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RESEARCH ARTICLE

Assessing the efficiency of bacterial and phytohormonal soybean (*Glycine max* L.) seed treatment under organic farming technology

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Abstract
Soybean (*Glycine max* L.) is one of the most sought-after legumes on the global market due to its high demand in the feed, food, and industrial sectors. Climate change necessitates the implementation of technological innovations to enhance plant resistance to adverse growing conditions. The aim of this study was to investigate the effect of pre-sowing seed treatment of soybean with the bacterial preparation Profix, the phytohormone-based preparation Violar, as well as their combined application through seed inoculation and crop spraying during the bud formation–bloom phase, on the development and productivity of soybeans under an organic farming. To examine the dynamics of leaf surface area, photosynthetic pigments, malondialdehyde and proline levels, and their relationship with yield, the ANOVA-Tukey test and principal component analysis were employed. The field experimental results showed that pre-sowing seeds treatment with the Profix inoculant, the phytohormonal preparation Violar, and their combined application contributes to an increase in leaf surface area, an enhancement in the content of photosynthetic pigments, a reduction in malondialdehyde levels, and an increase in proline content in soybean plant. These physiological improvements ultimately led to average soybean yield increases of 12.3%, 19.6%, and 29.2%, respectively, despite adverse weather conditions 2024. These results demonstrate the effectiveness of integrating bacterial and phytohormonal seed treatments as a sustainable and innovative approach to enhancing soybean productivity under organic farming conditions.

Keywords: leaf area, malondialdehyde, photosynthetic pigments, proline, stress biomarkers, yield

INTRODUCTION

Soybean (*Glycine max L.*) is one of the most highly demanded legumes on the global market due to its grain's chemical composition, which consists of 30–45% high quality protein with essential amino acids and 15–22% oil (Galben et al., 2022). This means that soybean crops contain more than 60% of various nutrients; therefore, the plant can be used in the food industry and other sectors (Kim et al., 2021). Soybean is a leader in vegetable oil production due to the highest lecithin content (2–3%) among vegetable oils. About 60% of the soybean harvest is processed into oil (Guo et al., 2022). Soybean has a significant agrotechnical effect. It fixes atmospheric nitrogen and leaves 60–90 kg/ha of biologically fixed nitrogen in the soil, which enables producers to reduce the use of nitrogen fertilizers and lowers the risk of water and environmental pollution. In addition, soybean suppresses weed growth and serves as an excellent preceding crop for many agricultural species (Peoples et al., 2021; Chen et al., 2022). Due to its strong root system, soybean absorbs hard-to-reach minerals even from deep soil layers (Kalra et al., 2024).

The demand for soybean in many industries has contributed to a significant growth in its global production, making it the sixth most widespread crop by production volume and the fourth by economic

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value and production area (Martignone et al., 2024). This growth follows a 5% increase in global soybean consumption, driven by feed, food and industrial demand (IGS, 2024). In Ukraine, soybean production in 2024 reached a record 6.0 million tonnes, compared to 5.2 million tonnes in 2023, despite abnormal heat and drought conditions. Soybean production in the EU increased by only 0.3% compared to the previous year. However, weather conditions, particularly in Central Europe, had a negative impact on the soybean harvest. Thus, Ukraine, one of the world's top ten soybean producers, has become a key factor in European soybean production this season (USDA: IPAD, 2025). In Europe, ongoing climate change and strong demand for genetically unmodified soybeans in domestic markets are driving the integration of soybean (*Glycine max* (L.) Merr.) into traditional organic farming systems. Since the EU market is the largest market for domestic production of soybeans (47% of total exports), domestic producers tend to follow the European Green Deal, which includes introducing organic production as one of its important areas (Rotundo et al., 2024).

Organic farming technology prohibits the use of pesticides and genetically modified organisms. It is based on biological approaches to improving natural soil fertility, agroecological cultivation methods, and biological tools for pest and disease control, which together promote biodiversity conservation (Benbrook et al., 2021; Kukol et al., 2024). The profitability of organic soybean in Ukraine is high despite the climatic risks, especially the uneven influence of rainfall and high temperatures, which requires technological innovations to increase its yield. Today, plants in 40% of the world's temperate climate zones are exposed to elevated stressful temperatures. A one-degree rise in temperature leads to a 3–8% reduction in yields of major crops. When high temperatures are combined with drought, more than half of the world's major crops can be lost (Zhao et al., 2017).

Considering the importance of soybean production under the changing climatic conditions, the study aim was to assess the role of pre-sowing seed treatment with bacterial and phytohormonal preparations, as well as their combined use by inoculating seeds with a bacterial preparation and spraying crops with a phytohormonal preparation in overcoming extremely high temperatures during the growing season and their impact on physiological and biochemical parameters of plants and yield.

MATERIALS AND METHODS

Field experiment

Field experiments were conducted in the experimental field of Poltava State Agrarian University (Kremenchuk district, Poltava region) in 2022–2024. The region is environmentally clean due to the nearby forests and Lake Sudebske which is a part of the nature reserve. The soil of the experimental plots is residual-saline black soil on loess rocks with moderate nitrogen, phosphorus and high potassium content. The total area of the land plot was 0.3 ha. The accounting area was 0.1 ha. The common agricultural technique for the growing zone was used in field experiment. The experiment was based on a randomized complete block design with four replications.

