MEMBRANE STABILITY AND ANTIOXIDANT ENZYME ACTIVITY OF VEGETABLE-TYPE SOYBEAN SEEDLINGS IN RESPONSE TO SHORT-TERM DROUGHT STRESS

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Abstract

Vegetable-type soybean (*Glycine max* L.) is gaining popularity in South Africa as a nutritious food crop, but its production is limited by periodic drought stress, which negatively impacts physiological processes and yield. Plant biostimulants, derived from natural resources, have been promoted for enhancing tolerance to abiotic stress, yet their effectiveness in improving drought tolerance in vegetable-type soybean has not been investigated. This study evaluated the efficacy of three biostimulant products (ComCat WP®, AgraAmino, and AgraBezuca) in activating drought tolerance mechanisms through cell membrane protection and antioxidant activity in two contrasting cultivars (drought-tolerant UVE5 and drought-susceptible UVE17). A glasshouse experiment was conducted at the University of the Free State in a factorial randomised complete block design with eight treatments and five replications. Plants were subjected to foliar biostimulant application, oxidative stress via hydrogen peroxide (H_2O_2), and a seven-day water-deficit treatment. Electrolyte leakage, antioxidant activity, chlorophyll content, and chlorophyll fluorescence were measured. Results showed significant cultivar effects, with UVE5 consistently exhibiting lower electrolyte leakage, higher antioxidant activity, and better photosynthetic performance (PIabs) compared to UVE17. Treatment effects were significant for antioxidant activity, with H_2O_2 combined with AgraAmino or ComCat enhancing antioxidant responses under combined oxidative and drought stress. However, biostimulant effects on electrolyte leakage, chlorophyll content, and fluorescence were not statistically significant. Overall, the study demonstrates that cultivar choice remains the primary determinant of drought-tolerance in vegetable-type soybean, while specific biostimulants, particularly amino acid- and brassinosteroid-based products, can enhance antioxidant defences under stress. These findings contribute to developing sustainable strategies for improving vegetable-type soybean production under semi-arid production conditions.

Resumen

La soja hortícola (*Glycine max* L.) está ganando popularidad en Sudáfrica como cultivo alimenticio nutritivo, pero su producción se ve limitada por el estrés hídrico periódico, que afecta negativamente los procesos fisiológicos y el rendimiento. Se han promovido bioestimulantes vegetales, derivados de recursos naturales, para mejorar la tolerancia al estrés abiótico; sin embargo, no se ha investigado su eficacia para mejorar la tolerancia a la sequía en la soja vegetal. Este estudio evaluó la eficacia de tres productos bioestimulantes (ComCat WP®, AgraAmino y AgraBezuca) para activar los mecanismos de tolerancia a la sequía mediante la protección de la membrana celular y la actividad antioxidante en dos variedades con características contrastantes (UVE5 tolerante a la sequía y UVE17 susceptible a la sequía). Se realizó un experimento en invernadero en la University of the Free State con un diseño factorial de bloques completos aleatorizados con ocho tratamientos y cinco repeticiones. Las plantas se sometieron a la aplicación de bioestimulantes foliares, estrés oxidativo mediante peróxido de hidrógeno (H_2O_2) y un tratamiento de déficit hídrico de siete días. Se midieron la fuga de electrolitos, la actividad antioxidante, el contenido de clorofila y la fluorescencia de la clorofila. Los resultados mostraron efectos significativos de la variedad, con UVE5 mostrando consistentemente menor fuga de electrolitos, mayor actividad antioxidante y mejor rendimiento fotosintético (PIabs) en comparación con UVE17. Los efectos del tratamiento fueron significativos en la actividad antioxidante, con H_2O_2 combinado con AgraAmino o ComCat mejorando las respuestas antioxidantes bajo estrés oxidativo y de sequía combinado. Sin embargo, los efectos de los bioestimulantes en la fuga de electrolitos, el contenido de clorofila y la fluorescencia no fueron estadísticamente significativos. En general, el estudio demuestra que la elección de la variedad sigue siendo el determinante principal de la tolerancia a la sequía en la soja de tipo hortícola, mientras que los bioestimulantes específicos, particularmente los productos basados en aminoácidos y brasinoesteroides, pueden mejorar las defensas antioxidantes bajo estrés. Estos hallazgos contribuyen al desarrollo de estrategias sostenibles para mejorar la producción de soja de tipo hortícola en condiciones de producción semiáridas.

Key words: Biostimulants, chlorophyll content, edamame, electrolyte leakage, photosynthetic potential

Introduction

Soybean is a member of the Fabaceae family and native to East Asia. The commodity type soybean is commonly produced for use as human food (oil and soy-based products) and livestock feed due to its high protein and oil content. Vegetable-type soybean, also known as “edamame”, is harvested at an immature stage (R6 growth stage) when the pods are fully filled but still green (Mentreddy et al. 2002). It is primarily promoted for human consumption and consumed as a vegetable. The immature beans have a sweet, nutty flavour which makes them suitable snack to be enjoyed either boiled in salt water or used in stews, stir-fries or soups (Mentreddy et al. 2002). Edamame consumption in Africa is being promoted because of its nutritional benefits (Djanta et al. 2020), as it contains plant-based protein, vitamins, minerals, dietary fibre and isoflavones.

