



Anatomical Modifications Modulated by Pretreatment with 24-Epibrassinolide Alleviate Boron Stress in Soybean Plants: Valuable Repercussions on Nutrient Contents, Photosynthesis, and Biomass

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Abstract

Boron (B) is an essential micronutrient for vascular plants, and its availability in the soil represents a limiting factor for world agricultural production. 24-Epibrassinolide (EBR) is a biodegradable and eco-friendly plant steroid hormone, with multiple benefits connected to growth and development. We hypothesized that inadequate B supplementation could limit plant development, causing damage to leaf and root structures. Therefore, the objective of this research was to verify the possible contributions of EBR in root and leaf structures and biomass accumulation in soybean plants under inadequate B supplies (deficiency or toxicity). The experiment followed a completely randomized factorial design with two concentrations of 24-epibrassinolide (0 and 100 nM EBR, described as –EBR and +EBR, respectively) combined with three B supplies (0.6, 30, and 1500 μ M B, described as low, control, and high supply of B). EBR alleviated the damages occasioned by the inadequate B supplies on root tissues, specifically maximizing the vascular cylinder, metaxylem, and epidermis, improving the nutritional status. This steroid also minimized the harmful effects of B stress on leaf anatomy, stimulating the epidermis on both leaf sides, palisade parenchyma, and spongy parenchyma; structures intrinsically related to protection and carbon dioxide availability to the photosynthetic process. Concomitantly, this steroid had a positive impact on biomass accumulation. These results are explained by beneficial actions on leaf structures and photosynthetic machinery. Therefore, our results demonstrate that the EBR application can improve soybean plants' tolerance under inadequate B supplementation.

Keywords Boron supplies · Brassinosteroids · *Glycine max* · Mesophyll · Metaxylem

1 Introduction

Soybean (*Glycine max*) is a leguminous species with high nutritional value, rich in proteins (Bamji and Corbitt 2017; Kim et al. 2006; Wang et al. 2008), being a commodity with broad importance in human and animal nutrition and industrial products (Goldsmith 2008; Nishinari et al. 2014). In the 2017/2018 harvest, world soybean production was around 336 million tons, with Brazil, the USA, and Argentina accounting for around 82% of world production. The root is the organ responsible for water and nutrient uptake, fixing the plant in the soil (Seago and Fernando 2013; Zeng et al. 2019), and is covered by the root epidermis. This tissue has direct contact with the soil solution, contributing to fluxes of ions (Du and Wei 2018; Javelle et al. 2011; Thomas et al.

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2007). The cortex is adjacent to the epidermis, consisting of parenchymal cells with spaces between them, working as storage tissue (Enstone et al. 2003; Pérez Chaca et al. 2014). The endodermis corresponds to the last layer of the cortex, acting as a barrier aiming to modulate the water and nutrient supplies due to the presence of Casparian strips (Barberon et al. 2016; Lux et al. 2011; Purushothaman et al. 2013).

The leaf is the main organ responsible for photosynthesis, frequently presenting stomata in abaxial and adaxial faces (Maia et al. 2018), and is covered by the epidermis that protects this organ against abiotic stresses (Gao et al. 2019; Nemeskéri and Helyes 2019; Wyka et al. 2019). Stomata modulate essential processes, such as CO₂ absorption, transpiration, and thermal regulation (Veza et al. 2018). In soybean plants, the mesophyll is formed by the palisade and spongy parenchyma (Gonçalves et al. 2017; Lansing and Franceschi 2000) being found in chloroplasts and other organelles that act during the photosynthesis process (Ju et al. 2017).

Boron (B) is an essential micronutrient for vascular plants, and its availability in the soil represents a limiting factor for world agricultural production (Kobayashi et al. 2018). B deficiency can be triggered by inadequate soil conditions, such as high pH and low organic matter content, and environmental factors, such as drought, high temperature, and light (Atique-ur-Rehman et al. 2018). Another problem is that B is susceptible to leaching in soil because it is highly soluble and mobile, which can easily lead to plant deficiency after excessive rainfall (Goli et al. 2019). On the other hand, in arid and semi-arid regions, there can be problems related to the toxicity of this micronutrient (Nawaz et al. 2020), where B can be taken to the surface layers of the soil due to the combined actions linked to the evaporation process of the soil water and capillary process (Yau and Ryan 2008).

In plants, B has structural and metabolic functions, being intrinsically related to calcium in cell wall development, acting in protein synthesis, sugar transport, respiration, and carbohydrate metabolism (Reid 2014; Shireen et al. 2018). Additionally, the mobility and permeability of this nutrient can vary by species (Wimmer and Eichert 2013). The B deficiency reflects mainly on meristematic tissues (Hänsch and Mendel 2009), affecting the root growth and the development of seeds, flowers, and fruits (Herrera-Rodríguez et al. 2010; Oiwa et al. 2013), also limiting absorption and transport of water and nutrients, due to damages to the root structures, including the xylem vessels (Li et al. 2017). On the other hand, B excess is associated with oxidative stress, in which B toxicity causes the overproduction of reactive oxygen species (ROS), such as superoxide (O₂⁻) and hydrogen peroxide (H₂O₂), causing an imbalance in metabolism, affecting the division and expansion of the plant cells (Farhaly et al. 2021; Sakamoto et al. 2011).

Brassinosteroids (BRs) are steroidal phytohormones that have multiple benefits on plant metabolism, including regulation of ion channels in the plasma membrane (Zhang et al. 2005), improvements in photosynthetic apparatus performance (Shahbaz et al. 2008), antioxidant metabolism (Zhang et al. 2008), and regulation of flowering and cell expansion (Clouse 2002). These steroids induce positive repercussions on root and leaf tissues (Maia et al. 2018; Oliveira et al. 2019; Ribeiro et al. 2019), being detected in plants exposed to nutritional stress (deficiency and/or excess) (Lima et al. 2018; Vriet et al. 2012; Zhang et al. 2008). Among the different types of BRs, 24-epibrassinolide (EBR) is the most used BR because it is considered the natural bioactive form and is biodegradable and highly efficient (Khrupach 2000; Maia et al. 2018).

