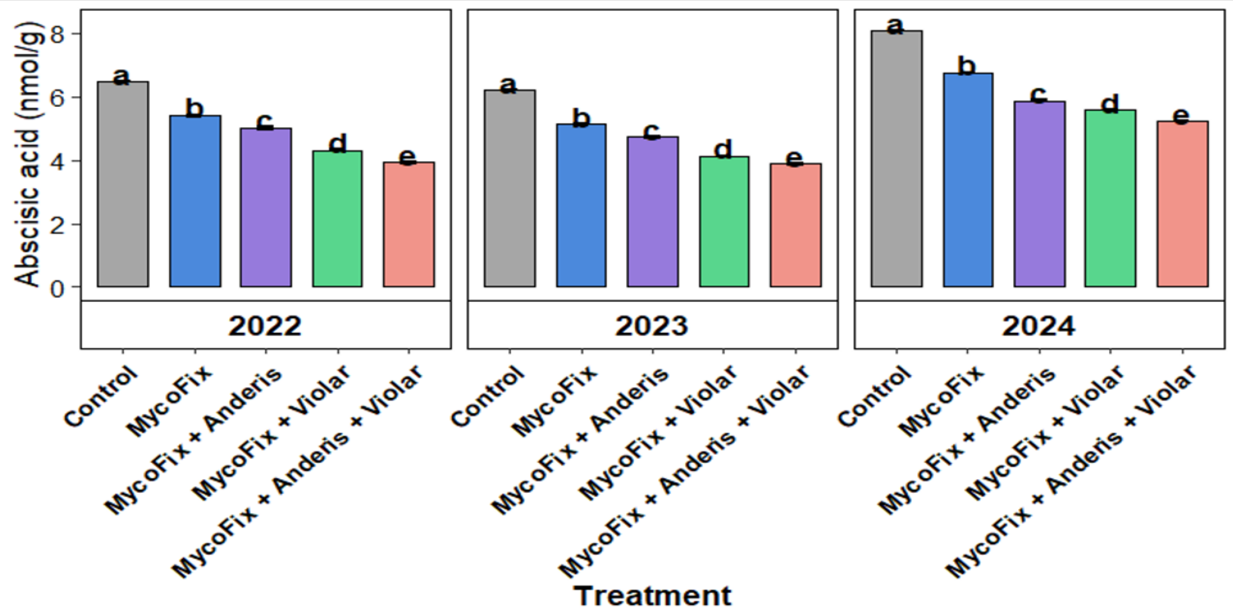

Fig. 2. Effect of biostimulant treatments on abscisic acid (ABA) concentration in soybean leaves. Bars represent standard errors; different letters indicate significant differences within each year according to Tukeys' HSD test (*p*≤0.05).

Relative water content as an indicator of plant water status

In the control variant, RWC showed a strong dependence on hydrothermal conditions (Table 1). In 2022, under moderately favourable conditions, RWC was 76.3 %. In 2023, under optimal moisture conditions, RWC slightly increased to 77.6 %. However, the drought in 2024 caused RWC to decrease sharply to 73.2 %. The application of MycoFix led to an increase in RWC to 81.7 % in 2022, 83.7 % in 2023 and 81.3 % in 2024, showing an improvement of 7.1–11.1 % points compared to the control. Combining MycoFix with the bacterial inoculant Anderis further optimised the water supply. Under the MycoFix + Anderis treatment, RWC increased to 87.9 %, 90.3 % and 88.1 % in the respective years, an increase of 15.2–23.2 percentage points compared to the control. In the MycoFix + Violar treatment, RWC reached 89.6 %, 92.0 % and 90.2 %, 17.4–23.2 percentage points higher than the control. The most effective treatment was the combination of MycoFix + Anderis + Violar, which maximised water retention. In this treatment, RWC was 94.8 % in 2022, peaked at 97.2 % in 2023 and remained at 95.4 % in 2024, a 24.2–30.3 % points increase compared to the control.