Technological soil tillage, taking into account the specifics of growing crops under the organic technology, included autumn ploughing with a plough, spring harrowing with a heavy harrow to preserve moisture, cultivation with a stubble cultivator, pre- and post-emergence harrowing with a Striegel mesh harrow, and two inter-row cultivations for weed control.

The study object was an early-ripening soybean variety of domestic selection Khorol, the originator of which is LLC Soybean Research Institute (Ukraine). Soybean was sown after spring barley at the optimal terms according to the climatic conditions of the research year to a depth of 5 cm, with row spacing of 38 cm and a seeding rate of 700 thousand seeds per hectare. To protect the crops from pests, the trichogram was manually applied three times (100-200 thousand individuals per ha) at 50 points per hectare: pre-sowing, one month later and as required, depending on the crop infestation level.

Climatic conditions in 2022 and 2023 were considerably more conducive to soybean growth and development, with average summer temperatures around 21°C and monthly precipitation levels ranging between 48 and 58 mm. In contrast, the 2024 growing season was characterized by episodes of extreme heat with temperatures reaching up to 24°C in July and periodically peaking at 30-32°C accompanied by critically low and unevenly distributed precipitation (ranging from 3 to 53 mm). These environmental stressors adversely impacted soybean plant development.

Soil analysis

Five soil samples were collected from the experimental plot with a 0-20 cm depth for analysis. A composite soil sample was made by mixing the collected samples. The sample was air-dried, crushed and passed through a 2 mm sieve. The main nutrients content in the soil and pH was determined using a Palintes SK500 multiparametric photometer (Palintest House, United Kingdom, 2020). Analyses were performed in 4 replicates. The humus content was 5.2%, P_2O_5 – 78.3 mg/kg, K_2O – 138.4 mg/kg, total nitrogen – 58.6 mg/kg of soil; pH_{KCl} = 6.3.

Experimental design and treatments

Four pre-sowing soybean seed treatment variants relevant to organic farming technology were investigated: (i) inoculation with the bacterial preparation Profix; (ii) seed treatment with a preparation containing a complex of phytohormones (Violar) and spraying of the leaf-stem mass during the bud formation–bloom phase; (iii) inoculation with the bacterial preparation Profix combined with spraying of the leaf-stem mass with Violar during the bud formation–bloom phase; (iv) no inoculation (control variant), where soybean seeds were treated with an equivalent amount of water. The Profix (Certis Belchim, Belchim) composition includes pure cultures of nitrogen-fixing bacteria *Bradyrhizobium Diazoefficiens* strain SEIMA 5079 and SEIMA 5080 + *Bradyrhizobium japonicum* strain USDA442 (532C), 5×10^9 CFU/g. Seeds were inoculated by dry method 48 hours before sowing at a rate of 1.25 kg of inoculant per 500 kg of seeds.

Biological preparation Violar (Bioinvest-Agro, LLC, Ukraine) is a multifunctional metabolic biological preparation based on soil streptomycetes containing a complex of natural phytohormones: auxins (3.6 mg/l), cytokinins (1.9 mg/l), gibberellins (1.6 mg/l), free amino acids (2.2 mg/l), lipids (5.5 mg/l), abscisic acid (0.02 mg/l), sterols (1.71 mg/l), including unsaturated fatty acids. Seeds were treated before sowing at a rate of 0.5 l/t, and foliar application was carried out at 10 ml/ha during the bud formation–bloom phase. In the combined treatment, seeds were inoculated with Profix as described above, while Violar was applied at 10 ml per 200 litres of water per hectare. All application rates of Profix and Violar followed the manufacturer's recommended concentrations, which represent the optimal doses for effective inoculation and phytohormonal stimulation under field conditions.

Laboratory methods

Proline and malondialdehyde content (MDA) in soybean plants was determined according to the method Fatema et al. (2023). The leaf surface area was calculated using the Easy Leaf Area software (Easlon and Bloom, 2014). Leaf area per hectare (m^2/ha) was calculated as the product of the leaf area of a single plant and the plant density per hectare. The material for determining photosynthetic pigments was treated in a fresh state immediately after collection. The pigments were extracted with 96% ethanol. The spectrophotometric measurement of the optical density of ethanol extracts to determine the chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*) content was carried out without prior separation at the absorption maxima of Chl *a* – 665 nm, Chl *b* – 649 nm on a ULAB 108 UV spectrophotometer (Ulab, China, 2021). The pigment content in the obtained extracts was measured according to the method Wellburn (1994).

Data analysis

Statistical data processing was performed using Statistica 12.0 (StatSoft Inc., USA, 2013) software. The results are expressed as the arithmetic mean ± standard error (SE) based on a sample size of $n = 10$. The least-square means differences between the values in the experimental variants were determined by the ANOVA-Tukey test multiple comparison method and considered significant at $P < 0.05$. A principal component analysis (PCA) biplots were constructed to visualize the impact of different inoculation technologies on plant physiological parameters using R statistical software (R Core Team, New Zealand, ver. 4.4.3, 2025-02-28).