We hypothesize that inadequate B supplementation can limit plant development, causing damage to leaf and root structures (Huang et al. 2014; Li et al. 2017; Mei et al. 2016). Recent studies have demonstrated that EBR can play an important role in plant development, especially under stressful conditions, because this steroid triggers benefits in root metaxylem (Santos et al. 2020), in nutritional status (Lima et al. 2018), also favoring the maintenance of chloroplastic pigments (Rodrigues et al. 2020) and improving the growth (Ribeiro et al. 2020). Therefore, the objective of this research was to verify the possible contributions of EBR in root and leaf structures and biomass accumulation in soybean plants under inadequate B supplies.

2 Materials and Methods

2.1 Location and Growth Conditions

The experiment was performed at the Campus of Paragominas of the Universidade Federal Rural da Amazônia, Paragominas, Brazil (2°55' S, 47°34' W). The study was conducted in a greenhouse with temperature and humidity-controlled. The minimum, maximum, and median temperatures were 24, 33, and 25.1 °C, respectively. The relative humidity during the experimental period varied between 60 and 80%.

2.2 Plants, Containers, and Acclimation

Seeds of *Glycine max* (L.) Merr. var. M8644RR Monsoy™ were germinated and grown in 1.2-L pots filled with a mixed substrate of sand and vermiculite at a ratio of 3:1. The plants were cultivated under semi-hydroponic conditions containing 500 mL of distilled water for 4 days. A nutritional solution described by Pereira et al. (2019) was used for plant nutrition, with ionic strength beginning at 50% (5th day) and later modified to 100% after 2 days (7th day). After

this period, the nutritional solution remained at total ionic strength.

2.3 Experimental Design

The experiment followed a completely randomized factorial design with two concentrations of 24-epibrassinolide (0 and 100 nM EBR, described as –EBR and +EBR, respectively) and three B supplies (0.6, 30, and 1500 μM B, described as low, control, and high supply of B). With five replicates for each of the six treatments, a total of 30 experimental units were used in the experiment, with one plant in each unit.

2.4 24-epibrassinolide Preparation and Application

Eight-day-old seedlings were sprayed with EBR or Milli-Q water (containing a proportion of ethanol equal to that used to prepare the EBR solution; described as –EBR) at 5-day intervals until day 28. Solution of EBR (Sigma-Aldrich, USA) (100 nM) was prepared by dissolving the solute in ethanol followed by dilution with Milli-Q water [ethanol: water (v/v) = 1:10000] (Ahammed et al. 2013a).

2.5 Plant Conduction and Boron Supplies

Plants received the following macro- and micronutrients contained in the nutrient solution in agreement with Pereira et al. (2019). For B treatments, H_3BO_3 was used at concentrations of 0.6 μM (low), 30 μM (control), and 1500 μM (high) applied over 12 days (days 16–28 after the start of the experiment). During the study, the nutrient solutions were changed at 07:00 h at 3-day intervals, with the pH adjusted to 5.5 using HCl or NaOH. On day 28 of the experiment, physiological and morphological parameters were measured for all plants, and leaf tissues were harvested for anatomical, biochemical, and nutritional analyses.

2.6 Chlorophyll Fluorescence, Gas Exchange, and Anatomical Variables

Chlorophyll fluorescence was measured as described by Maia et al. (2018). Gas exchange was evaluated following the calibration procedures described by Pereira et al. (2019). Samples were collected following the methodology of Oliveira et al. (2019) and O'Brien et al. (1964). Stomatal characterization was carried out according to Segatto et al. (2004).

2.7 Determination of Antioxidant Enzymes, Superoxide, and Soluble Proteins

Antioxidant enzymes (SOD, CAT, APX, and POX), superoxide, and soluble proteins were extracted from root tissues

according to the method of Badawi et al. (2004). Total soluble proteins were quantified using the methodology described by Bradford (1976). The SOD assay was measured at 560 nm (Giannopolitis and Ries 1977), and the SOD activity was expressed in unit mg^{-1} protein. The CAT assay was detected at 240 nm (Havir and McHale 1987), with CAT activity expressed as $\mu\text{mol H}_2\text{O}_2 \text{ mg}^{-1} \text{ protein min}^{-1}$. The APX assay was measured at 290 nm (Nakano and Asada 1981), and the APX activity was expressed in $\mu\text{mol AsA mg}^{-1} \text{ protein min}^{-1}$. The POX assay was detected at 470 nm (Cakmak and Marschner 1992), with the activity expressed in $\mu\text{mol tetraguaiacol mg}^{-1} \text{ protein min}^{-1}$. The determination of O_2^- was measured at 530 nm (Elstner and Heupel 1976).

2.8 Quantification of Hydrogen Peroxide, Malondialdehyde, and Electrolyte Leakage

Stress indicators (H_2O_2 and MDA) were extracted using the methodology described by Wu et al. (2006). H_2O_2 was measured with the procedures defined by Velikova et al. (2000). MDA was determined by the method of Cakmak and Horst (1991), using the extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$. EL was measured according to Gong et al. (1998) and is calculated by the formula $\text{EL} (\%) = (\text{EC}_1/\text{EC}_2) \times 100$.

2.9 Determination of Photosynthetic Pigments, Nutrient Contents, and Biomass

The chlorophyll and carotenoid determinations were performed using a spectrophotometer (model UV-M51; Bel Photonics), according to the methodology of Lichtenthaler and Buschmann (2001). The determination of B, K, Ca, S, Cu, Mn, and Mo was carried out using an inductively coupled plasma mass spectrometer (model ICP-MS 7900; Agilent). The growth of roots, stems, and leaves was measured based on constant dry weights (g) after drying in a forced-air ventilation oven at 65 °C.

2.10 Data Analysis

The normality of residues was checked using Shapiro–Wilk test. Data were subjected to two-way ANOVA, and significant differences between the means were determined using the Scott–Knott test at a probability level of 5% (Steel et al. 2006). All statistical procedures used the Assstat 7.7 software.

3 Results

Steroid application increased B contents in treatments control and with low B supply

The low and high B supplies promoted changes in B contents in the root, stem, and leaf tissues of soybean plants (Table 1). Plants sprayed with EBR and submitted to low B presented increases in B contents of 56%, 2%, and 19% in root, stem, and leaf, respectively, compared with the same treatment without EBR. The control + EBR treatment had increases of 36%, 26%, and 4% in root, stem, and leaf, respectively. However, plants submitted to high B and sprayed with EBR presented reductions of 26%, 12%, and 6% in root, stem, and leaf, respectively.