East Asian countries like China and Taiwan, have a long history of vegetable-type soybean production, resulting in a well-developed industry. However, the crop is still gaining global popularity with the demand increasing internationally (Wiliams et al. 2022). In the United States of America, the industry is still small, but there have been significant efforts to promote vegetable-type soybean over the years (Xu et al. 2015). In African countries like South Africa, vegetable-type soybean was introduced to address malnutrition, and the crop is gaining popularity locally. Current research is mainly focussed on agroecological adaptation of this crop (Djanta et al. 2020).

South Africa is a semi-arid country, which is associated with frequent erratic weather conditions. During the summer cropping season, brief periods of drought stress are common and have potential of significantly impacting production. Drought stress occurs when plants lose more water vapour than uptake (Seleiman 2021). In soybeans, seed yield reductions of 25 to 85% have been reported when plants are exposed to drought stress during the entire growth cycle (Van der Merwe et al. 2018). The reduction in yield can be directly attributed to flower and pod abortion; however, several metabolic processes further indirectly result in reduced production. Drought stress has been reported to disrupt photosynthetic metabolism with stomatal closure (Parent and Tardieu 2014), which limits the utilisation of carbon dioxide (CO_2), temperature pressure and increased photorespiration (Pinheiro and Chaves 2011). Metabolic pathways are pressured to decrease the production of substances like isoprenoids and phenols (Al-Gabbiesh et al. 2015). In addition, the reduction in water content affects chlorophyll content (Wang et al. 2019).

In plants, antioxidant metabolism is a crucial mechanism to cope with drought stress (Jaleel et al. 2009; Hameed et al. 2011). Rapid accumulation and sustained high activity of antioxidant enzymes can serve as a protective mechanism in plant cells during drought stress. Some plant species have demonstrated a correlation between drought tolerance and the level of antioxidant enzyme activity (Uzilday et al. 2012). Another drought tolerance mechanism involves preservation of cell-membrane stability (CMS). Drought stress induces cell membrane degradation, leading to severe metabolic dysfunctions in plants (Nir et al. 1969; Buttrose and Swift 1975). Maintaining membrane stability is critical for plants prone to drought stress, as it sustains physiological metabolism under low water conditions. Cell membrane stability serves as a physiological index which is widely recognised for evaluating drought tolerance. This, approach measures the amount of electrolyte leakage from leaf segments (Sullivan 1972).

The use of biotsimulants have been promoted to improve tolerance to abiotic stress in plants (European Biostimulant Industry 2019). Plant biostimulants are a diverse range of substances and microorganisms used to enhance plant growth and are derived from natural resources. Common components in biostimulants include beneficial mycorrhizae, trichoderma, vegetal sources, amino acids and peptide chains. The benefits of using biostimulants have been reported in several studies. They improve soil nutrient availability by reducing the need for inputs and reducing nutrient leaching, promote plant establishment and stimulate the plant's immune system (Alvarez et al. 2024). In vegetable-type soybean, the application of biostimulants to plants under ordinary field trial conditions has shown to improve nutrient (N, P, Mg and Ca) uptake, biomass and grain yield (Da Paixao 2024). However, the usefulness of biostimulants to improve drought tolerance in vegetable-type soybean has not been investigated previously. Therefore, the aim of this study was to determine the efficacy of various biostimulant products to activate the drought tolerance mechanism in vegetable-type soybean through cell membrane protection and antioxidative enzyme production. The specific objective is to investigate membrane stability and antioxidant enzyme activity in seedlings of two vegetable-type soybean cultivars exposed to hydrogen peroxide (H_2O_2) in combination with three biostimulant products under a short-term water-deficit treatment.

Material and Methods

Plant material

In this study, two vegetable-type soybean cultivars, UVE5 (AVSB0802) and UVE17 (AVSB1004) were used. These cultivars were selected from the drought-tolerance breeding programme, which included a drought-tolerant (UVE5) and drought-susceptible (UVE17) cultivar. Both cultivars belong to the same maturity grouping and fully matures at 115 days after sowing.

Experimental design and treatment application

A glasshouse experiment was conducted from July to September 2024 at the University of the Free State (UFS), South Africa. The factorial experiment was laid out in a randomised complete block design with two factors and five replications. Factor one included the two cultivars, while factor two comprised of eight treatments (table 1). Distilled water was used as the control treatment and hydrogen peroxide (H_2O_2) was used as the oxidiser. Three biostimulant products (ComCat WP®, AgraAmino and AgraBezuca) and combinations of these with H_2O_2 were used as treatments. The biostimulant products were obtained from Agraforum South Africa (SA) and are all organically certified and biodegradable. The trial comprised of 8o plots with one plant per plot.

