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Alleviation of Drought Stress in Soybean by Applying a Biostimulant Based on Amino Acids and Macro- and Micronutrients

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Abstract: Drought stress is one of the most predominant environmental factors hindering soybean productivity. Therefore, the study of stress-mitigating strategies, such as the use of biostimulants, is important in order to mitigate this problem. This study investigated the effects of an exogenous application of biostimulants based on amino acids and macro- and micronutrients in the physiological, biochemical and productive responses of soybean cultivated under drought stress. Treatments consisted of T1—dose 0.0 kg ha^{-1} (control); T2—dose 0.0 kg ha^{-1} (with water-deficit stress); T3—dose 0.25 kg ha^{-1}; T4—dose 0.5 kg ha^{-1}; T5—dose 0.75 kg ha^{-1}; T6—dose 1.0 kg ha^{-1} of biostimulant. Application of T4 maintained photosynthetic metabolism, with main action on stomatal conductance, and increased the activity of antioxidant enzymes superoxide dismutase by 420%, catalase by 167% and ascorbate peroxidase by 695%. In addition, it increased the levels of proline by 106%, leaf area by 279% and the dry matter mass of the plants by 26%, which was reflected in a 22% increase in productivity. Therefore, application of the studied biostimulant at a dose of 0.5 kg ha^{-1} is recommended to effectively alleviate the adverse effects of drought stress on soybean.

Keywords: *Glycine max* (L.) Merrill; water stress; biostimulants; photosynthetic metabolism; yield

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1. Introduction

Plants are often subjected to adverse environmental conditions, resulting in stresses that negatively affect their growth, development and/or yield [1–3]. Lack of water is the main limiting factor for soybean production worldwide [4,5]. Drought hinders the global production of soybean (*Glycine max* L. (Merr.)), which provides 71% and 29% of the world's protein and oil consumption, respectively [6].

The effects of water deficit on soybean germination, physiological processes [7], seed development and quality [8,9] and yield [10,11] have been reported. However, to meet the growing demand for food, it is necessary to increase soybean yield, even in environments with low water availability [12].

In the photosynthetic process, the lack of water leads to deleterious effects on important enzymes, such as ribulose-1 5-bisphosphate carboxylase/oxygenase (RUBISCO), phosphoenolpyruvate carboxylase, phosphoenolpyruvate carboxylase, pyruvate phosphate dikinase, NADP-malate dehydrogenase and NADP-malic enzyme [7,13,14]. This is due to imbalances of molecules or ions in cells, particularly in reactive oxygen species (ROS) [15,16], since these molecules are highly unstable and have a high reaction capacity, mainly damaging lipids, proteins and nucleic acids and affecting cell physiology [17].

Under drought conditions, there is an increase in ROS levels in the apoplast due to the activity of the NADPH oxidase enzymes in the respiratory burst of plants [17]. To counter the deleterious effects of ROS, plants have an antioxidant defense system [18]. Within this system, the activity of antioxidants enzymes superoxide dismutase (SOD), catalase (CAT),

peroxidase (POX) and ascorbate peroxidase (APX) stand out, eliminating the ROS and maintenance of homeostasis redox [14].

An alternative to improve crop yield under water deficiency is the application of biostimulants that act to protect plants, minimizing the adverse effects caused by environmental stresses [19,20]. Biostimulants are classified as products containing active ingredients capable of directly or indirectly enhancing plant development [21], consisting of macro- and micronutrients, as well as phytohormones and other beneficial substances for plant metabolism [22]. Their primary characteristic is the supplementation of nutrients and activation of physiological functions throughout the plant development process, and may be applied via soil, irrigation systems or foliar spraying [23,24].

Biostimulants are innovative agricultural techniques used to protect plants [12,20]. The use of biostimulants in agriculture has been gradually increasing, showing positive effects under stress conditions [12,20,25]. These products have been used in soybean crops to enhance plant response to stress and increase yield by preserving metabolism, nutrient and water absorption, as well as the activation of antioxidant activity mechanisms.

It is important to understand how biostimulants affect plants under water stress to understand the specific mechanisms of action. Therefore, the hypothesis of this study is that the use of biostimulants mitigates the effect of water deficit through improvements in morphology, maintenance of antioxidant defense and photosynthetic metabolism, which is reflected mainly in gains in crop productivity. To answer the question, biometric, physiological, biochemical and productive parameters were analyzed to investigate the capacity of a biostimulant based on essential nutrients and amino acids to mitigate the effects of water stress in soybean.

2. Materials and Methods

2.1. Experimental Layout

The experiment was conducted in greenhouse of the Department of Crop Production, School of Agricultural Sciences, São Paulo State University (UNESP), in Botucatu, São Paulo, Brazil, in summer 2018/19. The location's geographical coordinates are 22°50′31″ S, 48°25′29″ W at an altitude of 795 m. The experiment used seeds of the soybean cultivar 95R95-IPRO, 2018/19 season crop, sown in 24 pots, obtaining five plants per 14 L pot. The soil used was classified as Red–Yellow Latosol (RYL), consisting of 61% clay, 18% silt and 21% sand. Its nutritional characteristics were corrected, and the physicochemical characteristics are shown in Table 1.

Table 1. Chemical and physical analysis of the Red–Yellow Latosol (0–20 cm) used in the experiment.

pH	OM	P_{resin}	K	Ca	Mg	H + Al	SB	CEC	V	Clay	Silt	Sand
$CaCl_2$	g dm^{-3}	mg dm^{-3}				mmolc dm^{-3}			%		g kg^{-1}	
5.4	24	15	6.7	36	14	32	57	89	64	614	196	190

OM: Organic matter, SB: sum of bases, CEC: cation exchange capacity, V: base saturation.

Fertilization occurred according to the chemical analysis for fertility purposes (Table 1) and recommendation for the cultivation of soybean [26]. All tested treatments received a standard seed treatment with the recommended dose of *Bradyrhizobium*-based inoculant. In the sowing, 50 kg ha^{-1} of single super phosphate and 20 kg ha^{-1} of potassium chloride were applied.

Greenhouse climate conditions were logged throughout the experiment using Datalogger (Instrutherm, HT-500: São Paulo, Brazil) (Figure 1). Photosynthetically active radiation (PAR) within the greenhouse was monitored by a quantometer (QMSS-E Quantum Apogee PAR Meter: Logan, UT, USA), with an average daily reading of 833.5 µmol m^{-2} s^{-1}.

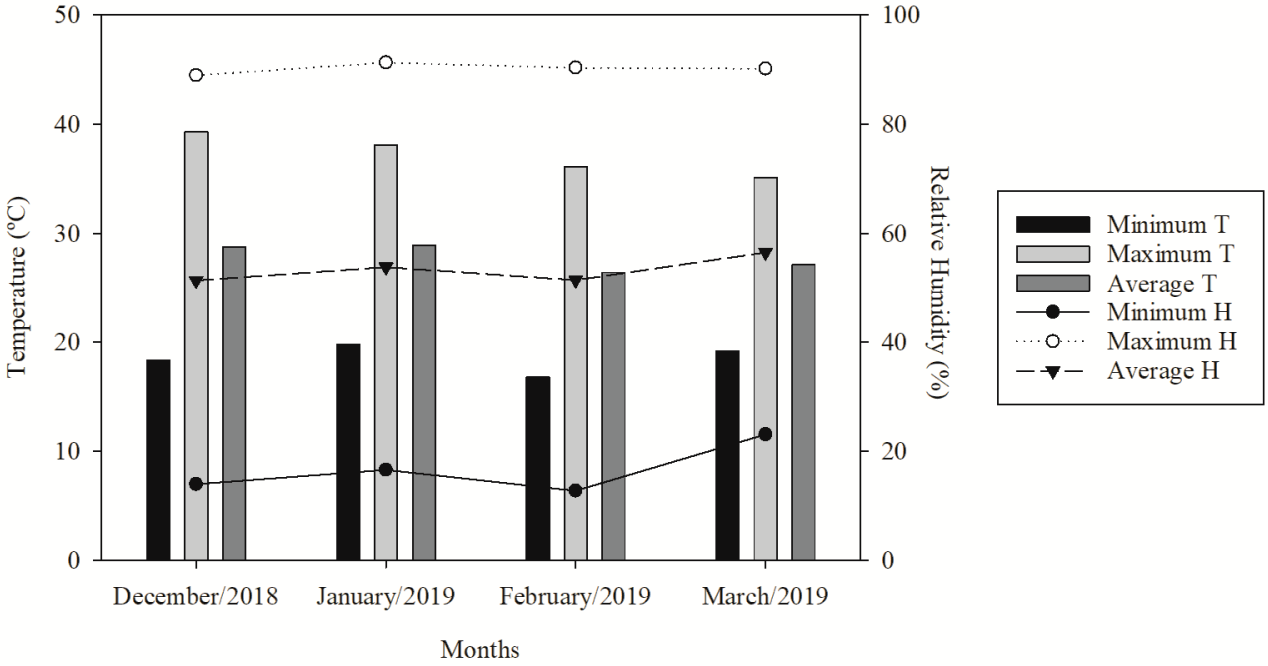

Figure 1. Minimum (Minimum T), maximum (Maximum T) and average (Average T) temperature and minimum (Minimum H), maximum (Maximum H) and average (Average T) air relative humidity in the greenhouse during the experiment.