EBR maximized the protection of roots and CO₂ availability in leaves under B stress.

Low and high B supplies occasioned decreases in root anatomy (Table 2 and Fig. 1). Plants sprayed with EBR and exposed to low B treatment had increased RET (root epidermis thickness), RDT (root endodermis thickness), RCD (root cortex diameter), VCD (vascular cylinder diameter),

and RMD (root metaxylem diameter) by 6%, 5%, 29%, 14%, and 4%, respectively when compared to the same treatment without EBR. While control + EBR treatment presented increases of 12% (RET), 15% (RDT), 21% (RCD), 6% (VCD), and 4% (RMD). To high B with EBR, the variables RET, RDT, RCD, VCD, and RMD had increases of 21%, 34%, 8%, 10%, and 12%, respectively. To leaf structures (Table 2 and Fig. 1), low and high B supplies promoted decreases, except for the PPT/SPT ratio. For ETAd, ETAb, PPT, and SPT plants sprayed with EBR under the low B treatment, we detected increases of 14%, 18%, 27%, and 32%, respectively, while the control treatment had increases of 3%, 18%, 6%, and 10%, respectively. High B presented increases of 20%, 8%, 14%, and 17% respectively. While for PPT/SPT, there were reductions of 4% (low B), 4% (control), and 4% (high B) in plants treated with EBR, if compared to equal treatment without EBR.

Table 1 Boron contents in soybean plants sprayed with EBR and exposed to different B supplies

EBR	B supply	B in root ($\mu\text{g g DM}^{-1}$)	B in stem ($\mu\text{g g DM}^{-1}$)	B in leaf ($\mu\text{g g DM}^{-1}$)
–	Low	14.34 ± 0.96 ^{Cb}	12.54 ± 0.64 ^{Ca}	13.85 ± 0.75 ^{Cb}
–	Control	17.77 ± 0.59 ^{Bb}	16.78 ± 0.70 ^{Bb}	24.45 ± 1.89 ^{Ba}
–	High	77.95 ± 3.79 ^{Aa}	40.71 ± 2.09 ^{Aa}	219.08 ± 2.06 ^{Aa}
+	Low	22.36 ± 1.00 ^{Ba}	12.74 ± 0.75 ^{Ca}	16.54 ± 0.53 ^{Ca}
+	Control	24.13 ± 1.03 ^{Ba}	21.18 ± 1.35 ^{Ba}	25.44 ± 0.63 ^{Ba}
+	High	57.71 ± 1.11 ^{Ab}	35.91 ± 1.40 ^{Ab}	204.92 ± 2.82 ^{Ab}

B = boron; EBR = 24-epibrassinolide. Columns with different uppercase letters between B supplies (low, control, and high B supply under equal EBR level) and lowercase letters between EBR level (with and without EBR under equal B supply) indicate significant differences from the Scott-Knott test ($P < 0.05$). Means ± SD, $n = 5$

Table 2 Root and leaf structures in soybean plants sprayed with EBR and exposed to different B supplies

EBR	B supply	RET (μm)	RDT (μm)	RCD (μm)	VCD (μm)	RMD (μm)
–	Low	9.3 ± 0.2 ^{Bb}	15.5 ± 0.7 ^{Ba}	282 ± 12 ^{Bb}	223 ± 8 ^{Bb}	54.2 ± 0.5 ^{Cb}
–	Control	10.6 ± 0.6 ^{Ab}	18.7 ± 0.7 ^{Ab}	358 ± 24 ^{Ab}	258 ± 14 ^{Aa}	64.1 ± 0.3 ^{Ab}
–	High	8.1 ± 0.3 ^{Cb}	12.5 ± 0.2 ^{Cb}	289 ± 7 ^{Bb}	237 ± 6 ^{Bb}	55.8 ± 0.3 ^{Bb}
+	Low	9.9 ± 0.3 ^{Ba}	16.3 ± 0.6 ^{Ba}	363 ± 20 ^{Ba}	255 ± 15 ^{Ba}	56.1 ± 0.7 ^{Ca}
+	Control	11.9 ± 0.7 ^{Aa}	21.5 ± 0.8 ^{Aa}	432 ± 10 ^{Aa}	273 ± 9 ^{Aa}	66.6 ± 0.6 ^{Aa}
+	High	9.8 ± 0.6 ^{Ba}	16.7 ± 0.4 ^{Ba}	313 ± 17 ^{Ca}	261 ± 9 ^{Ba}	62.7 ± 0.9 ^{Ba}
EBR	B supply	ETAd (μm)	ETAb (μm)	PPT (μm)	SPT (μm)	Ratio PPT/SPT
–	Low	16.1 ± 1.1 ^{Bb}	13.7 ± 0.9 ^{Bb}	74 ± 4 ^{Bb}	47 ± 2 ^{Bb}	1.57 ± 0.04 ^{Aa}
–	Control	20.4 ± 1.1 ^{Aa}	15.8 ± 0.6 ^{Ab}	107 ± 3 ^{Ab}	71 ± 4 ^{Ab}	1.51 ± 0.07 ^{Aa}
–	High	15.3 ± 1.1 ^{Bb}	14.2 ± 0.9 ^{Bb}	74 ± 3 ^{Bb}	47 ± 1 ^{Bb}	1.58 ± 0.09 ^{Aa}
+	Low	18.4 ± 0.9 ^{Ba}	16.1 ± 0.8 ^{Ba}	94 ± 4 ^{Ba}	62 ± 2 ^{Ba}	1.50 ± 0.06 ^{Aa}
+	Control	21.1 ± 1.4 ^{Aa}	18.6 ± 0.8 ^{Aa}	113 ± 4 ^{Aa}	78 ± 2 ^{Aa}	1.45 ± 0.08 ^{Aa}
+	High	18.4 ± 1.1 ^{Ba}	15.3 ± 0.7 ^{Ba}	84 ± 6 ^{Ca}	55 ± 3 ^{Ca}	1.52 ± 0.06 ^{Aa}