Pots were filled to 3 kg with dry sifted Bainsvlei type soil. The field water capacity (FWC) of the soil in pots was determined using the gravimetric method. Thereafter each pot was maintained at FWC through weighing of pots and watering pots to FWC by hand. The soil nutrient status was determined at the laboratory of the Department of Crop, Soil and Climate Sciences (at UFS) and this was used for fertiliser recommendation of the pots. Seeds from both cultivars were sown in polystyrene seed trays and transplanted at first trifoliate leave stage into the pots, as per layout in the trial design. The treatments (table 1) commenced three weeks after emergence and was applied using foliar applications (figure 1). The treatments were uniformly applied using a simulated tractor sprayer, delivering 300 l as a foliar spray.

Table 1. Treatment number, products and dosages used in the study.

Number	**Product**	**Dosage**	**Active ingredient**	**Type**
1	Water		H_2O	Control
2	Hydrogen peroxide (H_2O_2)	0.003%/ha	H_2O_2	Oxidiser
3	ComCat WP®	200 g/ha	24-Epibrassinolide (10.5 mg/kg)	Fertilizer group 3
4	AgraAmino	200 g/ha	Total amino acids (800 g/kg)	Fertilizer group 3
5	AgraBezuca	2 kg/ha	Glycine betaine	Fertilizer group 3
6	H_2O_2 & ComCat®		-	-
7	H_2O_2 & AgraAmino		-	-
8	H_2O_2 & AgraBezuca		-	-

Each pot received a separate treatment as indicated in table 1 and as per layout in trial design. The products were prepared and applied as specified on the label. After the foliar treatments were applied, a short-term water-deficit treatment was initiated to all pots by inhibiting hand watering for seven consecutive days. Pots were weighed daily to determine the FWC, and this was done to determine the percentage of drought stress (water loss) imposed during the seven-day period. Foliar data were collected at 48 h after foliar treatment application and again seven days after initiation of the short-term water-deficit treatment.

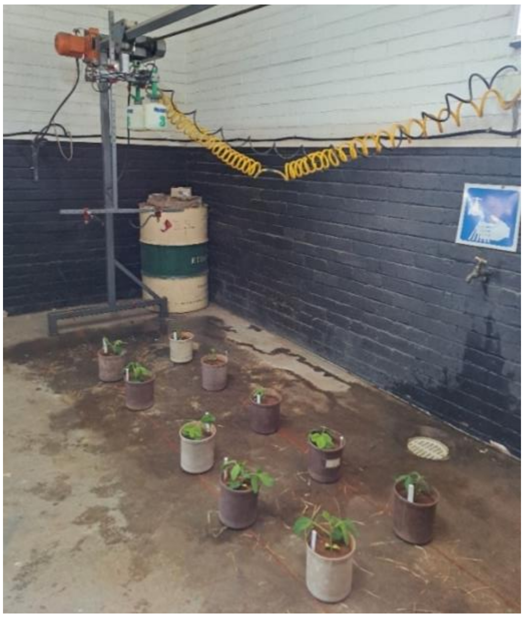

Figure 1. Leaf foliar applications of the eight treatments respectively applied using a simulated tractor sprayer.

Electrolyte leakage

Data on ion leakage was collected as a measure of cell membrane damage. Electrolyte leakage (EL%) was determined by using an EC-meter (InoLab® Cond 7110) based on Dionisio-Sese and Tobita (1998) method. In this procedure, fresh leaf samples (100 mg) were cut and placed in falcon tubes containing 10 ml distilled deionised water and stored in a water bath with a constant temperature (32°C) for 2 h. Thereafter, the initial electrical conductivity (EC1) of the leachate was determined and the falcon tubes containing samples were autoclaved at 121°C for 90 min. After cooling the samples at 25°C, EC2 of the leachate were measured. The EL was estimated by using EL (%) = [(EC2-EC1)/EC1] x 100.

Antioxidant activity

Antioxidant activity was measured to determine neutralisation of the H_2O_2 activity. The total antioxidant capacity of fresh leave samples was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) stable radicals, according to Maikai et al. (2010), with some modifications. For this purpose, 1 g leaf samples were homogenized with 30 mℓ methanol. Thereafter, 500 µℓ supernatant of methanolic extracts of each sample were mixed with 500 µℓ methanol and 500 µℓ DPPH solution (0.004%) in plastic vials. Thereafter, they were incubated at room temperature in the dark for 30 min. The absorbance of each sample was recorded by the UV-visible spectrophotometer against a blank at 517 nm. DPPH radical scavenging capacity was calculated using [(absorbance of blank – absorbance of the sample)/absorbance of blank] x 100. Calculation of antioxidant activity was determined using antioxidant activity (%) = [(Acontrol – Asample) / Acontrol] x 100.

Chlorophyll content

A single fresh leaf was selected per plant to measure relative chlorophyll content. The leaf was placed into the measurement slot of the Hansatech chlorophyll meter. The leaf must be placed flat in the chlorophyll meter and the sensor must be direct on the leaf surface. Readings were obtained by pressing the button on the meter. The same process was done for each plant.