RESULTS

Leaf area formation

The analysis of the dynamics of soybean leaf area formation allowed us to assess the impact of weather conditions during the growing season 2022–2024 on the plant morphological and photosynthetic parameters. The temperature and humidity conditions 2022 and 2023 were more favorable for the growth and development of soybean plants. Extremely high temperatures and low relative humidity were observed in 2024 due to insufficient precipitation and its uneven distribution during the growing season, which limited the formation of plant leaf area.

The leaf surface area of soybean plants in the hot year of 2024 was less in all experimental variants than in the previous years of 2022 and 2023: in the control by 17.1 and 23.3%; with Profix inoculation by 16.0 and 27.0%; with Violar treating by 18.8 and 27.0%; with Profix inoculation and spraying of plants with Violar preparation by 15.5 and 24.7%, respectively (Table 1).

Proline and malondialdehyde content

In response to water deficit, plants develop adaptive strategies to minimise water loss and increase its use efficiency and accumulate organic compounds, such as proline, to maintain osmotic balance under water deficit conditions. Besides, under high temperatures conditions, plants produce MDA, a marker of oxidative stress and lipid peroxidation. Our research used the proline and MDA content as stress biomarkers. The levels of these indicators accumulated by soybean plants in all experimental variants are shown in Table 2. Proline content in plants from non-inoculated seeds (control) was the lowest at every study year. Hot temperatures in 2024 correlated with the high proline content in plants (9.5 μg/g of fresh weight) due to Violar seed treatment. However, the proline content in plants growing from seeds inoculated by Profix with followed crop spraying by Violar in the bud formation-bloom phase was maximum −10.4 mg/g (Table 2).

Proline content in plants of 2024, as a result of Violar seed treatment, increased by 38.6% compared to the control and by 10.7% compared to the Profix seed treatment. Profix seed inoculation combined with Violar crop treatment resulted in a 50.6% increase in plant proline content compared to the control, and an 8.7% increase compared to plants treated Violar only. Soybean plants grown under more favorable weather conditions in 2022-2023 also had increased proline content but exceeding the control with the Violar seed treatment was an average of 35.0%, with the Profix treatment by 19.8% and under together used Profix and Violar – 44.0% (Table 2).

The highest MDA content was obtained in control plants, indicating the level of response to oxidative stress compared to other treatments. Soybean plants grown from inoculated seeds appeared to be more resistant to adverse temperature conditions, as evidenced by a decrease in MDA levels. MDA content in plants in 2024, which was characterized by extremely high temperatures during the growing season, decreased by ~37% and ~23% compared to control plants due to the use of Violar and Profix, respectively. The pre-sowing seed treatment with Profix and foliar fertilization with Violar in the bud formation-bloom phase proved to be the most effective measure in ensuring plant resistance to high temperatures. MDA content in plants decreased by 39% compared to control plants.

In 2022-2023, soybean plants had the lowest MDA content averaging 5.7 μg/g of fresh weight, which was 38.9% lower than the MDA content in control plants due to seed inoculation with Profix and foliar spraying by Violar. Soybean seeds treatment with Profix and Violar also showed an effective result in overcoming temperature stress, the MDA content in plants decreased by an average of 23.5 and 37.0%, respectively, compared to control plants. It can be assumed that seed inoculation with experimental preparations contributed to induction of soybean plant resistance to precipitation deficit and high-temperature effects.

Photosynthetic pigments content

To determine the influence of pre-sowing seed treatment with bacterial and phytohormonal preparations on the functioning of the photosynthetic apparatus of soybean plants under different weather conditions, the dynamics of the Chl *a* and Chl *b* content was analysed, since the efficiency of the pigment system affects the soybean crops yield and depends on environmental conditions. The study results are shown in Table 3.

Table 1. Dynamics of leaf area formation and soybean yield

Variant	Leaf surface area, thousand m^2/ha			Yield, t/ha		
	2022	2023	2024	2022	2023	2024
Control (water)	23.4±0.1^c	25.3±0.4^b	19.4±0.2^c	2.2±0.0^c	2.5±0.0^d	1.9±0.0^d
Profix	26.1±0.2^b	28.6±0.3^a	22.5±0.2^b	2.5±0.0^b	2.8±0.0^b	2.1±0.0^b
Violar	27.8±0.2^a	29.7±0.4^a	23.4±0.1^{ab}	2.7±0.0^b	2.9±0.0^b	2.3±0.0^b
Profix + Violar	27.6±0.7^a	29.8±0.6^a	23.9±0.5^a	2.9±0.1^a	3.1±0.1^a	2.5±0.1^a

Letters a, b, c and d indicate values which reliably differed one from another within one line of table according to the results of comparison using Tukey test at $p < 0.05$.

