Ascophyllum nodosum algae-based biostimulant mitigates heat stress in soybean

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Abstract

Heat stress is one of the environmental factors that most limit soybean productivity (*Glycine max* (L.) Merrill). The use of biostimulants based on *Ascophyllum nodosum* (L.) seaweed extract can mitigate the effects of heat stress. Therefore, the effects on the morphological, physiological, biochemical and productive variables of soybean grown at high temperature and under exogenous application of biostimulant based on seaweed was investigated in this research. The results showed that the application of 1 L ha^{-1} of the biostimulant increased the tolerance of soybean plants to heat stress, as it increased the rate of CO_2 assimilation, stomatal conductance, the rate of transpiration and carboxylation efficiency, in addition to reducing leaf temperature, but did not influence the efficiency of water use and relative chlorophyll content (SPAD index). In addition, the application of the biostimulant increased the activity of the antioxidant enzymes superoxide dismutase, catalase and ascorbate peroxidase; and maintained the activity of peroxidase and reductase nitrate, as well as the proline concentration. Furthermore, the foliar application of the biostimulant improved the biometric and production characteristics, resulting in greater productivity. Based on these beneficial properties, the biostimulant based on algae extract at a dose of 1 L ha^{-1} proved to be effective in alleviating the adverse effects of heat stress in soybeans.

Introduction

Climate change tends to promote drastic changes in the scope of agricultural production. With the increase in the concentration of greenhouse gases, such as carbon dioxide, the increase in temperature made it evident and worrying, given the recurrent possibility of heat stress during the production cycle of agricultural crops in the world (Hassan et al. 2020). These climatic changes threaten not only food security but also economic security for soybean producing countries (Ochuodho et al. 2016).

Plants are often subjected to adverse environmental conditions, that adversely affect their growth, development and/or productivity (Mousavi-Derazmahalleh et al. 2019). Heat stress negatively affects germination, initial and reproductive development of plants, in addition to photosynthetic activity and other factors linked to plant metabolism (Kai and Iba 2014; Taiz and Zeiger 2017; Hassan et al. 2020).

Soybean crop stands out for being the main agricultural activity in Brazil, which is the largest producer in the world (CONAB 2022). This commodity has a high economic value in the grain market and a significant share in exports, a fact that has generated interest in expanding its cultivation to other regions of the country subject to high temperatures. In soybean, high temperatures result in lower yields (Puteh et al. 2013).

Heat stress is characterized by an increase in temperature sufficient to alter metabolism through changes in protein structures and accumulation of reactive oxygen species (ROS) in cells, in addition to modifying membrane fluidity (Hasanuzzaman et al. 2013; Hamed et al. 2018). It is estimated that for each degree of average temperature increase, soybean yields decrease by 16% (Kucharik et al. 2008). Elevated

temperatures cause a reduction in CO_2 assimilation, due to changes in plant physiology and biochemistry, including increased photorespiration mediated by ribulose-1 5-bisphosphate carboxylase/oxygenase (RubisCO), in addition to higher concentrations of starch and soluble sugars, leading to reduced availability of organic substrates and compromised plant development (Sicher et al. 2015).

However, plants have different physiological, morphological and biochemical apparatus to attenuate abiotic stresses, mainly due to heat stress. Among them, we highlight the reduction of protein denaturation, maintenance of the biosynthesis of important proteins, ease of transport across the plasma membrane from protein activities and other factors (Hassan et al. 2020).

One of the ways to mitigate the negative effect of high temperatures on soybean crops is through the use of biostimulants, since they are macro and micronutrients, biopolymers, poly and oligosaccharides, vitamins, proteinogenic and non-proteinogenic amino acids, polyamines, and other substances beneficial to plant metabolism that act on plant metabolism and promote improvement nutritional efficiency, increased stress tolerance, increases in antioxidative and photoprotective defense mechanisms, increase the synthesis of essential metabolic agents and provide greater growth of the aerial part and root system of plants, in addition to provide higher photosynthetic rates and greater production (Du Jardin et al. 2015; Hamed et al. 2018; Kocira et al. 2018; Sharma et al. 2019; Mącik et al. 2020).

Biostimulants from seaweed extract stand out in agriculture due to the mitigating effect of abiotic factors (Povero et al. 2016). *Ascophyllum nodosum* is a large and common brown algae, that due to the presence of many bioactive components, its harvested biomass is a valuable resource for the human enterprise as it is used in food products, fertilizers, soil conditioners, biostimulants (for phyco-elicitators), among other uses (Pereira et al. 2020).

The genus *Ascophyllum* is more commonly used for agricultural purposes and most studied to increase efficiency in agriculture due to the diversity of molecules and mineral elements that can be extracted (Shukla et al. 2019; Hamed et al. 2018). Among such vitamins, mannitol, alginates, polyphenols, and other antioxidant compounds in addition to mineral nutrients such as potassium, phosphorus, calcium, boron, magnesium and zinc (Klarzynski et al. 2000; Jäger et al. 2010), which make the use as an important biostimulant for the relief of biotic stresses (Shukla et al. 2019) and abiotics (Mukherjee and Patel 2019; Rosa et al. 2021).

Brown seaweed of the species *A. nodosum* stimulates the production of hormones and betaines in the plant (Ali et al. 2016) and in the soil improve aeration, nutrient availability and cation exchange, and in the plant improve establishment, growth and development due to the effects anti-stress (Bulgari et al. 2019; Rosa et al. 2021). In addition, the biostimulants based on these marine algae contribute to other metabolic processes in plants, such as increased chlorophyll content in the leaves, greater photosynthetic and antioxidant enzyme activity, greater root and vegetative development, greater development of flowers and fruits and, consequently, greater productivity (Fan et al. 2013; Jithesh et al. 2019).