Chlorophyll fluorescence

In vivo, the photosynthetic efficiency is measured using a fluorimeter, which calculates the minimum and maximum quantum yield and maximum fluorescence intensity of light-adapted leaves (ΔFv/Fm) (Lysenko et al. 2022). The performance index on absorption basis (PIabs) is important in understanding the energy fluxes in photosystem II and photosystem I (Çiçek et al. 2015). The leaf was placed into a closed clip and left for 40 min, to inhibit photosynthesis. A Hansatech pocket chlorophyll fluorimeter was then placed on the clip and carefully opened while pressing onto the clip, to avoid light exposure. The chlorophyll fluorescence readings included PIabs and variable fluorescence by maximum fluorescence (Fv/Fm) and were recorded by pressing the button on the fluorimeter. The same process was done for each plant.

Statistical analysis

Statistical analysis was done using Genstat software. Data was subjected to analysis of variance (ANOVA) to determine cultivar and treatment effects as well as the occurrence of interaction between cultivars and the treatments on all data collected. The interaction was used as an indication whether treatments were cultivar specific or not. Skewness in the data was evaluated using the Shapiro Wilk test for normality. Cultivar and treatment mean values were presented in clustered column with standard bars in graphs using Excel.

Results and Discussion

Electrolyte leakage

Cell membrane damage was determined by measuring electrolyte conductivity. Low levels of electrolyte leakage mean that the plant is not severely damaged. Results from the ANOVA showed significant ($P<0.05$) cultivar effects for electrolyte leakage (EL%) at both 48 h and 7 days after treatment (table 2). Where significant cultivar effects are detected, it indicated that cultivar means were significantly different from each other. As a result, after 48 h the drought-susceptible cultivar UVE17 showed a significantly higher EL% of 467.13% (table 3) compared to the drought-tolerant cultivar UVE5 (387.17%). Similarly, 7 days after treatment the drought-susceptible cultivar UVE17 (1140.41%) showed a significantly higher EL% of 1140.41% compared to the drought-tolerant cultivar UVE5 (893.25%). This indicated that the drought-tolerant cultivar had the least cell membrane damage compared to the susceptible cultivar. In addition, the cultivar means for EL% at 48 h was less than at 7 days after treatment. This indicated that under oxidative stress alone (48 h after treatment) the EL% was less than when combined with a brief-drought stress treatment of seven days (7 days after treatment).

Results from the ANOVA further showed non-significant treatment effects for electrolyte leakage (EL%) at both 48 h and 7 days after treatment (table 2). This indicated that differences observed between treatment means (figure 2) are not significant and that the application of biostimulants to plants of these two cultivars subjected to both oxidative and drought stress will not statistically reduce cell membrane damage compared to the control (water) treatment. However, based on mean EL% values, the treatment H_2O_2 & AgraAmino resulted in the lowest cell membrane damage (EL of 817.73%) under combined oxidative and drought stress compared to the control (water only) treatment (EL% of 961.50%) at 7 days after treatment. In addition, biostimulant combinations H_2O_2 & AgraAmino (EL of 817.73%) and H_2O_2 & ComCat WP (EL of 974.68%) reduced cell membrane damage due to oxidative stress when compared with the H_2O_2 treatment (EL of 1002.87%). The active ingredient of AgraAmino is amino acids. It has been reported in literature that the application of amino acids as a biostimulant can protect the cell membrane by acting as osmoprotectants, which accumulate in cells to regulate osmotic balance and retain water (Franzoni et al. 2022). As a result, AgraAmino can reduce the impact of oxidative stress on vegetable-type soybean. No significant cultivar x treatment interaction was observed, indicating that the two cultivars responded the same across the eight treatments and that the treatments are not cultivar specific.

Antioxidant activity

Results from the ANOVA showed significant ($P<0.01$) cultivar effects for antioxidant activity after 7 days after treatment (table 2). Therefore, cultivar means were not significantly different when plants were exposed to oxidative stress alone (after 48 h), but in combination with drought stress (after 7 days) cultivar means were significantly different (table 3). Since antioxidant activity measures the ability of the plant to neutralise free radicals, a high level of antioxidant activity determines a healthy plant. At 7 days after treatment the drought-susceptible cultivar UVE17 (1.0%) showed a significantly lower antioxidant activity compared to the drought-tolerant cultivar UVE5 (22.1%). This indicated that the drought-tolerant cultivar had a very high antioxidant activity and was healthier compared to the susceptible cultivar when exposed to a combination of stresses. On the other hand, the drought-susceptible cultivar UVE17 constantly showed the lowest antioxidant activity across all eight treatments. Under oxidative stress alone (48 h) cultivars showed a higher antioxidant activity (ranging from 59 to 60%) than under combined oxidative and a brief drought stress (7 days; ranging from 1 to 22%). Results from the ANOVA further showed non-significant treatment effects for antioxidant activity at 48 h but significant ($P<0.01$) treatment effects at 7 days after treatment (table 2). This indicated that differences observed between treatment means (figure 3) are significant under combined oxidative and a brief drought stress.