Table 2. Proline and Malondialdehyde content in soybean plants

Variant	Proline, µg/g			MDA, µg/g		
	2022	2023	2024	2022	2023	2024
Control (water)	5.1±0.1^{d}	5.4±0.0^{c}	6.9±0.1^{d}	9.4±0.1^{a}	9.2±0.0^{a}	17.9±0.2^{a}
Profix	6.2±0.1^{c}	6.4±0.1^{b}	8.6±0.1^{c}	7.2±0.1^{b}	6.9±0.0^{b}	13.9±0.1^{b}
Violar	6.8±0.0^{b}	7.5±0.1^{a}	9.5±0.1^{b}	5.9±0.1^{c}	5.4±0.1^{d}	11.3±0.0^{c}
Profix + Violar	7.4±0.2^{a}	7.8±0.1^{a}	10.4±0.0^{a}	6.1±0.1^{c}	5.6±0.1^{c}	10.9±0.2^{c}

Letters a, b, c and d indicate values which reliably differed one from another within one line of table according to the results of comparison using Tukey test at $p < 0.05$.

Table 3. Chlorophyll *a* and *b* content in soybean plants in the bloom phase

Variant	Chl *a*, µg/g			Chl *b*, µg/g			Chl *(a+b)*, µg/g		
	2022	2023	2024	2022	2023	2024	2022	2023	2024
Control (water)	2.2±0.0^{c}	2.4±0.1^{c}	1.8±0.0^{c}	0.9±0.0^{c}	1.1±0.0^{c}	0.7±0.0^{c}	3.1±0.0^{c}	3.5±0.1^{c}	2.5±0.0^{c}
Profix	2.5±0.0^{b}	2.8±0.0^{b}	2.1±0.0^{b}	1.0±0.0^{a}	1.1±0.0^{b}	0.8±0.0^{a}	3.4±0.0^{b}	3.9±0.0^{b}	2.9±0.0^{b}
Violar	2.6±0.0^{b}	2.8±0.0^{b}	2.2±0.0^{ab}	1.0±0.0^{a}	1.2±0.0^{b}	0.8±0.0^{a}	3.6±0.0^{b}	4.0±0.0^{b}	3.1±0.0^{ab}
Profix + Violar	2.9±0.1^{a}	3.1±0.0^{a}	2.3±0.1^{a}	1.0±0.0^{a}	1.3±0.0^{a}	0.9±0.02^{a}	3.9±0.1^{a}	4.4±0.1^{a}	3.1±0.1^{a}

Letters a, b and c indicate values which reliably differed one from another within one line of table according to the results of comparison using Tukey test at $p < 0.05$.

In our study, the photosynthetic pigments content in soybean plants was significantly affected by seed treatment with biological preparations and weather conditions during the growing season. Adaptation to the insolation regime affected the photosynthetic pigments content. Comparison of Chl *a* and Chl *b* content in plants grown from seeds treated with Violar showed their high level in all study years. Chlorophyll Chl *a* and Chl *b* content in plants with Violar seed treatment exceeded the control by an average of 18.3 and 18.6%, respectively, and chlorophyll Chl *a* and Chl *b* content in plants from seeds inoculated with Profix – by 14.5 and 12.8%.

Soybean plants when using Profix in pre-sowing seed treatment followed by foliar fertilizer with Violar in the bud formation-bloom phase showed most photosynthetic system activity, Chl *a* content increased by 30% compared to the control and Chl *b* by 19.8% (Table 3). Photosynthetic pigments content in soybean plants increased in the more favorable year of 2023, which may be due to the seed treatment with biopreparations. Thus, Chl *a* content in plants from seeds inoculated with Profix increased by 11.7%, and in plants from seeds treated with the biological product Violar it increased by 10.2% compared to 2022. Chlorophyll *a* content in control plants almost did not change.

The increase of Chl *b* content in 2023 plants compared to 2022 correlated with the increase of Chl *a*. Chlorophyll *b* content in soybean plants with Violar using was 16.7% higher than the concentration of this pigment in plants of the 2022. Chlorophyll *b* concentration with Profix using exceeded this indicator by 17.5%. Chlorophyll *b* content in plants grown with the use of biological preparations in pre-sowing seed treatment and foliar fertilizer (Profix + Violar) compared to plants in 2022 increased the most significant by 22.5%. 2024, which was characterised by high temperatures and almost complete absence of precipitation, Chl *a* and Chl *b* content decreased in all experimental variants. However, the highest chlorophylls level was observed with the use of biological preparations Profix + Violar, and the lowest – in the control plants (Table 3). The total Chl *(a+b)* content in soybean plants with Profix seeds inoculation increased compared to the control by 11.7% in 2022 and by 13.3% in 2024. As a result of pre-sowing seed treatment with the Violar, the total Chl *(a+b)* content in 2022-2023 increased by ~ 16% relative to the control. As can be seen from the Table 3, the highest increase in Chl *(a+b)* occurred in plants when using the Profix (pre-sowing seed treatment) + Violar (spraying of crops) in the cultivation technology.