The adopted experimental design was casualized blocks, with six treatments and four repetitions. During the V4 growth stage of soybean cultivation, all treatments—except T1—were subjected to a continuous water deficit of 50% of field capacity until the moment of analysis.

The maintenance of the water requirements of the treatments was performed daily using the method of the soil water retention curve and weighing of the pots. Thus, water deficit was imposed by weighing the pots, saturating the sampling of pots with water, draining for 12 h to reach the field capacity (FC) and weighing again to determine the mass of water in this situation. From then on, and with the aid of a table of maximum soil retention capacity and of Equation (1):

$$W = Wfc - Wd \quad (1)$$

where W = water to be added to the pot (mL); Wfc = initial pot weight with soil moisture at field capacity or 50% (g); Wd = daily pot weight (g).

The pots were watered according to the treatment; that is, 100% of the FC for treatments without water deficiency and 50% of the FC with water deficiency. As a result, daily weighing and rehydration of the pots were carried out so that they reached the desired levels again.

Biostimulant based on amino acids and macro- and micronutrients foliar applications occurred during the R1 growth stage corresponding to the soybean reproductive phase. Applications were carried out using a high-pressure backpack sprayer (CO_2) equipped with a spraying boom with two nozzles 0.5 m apart, with a spray volume of 200 L ha^{-1}, constant pressure of 1.5 bar.

The treatments (T) consisted of the application of different doses of biostimulant, distributed as follows: T1—dose 0.0 kg ha^{-1} (without water-deficit stress); T2—dose 0.0 kg ha^{-1} (with water-deficit stress); T3—dose 0.25 kg ha^{-1}; T4—dose 0.5 kg ha^{-1}; T5—dose 0.75 kg ha^{-1}; T6—dose 1.0 kg ha^{-1}.

The biostimulant was obtained from the company Microquímica Tradecorp® (Microquímica Tradecorp: Hortolândia, São Paulo, Brazil) and consists of mineral and organic

components, such as macronutrients and micronutrients chelated with EDTA, and the amino acid glycine betaine, as shown in Table 2.

Table 2. Description of the characteristics of the biostimulant * used to relieve drought stress in soybean.

Composition	(*w w*$^{-1}$)
Nitrogen (N)	4.0%
Phosphorous (P_2O_5)	21.0%
Iron (Fe EDTA)	0.5%
Copper (Cu EDTA)	0.3%
Boron (B)	0.5%
Manganese (Mn EDTA)	3.0%
Zinc (Zn EDTA)	3.0%
Glycine betaine	12.0%
pH (1%)	3.6%

* Biostimulant in solid formulation; no granulometric specification.

2.2. Determination of Physiological Variables

Physiological evaluations were carried out during the phenological growth stage, referring to the period in which the pod contains green beans that completely fill its cavity, R6 (plant stress peak) and consisted of the following variables: leaf gas exchanges, based on the net CO_2 assimilation rate (A), stomatal conductance (g_s), transpiration rate (E), leaf temperature (Tl) and intercellular CO_2 concentration (C_i), using an Infrared Gas Analyzer (IRGA) (LI-COR Biosciences Inc., Li-6400xt: Lincoln, NE, USA), with measurements taken between 9:00 a.m. and 11:30 a.m., using the atmospheric CO_2 concentration, with room temperature and humidity, and constant photosynthetically active radiation (PAR) (1500 µmol photons m^{-2} s^{-1}). Water-use efficiency (WUE) was calculated based on the A/E ratio, and carboxylation efficiency (CE) was calculated based on the A/C_i ratio. The SPAD index was measured through a portable chlorophyll meter (SPAD-502®, Minolta, Konica Minolta Sensing, Inc.: Osaka, Japan).

2.3. Determination of Antioxidant Compound and Enzymes

To analyze the activity of antioxidant enzymes: SOD (EC 1.15.1.1), CAT (EC 1.11.1.6), APX (EC 1.11.1.1) and POX (EC 1.11.1), reductase nitrate (RN- EC 1.6.6.1), and the non-enzymatic compound proline (Prol), samples were collected during the R6 phenological growth stage.

For the activity of enzymes SOD, CAT, POX and APX, 300 mg samples of expanded leaves from the apex of soybean plants were milled in liquid nitrogen and added to a homogenization medium. The medium consisted of a potassium phosphate buffer 0.1 M, pH 6.8, ethylenediaminetetraacetic acid (EDTA) 0.1 mM, phenylmethylsulfonyl fluoride (PMSF) 1 mMe polyvinylpyrrolidone (PVPP) 1% (p/v). Next, homogenized samples were centrifuged in a refrigerated centrifuge (Hettich, Universal 320R: Tuttlingen, Germany) at 12,000× g at 4 °C for 15 min and the supernatant was used as crude enzyme extract.

For SOD activity, an aliquot of 50 µL of crude extract was added to 2950 µL of reaction medium, consisting of sodium phosphate buffer 50 mM (pH 7.8) containing methionine 13 mM, p-nitroblue tetrazolium (NBT) 75 µM, EDTA 0.1 mM and riboflavin 2 µM. The reaction was performed in a chamber with fluorescent light of 15 W at 25 °C for 10 min [27]. Subsequently, lighting was interrupted, and the absorbance of blue formazan resulting from NBT photoreduction was determined in a spectrophotometer at 560 nm (Shimadzu, UV-2700: Kyoto, Japan). The reaction blank consisted of a mixture between the plant sample and the reaction medium kept in the dark, under the same temperature and time conditions. A SOD unit was defined as the quantity of enzyme required to inhibit NBT photoreduction by 50%. Results were expressed in U min^{-1} mg^{-1} protein.

For CAT activity, in turn, an aliquot of 50 µL of crude extract was added to 950 µL of reaction medium, consisting of sodium phosphate buffer 50 mM (pH 7.0) and H_2O_2

12.5 mM [28]. Absorbance was obtained in a spectrophotometer (Shimadzu, UV-2700: Kyoto, Japan) at the wavelength of 240 nm after 1 min. Enzymatic activity was determined by using the absorbance and absorption coefficient of 36 M^{-1} cm^{-1}, and results were expressed in μmol of H_2O_2 min^{-1} mg^{-1} protein.

For POX activity, an aliquot of 100 μL of crude extract was added to 4900 μL of reaction medium, consisting of sodium phosphate buffer 25 mM (pH 6.8), pyrogallol 20 mM and H_2O_2 20 mM [29]. The production of purpurogallin was determined by the measure of spectrophotometer absorbance (Shimadzu, UV-2700: Kyoto, Japan) at the wavelength of 420 nm, at 25 °C. Enzymatic activity was calculated using absorbance and the molar extinction coefficient of 2.47 mM^{-1} cm^{-1} [30] and expressed in μmol of purpurogallin min^{-1} mg^{-1} protein.

For APX activity, an aliquot of 100 μL of crude extract was added to 900 μL of reaction medium, consisting of sodium phosphate buffer 0.05 M (pH 7.0), ascorbic acid 0.8 Mm and H_2O_2 1.0 Mm [31]. Enzymatic activity was determined by the measure of spectrophotometer absorbance (Shimadzu, UV-2700: Kyoto, Japan) at the wavelength of 290 nm, at 25 °C, considering the molar extinction coefficient of 2.8 Mm^{-1} cm^{-1}. Results were expressed in μmol of ascorbic acid min^{-1} mg^{-1} protein.

For Prol determination, 100 mg of leaf tissue was homogenized in 2 mL of sulfosalicylic acid 3% (*p*/*v*) and placed in the refrigerated centrifuge (Hettich, Universal 320R: Tuttlingen, Germany) at 6300 g for 10 min. Samples of 100 μL of the extract were added to 200 μL of acid ninhydrin solution (1.25 g ninhydrin, 30 mL glacial acetic acid and 20 mL of phosphoric acid 6 M), and the mixture was incubated at 100 °C for 1 h. The reaction was paralyzed in ice bath and supernatant absorbance was measured in a spectrophotometer (Shimadzu, UV-2700: Kyoto, Japan) at the wavelength of 520 nm. Absorbance results were compared to the standard curve of proline (0 to 100 μg mL^{-1}) [32], and results were expressed in μmol proline g^{-1} fresh matter $(FM)^{-1}$.