As a result, the application of biostimulants to plants subjected to a combination of oxidative and drought stress will result in statistically different mean responses for antioxidant activity (figure 3). This was observed in figure 3, where at 48 h after treatment the means ranged from 49 to 64%, while after 7 days the means ranged from 1.4 to 22%. In addition, the treatments H_2O_2 & AgraAmino (14.5%), H_2O_2 & ComCat WP (17.9%) resulted in significantly higher antioxidant activity under combined oxidative and drought stress compared to the H_2O_2 treatment (AA of 1.4%) at 7 days after treatment. Literature has shown that certain amino acids, like arginine and proline, can improve a plant's antioxidant defences (Heiderzadeh 2025). The biostimulant AgraAmino could play a role in enhancing the plant cell's ability to neutralise harmful reactive oxygen species (ROS) since amino acids indirectly protect the sensitive phospholipids in cell membranes from oxidative damage. In addition, the active ingredient of ComCat WP is 24-Epibrassinolide (EBR). Literature has shown that EBR promotes antioxidant activity in plants by activating antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX). These enzymes are critical for scavenging ROS and reducing oxidative damage under various stresses (Sharma et al. 2012). Significant cultivar x treatment interaction ($P<0.01$) was observed, indicating that the two cultivars responded differently across the eight treatments and that the treatments are cultivar specific. As a result, drought-tolerant cultivar UVE5 showed the highest antioxidant activity when treated with ComCat WP (43.8%) alone at 7 days after treatment, while the same cultivar gave the highest antioxidant activity when treated with both H_2O_2 & ComCat WP (34.1%). In contrast, UVE5 showed a very low antioxidant activity when treated with H_2O_2 & AgraBezuca (3.2%) after 7 days of treatment (data not shown).

Table 2. Analysis of variance showing degrees of freedom (df) and mean square values for electrolyte leakage and antioxidant activity after 48 h and 7 days of treatment respectively.

Source of variance	**df**	**Electrolyte leakage (48 h)**	**Electrolyte leakage (7 days)**	**Antioxidant activity (48 h)**	**Antioxidant activity (7 days)**
Replication	4	59442	2800277	1058.20	110.30
Cultivar (C)	1	127865*	1221838**	37.70	8824.20**
Treatment (T)	7	26869	140710	215.20	558.80**
CxT	7	20349	93967	715.20*	0560.10**
Residual	60	28712	86464	289.80	146.40
Coefficient of variation (%)		39.70	28.90	28.70%	64.00%

*, ** = significant at $P<0.05$ and $P<0.01$.

Table 3. Cultivar and treatment mean values for electrolyte leakage and antioxidant activity after 48 h and 7 days of treatment respectively.

Cultivar	**Electrolyte leakage % (48 h)**	**Electrolyte leakage % (7 days)**	**Antioxidant activity % (48 h)**	**Antioxidant activity % (7 days)**
UVE17	467.13^{a}	1140.41^{a}	58.60^{ns}	1.00^{b}
UVE5	387.17^{b}	893.25^{b}	59.90^{ns}	22.10^{a}
Mean difference	79.96	247.16	1.30	21.10
LSD ($P<0.05$)	75.79	131.52	7.61	5.41

Chlorophyll content

Data on chlorophyll content was collected after 48 h of treatment only. Chlorophyll content describes the ability of the plant to absorb and capture light energy. Healthy plants have high levels of chlorophyll content. Results from the ANOVA showed non-significant cultivar, treatment and cultivar x treatment interaction effects (table 4). Therefore, cultivar means were the same and that their responses across treatments were the same. As a result, for the two cultivars tested, the oxidative stress treatment at 48 h as well as the application of biostimulants did not result in a significant change in the cultivar or treatment means. However, it would appear that drought-susceptible cultivar UVE17 (10.81) had a slightly higher chlorophyll content then the drought-tolerant cultivar UVE5 (9.65), although this was not significantly higher (table 5). In addition, the control (water) treatment had the lowest chlorophyll content than the rest of the treatments, which indicated that oxidative stress due to the application of H_2O_2, will result in a non-significant increase in chlorophyll content (figure 4).