Soybean yield

Yield is the primary indicator of assessing the effectiveness of all agrotechnical practices applied in crop cultivation technology, taking into account weather conditions. Table 1 presents the average soybean seed yields for the studied agrotechnical variants over the years of the experiment. The yield variation correlated with weather conditions during the experiment. The higher yields were obtained under more favorable conditions in 2023. In contrast, the plots with non-inoculated seeds were found to yield significantly lower. Out of the all experimental variants, the most significant yield (3.1 t/ha) was obtained in 2023 with Profix seed inoculation and foliar application of the Violar preparation in the bud formation-bloom phase. The soybean yield in the control variant was at the level of 1.9−2.4 t/ha depending on the year, while the yield with the use of Profix inoculant and Violar biological preparation was 2.1-2.8 and 2.3-3.0 t/ha, respectively.

Considering the relationship between average yields and chlorophyll level in plants depending on the biological preparations application method, we can point to a certain trend in yield, which varies depending on the Chl *a*, Chl *b* content and the total Chl *(a+b)* content. Thus, the use of the inoculant Profix led to an increase in Chl (*a+b*) content by 14.7%, resulting in a 12.3% increase in average soybean yield. When using the bacterial preparation Violar, Chl (*a+b*) content increased by 18.4%, which caused a 19.6% yield increase compared to the control. The highest yields were obtained with application in growing technology of the Profix in pre-sowing seed treatment and Violar for the soybean crops spraying (Table 1). Under this cultivation variant, the yield increased by 29.2% compared to the control at the increase of the total chlorophyll by 27%.

Yield analysis

To explore in more detail the impact of different inoculation technologies (Control, Profix, Violar, and their combination) on plant physiological parameters, principal component analysis (PCA) was conducted with results presented in the biplot. The visualization demonstrates the relationships between Chl *(a+b)* content, leaf surface area, proline and MDA content, and soybean yield over the three-year observation period (2022–2024), allowing for a comprehensive assessment of the effectiveness of the studied preparations.

The PCA biplot (Figure 1) clearly demonstrates a complex picture of interactions between the studied plant physiological parameters and treatment variants with biologicals over a three-year period. The first dimension (Dim 1) explains 77.3% of variation, while the second dimension (Dim 2) accounts for 20.1%, together covering 97.4% of the total variation in the dataset, indicating the high informativeness of the analysis. The biplot distinctly displays four clusters corresponding to the applied technologies (control, Profix, Violar, Profix + Violar), with the position of each variant changing depending on the year of study, reflecting the influence of weather conditions on the effectiveness of the biologicals. Particularly revealing is the arrangement of physiological parameter vectors: the proline vector points toward variants with Profix + Violar treatment in 2024, confirming the activation of protective mechanisms under drought conditions, while the vectors of leaf area and chlorophyll a content are oriented toward high-productivity variants.

The control variant (without treatment) is consistently located in the lower part of the biplot throughout all years of the study, indicating the low plant's ability from untreated seed to maintain optimal physiological parameters. Variants with Profix treatment alone demonstrate an intermediate position, while the most productive combinations (Profix + Violar) are located in the zone of positive values for both components. Notably, the MDA vector (an indicator of oxidative stress) shows a negative correlation with yield and positively correlates with control variants, especially in 2024, indicating an increased level of oxidative stress in plants without pre-sowing treatment. Simultaneously, the yield vector points toward the zone of variants with complex Profix + Violar treatment, confirming its effectiveness in ensuring high productivity.

Biplot on Figure 1 not only illustrates the synergistic effect of the combined application of rhizobia and phytohormones but also allows for predicting the effectiveness of different technologies depending on the weather conditions of the year, which has significant practical importance for developing adaptive technologies for organic soybean cultivation.

Figure 2 better illustrates the final productivity indicators. The first principal component (Dim 1) explains an exceptionally high percentage of variation – 98.4%, indicating a dominant factor influencing all studied parameters.

The second component (Dim 2) accounts for only 1% of variability. According to this biplot, the combined Profix + Violar treatment demonstrates stability of effect in 2022–2023, with some deviation in 2024. The Violar treatments exhibit the greatest variability across years, with a particularly noticeable difference between 2022–2023 and 2024. Profix inoculation demonstrates relative stability, with a tendency to converge with the control in 2024. Thus, this biplot confirms that the combined application of biopreparations with different mechanisms of action (nitrogen-fixing bacteria and a phytohormonal complex) creates a synergistic effect, which is most evident under environmental stress conditions, ensuring the stability of organic soybean cultivation technology.

A comparison of both biplots allows for a deeper understanding of the causal relationships between physiological changes and productivity. The comparative analysis of point placement on both biplots leads to the conclusion that inoculation technologies offer significant advantages, particularly in the high stability of the combined Profix + Violar treatment for improving productivity and stress tolerance in the Khorol soybean variety under the conditions of a changing climate and drought, which have become characteristic of many temperate regions in recent years.