B, boron; EBR, 24-epibrassinolide; RET, root epidermis thickness; RDT, root endodermis thickness; RCD, root cortex diameter; VCD, vascular cylinder diameter; RMD, root metaxylem diameter; ETAd, epidermis thickness from adaxial leaf side; ETAb, epidermis thickness from abaxial leaf side; PPT, palisade parenchyma thickness; SPT, spongy parenchyma thickness. Columns with different uppercase letters between B supplies (low, control, and high B supply under equal EBR level) and lowercase letters between EBR level (with and without EBR under equal B supply) indicate significant differences from the Scott-Knott test ($P < 0.05$). Means ± SD, $n = 5$

3.1 Nutritional Status was Improved after EBR Pretreatment

The low and high B supplies promoted reductions in nutrient contents in root, stem, and leaf tissues (Table 3). Plants sprayed with EBR and exposed to low B presented increases in K, Ca, S, Cu, Mn, and Mo contents of 21%, 1%, 16%, 33%, 17%, and 3% in the root; 6%, 2%, 21%, 25%, 1%, and 21% in the stem; and 9%, 14%, 10%, 13%, 7%, and 10% in leaf, respectively, when compared to the same treatment without EBR. The control treatment with EBR also had increases in K, Ca, S, Cu, Mn, and Mo of 21%, 14%, 24%, 9%, 7%, and 7% (root); 4%, 1%, 5%, 12%, 33%, and 9% (stem); and 8%, 6%, 11%, 11%, 14%, and 18% (leaf) in this order, when compared to the same treatment without EBR. Plants submitted to high B with EBR presented increments in K, S, Cu, Mn, and Mo of 8%, 16%, 5%, 25%, and 5%, but Ca suffered a reduction of 2% in the root. In the stem, there were verified increases in K, Ca, S, Cu, Mn, and Mo of 5%, 7%, 9%, 14%, 8%, and 11%. To leaf, we detected increases of 11%, 9%, 7%, 9%, 5%, and 14%, respectively, when compared to the same treatment without EBR.

3.2 B Stressed Plants Suffered Minor Oxidative Damage on Photosynthetic Machinery

The low and high supplementation with B reduced the photosynthetic pigments considering Chl *a*, Chl *b*, Total Chl, and Car variables (Table 4), causing increases in Chl *a*/Chl *b* and Chl/Car when compared to the control treatment. Plants sprayed with EBR and exposed to low B treatment had increased Chl *b*, Total Chl, and Car (by 8%, 2%, and 6%, respectively) compared to the same treatment without EBR; the control treatment showed increases of 26%, 33%, 28%, and 27%, respectively; and in the high B supply, there were increases of 5%, 24%, 9%, and 16% in Chl *a*, Chl *b*, Total Chl, and Car, respectively. In plants sprayed with EBR, the Chl *a*/Chl *b*, and Chl/Car ratios showed reductions compared to non-pulverized plants. Concerning chlorophyll fluorescence (Fig. 2), low and high B treatments promoted reductions, except F_0 . For F_0 , plants sprayed with EBR had reductions of 11%, 13%, and 2% for the low, control, and high B treatments, respectively, compared to the same treatment without EBR (Fig. 2A). In relation to F_m (Fig. 2B), F_v (Fig. 2C), and F_v/F_m (Fig. 2D) under the application of EBR, the low B supplement had increases of 1%, 4%, and 3%, respectively; in the control B + EBR treatment there were increases of 1%, 4%, and 4%, respectively; and the high B with EBR treatment presented increments of 4%, 6%, and 2%, respectively. On photosystem II (Table 4), low and high B supplies induced reductions in Φ_{PSII} , q_p , and ETR, while increases in NPQ, EXC, and ETR/P_N presented increases compared to the control treatment. Plants sprayed

with EBR and exposed to low treatment had increased Φ_{PSII} , q_p , and ETR by 21%, 6%, and 19%, respectively, whereas for NPQ, EXC, and ETR/P_N decreased by 9%, 10%, and 32%, respectively. In the control treatment + EBR, Φ_{PSII} , q_p , and ETR presented increases of 12%, 10%, and 13%, respectively, whereas NPQ, EXC, and ETR/P_N presented reductions of 5%, 5%, and 7%, respectively. The high B supply + EBR treatment showed increases of 13%, 14%, and 2% for the variables Φ_{PSII} , ETR, and NPQ, respectively, but for q_p , EXC, and ETR/P_N , there were reductions of 7%, 6%, and 9%, respectively. Concerning gas exchange (Table 4), low and high B supplies caused reductions. Plants sprayed with EBR and exposed to low treatment resulted in increases for E , g_s , P_N , and P_N/C_i of 77%, 133%, 79%, and 123%, respectively, whereas for C_i , there was a reduction of 20%. Control plants with EBR, P_N , WUE, and P_N/C_i presented increases of 21%, 55%, and 24%, respectively, while C_i , E , and g_s had reductions of 3%, 21%, and 18%, respectively. The treatment with high B + EBR presented increases of 24%, 38%, and 25% in P_N , WUE, and P_N/C_i , respectively, and for C_i , E , and g_s , there were reductions of 2%, 4%, and 8%, respectively.

3.3 Stomatal Performance was Upregulated after EBR Application

The low and high B supplies reduced stomatal characteristics, except for PDS and EDS (Table 5). Plants sprayed with EBR under the low treatment B on the adaxial face presented increases for SD, SF, and SI of 30%, 6%, and 42%, respectively, compared to the same treatment without EBR. Under the control treatment, the adaxial face had increased for SD and SI by 9% and 18%, respectively; in high B treatment, there were increases of 23%, 5%, and 28% in SD, SF, and SI, respectively. For PDS and EDS in the abaxial face, EBR + low B induced decreases of 13% and 12%, respectively. Under control treatment combined with EBR suffered decreases of 2% (PDS) and 6% (EDS) and under the high B with EBR had reductions of 13% in PDS and 12% in EDS.