Stomatal resistance as a regulator of transpiration and gas exchange

Stomatal resistance is a key indicator of gas exchange regulation and transpiration. It reflects stomatal aperture and the intensity of water vapour diffusion from the leaf. In the control variant, Rs was highly influenced by weather conditions and plant water status (Table 1). In 2022, Rs was 3.35 s×m^2/mol, decreased to 3.04 s×m^2/mol in 2023 and sharply increased to 4.53 s×m^2/mol in the drought of 2024. The application of MycoFix reduced Rs by improving the plants' water supply. Stomatal resistance decreased to 3.02 s×m^2/mol in 2022, 2.68 s×m^2/mol in 2023 and 3.42 s×m^2/mol in 2024, reflecting a 9.9–24.5 % reduction compared to the control.

Combining MycoFix with Anderis further reduced Rs to 2.69, 2.32 and 3.13 s×m^2/mol in the respective years, 19.7–30.9 % lower than the control. In the MycoFix + Violar treatment, Rs decreased to 2.58, 2.10 and 2.87 s×m^2/mol, a 23.0–36.6 % reduction compared to the control. The most effective treatment was the combination of MycoFix + Anderis and Violar. In this treatment, Rs was 2.32 s×m^2/mol in 2022, decreased to a minimum of 1.87 s×m^2/mol in 2023 and remained at 2.39 s×m^2/mol in the drought of 2024, a 30.7–47.2 % reduction compared to the control.

Yield: An integral indicator of productivity

Soybean seed yield is a key indicator of the genetic potential of a variety under specific growing conditions and the influence of agronomic practices. The Adamos variety has a potential yield of 3.0–3.5 t/ha, but its realisation depends on weather conditions and applied agricultural practices. In the control variant, without biostimulants, the yield was 2.39 t/ha in 2022, increased to 2.63 t/ha in favourable 2023 conditions and decreased to 2.02 t/ha in the drought-stricken 2024 season (Fig. 3). These values reflect typical soybean responses to hydrothermal conditions.

The application of MycoFix significantly increased soybean yield to 2.91 t/ha in 2022, 3.22 t/ha in 2023 and 2.61 t/ha in 2024, demonstrating a 21.8–29.2 % increase compared to the control, highlighting the effectiveness of MycoFix effectiveness in organic farming. The combination of MycoFix with the nitrogen-fixing

Table 1. Effect of biostimulant treatments on RWC and Rs in soybean plants under contrasting hydrothermal conditions (2022–2024)

Treatment	RWC (%)			Rs (s×m^2/mol)		
	2022	2023	2024	2022	2023	2024
Control	76.3 ± 1.12e	77.6 ± 1.13e	73.2 ± 1.06e	3.35 ± 0.012a	3.04 ± 0.008a	4.53 ± 0.023a
MycoFix	81.7 ± 1.20d	83.7 ± 1.23d	81.3 ± 1.18d	3.02 ± 0.010b	2.68 ± 0.010b	3.42 ± 0.011b
MycoFix + Anderis	87.9 ± 1.29c	90.3 ± 1.32c	88.1 ± 1.29c	2.69 ± 0.012c	2.32 ± 0.010c	3.13 ± 0.011c
MycoFix + Violar	89.6 ± 1.31b	92.0 ± 1.35b	90.2 ± 1.31b	2.58 ± 0.010d	2.10 ± 0.005d	2.87 ± 0.010d
MycoFix + Anderis + Violar	94.8 ± 1.39a	97.2 ± 1.42a	95.4 ± 1.39a	2.32 ± 0.010e	1.87 ± 0.003e	2.39 ± 0.012e

Values followed by different letters differ significantly according to Tukeys' HSD test (*p*≤0.05). Data are presented as mean ± SE. RWC: Relative water content, Rs: Stomatal resistance.