There is an urgent need to understand the response and the potential for adaptation to the future scenario of climate change and, consequently, increase in temperatures, about the magnitude of the loss of productivity and impact on the physiological processes of the soybean crop. Based on these arguments, the hypothesis of this research is that the adequate concentration of the biostimulant based on *A. nodosum* mitigates the effect of heat stress on soybeans and increases productivity. To answer the hypothesis raised, morphological, physiological, biochemical and production parameters were used to evaluate the attenuating effect of the application of algae extract in soybean plants under heat stress.

Materials And Methods

Location, experimental design and treatments

The experiment was conducted in a greenhouse in the Department of Crop Production of the School of Agricultural Sciences, São Paulo State University (UNESP), Botucatu, SP, Brazil (22°50'31" S, 48° 25'29" W, and 786 m above sea level). The greenhouse was completely covered with transparent plastic film of low density polyethylene, 150 microns thick, without an air cooling system, which allowed to check high temperatures during the development of the experiment. Air temperature and humidity data (Fig. 1) during the experiment were obtained using the datalogger (Instrutherm, HT-500, São Paulo, Brazil).

Soybean seeds of cultivar 95R95-IPRO were used were sown on December 7, 2019, in order to obtain 5 plants per 14 L. pots. The soil used was classified as Red-Yellow Latosol (RYL), with 61% clay, 18% silt, and 21% sand, and its nutritional characteristics were corrected and the physicochemical characteristics are shown in Table 1.

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

pH	O.M.	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-----		
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 (Raij et al. 1996). All tested treatments received a standard seed treatment with the recommended dose of *Bradyrhizobium*-based inoculant. In the sowing, 50 kg ha^{-1} of simple super phosphate and 20 kg ha^{-1} of potassium chloride were applied.

The experiment was conducted under a natural photoperiod, during the summer, and the maintenance of the water requirement of the treatments was carried out daily by the method of water retention curve in the soil and weighing of the vessels. The experimental design used was in randomized blocks, with six treatments and four replications.

Since the beginning of the development of soybean plants, treatments T2 to T6 were subjected to stress by high temperature, by means of an average temperature of 40°C throughout the cycle until harvest (Fig. 1a), with the exception of treatment T1 (control without heat stress and without application of the biostimulant, 0 L ha^{-1}), which was kept in an attached greenhouse with temperature control, which averaged 26.35°C during the experiment (Fig. 1b). As a positive control for heat stress, T2 was subjected to high temperatures without the application of the biostimulant (0 L ha^{-1}). The treatments with biostimulant consisted of four increasing doses: T3–0.25 L ha^{-1} dose; T4 - dose of 0.50 L ha^{-1}; T5 - dose of 0.75 L ha^{-1}; T6 - dose of 1 L ha^{-1}. Foliar applications occurred at the stage of development R1, at 43 days after sowing (DAS) (Fig. 1). In T1 and T2, only water was applied,. The applications were carried out by means of a pressurized backpack sprayer (CO_2) equipped with a spray bar with two nozzles spaced at 0.5 m, with a spray volume of 200 L ha^{-1}, constant pressure of 1.5 bar.

The biostimulant used is a formulation based on seaweed extract *A. nodosum*, with cold extraction by the exclusive Gentle Extraction® method from Microquímica Tradecorp company (Table 2).

Table 2
Description of the characteristics of the biostimulant used for application in the relief of heat stress in soybeans.

NUTRIENTS	CONCENTRATION
Seaweed extract – *Ascophyllum nodosum*	99%
Nitrogen (N)	0.5%
Organic carbon	8.0%
pH (1%)	4.4
Density	1.1 $g.cm^{-3}$
* Biostimulant in fluid formulation - homogeneous suspension	

The maintenance of the water requirements of the treatments was performed daily by 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 hours 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 the equation:

$$W = Wfc - Wd$$

Where:

W = water to be added to the pot (mL);

Wfc = initial pot weight with soil moisture at field capacity (g);

Wd = daily pot weight (g).

Determination of physiological variables

The physiological evaluations were collected at the phenological development stage R1, at 43 DAS (Fig. 1), and consisted of the leaf gas exchange variables, in which they were obtained by rate of CO_2 assimilation (A), stomatal conductance (g_s), internal leaf carbon concentration (C_i), transpiration (E) and leaf temperature (LT) using an infrared gas analyzer (IRGA) (LI-COR Biosciences Inc., Li-6400xt, Lincoln, NE, USA), whose measurements took place between 09:00 am and 11:30 am, using atmospheric CO_2 concentration, with ambient temperature and humidity and photosynthetically active radiation (PAR) constant (1500 µmol photons m^{-2} s^{-1}). Water use efficiency (WUE) was calculated by the A/E ratio and carboxylation efficiency (CE) by the A/C_i ratio. SPAD index was measured using a portable chlorophyll meter (SPAD-502®, Minolta, Konica Minolta Sensing, Inc., Osaka, Japan). Leaf area was quantified using a meter (Li-COR Biosciences Inc., Li-3100C, Lincoln, NE, USA).

Determination of enzymes and antioxidant compounds

Enzymes activity was determined superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD) and nitrate reductase (RN), in addition to the non-enzymatic antioxidant proline (Pro). The samples were collected at the stage of phenological development R6, at 80 DAS (Fig. 1).

For the activity of the enzymes SOD, CAT, POD and APX it was determined through the sample of 300 mg expanded leaves of the middle third of the plants and macerated in liquid nitrogen and added to a homogenization medium. The medium consists of potassium phosphate buffer 0.1 M, pH 6.8, ethylenediaminetetraacetic acid (EDTA) 0.1 mM, 1 mMe phenylmethylsulfonic fluoride (PMSF) 1% polyvinylpyrrolidone (PVPP) 1% (w/v). Then, the homogenates were centrifuged in a refrigerated centrifuge (Hettich, Universal 320R, Tuttlingen, Germany) at 12,000g at 4ºC for 15 minutes and the supernatants used as crude enzyme extract.