3.4 EBR Spray Increased the Activities of Antioxidant Enzymes in Plants Exposed to B Stress

Low and high B supplies increased enzyme activities (Fig. 3). For SOD, plants sprayed with EBR had increases of 176%, 115%, and 73% in supplements low, control, and high B, respectively, relative to the same treatment without EBR (Fig. 3A). Concerning CAT, plants exposed to EBR presented increases of 9%, 17%, and 27% in the low, control, and high B treatments, respectively (Fig. 3B). To APX, plants pretreated with EBR presented elevations of 21%, 13%, and 42% in the low, control, and high B treatments, respectively (Fig. 3C). In POX, plants with EBR had increases of 21%

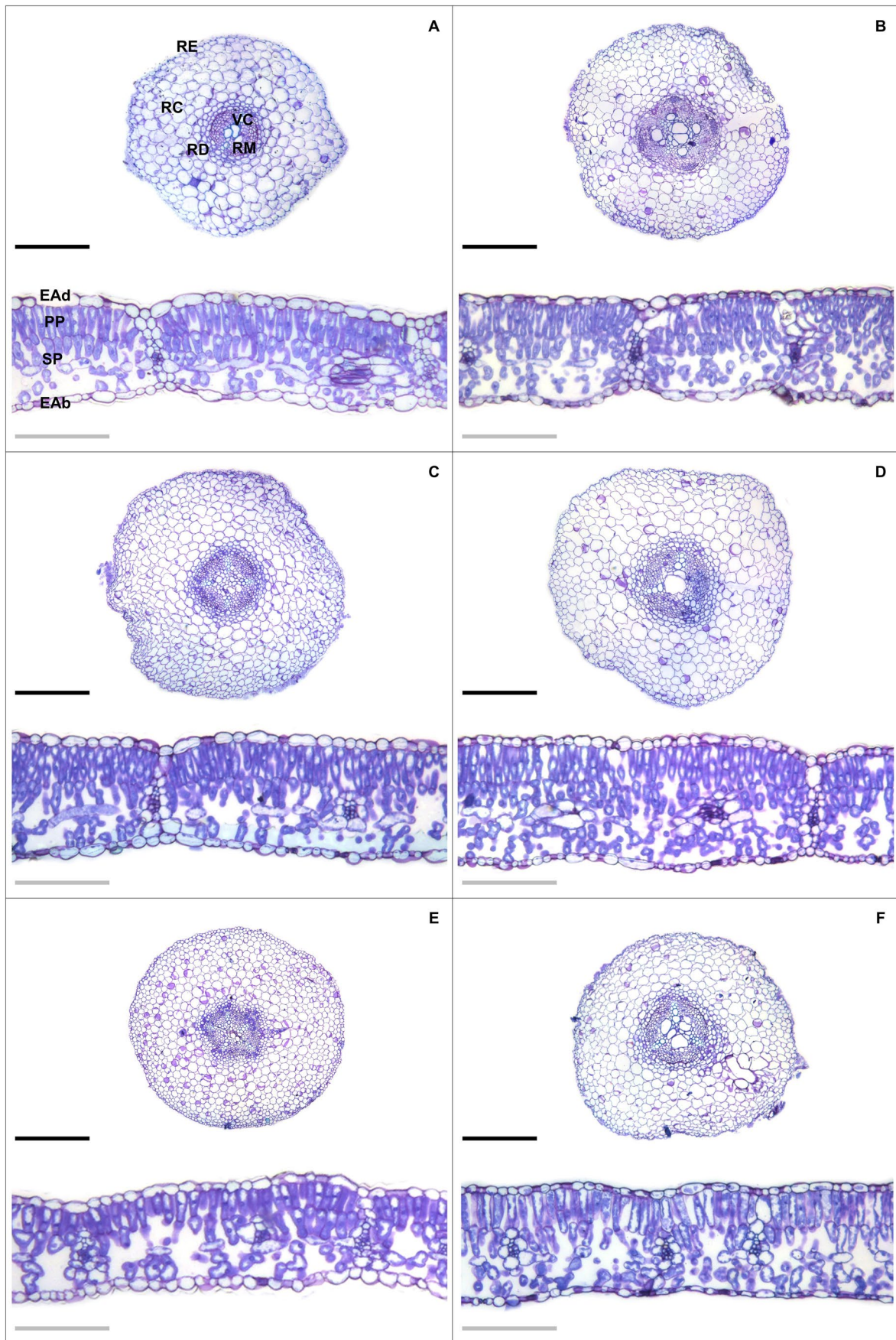


Fig. 1 Root and leaf cross sections in soybean plants sprayed with 24-epibrassinolide (EBR) and exposed to different boron (B) supplies. B low/–EBR (A), B low/+EBR (B), B control/–EBR (C), B control/+EBR (D), B high/–EBR (E), and B high/+EBR (F). RE root epidermis, RC root cortex, RD root endodermis, VC vascular cylinder, RM root metaxylem, EAd adaxial epidermis, EAb abaxial epidermis, PP palisade parenchyma, SP spongy parenchyma. Black bars, 300 μm ; grey bars, 150 μm

(low B), 48% (control), and 48% (high B) (Fig. 3D). On stress indicators (Fig. 4), supplies with low and high B levels caused increases. For O_2^- , plants sprayed with EBR had reductions of 1%, 2%, and 26% in low, control, and high B, respectively, compared to the same treatment without EBR (Fig. 4A). Concerning H_2O_2 , plants sprayed with EBR had decreases of 2%, 14%, and 10% in the low, control, and high B treatments, respectively (Fig. 4B). To MDA, plants pre-treated with EBR presented reductions of 9%, 3%, and 33% in treatments using low, control, and high B, respectively (Fig. 4C). EL (plants with EBR) was decreased by 1%, 19%, and 5% under low, control, and high B supplies, respectively (Fig. 4D).

3.5 EBR Mitigated the B Stress-Induced Effects on Biomass

The low and high B supplies promoted significant reductions in growth compared to the control treatment (Fig. 5), except for RDM. For LDM, plants sprayed with EBR and exposed to the control treatment had a 6% increase compared with equal treatment without EBR (Fig. 5A). Concerning RDM, the treatments under low, control, and high B + EBR had increases of 15%, 8%, and 61%, in this order (Fig. 5B). To SDM (Fig. 5C) and TDM (Fig. 5D), the low B supply + EBR promoted increases of 7% and 1%, respectively; whereas the control supply with EBR presented increases of 1% and 5%, respectively; and under high B + EBR, increases of 5% and 10%, respectively.