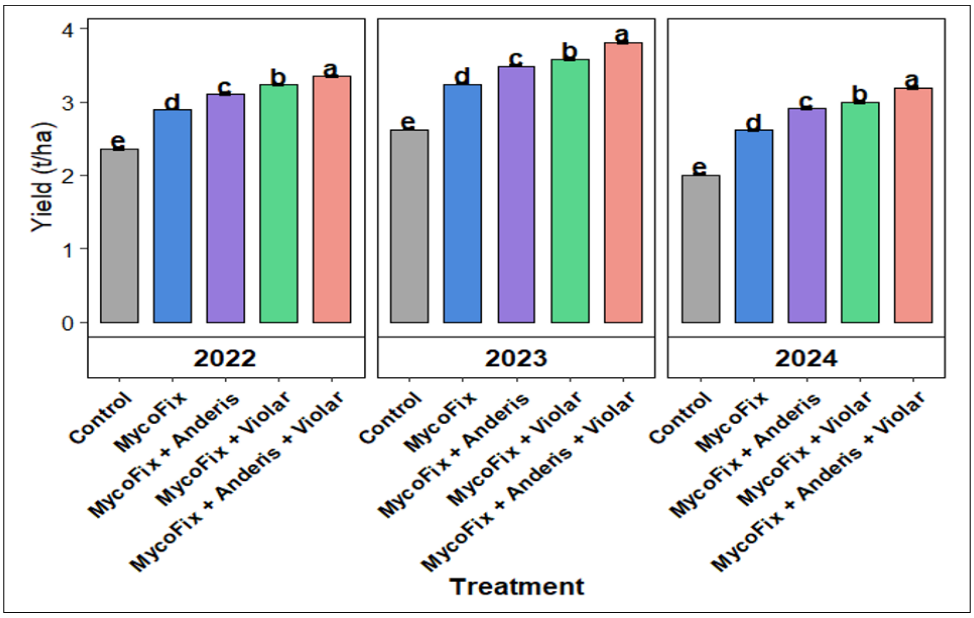

Fig. 3. Soybean grain yield under various biostimulant treatments across three growing seasons. Bars represent standard errors; different letters indicate significant differences within each year according to Tukeys' HSD test (*p*≤0.05).

inoculant Anderis further increased yield. Under the MycoFix + Anderis treatment, the yield was 3.12 t/ha in 2022, 3.47 t/ha in 2023 and 2.88 t/ha in 2024, a 30.5-42.6 % increase compared to the control. The addition of the phytohormonal preparation Violar further enhanced yield. In the MycoFix + Violar treatment, the yield reached 3.22 t/ha in 2022, 3.60 t/ha in 2023 and 2.99 t/ha in 2024, a 34.7-48.0 % increase compared to the control.

The highest yield was achieved through the comprehensive use of MycoFix + Anderis and Violar, combining the synergistic effects of these biostimulants. The yield was 3.36 t/ha in 2022, peaked at 3.82 t/ha in optimal 2023 conditions and remained high at 3.21 t/ha in the drought-prone 2024 season, an increase of 40.6–58.9 % compared to the control. This demonstrates the exceptional effectiveness of integrated biostimulant use in organic soybean cultivation.

Principal component analysis of the impact of climatic conditions and biostimulants

Principal component analysis (PCA) was applied to analyse the effects of biostimulants on the soybean Adamos variety under organic farming conditions during 2022–2024 (Fig. 4). The total explained variance was 98.0 % (Dim1 - 93.6 %, Dim2 - 4.4 %), indicating strong treatment effects over temporal variation. Dim1 differentiated treatments based on effectiveness and plant physiological status. Positive Dim1 values corresponded to high ABA levels and Rs, indicating stress and water deficit. Control treatments across all years were positioned in the right part of the biplot, with control-2024 showing the highest stress response under