To determine RN activity, 200 mg of leaf sample was placed in a tube with penicillin and added to 10 mL of the extraction solution; subsequently, plants were vacuum incubated for 3 cycles of 2 min each. After incubation, samples were placed in a water bath for another 30 °C for 1 h. Next, 1 mL of the extracted solution was collected and transferred to tubes, where 1 mL of the sulfanilamide solution and 1 mL of the *N*-Naphthyl solution were added; readings were made through spectrometry at 540 nm, in accordance with the methodology proposed by [33].

2.4. Determination of Biometric Parameters of Plants

Biometric evaluations were collected during the R6 phenological growth stage (plant stress peak) and consisted of variables of leaf area (LA) (cm^2 $plant^{-1}$), number of branches (NB); shoot dry matter mass (SDM) (g $plant^{-1}$); root dry matter mass (RDM) (g $plant^{-1}$); height of plants (PH) (cm); diameter of plant stem (SD) (cm); and number of primary stem nodes (NN).

LA was quantified using a meter (Li-COR Biosciences Inc., Li-3100C: Lincoln, NE, USA). The height of plants was calculated using measuring tape from the base to the apex of plants. NB by direct count. The diameter of the base stem was obtained with a digital caliper (MeterMall, 150 mm and reading 0.1 mm: Marysville, OH, USA).

SDM and RDM was obtained by collecting a plant sample and inserting it in a forced air circulation drying incubator (Fanem, 330/5: São Paulo, SP, Brazil) at 65 °C until reaching constant mass, and each sample was later weighed separately in a precision analytical scale (Shimadzu, BL-3200H: Kyoto, Japan).

NN was counted after washing the roots with water.

2.5. Determination of Production Components

The evaluations of production components were collected during the harvesting stage, when grains had a humidity of approximately 13%. They consisted of counting the average number of pods per plant (NPP), average number of pods with 1 grain (NP1), average number of pods with 2 grains (NP2) and average number of pods with 3 grains (NP3),

and productivity (P). P (g plant^{-1}) was obtained through the mass of grains measured in a precision analytical scale (Shimadzu, BL-3200H: Kyoto, Japan), adjusting humidity to 13%.

2.6. Statistical Analysis

Results were submitted to variance analysis, polynomial reduction to assess product doses under water deficit and Tukey's test to compare doses with the control without water deficit and without biostimulant application, at a level of 0.05 of probability. The non-significance of the regression deviation and/or higher value of the determination coefficient (R^2) express the significance of parameters of the statistical model, using the statistics software SISVAR® [34]. Pearson's correlation analysis was performed with normalized data from the treatments adopted to verify the relationship among analyzed variables. Pearson's correlation heatmap was generated with software RStudio® (R Software, R Development Core Team, Vienna, Austria).

3. Results

3.1. Physiological Variables

Highest *A* was observed without water deficit. However, under water-deficit conditions, the dose of 0.5 kg ha^{-1} of the biostimulant reached a similar net CO_2 assimilation rate (*A*) compared to 0, 0.75 and 1.00 kg ha^{-1} doses. Under water-deficit conditions and biostimulant application conditions, *A* results were adjusted to the quadratic model and increased by 34.75% up to a dose of 0.5 kg ha^{-1} compared to a dose of 0 kg ha^{-1}, with subsequent reduction in larger doses (Figure 2A).

A dose of 0.25 kg ha^{-1} of biostimulant reduced the stomatal conductance by 266.38% in relation to the control. The other doses tested did not differ from each other (Figure 2B).

The use of biostimulant under water-deficit conditions increased intercellular CO_2 concentration by 197.96% with the dose of 0.5 kg ha^{-1} compared to the dose 0 kg ha^{-1} (Figure 2C). However, there was no significant effect of biostimulant application on transpiration rate (Figure 2D), leaf temperature (Figure 2E) and water-use efficiency (Figure 2F).

Higher carboxylation efficiency (CE) was observed under the application of 0 kg ha^{-1} of biostimulant under water-deficit conditions, but without significant difference from the control and at doses 0.25 kg ha^{-1} and 0.75 kg ha^{-1}. Lower CE was observed under the effect of the 0.5 kg ha^{-1} and 1.0 kg ha^{-1} doses, which were similar to the 0.25 kg ha^{-1} and 0.75 kg ha^{-1} doses (Figure 2G).

The relative chlorophyll content increased by 24% following the 0.5 kg ha^{-1} dose compared to the 0 kg ha^{-1} dose of the biostimulant in plants subjected to water-deficit conditions (Figure 2H).

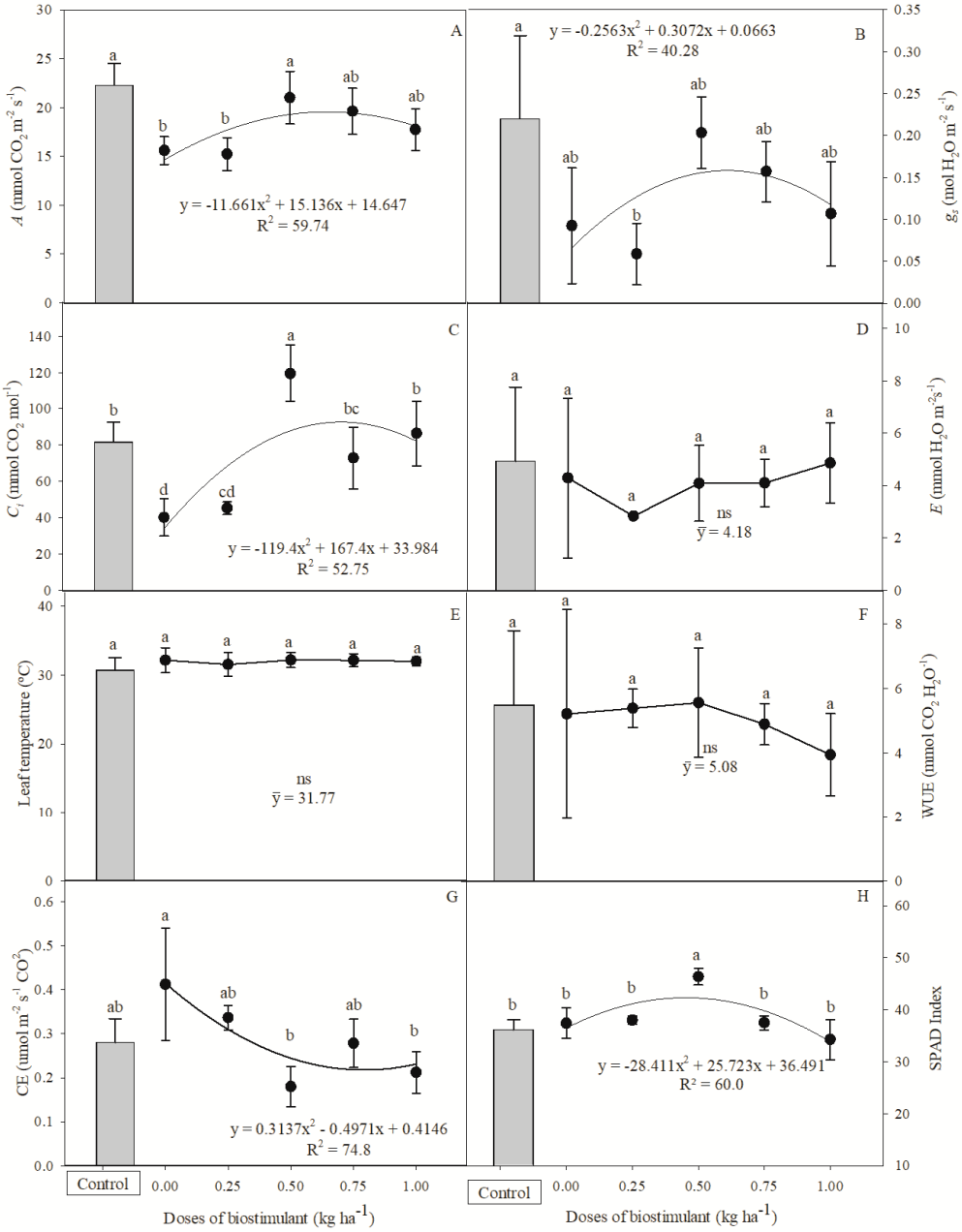

Figure 2. Physiological characteristics of soybean plants subjected to the application of different doses of biostimulant under water deficit. CO_2 assimilation—*A* (**A**), stomatal conductance—g_s (**B**), internal CO_2 concentration—C_i (**C**), transpiration—*E* (**D**), leaf temperature (**E**), water-use efficiency—WUE (**F**), carboxylation efficiency—CE (**G**) and SPAD index (**H**). Means followed by the same letter do not differ from each other according to the Tukey test at 5% probability. ns = not significant.