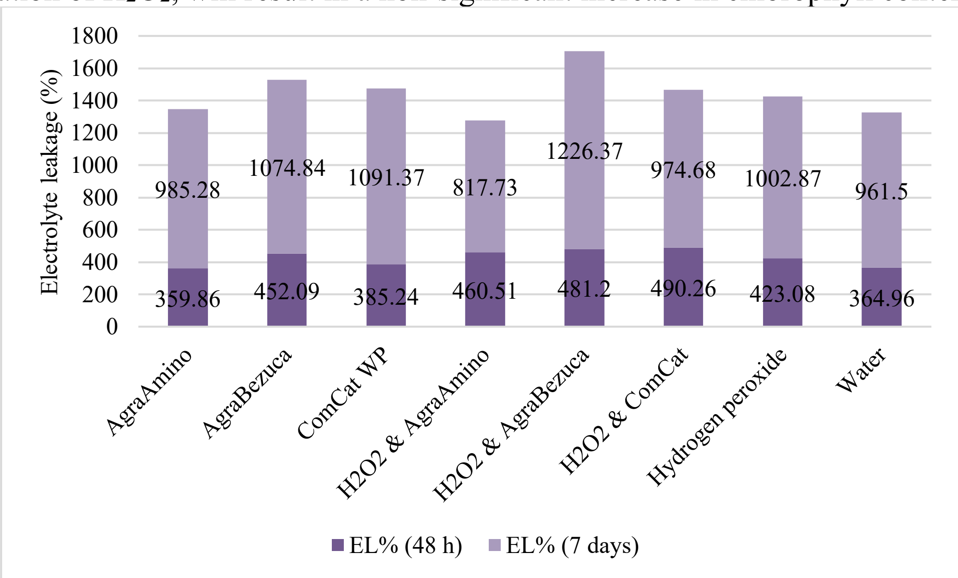

Figure 2. Mean values for electrolyte leakage (EL%) of the eight treatments after 48 h and 7 days of treatment respectively.

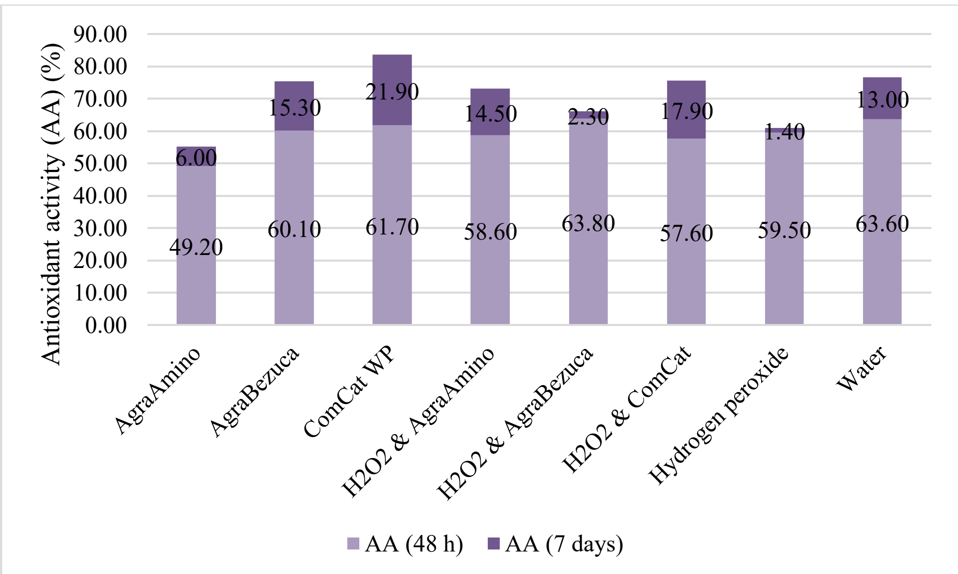

Figure 3. Mean values for antioxidant activity (AA%) of the eight treatments after 48 h and 7 days of treatment respectively.

Literature has shown that broccoli (*Brassica oleracea* var. *italica*) plants treated with biostimulants, obtained from *Ascophyllum nodosum*, showed a higher chlorophyll content under stress conditions than non-stress conditions, which resulted in increased biosynthesis and reduced degradation of chlorophyll (Kałuzewicz et al. 2017). In addition, the combination treatments that received both a biostimulant and H_2O_2, did not differ significantly from plants that only received the biostimulant (without the oxidative stress). As a result, the chlorophyll content was either not responsive to the treatments, the oxidative stress was not severe enough or the waiting period of 48 h after treatment was insufficient.

Table 4. Analysis of variance showing degrees of freedom (d.f.) and mean square values for chlorophyll content and chlorophyll fluorescence after 48 h of treatment.

Source of variance	**d.f.**	**Chlorophyll content**	**Fv/Fm**	**PIabs**
Replication	4	6.29	0.008	2.03
Cultivar (C)	1	26.83	0.003	4.04*
Treatment (T)	7	6.10	0.001	1.04
CxT	7	2.07	0.003	1.20
Residual	60	11.21	0.002	0.78
Coefficient of variation (%)		32.70	5.50	59.90

*, ** = significant at P<0.05 and P<0.01.

Table 5. Cultivar and treatment mean values for chlorophyll content and chlorophyll fluorescence after 48 h of treatment.