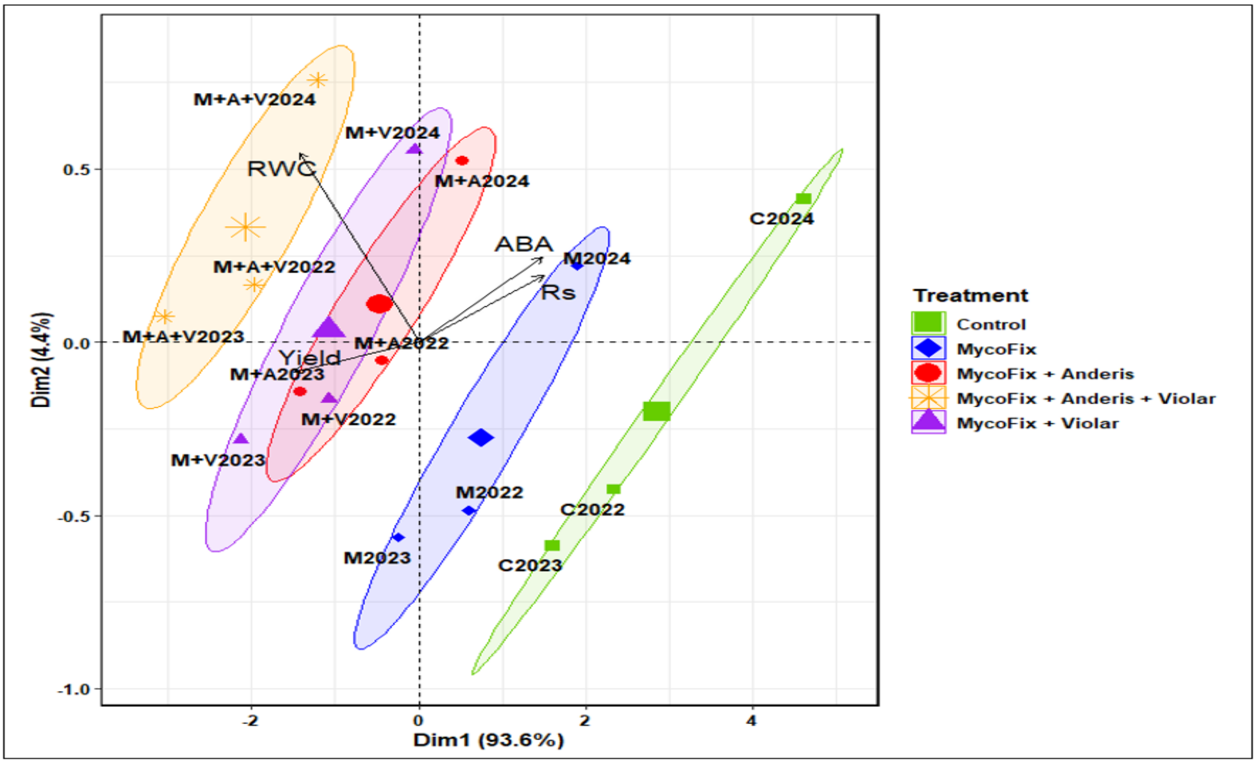

Fig. 4. Principal component analysis biplot of biostimulant treatments, physiological parameters and grain yield (2022–2024). Ellipses denote 95 % confidence intervals; labels indicate treatment-year combinations.

drought. Negative Dim1 values reflected improved water status, reduced stress hormones and enhanced productivity. Biostimulants, especially the MycoFix + Anderis + Violar combination, shifted leftward, indicating effective stress mitigation and physiological optimisation. Yield was negatively correlated with Dim1 (r = -0.95), confirming the relationship between stress load and productivity.

Dim2 (4.4 % variance) reflected inter-annual variability linked to hydrothermal conditions. The 2024 data, marked by extreme drought, shifted upward on Dim2. In contrast, 2022 and 2023 data points were positioned in negative zones, with 2023 showing the most favourable conditions. The low explanatory power of Dim2 suggests that biostimulants had a greater impact than inter-annual variability.

The ABA vector was oriented positively along Dim1, reflecting stress hormone accumulation in control treatments, especially in 2024. Stomatal resistance showed a similar orientation, strongly correlated with ABA (r = 0.970, p<0.001). The RWC vector was negatively oriented along Dim1, confirming its inverse relationship with stress indicators (ABA and Rs) and showing a strong correlation with yield (r = 0.992, p<0.001). Five distinct clusters were identified, with the control cluster separated on the right, reflecting weather impact. The MycoFix cluster was positioned between control and combined treatments, showing partial physiological modification. Combined treatments (MycoFix + Anderis and MycoFix + Violar) overlapped slightly, while the MycoFix + Anderis + Violar cluster was distinctly separated on the left, demonstrating a unique physiological system. The confidence ellipses indicated the highest stability in the MycoFix + Anderis + Violar group, which maintained optimal parameters even under extreme drought in 2024.