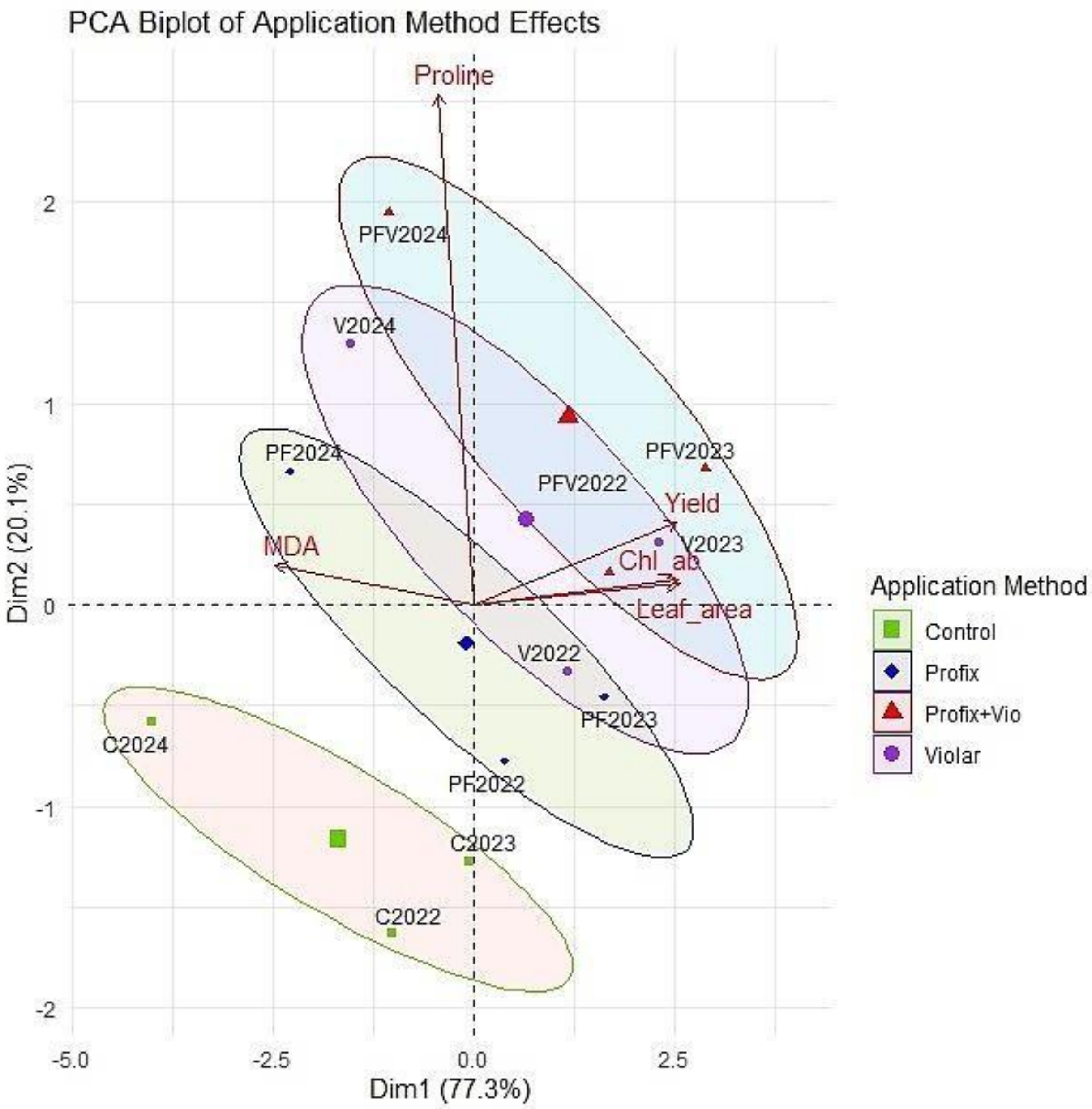

Figure 1. Principal component analysis the relationship between inoculation technologies and plant physiological parameters (full variable set) across three study years (2022–2024): Chl_*ab* – Chl *(a+b)*, μg/g; Leaf_area – leaf surface area, thousand m²/ha; Proline – proline content, μg/g; MDA – malondialdehyde content, μg/g; Yield – yield, t/ha. Labels correspond to treatment-year combinations (C – Control, PF – Profix, V – Violar, PFV – Profix + Violar).