4 Discussion

Our findings provide evidence supporting the ability of EBR to alleviate the negative effects triggered by B stress (deficiency or toxicity) in soybean plants. The EBR application reduced B contents in plants subjected to toxicity and increased B contents in control and low B treatments. The positive effects detected in the control and low B can be attributed to improvements in the transport mechanisms of this element in the plant, especially to inflow channels and transporters BOR1, BOR2, and NIP5. The BOR2 transporter promotes the cross-linking of the pectin polysaccharide rhamnogalacturonan II (RG-II) and root elongation, maximizing nutrient uptake, including B (Takada et al. 2014).

The NIP5 influx channels are located in the epidermis, cortex, and endoderm cells, while NIP6 is required for transport between the xylem and phloem (Robert and Friml 2009; Takano et al. 2008; Tanaka et al. 2008).

On the other hand, under B toxicity, EBR probably stimulated the BOR4 transporter, which is located in the endoderm and pericycle, being responsible for the detoxification mechanism of B in the plant via secretion (Miwa et al. 2007; Takano et al. 2008). Concomitantly, EBR-induced benefits on antioxidant metabolism, contributing to homeostasis and reducing reactive oxygen species (ROS) often found in plants exposed to stress conditions, including B toxicity (Bajguz and Hayat 2009). B is a micronutrient with high plant mobility (Takano et al. 2008), and multiple B transport mechanisms have been described in the literature (Robert and Friml 2009). This element is a small molecule presenting high permeability relative to other nutrients, with uptake linked to several transporters and broad distribution in plant tissues (Reid 2014). Landi et al. (2012) reported that B toxicity causes oxidative stress due to B accumulation in leaf cell walls, causing imbalances of cytoplasmic metabolism. Devi et al. (2012), evaluating soybean responses under different concentrations of B (0.5, 1.0, 1.5, and 2.0 kg ha^{-1}), observed increases in B contents in leaves and stems.

Soybean plants exposed to deficiency and toxicity of B suffered reductions related to root anatomy variables, but the use of EBR mitigated these effects, reflected in increases in RET, RDT, RCD, VCD, and RMD. The epidermis, endodermis, and cortex are specialized tissues linked to the protection and ionic transport of the apoplast in the direction of the symplast (Scheres et al. 2002). The endoderm still acts as an apoplastic barrier controlling water and nutrient uptake (Enstone et al. 2003; Lux et al. 2004). Increases in RET, RDT, and RCD indicate that EBR spray reduced the damages caused by the B stresses, promoting the increase of the cell expansion rate in these tissues and stimulating the mitotic cycle to maintain cell proliferation in the meristem (Hacham et al. 2011). The effects of EBR on RMD indicate that it promotes benefits on root protection, with gradual increase and consequent improvement in hydraulic conductivity (Hameed et al. 2009). With the thickening of the root, higher absorption of water and nutrients can occur due to positive repercussions on vascularization (Meyer et al. 2011). Ghanati et al. (2005) demonstrated that soybean seedlings exposed to B toxicity (5 mM) had inhibition of root growth due to hypodermis formation and suberin deposition in cortical cell walls corroborated by our research. A study conducted by Aquea et al. (2012) investigating the effects of B toxicity on *Arabidopsis thaliana* demonstrated that there were cellular changes in the root meristem, inhibiting root growth.

EBR had positive effects on leaf anatomy (ETAd, ETAb, PPT, and SPT). These results evidenced the ability of this

Table 3 Nutrient contents in soybean plants sprayed with EBR and exposed to different B supplies