3.2. Antioxidant Compound and Enzymes

SOD activity was higher under application of 0.5 kg ha^{-1} under water-deficit conditions, with an increase of 420% compared to plants that did not receive biostimulant and 86.57% compared to the control (Figure 3A).

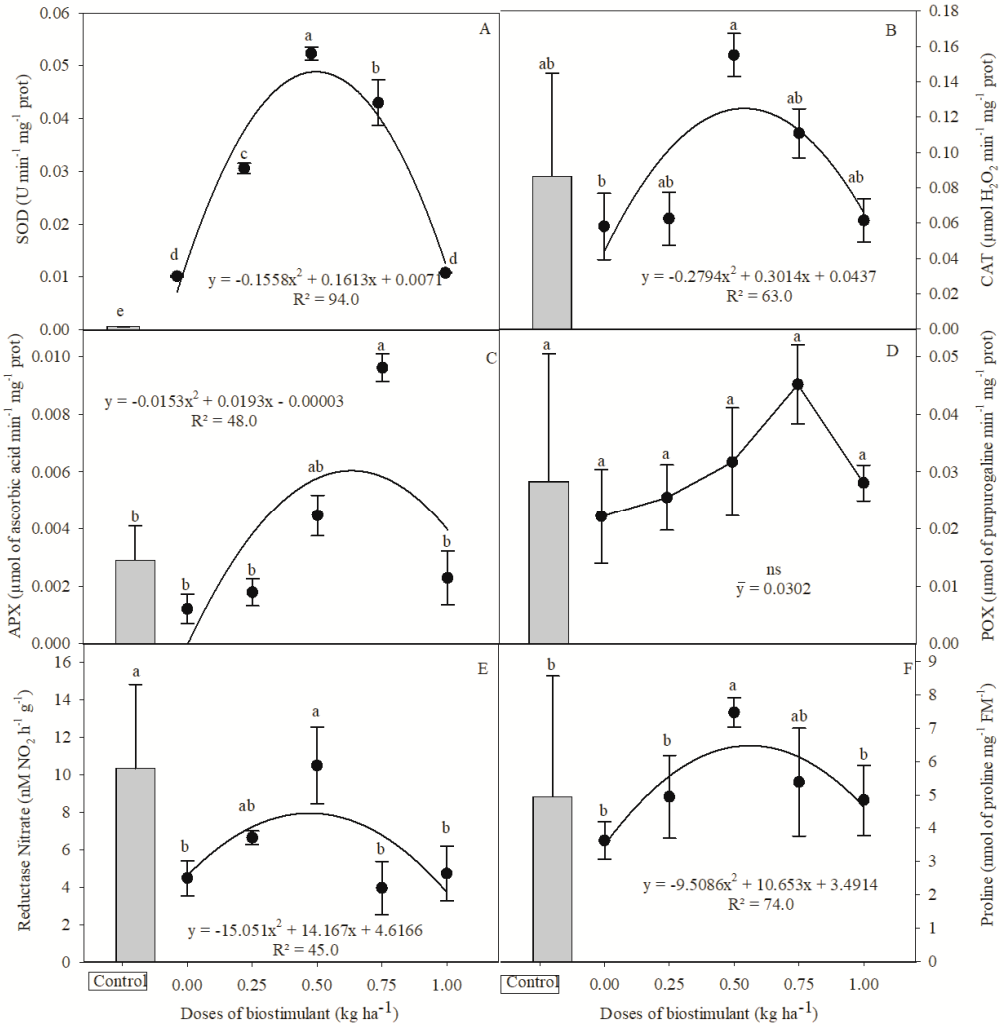

Figure 3. Activity of antioxidant enzymes and compound in soybean plants subjected to the application of different doses of biostimulant under water deficit. Superoxide dismutase—SOD (**A**); catalase—CAT (**B**); ascorbate peroxidase—APX (**C**); peroxidase—POX (**D**); reductase nitrate (**E**) and proline (**F**). Means followed by the same letter do not differ from each other according to the Tukey test at 5% probability. ns = not significant.

CAT activity increased by 167.24% under application of 0.5 kg ha^{-1} of biostimulant and water deficit compared to 0 kg ha^{-1}, but it did not differ statistically from the control and doses 0.25, 0.75 and 1.00 kg ha^{-1} (Figure 3B).

Higher APX activity was observed under the application of 0.75 kg ha^{-1} of biostimulant, with an increase of 695.04% in relation to the 0 kg ha^{-1} dose, but with no significant difference from the 0.5 kg ha^{-1} dose (Figure 3C). The POX activity was not influenced by the evaluated treatments (Figure 3D).

Under water deficit, RN activity increased by 134.15% with application of 0.5 kg ha^{-1} compared to the dose of 0 kg ha^{-1}; however, it did not differ from control and dose 0.25 kg ha^{-1} (Figure 3E).

Higher accumulation of Prol was observed at the dose of 0.5 kg ha^{-1}, with an increase of 105.79% in relation to the dose of 0 kg ha^{-1}, but it was similar to the dose of 0.75 kg ha^{-1} (Figure 3F).

3.3. Biometric Components

The highest PH was seen in the control without water deficiency. Under water-deficit conditions, the highest plant height was observed in the biostimulant dose of 0.5 kg ha^{-1}, which did not differ from the doses of 0 kg ha^{-1} and 0.75 kg ha^{-1} (Figure 4A).

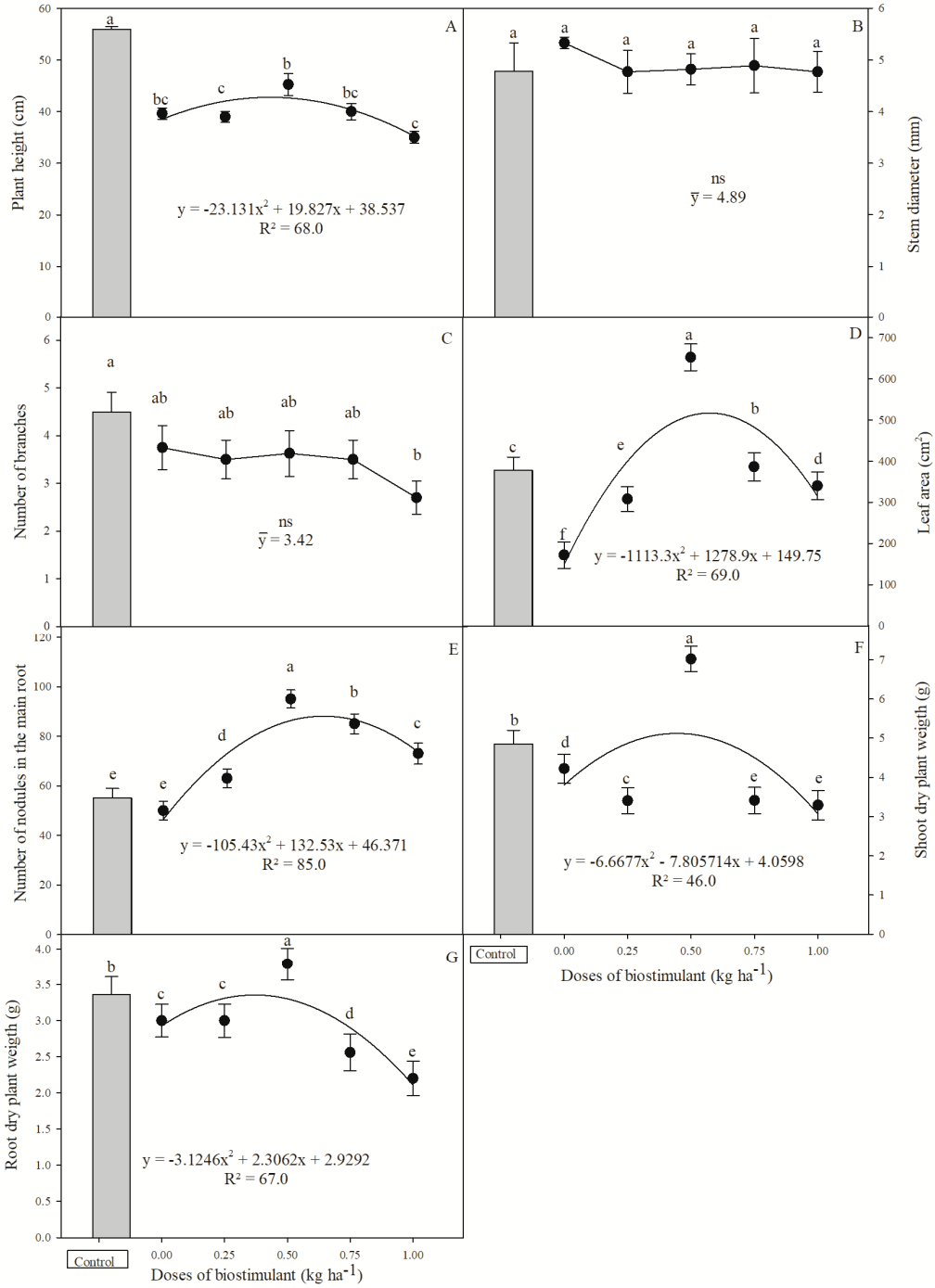

Figure 4. Biometric characteristics of soybean plants subjected to the application of different doses of biostimulant under water deficit. Plant height (**A**), stem diameter (**B**), number of lateral branches (**C**), leaf area (**D**), number of nodules in the main root (**E**), shoot dry matter weight (**F**) and root dry matter weight (**G**). Means followed by the same letter do not differ from each other by the Tukey test at 5% probability. ns = not significant.