Cultivar	**Chlorophyll content**	**Fv/Fm**	**PIabs**
UVE17	10.81^{ns}	0.77^{ns}	1.25^{b}
UVE5	9.65^{ns}	0.78^{ns}	1.69^{a}
Mean difference	1.16	0.01	0.44
LSD (P<0.05)	1.50	0.02	0.39

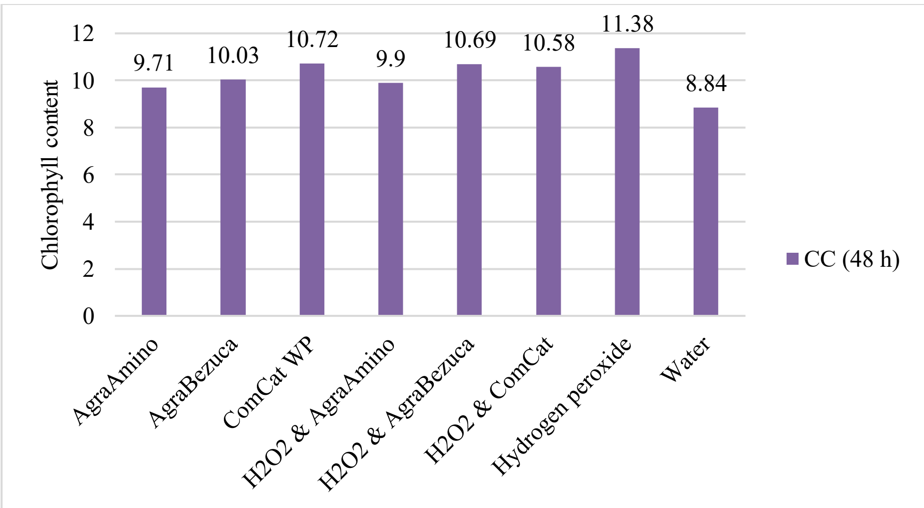

Figure 4. Mean values for chlorophyll content (CC) of the eight treatments after 48 h of treatment.

Chlorophyll fluorescence

Data on chlorophyll fluorescence was collected after 48 h of treatment only. Chlorophyll fluorescence is used to detect changes in plant's photosynthesis over time and is widely used in agronomy and applied plant physiology to monitor plant health under environmental stresses (Küpper et al. 2019). Fluorescence (Fv/Fm) measures the number of excited molecules due to light emission. High fluorescence level indicate that the plant is under less stress, while a low Fv/Fm signals that the plant is under stress and has experienced damage to its photosystem (Bartold and Kluczek 2024). Results from the ANOVA showed non-significant cultivar, treatment and cultivar x treatment interaction effects and that cultivar means were the same and that their responses across treatments were the same (table 4). As a result, for the two cultivars tested, the oxidative stress treatment at 48 h as well as the application of biostimulants did not result in a significant change in the cultivar or treatment means (table 5 and figure 5).

In addition, the combination treatments that received both a biostimulant and H_2O_2, did not differ significantly from plants that only received the biostimulant (without the oxidative stress). As a result, the florescence level was either not responsive to the treatments, the oxidative stress was not severe enough or the waiting period of 48 h after treatment was insufficient. However, since the Fv/Fm levels ranged between 0.76 to 0.79, the Fv/Fm values were relatively low (less than the optimal range of 0.79 to 0.83 as for most terrestrial plant species (Bartold and Kluczek 2024). This indicated that plants were under stress, although the use of ComCat WP (with a Fv/Fm of 0.79), under non-stress conditions, can aid in the prevention of damage to the photosystem. This is supported by the findings of (Hu et al. 2013) who found that drought-induced photoinhibition and drought-reduced photosynthesis were ameliorated by the exogenous application of EBR, which is an active ingredient of ComCat WP.

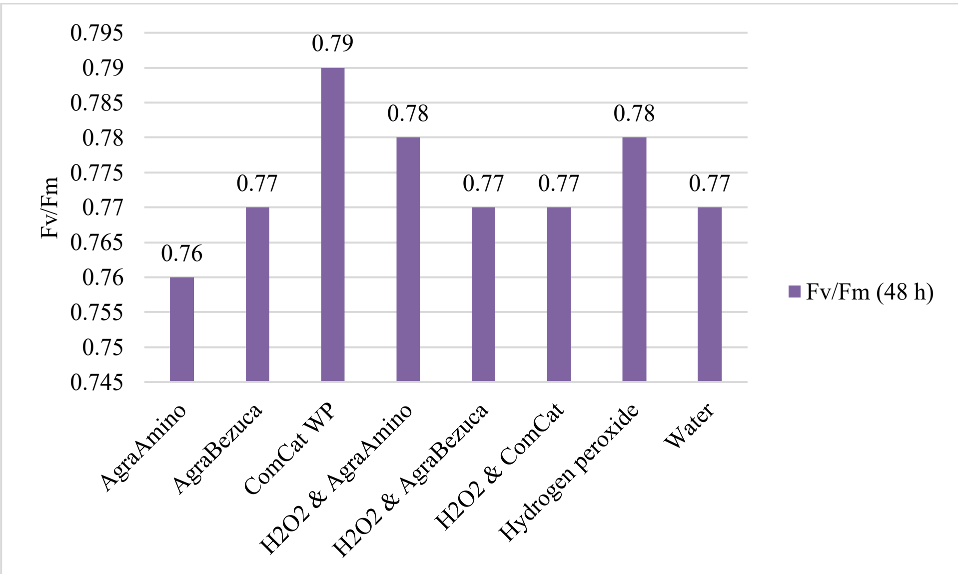

Figure 5. Mean values for variable fluorescence by maximum fluorescence (Fv/Fm) of the eight treatments after 48 h of treatment.