EBR	B supply	K (mg g DM ⁻¹)	Ca (mg g DM ⁻¹)	S (mg g DM ⁻¹)	Cu (µg g DM ⁻¹)	Mn (µg g DM ⁻¹)	Mo (µg g DM ⁻¹)
Contents in root							
–	Low	24.26 ± 0.72 ^{Cb}	8.38 ± 0.09 ^{Ba}	2.25 ± 0.08 ^{Cb}	5.70 ± 0.37 ^{Cb}	281.30 ± 8.59 ^{Bb}	4.93 ± 0.18 ^{Ca}
–	Control	29.86 ± 0.93 ^{Ab}	11.01 ± 0.68 ^{Ab}	3.00 ± 0.13 ^{Ab}	8.80 ± 0.06 ^{Ab}	326.67 ± 5.67 ^{Ab}	5.38 ± 0.19 ^{Ab}
–	High	26.81 ± 0.51 ^{Bb}	10.29 ± 0.20 ^{Aa}	2.41 ± 0.07 ^{Bb}	8.28 ± 0.07 ^{Bb}	259.66 ± 12.83 ^{Cb}	5.15 ± 0.10 ^{Bb}
+	Low	29.43 ± 0.64 ^{Ba}	8.44 ± 0.31 ^{Ca}	2.60 ± 0.06 ^{Ca}	7.58 ± 0.17 ^{Ca}	328.21 ± 11.96 ^{Ba}	5.09 ± 0.12 ^{Ca}
+	Control	34.90 ± 1.14 ^{Aa}	12.53 ± 0.73 ^{Aa}	3.71 ± 0.11 ^{Aa}	9.58 ± 0.11 ^{Aa}	349.21 ± 6.68 ^{Aa}	5.75 ± 0.11 ^{Aa}
+	High	28.94 ± 1.12 ^{Ba}	10.10 ± 0.84 ^{Ba}	2.79 ± 0.11 ^{Ba}	8.68 ± 0.19 ^{Ba}	323.95 ± 14.56 ^{Ba}	5.41 ± 0.19 ^{Ba}
Contents in stem							
–	Low	46.33 ± 0.74 ^{Cb}	8.50 ± 0.09 ^{Ca}	1.23 ± 0.20 ^{Bb}	0.84 ± 0.03 ^{Cb}	9.97 ± 0.51 ^{Ba}	4.44 ± 0.20 ^{Cb}
–	Control	55.12 ± 0.54 ^{Ab}	10.41 ± 0.34 ^{Aa}	1.63 ± 0.08 ^{Aa}	1.21 ± 0.06 ^{Ab}	14.53 ± 0.77 ^{Ab}	5.56 ± 0.11 ^{Ab}
–	High	52.30 ± 1.38 ^{Bb}	8.97 ± 0.27 ^{Bb}	1.37 ± 0.05 ^{Ba}	1.11 ± 0.04 ^{Bb}	13.81 ± 0.46 ^{Ab}	5.13 ± 0.10 ^{Bb}
+	Low	48.93 ± 1.11 ^{Ca}	8.71 ± 0.15 ^{Ca}	1.49 ± 0.08 ^{Ba}	1.05 ± 0.05 ^{Ca}	10.05 ± 0.34 ^{Ca}	5.39 ± 0.14 ^{Ca}
+	Control	57.41 ± 1.83 ^{Aa}	10.54 ± 0.10 ^{Aa}	1.71 ± 0.09 ^{Aa}	1.35 ± 0.05 ^{Aa}	19.34 ± 0.83 ^{Aa}	6.07 ± 0.14 ^{Aa}
+	High	55.15 ± 1.32 ^{Ba}	9.59 ± 0.13 ^{Ba}	1.49 ± 0.03 ^{Ba}	1.27 ± 0.05 ^{Ba}	14.86 ± 0.46 ^{Ba}	5.69 ± 0.06 ^{Ba}
Contents in leaf							
–	Low	22.05 ± 0.85 ^{Cb}	8.09 ± 0.16 ^{Cb}	2.37 ± 0.09 ^{Cb}	0.88 ± 0.04 ^{Bb}	25.04 ± 0.43 ^{Cb}	2.92 ± 0.07 ^{Cb}
–	Control	27.23 ± 1.38 ^{Ab}	10.06 ± 0.45 ^{Ab}	2.81 ± 0.12 ^{Ab}	1.27 ± 0.08 ^{Ab}	32.83 ± 0.83 ^{Ab}	4.04 ± 0.11 ^{Ab}
–	High	24.94 ± 0.63 ^{Bb}	9.56 ± 0.25 ^{Bb}	2.62 ± 0.08 ^{Bb}	1.19 ± 0.08 ^{Ab}	31.53 ± 0.48 ^{Bb}	3.85 ± 0.10 ^{Bb}
+	Low	23.94 ± 0.63 ^{Ca}	9.20 ± 0.95 ^{Ba}	2.60 ± 0.07 ^{Ca}	0.99 ± 0.06 ^{Ca}	26.80 ± 1.17 ^{Ca}	3.21 ± 0.14 ^{Ca}
+	Control	29.30 ± 0.68 ^{Aa}	10.70 ± 0.21 ^{Aa}	3.11 ± 0.18 ^{Aa}	1.41 ± 0.05 ^{Aa}	37.36 ± 0.74 ^{Aa}	4.76 ± 0.19 ^{Aa}
+	High	27.62 ± 1.31 ^{Ba}	10.44 ± 0.17 ^{Aa}	2.81 ± 0.12 ^{Ba}	1.30 ± 0.04 ^{Ba}	32.97 ± 0.99 ^{Ba}	4.40 ± 0.12 ^{Ba}

B, boron; EBR, 24-epibrassinolide; K, potassium; Ca, calcium; S, sulfur; Cu, copper; Mn, manganese; Mo, molybdenum. Columns with different uppercase letters between boron (B) supplies (low, control, and high B supply under equal 24-epibrassinolide (EBR) level) and lowercase letters between EBR level (with and without EBR under equal B supply) indicate significant differences from the Scott-Knott test ($P < 0.05$). Means ± SD, $n = 5$

steroid to mitigate the damage to leaf structures exposed to B toxicity. On the other hand, B deficiency interferes negatively with cell wall structures (Meriño-Gergichevich et al. 2017), causing imbalances in water relations and reductions in leaf elongation rate (Wimmer and Eichert 2013). EBR spray on plants with B deficiency, or toxicity promoted leaf integrity and anatomy (Shahbaz and Ashraf 2007). Sotiropoulos et al. (2002) studied *Actinidia deliciosa* and *Actinidia arguta* species submitted to five B treatments (20, 50, 100, 200, and 500 µM B) and observed a reduced thickness of the leaf cross-section due to the reduction of PPT and SPT.

In general, EBR treatment causes increases in macro-nutrient and micronutrient contents in low, adequate, and high B supplies because EBR is efficient in regulating the absorption of ions in the plant cell (Khrpach 2000; Shahbaz and Ashraf 2007). B modulates the secretory activities in the membranes, exerting influence on proton extrusion and electric potential generation, which is essential for ATPase enzyme activities, causing membrane hyperpolarization and consequent stimulation in the absorption of K and Ca ions (Ahmad et al. 2009; Brown et al. 2002). In plants under B deficiency and toxicity, the membrane properties linked to nutrient uptake often modified, explaining the reductions in nutritional contents (K, Ca, S, Cu, Mn, and Mo). Davis et al.

(2003), evaluating the responses of tomato plants under the soil and leaf application of B, observed increases in K and Ca contents in the shoot, similar to our results.

EBR spray in plants under B stress (low and high supplies) increased the photosynthetic pigments (Chl *a*, Chl *b*, Total Chl, and Car), suggesting that the EBR helped to pigment membrane damages, which was confirmed by the reductions of MDA and EL. In other words, ROS accumulation causes oxidative damage and lipid peroxidation, producing MDA with subsequent increments of EL, signaling deleterious effects on membrane integrity (Genisel et al. 2013; Li et al. 2018; Mito et al. 2019; Sun et al. 2015; Yao et al. 2017). Similar results were found by Zhang et al. (2014) and Dong et al. (2017), where EBR spraying increased the levels of Chl *a*, Chl *b*, and Total Chl in melon plants subjected to high temperature and in wheat plants under saline stress (120 mM NaCl).