For SOD activity, a 50 µL aliquot of crude extract was added to 2.950 µL of reaction medium consisting of sodium phosphate buffer 50 mM (pH 7.8) containing methionine 13 mM, p-nitro tetrazolium blue (NBT) 75 µM, EDTA 0.1 mM and riboflavin 2 µM. The reaction was carried out in a chamber with fluorescent light of 15 W at 25 ºC for 10 minutes (Del Longo et al. 1993). Then, the lighting was interrupted and the absorbance of the blue formazan resulting from the photoreduction of the NBT was determined in a spectrophotometer at 560 nm (Shimadzu, UV-2700, Kyoto, Japan). The blank solution consisted of the mixture between plant sample and reaction medium kept in the dark under the same temperature and weather conditions. One unit of SOD was defined as the amount of enzyme required to inhibit NBT photoreduction by 50%. The result was expressed in U min^{-1} mg^{-1} protein.

For CAT activity, a 50 µL aliquot of crude extract was added to 950 µL of reaction medium containing 50 mM (pH 7.0) potassium phosphate buffer and H_2O_2 12.5 mM (Havir and McHale 1987). The absorbance was obtained in a spectrophotometer (Shimadzu, UV-2700, Kyoto, Japan) at a wavelength of 240 nm

after 1 minute. The enzymatic activity was determined by using the absorbance and the absorption coefficient of 36 M^{-1} cm^{-1} and the result expressed in µmol of H_2O_2 min^{-1} mg^{-1} protein.

For APX activity, a 100 µL aliquot of crude extract was added to 900 µL of the reagent medium containing potassium phosphate buffer 0.05M (pH 7.0), ascorbic acid 0.8 mM and H_2O_2 1.0 mM (Nakano and Asada 1981). The enzymatic activity was determined by measuring the spectrophotometer absorbance (Shimadzu, UV-2700, Kyoto, Japan) at a wavelength of 290 nm at 25 ºC considering the molar extinction coefficient of 2.8 mM^{-1} cm^{-1}. The result was expressed in µmol of ascorbic acid min^{-1} mg^{-1} protein.

For POD activity, an aliquot of 100 µL of crude extract was added to 4900 µL of the reaction medium consisting of potassium phosphate buffer 25 mM (pH 6.8), pyrogallol 20 mM and H_2O_2 20 mM (Kar and Mishra 1976). Purpurogallin production was determined by measuring the spectrophotometer absorbance (Shimadzu, UV-2700, Kyoto, Japan) at a wavelength of 420 nm, at 25°C. The enzymatic activity was calculated using the absorbance and the molar extinction coefficient of 2.47 mM^{-1} cm^{-1} (Chance and Maehley 1955) and expressed in µmol of purpurogallin min^{-1} mg^{-1} protein.

For the determination of Pro, 100 mg of leaf tissue were homogenized in 2 mL of sulfosalicylic acid 3% (w/v), and refrigerated centrifuged (Hettich, Universal 320R, Tuttlingen, Germany) at 6300 g for 10 min. Samples of 100 µL of the extract were added to 200 µL of acidic ninhydrin solution (1.25 g of ninhydrin, 30 mL of glacial acetic acid and 20 mL of 6M phosphoric acid) and the mixture was incubated at 100 ºC for 1 hour. The reaction was stopped in an ice bath and the absorbance of the supernatant was measured on a spectrophotometer (Shimadzu, UV-2700, Kyoto, Japan) at a wavelength of 520 nm. The absorbances were compared to the standard proline curve (0 to 100 µg mL^{-1}) (Bates 1973) and the results expressed in µmol proline g^{-1} fresh matter $(FM)^{-1}$.

For the determination of RN activity, 200 mg of the leaf were collected and inserted in penicillin tube, after which 10 mL of the extracting solution was added, afterwards the samples were incubated under vacuum for a duration of 3 cycles of 2 minutes. After incubation, the samples were placed in a water bath for an additional 30°C for an interval of 1 hour. Then 1 mL of the extracted solution was collected and transferred to tubes, where afterwards 1 mL of the sulfanilamide solution and 1 mL of the N-Naftil solution were added. The readings were performed by spectrophotometry at 540 nm, according to the methodology proposed by Jaworski (1971).

Determination of plant biometric variables

Biometric assessments were carried out at the phenological development stage R6, at 80 DAS (Fig. 1) and consisted of the variables of leaf area (LA), number of lateral branches (NLB); shoot dry plant weight (SDW); root dry weight (RDW); average plant height (PH); average plant stem diameter (SD) and average number of nodules in the main root (NNR).

The NLB was obtained by direct counting, the PH (cm) was obtained with the aid of a tape measure from the base to the apex of the plants. The SD (mm) in the neck of the plant was obtained by means of a

digital caliper (MeterMall, 150 mm and reading 0.1 mm, Marysville, OH, USA).

LA (cm^2 $plant^{-1}$) was quantified using a meter (Li-COR Biosciences Inc., Li-3100C, Lincoln, NE, USA). SDW and RDW (g $plant^{-1}$) were obtained by collecting one plant per plot, which was placed in a drying oven by forced air circulation (Fanem, 330/5, São Paulo, SP, Brazil) at 65 ºC until constant mass and subsequent weighing of each sample separately on a precision analytical balance (Shimadzu, BL-3200H, Piracicaba, SP, Brazil).

The NNR was counted after washing the roots to loosen the soil in the pot.

Determination of production components

The evaluations of the production components were collected at the harvest stage, at 106 DAS (Fig. 1) and consisted of the variables of average number of pods per plant (PP), average number of pods with 1 grain (G1), average number of pods with 2 grains (G2), average number of pods with 3 grains (G3) and productivity (P). P (g $plant^{-1}$) was obtained through the mass of the grains that were measured in a precision analytical balance (Shimadzu, BL-3200H, Piracicaba, SP, Brazil), correcting the humidity level to 13%.