SD was not affected by treatments (Figure 4B). However, NB reduced as the biostimulant dose increased. The highest NB was observed in the sample with no water deficit, which did not differ from samples under water deficit and biostimulant application from dose 0 to dose 0.75 kg ha^{-1}. The smallest NB was observed in the dose of 1.0 kg ha^{-1} (Figure 4C).

The largest LA was observed under a dose of 0.5 kg ha^{-1}, with an increase of 278.75% compared to the dose of 0 kg ha^{-1} (Figure 4D). The NN was highest under the application

of 0.5 kg ha^{-1}, with an increase of 90% compared to dose 0 kg ha^{-1} and 73% compared to the control (Figure 4E).

SDM under a dose of 0.5 kg ha^{-1} under water deficit conditions showed an increase of 66.35% compared to plants that did not receive biostimulant, with subsequent reduction at higher doses, and an increase of 44.74% compared to the control (Figure 4F). This performance was also observed in RDM; however, the increase in this case was 26.33% in the dose of 0.5 kg ha^{-1} compared to plants that did not receive biostimulant, and 12.00% compared to the control (Figure 4G).

3.4. Production Components

NPP was negatively impacted by water deficit conditions even with the application of the biostimulants; thus, the control treatment presented higher NPP (Figure 5A). The NP1 was not affected by treatments, resulting in an average of 2.11 pods plant^{-1} (Figure 5B).

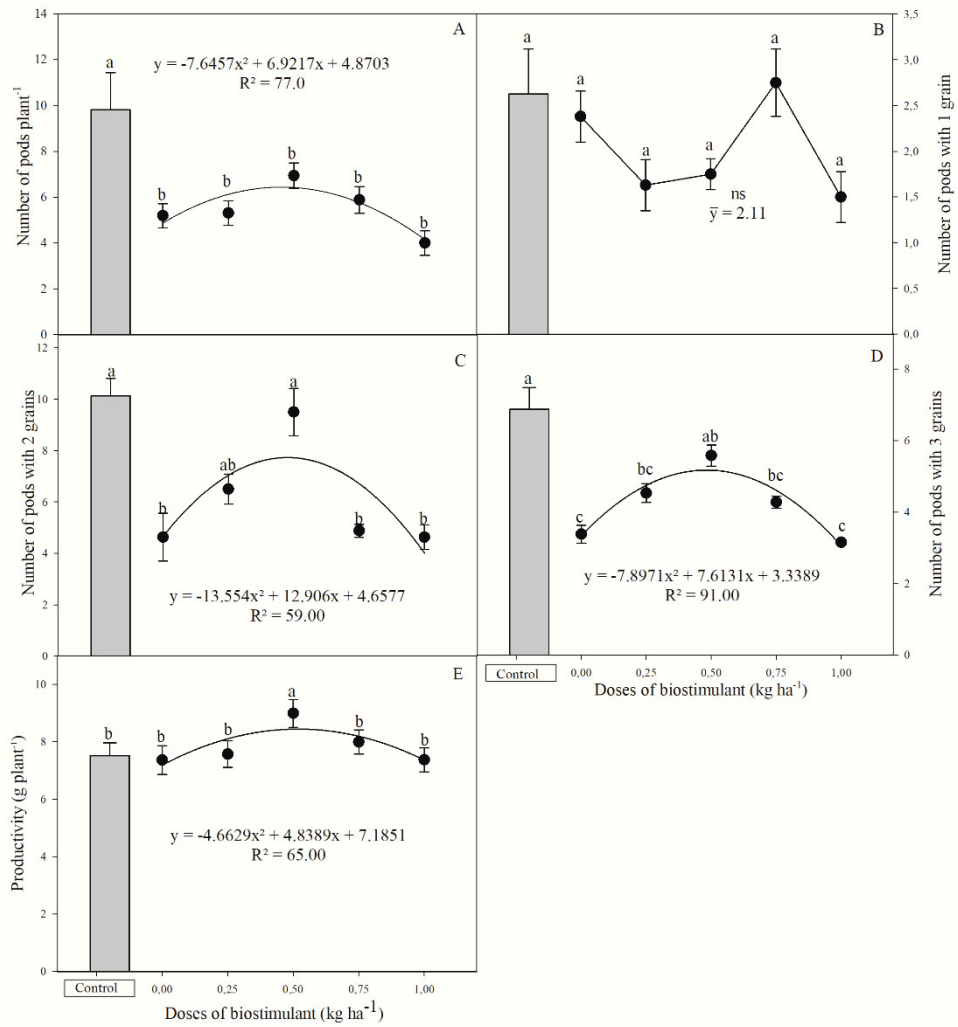

Figure 5. Components of production of soybean plants subjected to the application of different doses of biostimulant under water deficit. Number of pods per plant (**A**), number of pods with 1 grain (**B**), number of pods with 2 grains (**C**), number of pods with 3 grains (**D**) and productivity (**E**). Means followed by the same letter do not differ from each other according to the Tukey test at 5% probability. ns = not significant.

NP2 increased 105.18% with the dose of 0.5 kg ha^{-1} compared to the dose of 0 kg ha^{-1}; however, it did not differ in the control and dose 0.25 (Figure 5C). This tendency was also observed in NP3, with an increase of 65.09% compared to the dose of 0 kg ha^{-1}, but without significant difference in relation to control, doses 0.25 and 0.75 kg ha^{-1} (Figure 5D).

P increased 22.15% under 0.5 kg ha^{-1} of biostimulant, compared to dose 0 kg ha^{-1}, in addition to 19.55% compared to the control (Figure 5E).

There was a greater correlation between productivity and LA, NN, SOD, SDM, RDM, SPAD index, Ci and CAT (Figure 6).