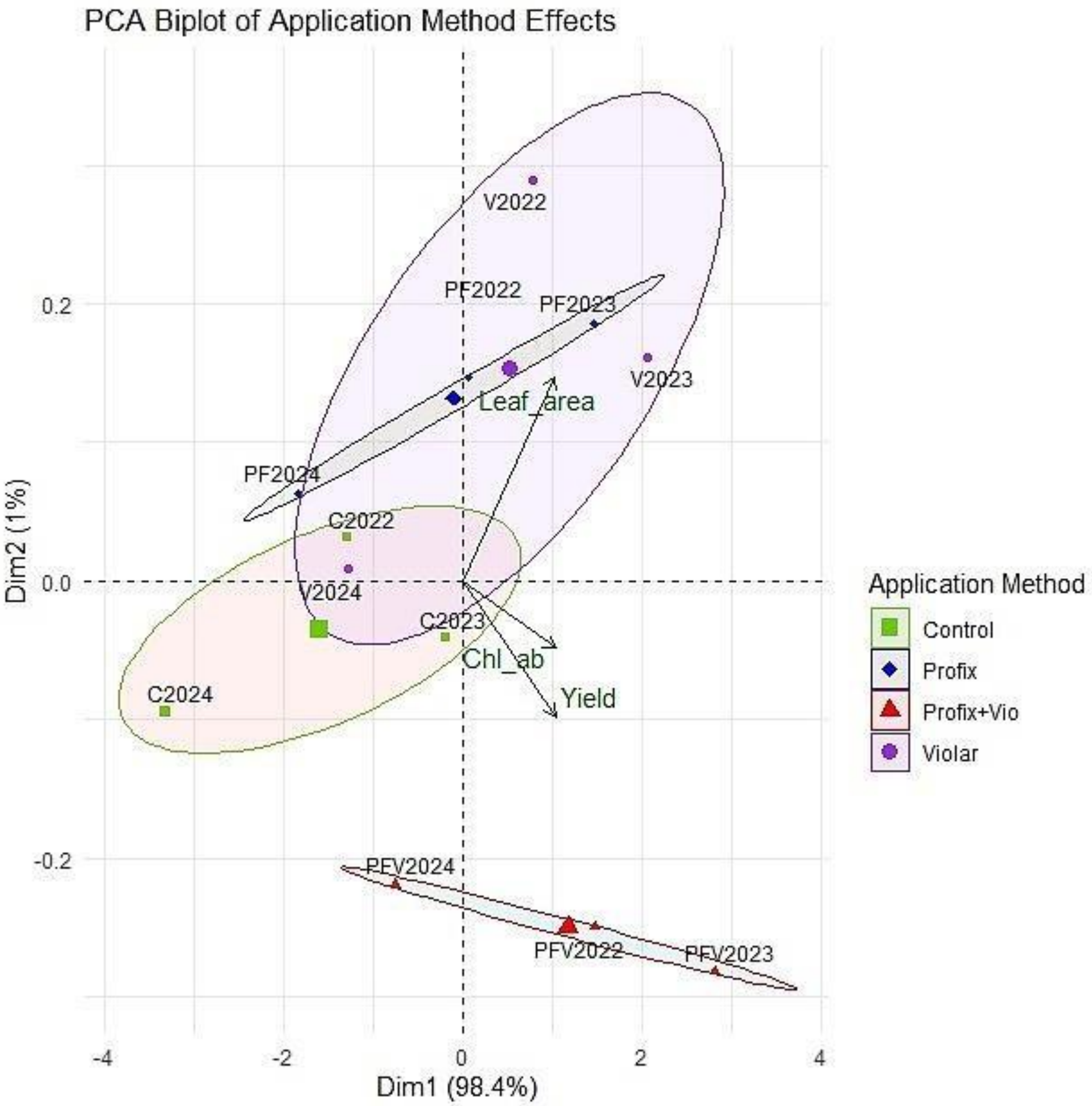

Figure 2. Principal component analysis the relationship between inoculation technologies and plant physiological parameters (reduced variable set) across three study years (2022–2024): Chl_*ab* – Chl *(a+b)*, μg/g; Leaf_area – leaf surface area, thousand m²/ha; Yield – yield, t/ha. Labels correspond to treatment-year combinations (C – Control, PF – Profix, V – Violar, PFV – Profix + Violar).

DISCUSSION

It is difficult to obtain high yields and quality soybean seeds despite good soil properties, even in organic farming, due to a lack of water and prevailing high temperatures. One of the most promising areas of managing the process of forming sustainable agrocenoses of legumes, especially soybeans, is the use of preparations that would protect the physiological state of plants from excessive temperature and rainfall deficits. Today, the most common technological measures are seed treatments with various biostimulants, hormones and osmoprotectants, which have already been tested on crops such as wheat, corn and chickpeas (Khan et al., 2019).

Despite its adaptability, soybeans also face the challenges of climate variability, including unpredictable rainfall regime, which threatens growth and yields. The degree of impact depends on the timing of high temperatures and the intensity of water shortages. The loss of water from the soil through evaporation caused by high temperatures, high light intensity and dry winds can further intensify this effect (Cohen et al., 2021). Such changes in weather conditions can lead to a reduction in soybean yields of up to 40% (Hossain et al., 2014).

Siebers et al. (2015) and Nakagawa et al. (2020) reported that soybean yield decreases and seed composition changes when plants exposed to high temperatures during the seed filling phase. The combined effects of high temperature and drought were shown to have the most detrimental effect on soybean yield (42–64% reduction) compared to their individual effects (Dreesen et al., 2012; Ogunkanmi et al., 2022).

To determine a potential strategy to reduce the influence of abnormal climatic conditions on the soybean growing process with organic farming, the effect of a bacterial preparation Profix and a preparation with natural phytohormones Violar, which using in pre-sowing seed treatment was investigated in present work. Numerous studies have confirmed the significant role of pre-sowing seed treatment with natural preparations in organic farming technologies to increase yields of emmer wheat (Korotkova et al., 2022), soybean (Novytska et al., 2020) and enhanced plant resistance to adverse weather conditions (such as drought and sharp fluctuations in air temperature).