Statistical analysis

The results were submitted to analysis of variance (ANOVA), polynomial regression to evaluate product doses under heat stress of plants and Tukey test to compare doses with control without heat stress and without application of biostimulant, at the level of 0.05 probability. Homogeneity of variance was verified by the Levene's test and normality by the Shapiro-Wilk test to determine the appropriate coefficient of correlation to be applied for $p < 0.05$. The non-significance of the regression deviation and/or greater value of the coefficient of determination (R^2) expresses the significance of the parameters of the statistical model, using the SISVAR® statistical software (Ferreira 2014). 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)).

Results

Physiological variables

Most of the physiological variables were influenced by heat stress and doses of the biostimulant (Fig. 2). Greater *A* was observed in plants under application of 1 L ha^{-1} with an increase of 56.56% in relation to the control with heat stress and 100% in relation to the control without heat stress (Fig. 2a). The dose of 1 L ha^{-1} of the biostimulant provided greater g_s, however, similar to doses 0.50 and 0.75 L ha^{-1}, with an increase of 94.97% in relation to T2 (dose 0 L ha^{-1} with stress) and 175.56% in relation to T1 (dose 0 L ha^{-1} without heat stress) (Fig. 2b).

A lower C_i was observed in plants that received 1 L ha^{-1}, which did not differ from the control without heat stress, and reduced 6.47% in relation to the stressed control (Fig. 2c). There was no significant difference in *E* between doses 0.50, 0.75 and 1 L ha^{-1}, however the highest dose provided increases of 71.59 and 150.82% in relation to controls with and without heat stress, respectively (Fig. 2d).

The LT did not differ between the control without stress and the dose of 1 L ha^{-1}, with a reduction of 10.46% in relation to the control with stress (Fig. 2e). WUE was not influenced by heat stress and biostimulant doses with an average of 1.54 mmol CO_2 H_2O^{-1} (Fig. 2f).

Plants that received 1 L ha^{-1} of the biostimulant had their CE increased by 67.47 and 100% compared to controls with and without stress, respectively (Fig. 2g). The relative chlorophyll content did not differ between treatments, both for the doses of the biostimulant and for the treatment without stress (Fig. 2h).

Higher positive correlation among *A*, g_s, *E* and CE and P was observed (Fig. 6).

Enzymes And Antioxidant Compounds

SOD activity increased by 1177% with the application of 0.75 L ha^{-1} in the condition of thermal stress in relation to plants that did not receive the application of the biostimulant and were not stressed and 837% in relation to the treatment without stress and with product application (Fig. 3a).

The dose of 0.75 L ha^{-1} also favored CAT activity, which responded with an increase of 171.81% in relation to plants under stress and which did not receive product application and 103.60% in relation to plants without heat stress (Fig. 3b).

The APX (0.0030 µmol of ascorbic acid min^{-1} mg^{-1} protein), POD (0.0259 µmol of purpurogallin min^{-1} mg^{-1} protein), NR (0.3380 nM NO_2 h^{-1} g^{-1}) activity and Pro concentration (5.0418 nmol proline mg^{-1} FM^{-1}), there was no significant difference between the applied and control doses (Fig. 3c, 3d 3e and 3f).

According to Pearson's correlation, SOD and CAT showed higher positive correlations with P in relation to other biochemical variables (Fig. 6).

Biometric Components

The HP was favored with the application of the 1 L ha^{-1} dose of the biostimulant in the heat stress condition, however this dose did not differ from the 0.50 and 0.75 L ha^{-1} doses and the control without stress, in the however, the dose of 1 L ha^{-1} showed an increase of 23.93% in relation to the treatment with stress and dose 0 L ha^{-1} (Fig. 4a).

The doses of the biostimulant were not adjusted for regression in relation to SD, but the Tukey test indicates superiority in doses 0.50 and 0.75 L ha^{-1} with an increase of 9.51 and 11.46% in relation to the control without stress (Fig. 4b). The NLB was superior in the control without stress, however similar to the doses the doses 0.25, 0.50 and 0.75 L ha^{-1} of the biostimulant (Fig. 4c).

Higher LA was observed at doses of 0.50, 0.75 and 1 L ha^{-1}, resulting in an increase of 5.41% in the highest dose compared to the dose 0 L ha^{-1} under stress (Fig. 4d).

The NNR was favored by the dose of 1 L ha^{-1} of the biostimulant (73 nodules) and showed a significant difference of 22 nodules in relation to the stress treatment and dose 0 L ha^{-1} of the biostimulant (51 nodules), which represents an increase of 43%, however when compared to the dose of 1 L ha^{-1} applied under stress conditions with the treatment that was not subjected to stress, there is an increase of 18 nodules, representing 33% more than nodules in the main root of the plants (Fig. 4e).

SDW increased by 14.84% with the application of 1 L ha^{-1} in the heat stress condition in relation to plants that did not receive the application of the biostimulant (Fig. 4f). This performance is also observed in the RDW, however this increase was 19.93% in the dose 1 L ha^{-1} in relation to plants that did not receive the application of the biostimulant, and an increase of 12.46% in comparison with the treatment control without stress (Fig. 4g).

PH, LA, NNR, SDW and RDW showed higher positive correlations with P (Fig. 6).

Production Components

The PP was higher in the dose 1 L ha^{-1} with an increase of 75.72 and 18.51% in relation to controls with and without heat stress (Fig. 5a). G1 did not differ between the treatments tested with an average of 2.69 (Fig. 5b). G2 was superior in the control without stress, however, similar to the 1 L ha^{-1} dose of the biostimulant (Fig. 5c).

The G3 increased 131.78 and 51.60% with the application of 1 L ha^{-1} of the biostimulant, in relation to controls with and without stress, respectively (Fig. 5d). P was similar between doses 0.75 and 1 L ha^{-1}, but higher than the others, with increases of 19.21 and 27.14% in the highest dose compared to the dose 0 L ha^{-1} with and without heat stress, respectively (Fig. 5e).

The variables that most contributed to P were *A*, CE, SOD, PH, LA, NNR, PP and G3, with a positive correlation above 60%. On the other hand, P showed a negative correlation with LT. (Fig. 6).