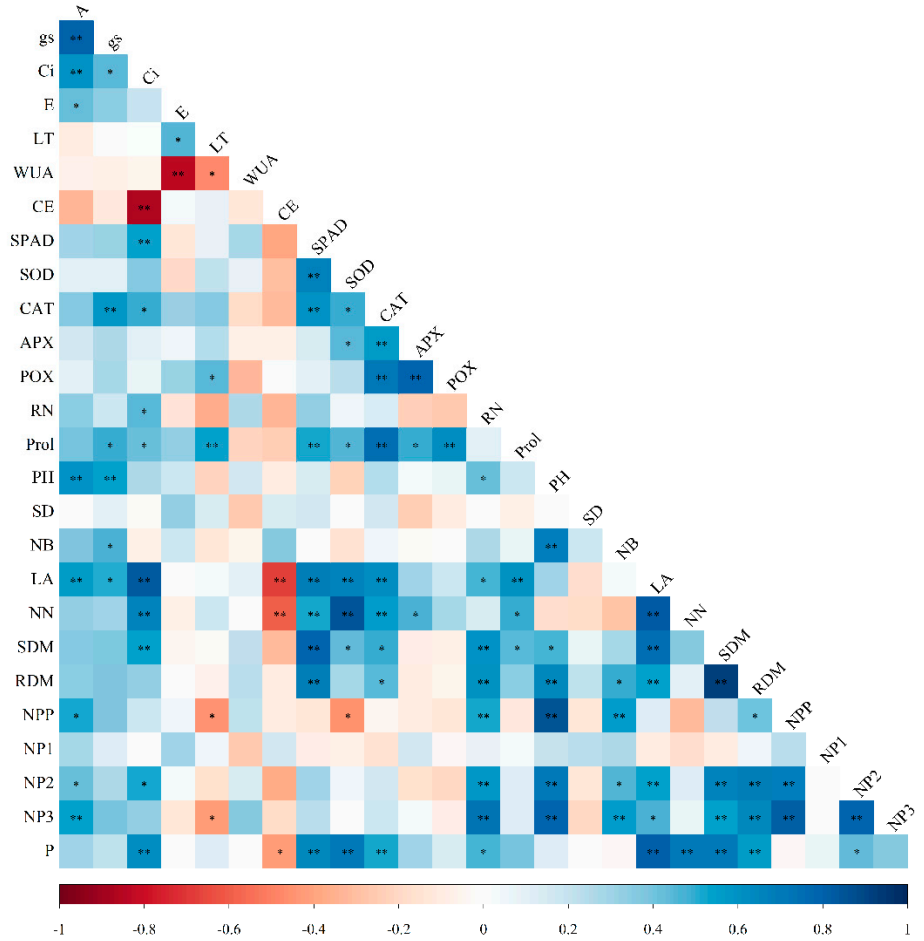

Figure 6. Pearson's correlation between CO_2 assimilation rate (A), stomatal conductance (g_s), transpiration rate (E), leaf temperature (Tl), intercellular CO_2 concentration (C_i), Water-use efficiency (WUE), carboxylation efficiency (CE), SPAD index (SPAD), superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), peroxidases (POX), nitrate reductase (RN), proline (Prol), leaf area (LA), number of branches (NB), shoot dry matter mass (SDM), root dry matter mass (RDM), height of plants (PH), diameter of plant stem (SD), number of primary stem nodes (NN), number of pods per plant (NPP), average number of pods with 1 grain (NP1), average number of pods with 2 grains (NP2), and average number of pods with 3 grains (NP3), and productivity (P). ** Significant at 5% * Significant at 1%.

4. Discussion

Photosynthesis is one of the processes most impacted by drought stress [35,36]. The ability of plants to adapt to stress conditions is an important factor used to mitigate the effects of drought stress [12,37]. Such adaptation can influence the maintenance or increase in productivity and biological processes, such as photosynthesis and, as a result, the growth and development of plants. In this study, under water-deficit conditions, the use of biostimulant in the dose of 0.5 kg ha^{-1} increased the assimilation of CO_2 compared to the sample not treated with biostimulant, possibly due to the maintenance of partially open stomata, and favored the entry of CO_2 in the leaves. This was converted into energy for the plant with greater water savings. In fact, the correct biostimulant dosage directly contributes to enhancing the efficiency of the physiological process [20,25].

The relief of water deficiency effects in soybean plants promoted by the biostimulant was also seen in stomatal conductance. The increase in stomatal conductance based on biostimulant dose resulted in keeping stomata open, which enabled the continuous addition of CO_2 in the Calvin cycle, contributing to a higher flow of Ca^{2+} and K^+ at stomatal level, essential ions to protect ionic and osmotic stress, in addition to the improvement of the water rate and photosynthesis through photosynthetic efficiency [38]. On the other hand, under conditions of scarce water supply, abscisic acid quickly accumulates and closes the stomata, reducing mesophyll conductance, CO_2 assimilation and the electron transport rate [13,39].

Despite the maintenance of A and g_s under water-deficit conditions and with biostimulant application at the dose of 0.5 kg ha^{-1}, there was an increase in C_i and reduction of CE. Lower CE values associated with higher C_i reflect biochemical alterations in the photosynthesis machinery of soybean, and lower activity of the RUBISCO enzyme [40]. Therefore, despite the potential damage to the photosystem, the biostimulant used in this study helped to maintain the stomatal control of plants and gas exchange, mainly under the effect of the 0.5 kg ha^{-1} dose. The action of biostimulants varies according to the species, growth stage and doses [41].

The similar effect on plant transpiration in the different treatments may be related to the maintenance of photosynthetic efficiency and, consequently, to the production of sugars that play an important role in the osmotic balance in conditions of water deficit. In fact, under water deficit, plants promote changes in the accumulation of soluble sugars, in order to maintain the hydric potential, mitigate oxidative damage and other functions to help maintain plasma membrane stability during extended stress periods [42].

However, the application of biostimulant had a beneficial effect on plants under water stress, which showed WUE values similar the control sample without stress [43]. This effect could be associated with the maintenance of g_s, without prejudice to E, by maintaining the osmotic potential due to the biostimulant's application. [44] demonstrated that the leaf hydric conductance is regulated by the osmotic permeability coefficient of cell membranes. In addition, applying the biostimulant could reduce the concentration of ABA and help prevent ABA production from resulting in stoma closure, under stress conditions, ultimately reducing g_s, directly influencing water use efficiency [45].

The reduction in the chlorophyll level in plants under water-deficit conditions is broadly known [2,46]. The biostimulant's mitigating effect in the dose of 0.5 kg ha^{-1} in plants submitted to water-deficit stress can be seen by the increase in the SPAD index. This increase may be related to the composition of the biostimulant, which consists of N, Mn, Fe, Zn, Cu and glycine betaine, which provide substrates for metabolic syntheses, such as chlorophyll molecules, which may be related to the increase in the activity of antioxidant complex enzymes [38].

According to [47], plants use different mechanisms, such as antioxidant enzymes and protein, simultaneously to keep the photosynthetic machinery active even during drought stress conditions. In this study, the biostimulant induced an increase in antioxidant enzymes SOD, CAT and APX in soybean plants submitted to water deficit. SOD acts in the dismutation of $O_2^{\bullet -}$ into O_2 and H_2O_2, as one of the first enzymes in ROS elimination in plants under stress conditions [18]. This increase is possibly related to a higher availability of ions in the biostimulant's composition, since SODs are categorized by a connection with ions in different cell compartments.

The use of biostimulant improved the activity of antioxidant enzymes, especially when using 0.5 kg ha^{-1}. SOD is the first line of defense against accumulation of ROS resulting from stresses. It acts in the dismutation of the superoxide anion (O_2^-) to form H_2O_2 in order to reduce the level of ROS generated by oxidative stress [48]. Meanwhile, CAT is responsible for removing H_2O_2, reducing it to two molecules of H_2O, and its activity is associated with increased levels of photorespiration [48,49]. APX breaks down this ROS using ascorbic acid + H_2O as a reducing agent and is part of the glutathione cycle pathway which, together with NAPDH, forms redox pairs that are essential for maintaining homeostasis and combating oxidative damage [18,50].

Our results demonstrate that the use of biostimulant similarly influenced the activity of POX in all tested treatments, suggesting a greater effect of the product on the other enzymes of the antioxidant complex mentioned above. In fact, POX was more correlated with Prol (Figure 6).

Biostimulants improve plant metabolism by increasing the activity of antioxidant compounds and enzymes. Therefore, plants show greater tolerance to stress throughout their life cycle, as well as improved growth. The authors of [19,51] reported a considerable increase in soy antioxidant activity and productivity following the foliar application of a biostimulant based on amino acids and micronutrients.

The application of biostimulant at the dose of 0.5 kg ha^{-1} promoted nitrogen use under water-deficit conditions, similar to the samples with no drought stress conditions, due to the maintenance of the nitrate reductase enzyme's activity. Prolonged water-deficit periods can be harmful to plants, since they reduce the absorption and transport of water and nutrients, altering the concentration of several metabolic processes, such as the production of amino acids and carbohydrates [52]. The maintenance of CO_2 assimilation under water-deficit conditions promoted by the dose of 0.5 kg ha^{-1} of the biostimulant may be related to the exogenous application of nutrients and amino acids [53]. Therefore, plants showed a reduction in the stress effects and, as a result, improved growth conditions.

During a drought, the accumulation of osmoprotective molecules helps maintain cell osmotic balance and reduces the plant water potential, maintaining soil water absorption and therefore assuring the continuity of metabolic and growth processes [16]. The increased proline concentration in soy plants that received the biostimulant dose of 0.5 kg ha^{-1} indicates increased tolerance to drought and may be associated with the number of compounds of the proline biosynthesis metabolic pathway, based on the use of the biostimulant.

Proline biosynthesis generally occurs based on the phosphorylation of glutamate, which is dependent upon nitrogen [54], a nutrient present in the composition of the biostimulant analyzed in this research. Proline maintains turgidity under stress, enhances the activities of antioxidant enzymes and allows stomata to remain partially open, allowing photosynthesis to continue under water-deficit conditions [16,54,55].

Water-deficit conditions change the physiological and biochemical processes of plants, reducing plant growth and, as a result, the productive capacity of crops [12,36]. Stem diameter did not show any significant difference compared to the control sample, corroborating the preventive effect of the biostimulant under drought conditions. On the other hand, the lower plant height resulted in a greater leaf area under drought conditions and with biostimulant application (i.e., there was a biomass partition with investment in photosynthesizing tissues, evidenced by the greater leaf area). In general, plants presented a reduction in leaf area under drought conditions. However, the use of biostimulant at the dose of 0.5 kg ha^{-1} induced the increase in leaf area, potentially assisting in the stress adaptation process, reducing energy demand, despite the increase in leaf area.