Data on PIabs was collected after 48 h of treatment only. A high PIabs value signifies that the plant photosynthetic machinery is functioning well, with efficient energy capture and electron transport. This indicates good primary photochemical reactions and overall plant performance (Živčák et al. 2008). Results from the ANOVA showed significant ($P<0.05$) cultivar effects for PIabs after 48 h of treatment (table 4). At 48 h after treatment the drought-susceptible cultivar UVE17 (1.25%) showed a significantly lower performance index compared to the drought-tolerant cultivar UVE5 (1.69%) and as a result, UVE5 was more able to withstand the oxidative stress imposed due to H_2O_2 (table 5). However, non-significant treatment and cultivar x treatment interaction effects were observed, indicating that treatment means were statistically the same and that cultivar responses across the eight different treatments were the same (table 4). As a result, the oxidative stress treatment at 48 h as well as the application of biostimulants did not result in a significant change in the treatment means. This was not as expected since oxidative stress can result in a reduced PIabs. However, across the means of the eight treatments, some of the biostimulants (AgraBezuca and ComCat WP) and their combination treatments (AgraAmino and AgraBezcua) with H_2O_2 (ranging between 1.59-1.66), performed better than the control (water) treatment (1.11). This indicated that the application of biostimulants (together with the application of H_2O_2) can increase the photosynthetic performance of the plants, compared to the control. In addition, the H_2O_2 treatment alone gave the highest PIabs value of 2.02.

A number of studies have suggested that pre-treatment of seedlings with H_2O_2, or the combined application of H_2O_2 and abiotic stress, induces an inductive pulse that helps to protect plants under abiotic stresses. This is done by restoration of redox-homeostasis and mitigation of oxidative damage to membranes, proteins and lipids and by modulating stress signalling pathways (He et al. 2017). In addition, Hossain et al. (2015) reported that H_2O_2 can increase photosynthetic activity in plants. The pre-treatment of plants with an appropriate level of H2O2 can enhance abiotic stress tolerance through the modulation of multiple physiological processes, such as photosynthesis, and modulating multiple stress-responsive pathways stress-responsive pathways. This could explain why the PIabs was higher under H_2O_2 treatment than the control.

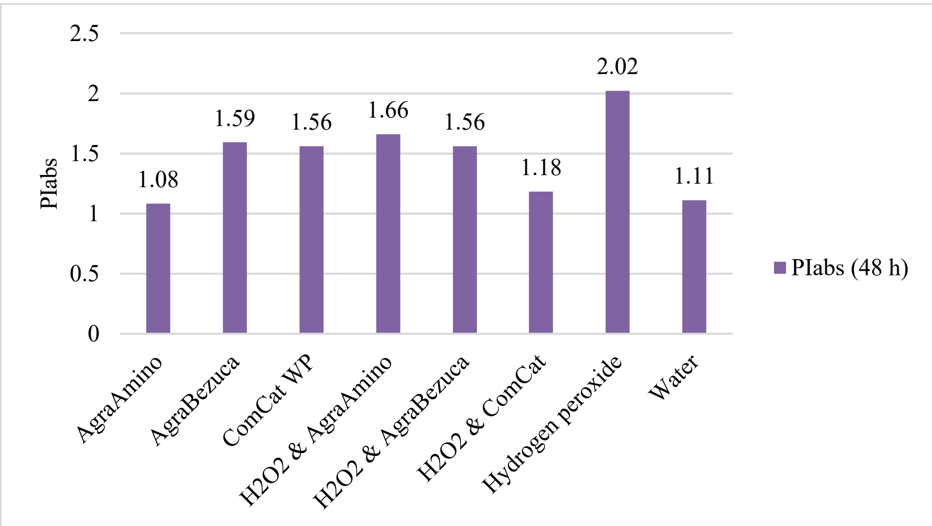

Figure 6. Mean values for performance index (PIabs) of the eight treatments after 48 h of treatment.

Conclusions

This study demonstrated that drought tolerance in vegetable-type soybean is primarily determined by cultivar differences, with UVE5 having higher tolerance than UVE17 under both oxidative and drought stress conditions. While the overall effects of biostimulants on membrane stability, chlorophyll content, and fluorescence were limited, treatments combining H_2O_2 with AgraAmino or ComCat WP enhanced antioxidant activity, suggesting a potential role in strengthening stress-responsive pathways. These findings highlight the importance of selecting drought-tolerant cultivars for production, while also indicating that targeted biostimulant use may provide additional physiological benefits under stress conditions. Further research should investigate longer-term field responses, optimal application regimes, and the integration of biostimulants with other agronomic practices to support sustainable vegetable-type soybean production in South Africa.

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