The high explanatory power of Dim1 high explanatory power (93.6 %) suggests that biostimulant effects mainly optimise water status and reduce stress load. The synergistic interactions among biostimulants were evident, as combined treatments exceeded the sum of individual effects, indicating the formation of an integrated biological system with emergent properties. Comparing 2023 and 2024, biostimulants improved productivity and water status under favourable conditions, while enhancing physiological adaptation under drought. For instance, MycoFix + Anderis + Violar in 2024 maintained high RWC (95.4 %) compared to the control (73.2 %), with reduced stress hormone levels. The PCA results confirm that complex biostimulants (MycoFix + Anderis + Violar) significantly enhance soybean stress tolerance and productivity in organic farming. The treatment ensured stable yields across years with contrasting hydrothermal conditions, with a 40.6–58.9 % yield increase compared to the control (3.36–3.82 t/ha vs. 2.02–2.63 t/ha). This highlights the potential of biostimulants for developing adaptive technologies for soybean cultivation under changing climate conditions. The high explained variance (98.0 %) and clear treatment differentiation validate the experimental design, emphasising the role of biostimulants in optimising soybean cultivation in water-limited environments. These findings contribute to both understanding plant adaptation mechanisms and improving farming practices under stress conditions.

Discussion

Correlation relationships among experimental indicators

Based on the results of a comprehensive correlation analysis, 2 major clusters of variables were identified: (1) stress-related factors (ABA and Rs), which exhibited a strong positive intercorrelation; and (2) productivity-related parameters (RWC and yield), which demonstrated an extremely strong positive relationship. The strongest negative relationship was observed between Rs and yield (r = -0.976), indicating the impact of restricted gas exchange on productivity. Abscisic acid content was strongly positively correlated with Rs (r = 0.970) and negatively correlated with yield (r = -0.910) and RWC (r = -0.871), confirming the association between stress hormone accumulation, stomatal closure and plant water status. Conversely, RWC showed a very strong positive correlation with yield (r = 0.992) and a strong negative correlation with Rs (r = -0.963), highlighting the importance of water status in yield formation under hydrothermal stress. Collectively, these identified correlations underscore the effectiveness of the applied treatments in optimising the plants' physiological response by suppressing the stress cascade (ABA and Rs increase) and promoting a favourable water balance (high RWC), which is the most critical determinant of yield formation under hydrothermal stress.

Role of ABA in the plants' physiological adaptation to stress conditions

The obtained results are consistent with current scientific data demonstrating the role of biostimulants in modulating ABA levels (31, 32). Abscisic acid is a key signalling molecule that regulates numerous aspects of plant growth and development and coordinates various physiological processes, particularly responses to abiotic stresses (33). The strong positive correlation observed in our study between ABA content and Rs (r = 0.970) confirms the pivotal role of ABA in regulating stomatal function. Increased ABA levels lead to stomatal closure, as stomata are microscopic pores formed by pairs of guard cells that regulate gas exchange (9). This physiological mechanism is highly adaptive, as it substantially reduces water loss through transpiration and mitigates the detrimental effects of dehydration on the plant. The pronounced negative correlation between ABA and RWC (r = -0.871) further indicates that ABA accumulation serves as a sensitive indicator of developing water deficit. Such strong correlations demonstrate the functional activity of ABA-dependent signalling pathways in the studied soybean plants under hydrothermal stress.

Effect of biostimulants on the plant hormonal status

Special attention should be paid to the ability of rhizosphere and endosphere microbiota, including rhizobia and mycorrhizal fungi, to directly or indirectly modulate the plant hormonal profile, particularly ABA levels. The biostimulants tested in this study - MycoFix (arbuscular mycorrhizal fungus *G. intraradices*) Anderis (*B. japonicum* and other rhizobia) and Violar (metabolites of *S. violaceus*) - have different modes of action, including the modulation of phytohormone levels, particularly ethylene and ABA, which collectively enhance plant productivity and stress tolerance (34).

Experimental evidence confirms that AMF and rhizobacteria can modulate ABA-related signalling and stress responses in plants; AMF act as natural biostimulants, influencing physiological pathways associated with ABA regulation under abiotic stress (35). This is particularly relevant for MycoFix, which contains *G. intraradices* (1 %, minimum 2000 spores/g) combined with seaweed extract from *A. nodosum* (99 %). Recent studies on *R. intraradices* have demonstrated its effectiveness in enhancing drought tolerance through improved water and nutrient uptake, enhanced photosynthetic efficiency, elevated osmoprotectant levels (proline, soluble sugars) and activation of antioxidant systems, including increased ascorbate and glutathione content (23). The seaweed component provides additional benefits through bioactive compounds that enhance osmoregulation and stress tolerance (18). This dual-action mechanism – the symbiosis of mycorrhiza and seaweed metabolites - explains the significant increase in RWC and reduction in ABA accumulation observed in MycoFix-treated plants in our study, consistent with a study on the effect of the combined application of AMF with biochar and other organic amendments, which enhanced wheat yield (36).