However, not many studies are devoted to compare the effectiveness of soybean seed treatment with microorganisms and phytohormones. Meanwhile, pre-sowing seed inoculation with bacterial preparations is an integral element of cultivation technology, which is important for organic farming. Preparations based on a variety of symbiotic and non-symbiotic rhizobacteria are used as bioinoculants to stimulate plant growth and development through various mechanisms, including nitrogen fixation, production of siderophores, synthesis of phytohormones, etc. (Marinkovic et al., 2018).

Effect of Profix, Violar and its combination on the photosynthetic pigments' content

Many studies supported that seed inoculation can positively affect photosynthetic pigments' content in plants under insufficient moisture supply. Zhang et al. (2013) and Cardarelli et al. (2022) showed that seed inoculation led to an increase in Chl *(a+b)* content. This effect can be explained by the direct relationship between chlorophyll concentration in plants and the intensity of nitrogen fixation, which significantly depends on the symbiotic properties of nodule bacteria that determine plant nitrogen nutrition. Seed inoculation with *B. subtilis* contributed to increased Chl *a* and Chl *b* levels by 1.52 and 1.46 times, respectively, compared to non-inoculated seeds due to an increase in proline levels, which ensures the neutralisation of reactive oxygen species (ROS) production in plants under drought conditions (Lastochkina et al., 2020). At the same time, there is scientific evidence that different results can be obtained with respect to soybean development and yield depending on the strain used (Marchão et al., 2025).

Pre-sowing seed treatment with biologically active substances such as phytohormones (Jaybhaye et al., 2024), vitamins (Koziuchko et al., 2024), amino acids (Dörr et al., 2018), etc. plays an equally important role in increasing soybean productivity. Biologically active substances create a favorable microbiological and biochemical environment around germinating seeds, stimulating growth, increasing seedling resistance to adverse weather conditions, and activating beneficial soil microflora (Chakraborti et al., 2022). According to research Furman et al. (2023), pre-sowing seed treatment with growth regulators significantly increased the Chl (*a*+*b*) content in soybean leaves.

The purpose of various preparations used for pre-sowing seed treatment is to enhance natural plant resistance mechanisms. Phytohormonal preparations help to reduce the adverse effects of growing conditions by enhancing photosynthesis, strengthening antioxidant defense, reducing ROS and lipid peroxidation (Chen et al., 2018), and modulating osmolytes level and redox potential (Fahad et al., 2017; Razzak et al., 2022). Bacterial preparations also play a strategic role in mitigating the harmful effects of ROS by producing various phytohormones that ensure plant resistance (Sheteiwy et al., 2021).

Our results well correlate with other authors. Zimmer et al. (2016), Leggett et al. (2017), Serafin-Andrzejewska et al. (2024) have confirmed the relationship between pre-sowing seed treatment and the growth of biological soybean yields. Tsygankova et al. (2017) showed that the use of Violar in the spring wheat cultivation contributed to an increase in the Chl *a*, Chl *b* and Chl *(a+b)* content by 20.0, 40.0 and 19.2%, respectively, which increased grain yield by 20.3%. These findings are consistent with our results. On average across all study years, an increase Chl *(a+b)* content by 14.3% with Profix seed inoculation and by 18% with the use of the Violar biological preparation led to an increase in soybean yield by 12.3% and 19.6%, respectively.

Effect of Profix, Violar and its combination on proline and MDA levels

Jaybhaye et al. (2024), Almakas et al. (2025) have proposed to combine the bacterial and phytohormonal preparations in pre-sowing seed treatment. The ability of these treatments to maintain higher antioxidant enzyme activity and chlorophyll content while reducing MDA levels and increasing proline levels demonstrates their effectiveness in protecting cellular structures and maintaining photosynthetic efficiency under drought and high-temperature conditions (Sheteiwy et al., 2021). The observed reduction in MDA levels and the increase in proline levels support the hypothesis that these treatments reduce membrane damage associated with oxidative stress and promote osmotic regulation, thereby maintaining plant photosynthetic activity under drought conditions.

CONCLUSION

Pre-sowing treatment of soybean seeds with biological products in organic farming positively affected plant development and productivity under stressful conditions such as high temperatures and low rainfall. Quantitative evaluation of leaf area, chlorophyll content, MDA, and proline levels proved to be reliable indicators of stress tolerance and yield potential. All treatment methods contributed to increased photosynthetic pigment content and leaf surface area, while reducing MDA and increasing proline levels. PCA analysis identified the combined application of Profix (1.25 kg per 500 kg seeds) and Violar (10 ml/ha) as the most effective strategy for enhancing stress resistance and yield. Under projected drought conditions, optimal results may be achieved by increasing the Profix rate to 1.5 kg per 500 kg of seeds, applying Violar at 5 ml/ha during the three-leaf stage, and reducing seeding density to 650.000 seeds/ha. These findings highlight the potential of bacterial and phytohormonal treatments for improving soybean resilience in organic systems. Future research should focus on integrating these treatments with arbuscular mycorrhizal fungi (AMF) to strengthen adaptation to ongoing climate change.

CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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