Discussion

Ambient temperatures are increasing at a considerable rate as part of the current climate change, and agriculture has been impacted through losses in crop yields that compromise food security (Nadeem et

al. 2018; Carmody et al. 2020). Our results demonstrate that soybean development was affected by heat stress at an average temperature of 40 ºC throughout the soybean cycle.

Temperatures are categorized as high for soybeans when they are above 27°C in the long term, both in the vegetative and reproductive phases (Kai and Iba 2014). In high temperature situations the photosynthetic process is impaired due to the activity of oxidative stress. Severe heat stress induces programmed cell death by enzymatic denaturation (Hassan et al. 2020).

The increase in temperature directly affected the photosynthetic metabolism, however, under the application of the biostimulant, especially at the 1 L ha^{-1} dose, there was mitigation of the harmful effect of stress, which may be related to the action of *A. nodosum* extract in soybean. Algae extract promotes an increase in the absorption and assimilation of nutrients, with emphasis on nitrogen resulting from increased activity of enzymes such as nitrate reductase and glutamine synthetase (Fan et al. 2013). It can also increase up to 30.5 and 20% the concentration of nutrients such as phosphorus and potassium, respectively, in plant tissue (Di Stasio et al. 2017). In this way, the organic composition of biostimulants contributes to improving physiological processes, inducing tolerance to abiotic stress, and improving production quality (Di Stasio et al. 2017; Langowski et al. 2019). The reduction of A in the other treatments evaluated may be due to the photoprotection mechanism, since, in Arabidopsis model plants, a reduction in the expression of the AtRBCS1A and AtRCA genes, responsible for catabolism and activation of the RubisCO enzyme complex, was observed (Santaniello et al. 2017), which does not favor the assimilation of CO_2, evidenced by the high C_i in these treatments.

Higher g_s and E in plants under application of 1 L ha^{-1} of the biostimulant demonstrate a positive effect. The increase in g_s favored A and CE. In addition, greater E provided a reduction in LT, indicating photochemical efficiency of the photosynthetic apparatus even under heat stress, corroborated by the low C_i (Martynenko et al. 2016; Santaniello et al. 2017; Ergo et al. 2018). In fact, biostimulants induce photoprotective plant defence systems under periods of drought stress (Goñi et al. 2018). However, there was no influence of the biostimulant added to the heat stress in the WUE, indicating that in these conditions, soybean maintained the metabolic processes essential for its development (Flexas et al. 2016).

In fact, the application of seaweed extract contributed to attenuating the effect of stress due to the maintenance of the gene expression of RBCS1A related to photosynthesis (*At1g67090*) and RCA (*At2g39730*) and PIP1; 2 (*At2g45960*) and βCA1 (*At3g01500*) that are involved in controlling the diffusion of CO_2 in the mesophile in *Arabidopsis thaliana* (De Saeger et al. 2020).

Under conditions of heat stress, the high temperature can decrease the internal concentration of CO_2, causing the blocking of enzymes of photosynthetic activity and ATP synthesis (Iqbal et al. 2019). However, our results demonstrate that differences in photosynthetic efficiency between the evaluated doses are likely to be large enough to be the main factor related to increases in P.

Among the harmful effects of heat stress, the reduction in membrane permeability is noteworthy, as it promotes drastic changes in the process of photosynthesis and respiration, which come from the increase in the energetic kinetics of the molecules, resulting in the denaturation of proteins, porosity of the cell membrane and increased fluidity (Jedmowski et al. 2015; Hassan et al. 2020).

The relative chlorophyll content of plants under heat stress did not differ from control plants. This maintenance of chlorophyll concentration may be related to the plant's self-protection system, as stress due to high temperatures induces the reduction of chlorophyll biosynthesis, which may have the effect combined with the degradation of chlorophyll molecules (Vass 2012; Fahad et al. 2017), however chlorophylls perish have not been influenced by the factors studied in this research, unlike the antioxidant enzymes SOD and CAT.

SOD activity increased due to the dose of the biostimulant, enabling the attenuating effect of heat stress, as it is the first enzyme to act in the defense of the antioxidant system. SOD acts in the dismutation of the superoxide anion to form H_2O_2, thus minimizing the damage caused by ROS through the breakdown of superoxide radicals that are generated by oxidative stress (Shafi et al. 2015; Caverzan et al. 2016).

CAT also showed an increase in activity due to the application of biostimulant and acts by removing H_2O_2, reducing it to two H_2O molecules (Barbosa et al. 2014; Caverzan et al. 2016). According to the study by Chakraborty and Pradhan (2011), enzymes such as CAT, APX and SOD increased activity up to a temperature of 50 ºC. However, the activity of APX, POD, RN and Pro concentration were not influenced by the factors studied in this research, indicating that SOD and CAT were the main contributors in the elimination of ROS in soybean, which favored the maintenance of soybean development. In fact, SOD and CAT are directly related to productivity gains (Fig. 6).

The application of the biostimulant promoted an increase in HP, SD and LA, especially under the dose of 1 L ha^{-1}, which may be related to the greater availability of molecules and nutrients from the algae extract. The larger the leaf area, the greater the photosynthetic area of the plant, with a direct influence on crop yield (Devi et al. 2015). Another factor that corroborates the stress-reducing effect was the increase in NNR, SDW and RDW.

Rosa et al. (2021) observed that the biostimulant based on *A. nodosum* (L.) seaweed extract and fulvic acids induced soybean plants better recovery after water deficit by providing faster reestablishment of cellular water potential, osmotic adjustment, increased stomatal conductance, photosynthetic activity and production of photoassimilates, higher efficiency in energy dissipating mechanisms, which reduced ROS generation, and higher activity of antioxidant enzymes. Similar results were obtained in this research on heat stress in soybean.