The number of pods per plant was significantly affected without the application of biostimulant, presenting values below the control condition. The mitigating effect of the biostimulant resulted in a production increase under drought stress conditions, related to the increase in tolerance mechanisms due to the modulation of ROS concentration and regulation of the concentration of phytohormones and lipids [56].

The use of biostimulant at the dose of 0.5 kg ha^{-1} contributed to an increase in productivity, also exceeding the conditions with no drought stress, as it provided an increase in leaf area to increase the surface available to absorb sunlight and transform it into energy chemical, the SPAD index and the number of nodules for better use in N assimilation, increasing the activity of antioxidant enzymes and dry matter mass.

The effect on production components was most expressive in the number of pods with two and three grains, indicating that the product maintained the pod formation period similar to that of the control sample with no drought stress, contributing to plant productivity. Final plant productivity depends on the remobilization of photosynthetic products to form pods and fill grains [10], evidenced by the increased antioxidant activity

and accumulation of the metabolic osmoprotective agent proline [57]. Therefore, the use of biostimulant helps increase drought tolerance in a more sustainable and economically feasible manner compared to genetic enhancement [25].

Our results agree with [12], who observed induction of soybean plants to recovery after water deficit under the action of a biostimulant based on *Ascophyllum nodosum* (L.) seaweed extract and fulvic acids, through improvements in the physiological and biochemical aspects of the plants, which was reflected in income gains.

Soybean plants can enhance the efficiency of physiological, biochemical and yield characteristics when treated with a biostimulant based on macro- and micronutrients and amino acids under drought conditions.

5. Conclusions

Soybean plants showed inhibition of photosynthetic metabolism during drought stress, and the response to this condition was dependent on applications of a biostimulant based on amino acids and macro- and micronutrients. The decline in physiological processes was due to cellular damage caused by the drought condition. The application of 0.5 kg ha^{-1} of the studied biostimulant improved photosynthesis and increased the antioxidant defense in drought-stressed plants, as was reflected in the higher soybean yield. Therefore, our findings shed new light onto the processing of biostimulants based on macro- and micronutrients and amino acid in soybean crop and provides guidance for further investigations into different soybean varieties and water regimes under field conditions, considering the current climate change scenario.

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References

1. Farouk, S.; Al-Huqail, A.A. Sodium nitroprusside application regulates antioxidant capacity, improves phytopharmaceutical production and essential oil yield of marjoram herb under drought. *Ind. Crops Prod.* **2020**, *158*, 113034. [CrossRef]
2. Farouk, S.; Al-Ghamdi, A.A.M. Sodium nitroprusside application enhances drought tolerance in marjoram herb by promoting chlorophyll biosynthesis and enhancing osmotic adjustment capacity. *Arab. J. Geosci.* **2021**, *14*, 430. [CrossRef]
3. El-Gamal, S.M.A.; El-Din, W.M.S.; Farouk, S.; Moktar, N.A.Y.O. Integrated effects of biochar and potassium silicate on borage plant under different irrigation regimes in sandy soil. *J. Hortic. Sci. Ornam. Plants* **2021**, *13*, 60–76. [CrossRef]
4. Dong, S.; Wang, W.; Jiang, Y.; Ma, Z.; Yan, C.; Liu, L.; Cui, G. Antioxidant and proteomic analysis of soybean response to drought during soybean flowering. *Ekoloji* **2019**, *28*, 2041–2052.
5. Anda, A.; Soós, G.; Menyhárt, L.; Kucserka, T.; Simon, B. Yield features of two soybean varieties under different water supplies and field conditions. *Field Crops Res.* **2020**, *245*, 107673. [CrossRef]

6. Wijewardana, C.; Alsajri, F.A.; Irby, J.T.; Golden, B.R.; Henry, W.B.; Reddy, K.R. Water deficit effects on Soybean root morphology and early-season vigor. *Agronomy* **2019**, *9*, 826. [CrossRef]
7. Farooq, M.; Wahid, A.; Kobayashi, N.; Fujita, D.; Basra, S.M.A. Plant drought stress: Effects, mechanisms and management. *Agron. Sustain. Dev.* **2009**, *29*, 185–212. [CrossRef]
8. Bellaloui, N.; Mengistu, A.; Fisher, D.K.; Abel, C.A. Soybean seed composition constituents as affected by drought and phomopsisin phomopsis susceptible and resistant genotypes. *J. Crop Improv.* **2012**, *26*, 428–453. [CrossRef]
9. Wijewardana, C.; Reddy, K.R.; Krutz, L.J.; Gao, W.; Bellaloui, N. Drought stress has transgenerational effects on soybean seed germination and seedling vigor. *PLoS ONE* **2019**, *14*, e0214977. [CrossRef]
10. Wijewardana, C.; Reddy, K.R.; Alsajri, F.A.; Irby, J.T.; Krutz, J.; Golden, B.R. Quantifying soil moisture deficit effects on soybean yield and yield component distribution patterns. *Irrig. Sci.* **2018**, *36*, 241–255. [CrossRef]
11. Wijewardana, C.; Reddy, K.R.; Bellaloui, N. Soybean seed physiology, quality, and chemical composition under soil moisture stress. *Food Chem.* **2019**, *278*, 92–100. [CrossRef]
12. Rosa, V.R.; Santos, A.L.F.; Silva, A.A.; Sab, M.P.V.; Germino, G.H.; Cardoso, F.B.; Silva, M.A. Increased soybean tolerance to water deficiency through biostimulant based on fulvic acids and *Ascophyllum nodosum* (L.) seaweed extract. *Plant Physiol. Biochem.* **2021**, *158*, 228–243. [CrossRef] [PubMed]
13. Taiz, L.; Zeiger, E. *Fisiologia e Desenvolvimento Vegetal*, 6th ed.; Artmed: Porto Alegre, Brazil, 2017; 888p.
14. Shukla, P.S.; Shotton, K.; Norman, E.; Neily, W.; Critchley, A.T.; Prithiviraj, B. Seaweed extract improve drought tolerance of soybean by regulating stress-response genes. *AoB PLANTS* **2018**, *10*, 051. [CrossRef]
15. Schmidt, R.R.; Weits, D.A.; Feulner, C.F.J.; Dongen, J.T.V. Oxygen sensing and integrative stress signaling in plants. *Plant Physiol.* **2018**, *176*, 1131–1142. [CrossRef]
16. Farouk, S.; Al-Huqail, A.A. Sustainable biochar and/or melatonin improve salinity tolerance in borage plants by modulating osmotic adjustment, antioxidants, and ion homeostasis. *Plants* **2022**, *11*, 765. [CrossRef]
17. Podgórska, A.; Burian, M.; Szal, B. Extra-cellular but extra-ordinarily important for cells: Apoplastic reactive oxygen species metabolism. *Front. Plant Sci.* **2017**, *8*, 1353. [CrossRef]
18. Das, K.; Roychoudhury, K.D. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Front. Environ. Sci.* **2014**, *2*, 53. [CrossRef]
19. Calvo, P.; Nelson, L.; Kloepper, J.W. Agricultural uses of plant biostimulants. *Plant Soil* **2014**, *383*, 3–41. [CrossRef]
20. Repke, R.A.; Silva, D.M.R.; Santos, J.C.C.; Silva, M.A. Increased soybean tolerance to high-temperature through biostimulant based on *Ascophyllum nodosum* (L.) seaweed extract. *J. Appl. Phycol.* **2022**, *11*, 1–14. [CrossRef]
21. Mącik, M.; Gryta, A.; Frąc, M. Biostimulants in agriculture: An overview on concepts, strategies and effects on soil microorganisms. *Adv. Agron.* **2020**, *162*, 31–87. [CrossRef]
22. Kocira, S.; Szparaga, A.; Kocira, A.; Czerwińska, E.; Wójtowicz, A.; Bronowicka-Mielniczuk, U.; Koszel, M.; Findura, P. Modeling biometric traits, yield and nutritional and antioxidant properties of seeds of three soybean cultivars through the application of biostimulant containing seaweed and amino acids. *Front. Plant Sci.* **2018**, *9*, 388. [CrossRef] [PubMed]
23. Mariani, L.; Ferrante, A. Agronomic management for enhancing plant tolerance to abiotic stresses-drought, salinity, hypoxia, and lodging. *Horticulturae* **2017**, *4*, 52. [CrossRef]
24. Wozniak, E.; Blaszczak, A.; Wiatrak, P.; Canady, M. Mode of Action Biostimulant. In *The Chemical Biology of Plant Biostimulants*; Geelen, D., Xu, L., Eds.; John Wiley & Sons Ltd.: Hoboken, NJ, USA, 2020. [CrossRef]
25. Fleming, T.R.; Fleming, C.C.; Levy, C.C.B.; Repiso, C.; Hennequart, F.; Nolasco, J.B.; Liu, F. Biostimulants enhance growth and drought tolerance in Arabidopsis thaliana and exhibit chemical priming action. *Ann. Appl. Biol.* **2019**, *174*, 153–165. [CrossRef]
26. Raij, B.V.; Cantarella, H.; Quaggio, J.A.; Furlani, A.M.C. *Recomendações de Adubação e Calagem Para o Estado de São Paulo*; Instituto Agronômico & Fundação IAC: Campinas, Brasil, 1996; 154p.
27. Del Longo, O.T.; Gonzáles, C.A.; Pastori, G.M.; Trippi, V.S. Antioxidant defenses under hyperoxygenic and hyperosmotic conditions in leaves of two lines of maize with differential sensitivity to drought. *Plant Cell Physiol.* **1993**, *34*, 1023–1028. [CrossRef]
28. Havir, E.A.; McHale, N.A. Biochemical and Developmental Characterization of Multiple Forms of Catalase in Tobacco Leaves. *Plant Physiol.* **1987**, *84*, 450–455. [CrossRef]
29. Kar, M.; Mishra, D. Catalase, Peroxidase and Polyphenol Oxidase Activities during Rice Leaf Senescence. *Plant Physiol.* **1976**, *57*, 315–319. [CrossRef]
30. Chance, B.; Maehly, A.C. Assay of Catalase and Peroxidase. *Methods Enzymol.* **1955**, *2*, 764–775. [CrossRef]
31. Nakano, Y.; Asada, K. Hydrogen Peroxide Is Scavenged by Ascorbate-Specific Peroxidase in Spinach Chloroplasts. *Plant Cell Physiol.* **1981**, *22*, 867–880. [CrossRef]
32. Bates, L.S.; Waldren, R.P.; Teare, I.D. Rapid Determination of Free Proline for Water Stress Studies. *Plant Soil* **1973**, *39*, 205–207. [CrossRef]
33. Jaworski, E.G. Nitrate reductase assay in intact plant tissues. *Biochem. Biophys. Res. Commun.* **1971**, *43*, 1274–1279. [CrossRef]
34. Ferreira, D.F. Sisvar: A guide for its bootstrap procedures in multiple comparisons. *Ciência Agrotecnologia* **2014**, *38*, 109–112. [CrossRef]
35. Pinheiro, C.; Chaves, M.M. Photosynthesis and drought: Can we make metabolic connections from available data? *J. Exp. Bot.* **2011**, *62*, 869–882. [CrossRef] [PubMed]