Rhizobacteria have been shown to metabolise ABA, reducing its levels in plants and influencing growth parameters (37). This mechanism may explain the progressive decrease in ABA content observed with increasing complexity of biostimulant combinations in our study, especially in treatments involving Anderis. The Anderis inoculant contains nitrogen-fixing *B. japonicum* and phosphorus-mobilising *Penicillium bilaii* (*P. bilaiae*), which enhance nutrient availability and root development, indirectly alleviating drought stress and reducing ABA accumulation. Certain *Streptomyces* strains can synthesise phytohormones *in vitro*, indicating their potential in regulating plant hormonal status (38). The Violar biostimulant, based on *S. violaceus* metabolites, contains a complex of natural phytohormones including auxins, cytokinins, gibberellins and trace amounts of ABA (0.02 mg/L). This composition suggests that Violar may influence endogenous ABA levels through multiple pathways: direct hormonal supplementation and modulation of plant hormonal biosynthesis through bacterial metabolites.

Recent research has confirmed that dual inoculation with PGPR (*Bacillus subtilis*) and AMF (*R. intradices*) enhances plants' tolerance to salinity stress by increasing ABA levels and activating antioxidant enzyme systems (39). In line with these findings, our results indicate that more complex microbial and biostimulant consortia can further modulate physiological stress responses. This aligns with reports indicating that double and triple microbial associations (e.g., *Bradyrhizobium*, *Azospirillum* and AMF) induced metabolic changes and soybean differential growth, highlighting both the synergistic benefits and the competitive dynamics during the establishment of multiple symbionts (40). Similarly, field trials have demonstrated that multifunctional microbial consortia successfully sustain soybean grain yield and physiological resilience (41). The importance of such multi-strain interactions has been further supported by evidence showing that rhizosphere-borne beneficial microbes play an important role in mitigating abiotic stress by restoring nutrient and hormonal balance in plants (42). Furthermore, the inclusion of organic extracts or biostimulants alongside beneficial microbes amplifies these natural defence mechanisms. For instance, the application of seaweed extracts has been reported to enhance drought tolerance in soybean through improved water status and upregulation of stress-responsive genes (43). This is consistent with reports indicating that exogenous growth-regulating biostimulants (e.g., brassinosteroids) modulate physiological parameters and enhance soybean yield under environmental stress conditions (44). These hormonal and physiological responses correspond to the improved RWC and ABA regulation observed in our combined treatments. Complex biostimulant extracts have been shown to stimulate AMF growth and upregulate plant genes involved in symbiotic accommodation (45). Thus, the enhanced physiological status (e.g., optimised Rs and RWC) and higher yield observed in our combined treatments may result from this dual effect: direct physiological priming and stimulation of the beneficial rhizosphere network.

Conclusion

The integrated application of mycorrhizal, rhizobial and phytohormonal biostimulants (MycoFix + Anderis + Violar) proved highly effective in enhancing soybean stress tolerance and productivity under organic farming conditions across different hydrothermal regimes. The synergistic effects of biostimulants optimised ABA-mediated stress responses, maintained favourable plant water status and ensured stable yields, even under extreme drought conditions. The strong correlations between ABA content, Rs, RWC and yield highlight the coordinated nature of plant adaptive mechanisms modulated by biostimulant treatments. Thus, the study results support the need to implement biostimulant-based strategies as sustainable solutions to maintain soybean productivity under increasingly challenging climatic conditions.

Authors' contributions

CT conceived of the study, participated in its design and coordination and performed the statistical analysis. KI carried out the biochemical studies, participated in the sequence alignment and drafted the manuscript. RA and SM participated in the sequence alignment. LV and PG participated in the design of the study. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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