The seaweed *A. nodosum* has in its composition hormones such as auxins, cytokinins, gibberellins and abscisic acid that contribute to plant growth and development and adaptation to stress (Mori et al. 2017, Rouphael and Colla 2018, Ghaderiardakani et al. 2019, De Saeger et al. 2020). Studies suggest an effective participation in the regulation of homones using biostimulants based on algae extract, such as

regulation in the promoters of auxin activity (AuxRE), activation of the cytokinin promoter (ARR5) and modulation of the response of the GA24 genes, GASA4, GASA1 of gibberellins (Wally et al. 2013; Goñi et al. 2016).

Our results suggest that increases in PP, G2, G3 and P under the dose of 1 L ha^{-1} are related to the attenuating effect of the biostimulant during the period of heat stress due to the maintenance of photosynthetic metabolism and antioxidant defense that allowed continuity of soybean development under stressful conditions.

As previously observed, the production components were positively influenced by most of the variables studied (Fig. 6). Thus, there are indications that the increase in LA provided greater surface for absorption of radiation necessary for CO_2 assimilation, which was reflected in gains in dry matter mass in soybean plants and energy required for pod formation, mainly of 3 grains that directly influenced the return of culture. However, negative correlation between yield parameters and LT demonstrates the harmful effect of heat stress on soybean production.

Activity of proteins and molecules that respond to stress, directing less energy to defense mechanisms and increasing productive components under biostimulant action (Zang et al. 2010; Tandon and Dubey 2015; Hamed et al. 2018; Mukherjee and Patel 2020). All these ways of inducing different mechanisms promoted the process of adaptation to stress faster, which contributed to the gain in productivity even under conditions of thermal stress.

Synergistic effect of *A. nodosum* based biostimulants as mitigator of abiotic stress has been reported in the literature on heat stress in tomato (Carmody et al. 2020) and water deficit in soybean (Rosa et al. 2021). In fact, biostimulants with *A. nodosum* stimulate plant growth and adaptation to stress (Rouphael et al. 2017, De Saeger et al. 2020).

This research was focused on challenging soybean plants to several degrees of temperatures above their ideal conditions for development, i.e., 27°C (Kai and Iba 2014). Unlike thermal shocks applied for a short period of time (hours), this experimental design was more representative of naturally occurring stress conditions in the field, as moderate heat stress regimes can affect the function of vegetative tissues and further impair production parameters. Given the gap on the effect of this biostimulant on high temperatures in soybeans and its effects on crop yield, this research guides the search for a viable solution, creating more sustainable and environmentally acceptable agricultural practices.

Conclusion

The treatment of soybean plants with biostimulant based on *Ascophyllum nodosum* improves the efficiency of the morphological, physiological, biochemical and yield characteristics under high temperature conditions. In our results, we show that the use of this biostimulant can mitigate the effects caused by high temperature stress maintaining and assimilating carbon and stomatal conductance, reducing leaf temperature and effective production of antioxidant compounds, reflecting greater

accumulation of biomass and grain production in soybean. This positive effect was observed in plants treated with a dose of 1 L ha^{-1}, which allowed a better adaptation process of soybean plants to high temperature stress. It is important to note that our results guide new research on the management of biostimulants based on *A. nodosum* seaweed extract in soybean culture in the face of the current climate change scenario for sustainable use in agriculture.

Declarations

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Authors contributions RAR, DMRS and JCCS performed the experiments, collected and analyzed data and wrote the original draft; RAR and MAS contributed to the conceptualization of research goals, experimental design, acquisition of funding, project administration, and manuscript review.

Data availability Contact corresponding author.

Conflict of interest The authors declare that there are no conflicts of interest.

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Figures

Figure 1

Maximum, average, and minimum temperature (T) and maximum, average, and minimum relative humidity (RH) in the greenhouse during the execution of the experiment (A) and in the conditions in which the control (without application of the biostimulant and without heat stress) was maintained (B).

Figure 2

Physiological parameters, CO_2 assimilation - A (A), stomatal conductance - g_s (B), Internal carbon concentration - C_i (C), transpiration - E (D), leaf temperature (E), water use efficiency - WUE (F), carboxylation efficiency - CE (G) and SPAD Index (H), of soybean plants submitted to the application of different doses of biostimulant under heat stress. ns = not significant, * significant at 5% and ** significant at 1% and 5% by regression analysis. Averages followed by the same letter do not differ from each other by the Tukey test ($p \leq 0.05$).