36. Aires, E.S.; Ferraz, A.K.L.; Carvalho, B.L.; Teixeira, F.P.; Putti, F.F.; Souza, E.P.; Rodrigues, J.D.; Ono, E.O. Foliar application of salicylic acid to mitigate water stress in tomato. *Plants* **2022**, *11*, 1775. [CrossRef] [PubMed]
37. Basu, S.; Ramegowda, V.; Kumar, A.; Pereira, A. Plant adaptation to drought stress. *F1000 Res.* **2016**, *5*, 1554. [CrossRef] [PubMed]
38. Van Oosten, M.J.; Pepe, O.; De Pascale, S.; Silletti, S.; Maggio, A. The role of biostimulants and bioeffectors as alleviators of abiotic stress in crop plants. *Chem. Biol. Technol. Agric.* **2017**, *4*, 5. [CrossRef]
39. Siddique, Z.; Jan, S.; Imadi, S.R.; Gul, A.; Ahmad, P. Drought stress and photosynthesis in plants. In *Water Stress and Crop Plants: A Sustainable Approach*; John Wiley & Sons Ltd.: Hoboken, NJ, USA, 2016; Volume 10, pp. 1–11. [CrossRef]
40. Zhang, J.; Liu, J.; Yang, C.; Du, S.; Yang, W. Photosynthetic performance of soybean plants to water deficit under high and low light intensity. *S. Afr. J. Bot.* **2016**, *105*, 279–287. [CrossRef]
41. Du Jardin, P. Plant biostimulants: Definition, concept, main categories and regulation. *Sci. Hortic.* **2015**, *196*, 3–14. [CrossRef]
42. Arabzadeh, N. The effect of drought stress on soluble carbohydrates (sugars) in two species of *Haloxylon persicum* and *Haloxylon aphyllum*. *Asian J. Plant Sci.* **2012**, *11*, 44–51. [CrossRef]
43. Santaniello, A.; Scartazza, A.; Gresta, F.; Loreti, E.; Biasone, A.; Di, T.D.; Piaggesi, A.; Perata, P. *Ascophyllum nodosum* seaweed extract alleviates drought stress in *Arabidopsis* by affecting photosynthetic performance and related gene expression. *Front. Plant Sci.* **2017**, *8*, 1362. [CrossRef]
44. Prado, K.; Boursiac, Y.; Tournaire-Roux, C.; Monneuse, J.M.; Postaire, O.; Ines, O.; Maurel, C. Regulation of *Arabidopsis* leaf hydraulics involves light-dependent phosphorylation of aquaporins in veins. *Plant Cell* **2013**, *25*, 1029–1039. [CrossRef]
45. Cohen, A.S.; Attia, Z.; Moshelion, M. Bundle-sheath cell regulation of xylem-mesophyll water transport via aquaporins under drought stress: A target of xylem-borne ABA? *Plant J.* **2011**, *67*, 72–80. [CrossRef]
46. Silva, M.A.; Santos, C.M.; Vitorino, H.S.; Rhein, A.F.L. Pigmentos fotossintéticos e índice SPAD como descritores de intensidade do estresse por deficiência hídrica em cana-de-açúcar. *Biosci. J.* **2014**, *30*, 173–181.
47. Oliver, M.J.; Farrant, J.M.; Hilhorst, H.W.M.; Mundree, S.; Brett, W.; Bewley, J.D. Desiccation tolerance: Avoiding cellular damage during drying and rehydration. *Annu. Rev. Plant Biol.* **2020**, *71*, 435–460. [CrossRef]
48. Caverzan, A.; Casassola, A.; Brammer, S.P. Reactive oxygen species and antioxidant enzymes involved in plant tolerance to stress. In *Abiotic and Biotic Stress in Plants—Recent Advances and Future Perspectives*; Shanker, A.K., Shanker, C., Eds.; InTech: Rijeka, Croatia, 2016; pp. 463–480.
49. Rivas, R.; Frosi, G.; Ramos, D.G.; Pereira, S.; Benko-Iseppon, A.M.; Santos, M.G. Photosynthetic limitation and mechanisms of photoprotection under drought and recovery of *Calotropis procera*, an evergreen C3 from arid regions. *Plant Physiol. Biochem.* **2017**, *118*, 589–599. [CrossRef]
50. Noctor, G.; Mhamdi, A.; Foyer, C.H. Oxidative stress and antioxidative systems: Recipes for successful data collection and interpretation. *Plant Cell Environ.* **2016**, *39*, 1140–1160. [CrossRef]
51. Kocira, S. Effect of amino acid biostimulant on the yield and nutraceutical potential of soybean. *Chil. J. Agric. Res.* **2019**, *79*, 17–25. [CrossRef]
52. Dat, J.F.; Foyer, C.H.; Scott, I.M. Changes in salicylic acid and antioxidants during induced thermotolerance in mustard seedlings. *Plant Physiol.* **1998**, *118*, 1455–1461. [CrossRef]
53. Teixeira, W.F.; Soares, L.H.; Fagan, E.B.; Mello, S.C.; Reichardt, K.; Dourado-Neto, D. Amino acids as stress reducers in soybean plant growth under different water-deficit conditions. *J. Plant Growth Regul.* **2019**, *39*, 905–919. [CrossRef]
54. Meena, M.; Davyanshu, K.; Kumar, S.; Swapnil, P.; Zehra, A.; Shukla, V.; Yadav, M.; Upadhyay, R.S. Regulation of L-proline biosynthesis, signal transduction, transport, accumulation and its vital role in plants during variable environmental conditions. *Heliyon* **2019**, *5*, 02952. [CrossRef]
55. Merward, A.-R.M.A.; Desoky, E.-S.M.; Rady, M.M. Response of water deficit-stressed *Vigna unguiculata* performances to silicon, proline or methionine foliar application. *Sci. Hortic.* **2018**, *228*, 132–144. [CrossRef]
56. Rouphael, Y.; Colla, G. Editorial: Biostimulants in Agriculture. *Front. Plant Sci.* **2020**, *11*, 40. [CrossRef] [PubMed]
57. Nishikawa, F.; Kato, M.; Hyodo, H.; Ikoma, Y.; Sugiura, M.; Yano, M. Effect of sucrose on ascorbate level and expression of genes involved in the ascorbate biosynthesis and recycling pathway in harvested broccoli florets. *J. Exp. Bot.* **2005**, *56*, 65–72. [CrossRef] [PubMed]