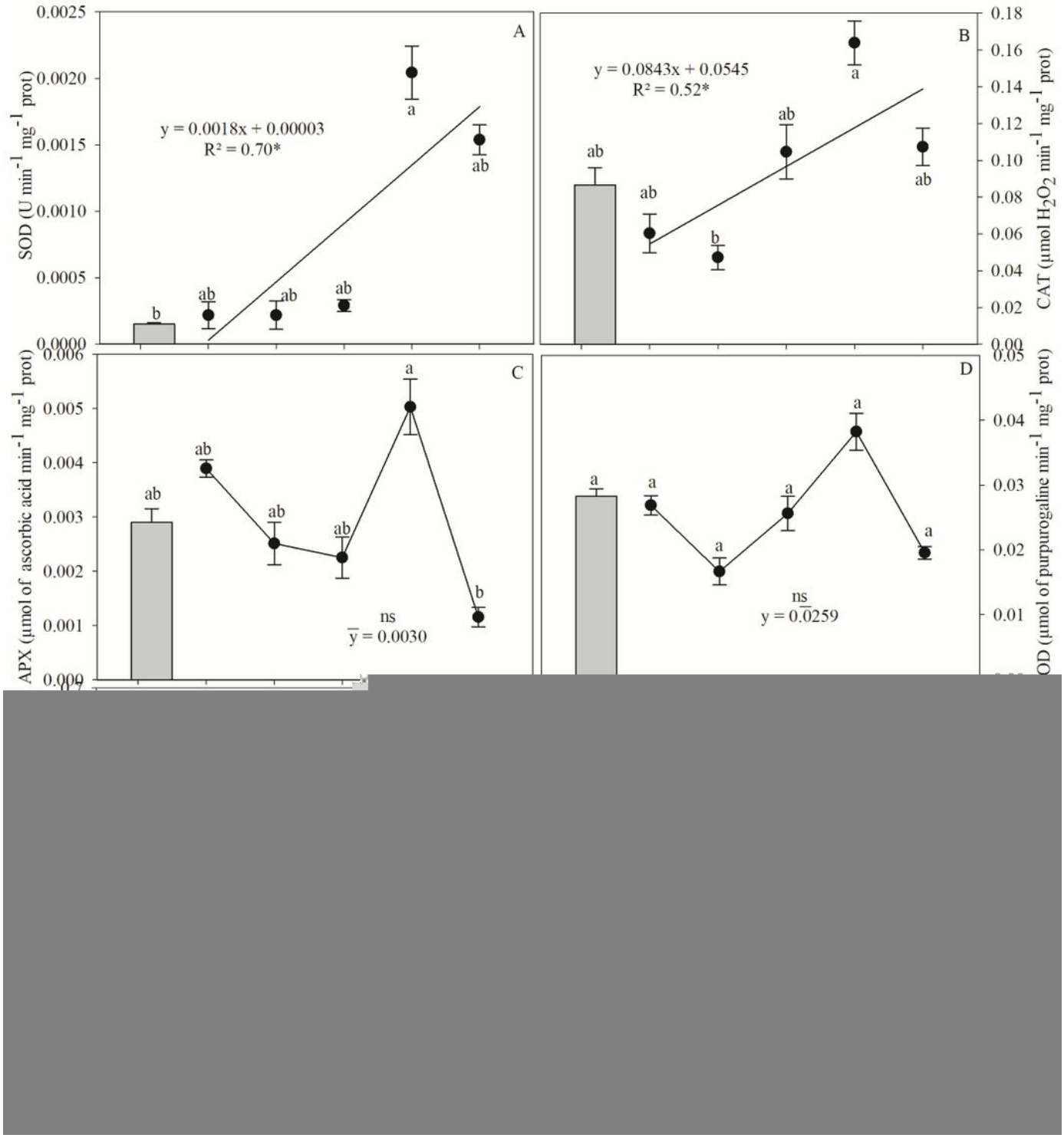

Figure 3

Activity of the enzymes Superoxide dismutase - SOD (A), catalase - CAT (B), ascorbase peroxide - APX (C), peroxidase - POD (D), reductase nitrate (E) and proline concentration (F) in soybean plants submitted application of different doses of biostimulant under heat stress. ns = not significant, * significant at 5% and ** significant at 1% and 5% by regression analysis. Averages followed by the same letter do not differ from each other by the Tukey test ($p \leq 0.05$).

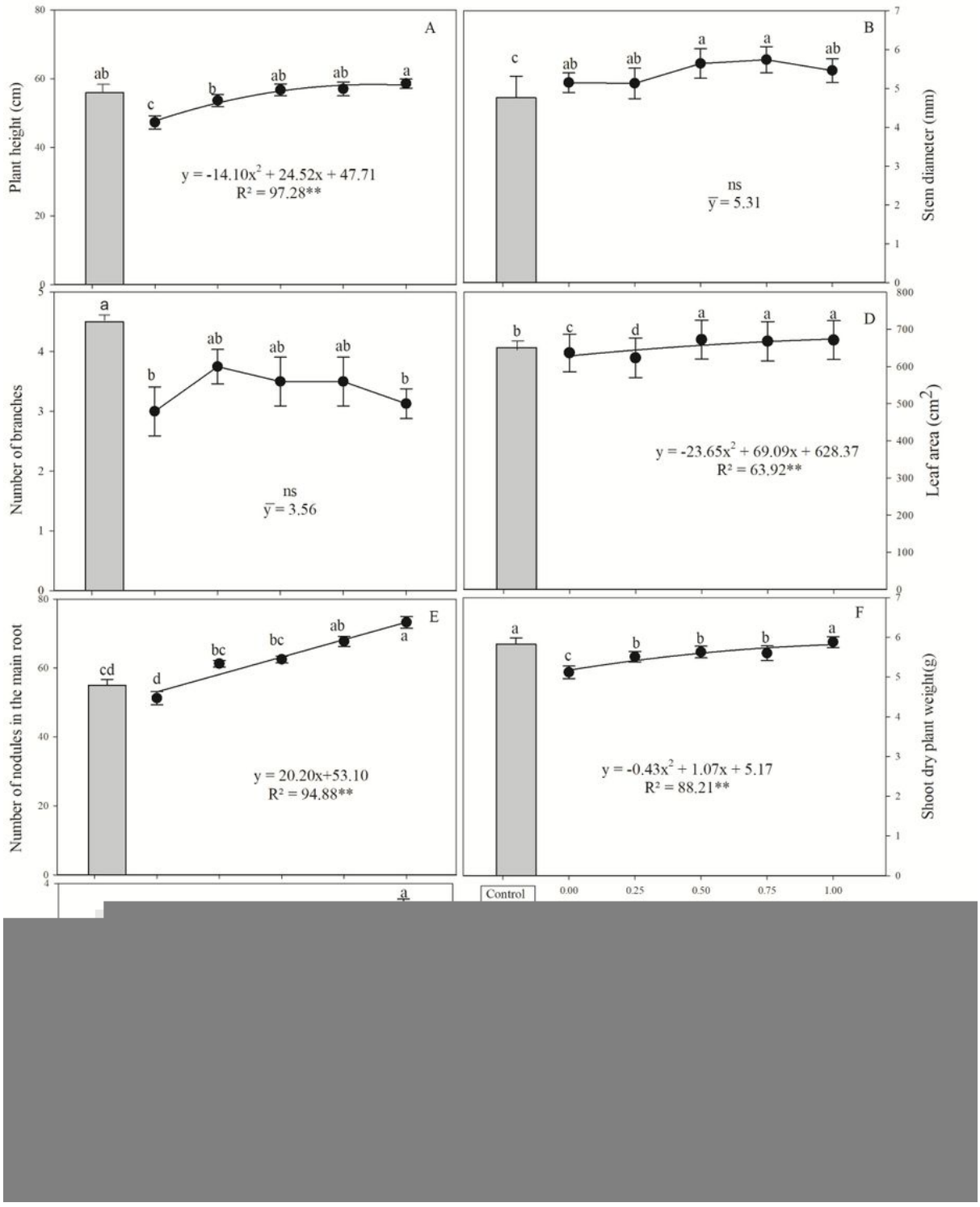

Figure 4

Biometric parameters plant height (A), stem diameter (B), number of branches (C), leaf area (D), number of nodules in the main root (E), shoot dry weight (F) and root dry weight (G) of soybean plants submitted to the application of different doses of biostimulant under heat stress. ns = not significant, * significant at

5% and ** significant at 1% and 5% by regression analysis. Averages followed by the same letter do not differ from each other by the Tukey test ($p \leq 0.05$).

Figure 5

Production components 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 (C) and productivity (E) of soybean plants submitted to the application of different doses of biostimulant under heat stress. ns = not significant, * significant at 5% and ** significant at 1% and 5% by regression analysis. Averages followed by the same letter do not differ from each other by the Tukey test ($p \leq 0.05$).

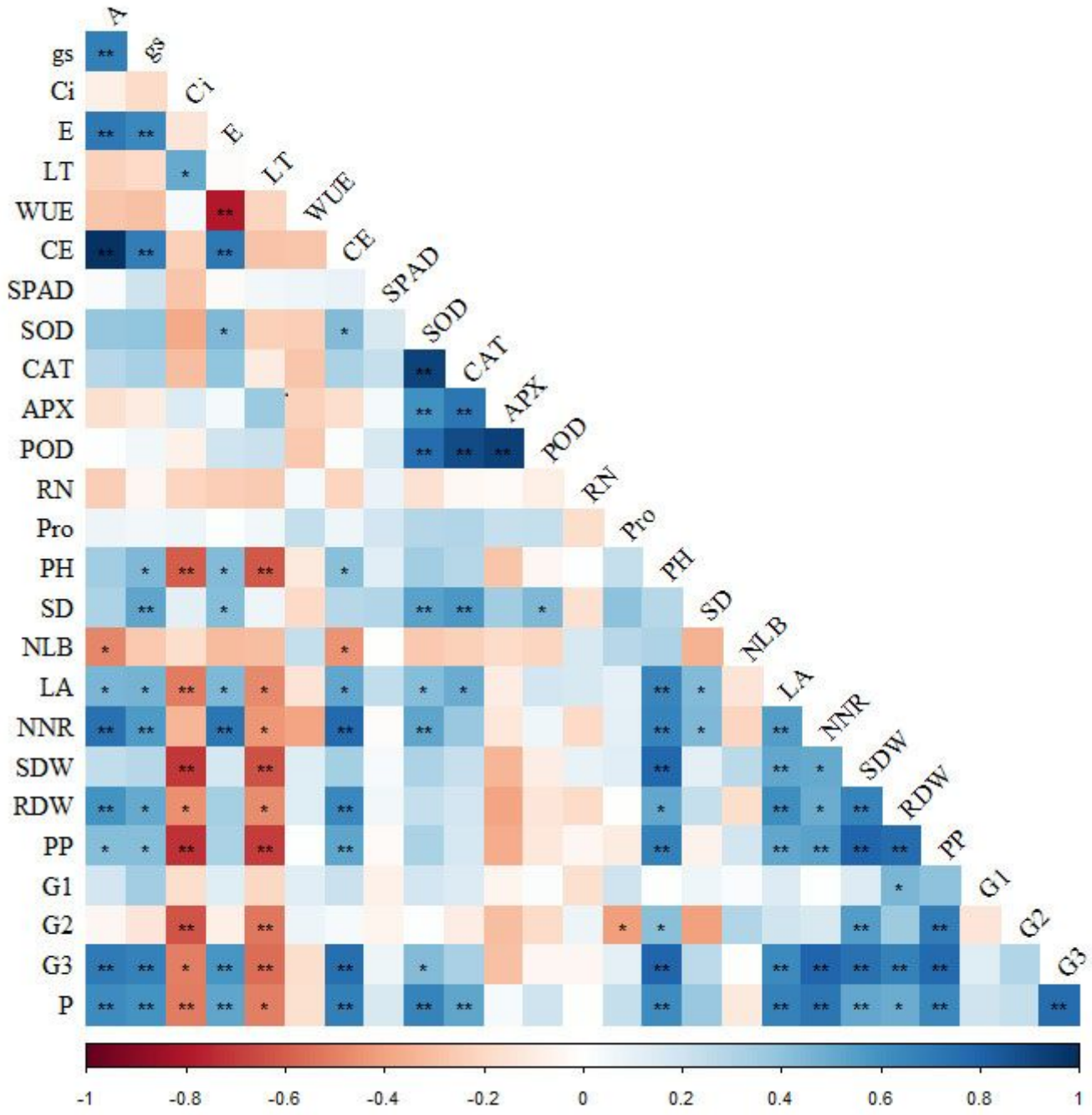

Figure 6

Pearson's correlation between CO_2 assimilation rate (A), stomatal conductance (g_s), transpiration rate (E), intercellular CO_2 concentration (C_i), leaf temperature (LT), Water-use efficiency (WUE), carboxylation efficiency (CE), SPAD index (SPAD), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidases (POD), nitrate reductase (RN), proline (Pro), height of plants (PH), diameter of plant stem (SD), number of lateral branches (NBL), leaf area (LA), number of nodules in the main root (NNR) , shoot dry plant weight (SDW), root dry weight (RDW), number of pods per plant (PP), average number of

pods with 1 grain (G1), average number of pods with 2 grains (G2), and average number of pods with 3 grains (G3), and productivity (P). **Significant at 5% *Significant at 1%