EBR application in plants exposed to different B concentrations promoted better results concerning chlorophyll fluorescence. Low and high B supplies caused inhibition of the processes linked to the electron transport chain. However, our results revealed that the EBR treatment was able to increase F_m , F_v , and F_v/F_m values and reduce F_0 , demonstrating beneficial effects on photochemical reactions of

Table 4 Photosynthetic pigments, chlorophyll fluorescence, and gas exchange in soybean plants sprayed with EBR and exposed to different B supplies

EBR	B supply	Chl <i>a</i> (mg g ⁻¹ FM)	Chl <i>b</i> (mg g ⁻¹ FM)	Total Chl (mg g ⁻¹ FM)	Car (mg g ⁻¹ FM)	Ratio Chl <i>a</i> /Chl <i>b</i>	Ratio Total Chl/Car
-	Low	4.99 ± 0.33 ^{Aa}	1.58 ± 0.09 ^{Ba}	6.57 ± 0.31 ^{Ba}	1.27 ± 0.08 ^{Ba}	3.16 ± 0.18 ^{Ba}	5.17 ± 0.09 ^{Ba}
-	Control	5.40 ± 0.45 ^{Ab}	1.87 ± 0.15 ^{Ab}	7.27 ± 0.42 ^{Ab}	1.45 ± 0.06 ^{Ab}	2.89 ± 0.26 ^{Ba}	5.01 ± 0.45 ^{Ba}
-	High	5.23 ± 0.50 ^{Aa}	1.47 ± 0.07 ^{Bb}	6.70 ± 0.50 ^{Bb}	1.05 ± 0.03 ^{Cb}	3.56 ± 0.20 ^{Aa}	6.38 ± 0.56 ^{Aa}
+	Low	5.00 ± 0.31 ^{Ba}	1.71 ± 0.10 ^{Ba}	6.71 ± 0.40 ^{Ca}	1.34 ± 0.07 ^{Ba}	2.92 ± 0.12 ^{Aa}	5.00 ± 0.33 ^{Ba}
+	Control	6.82 ± 0.36 ^{Aa}	2.48 ± 0.13 ^{Aa}	9.30 ± 0.28 ^{Aa}	1.84 ± 0.14 ^{Aa}	2.75 ± 0.27 ^{Aa}	5.05 ± 0.37 ^{Ba}
+	High	5.51 ± 0.32 ^{Ba}	1.82 ± 0.02 ^{Ba}	7.33 ± 0.33 ^{Ba}	1.22 ± 0.10 ^{Ca}	3.03 ± 0.17 ^{Ab}	6.01 ± 0.36 ^{Aa}
EBR	B supply	Φ _{PSII}	q _p	NPQ	ETR (μmol m ⁻² s ⁻¹)	EXC (μmol m ⁻² s ⁻¹)	ETR/P _N
-	Low	0.29 ± 0.00 ^{Bb}	0.69 ± 0.05 ^{Ba}	1.07 ± 0.07 ^{Aa}	42.9 ± 0.6 ^{Bb}	0.62 ± 0.01 ^{Aa}	10.78 ± 0.63 ^{Aa}
-	Control	0.33 ± 0.01 ^{Ab}	0.77 ± 0.02 ^{Ab}	0.97 ± 0.06 ^{Ba}	48.5 ± 1.6 ^{Ab}	0.57 ± 0.02 ^{Ba}	5.16 ± 0.26 ^{Ba}
-	High	0.24 ± 0.01 ^{Cb}	0.74 ± 0.03 ^{Aa}	0.98 ± 0.06 ^{Ba}	35.5 ± 1.2 ^{Cb}	0.64 ± 0.02 ^{Aa}	5.37 ± 0.29 ^{Ba}
+	Low	0.35 ± 0.01 ^{Ba}	0.73 ± 0.03 ^{Ba}	0.97 ± 0.04 ^{Ab}	51.1 ± 2.0 ^{Ba}	0.56 ± 0.02 ^{Bb}	7.30 ± 0.49 ^{Ab}
+	Control	0.37 ± 0.01 ^{Aa}	0.85 ± 0.06 ^{Aa}	0.92 ± 0.06 ^{Aa}	54.8 ± 1.3 ^{Aa}	0.54 ± 0.01 ^{Bb}	4.79 ± 0.28 ^{Ba}
+	High	0.27 ± 0.01 ^{Ca}	0.69 ± 0.04 ^{Ba}	1.00 ± 0.04 ^{Aa}	40.3 ± 1.8 ^{Ca}	0.60 ± 0.03 ^{Ab}	4.91 ± 0.22 ^{Ba}
EBR	B supply	P _N (μmol m ⁻² s ⁻¹)	E (mmol m ⁻² s ⁻¹)	g _s (mol m ⁻² s ⁻¹)	C _i (μmol mol ⁻¹)	WUE (μmolmmol ⁻¹)	P _N /C _i (μmol m ⁻² s ⁻¹ Pa ⁻¹)
-	Low	3.9 ± 0.2 ^{Cb}	1.01 ± 0.05 ^{Cb}	0.03 ± 0.01 ^{Bb}	306 ± 25 ^{Aa}	3.9 ± 0.3 ^{Aa}	0.013 ± 0.001 ^{Cb}
-	Control	9.4 ± 0.7 ^{Ab}	2.32 ± 0.14 ^{Ba}	0.11 ± 0.01 ^{Aa}	227 ± 12 ^{Ba}	4.0 ± 0.2 ^{Ab}	0.042 ± 0.002 ^{Ab}
-	High	6.6 ± 0.4 ^{Bb}	2.71 ± 0.17 ^{Aa}	0.12 ± 0.01 ^{Aa}	241 ± 14 ^{Ba}	2.4 ± 0.1 ^{Bb}	0.028 ± 0.001 ^{Bb}
+	Low	7.0 ± 0.5 ^{Ca}	1.79 ± 0.07 ^{Ba}	0.07 ± 0.01 ^{Ca}	246 ± 18 ^{Ab}	3.9 ± 0.2 ^{Ba}	0.029 ± 0.001 ^{Ca}
+	Control	11.4 ± 0.5 ^{Aa}	1.83 ± 0.08 ^{Bb}	0.09 ± 0.01 ^{Bb}	220 ± 16 ^{Aa}	6.2 ± 0.4 ^{Aa}	0.052 ± 0.002 ^{Aa}
+	High	8.2 ± 0.4 ^{Ba}	2.61 ± 0.66 ^{Aa}	0.11 ± 0.01 ^{Aa}	235 ± 7 ^{Aa}	3.3 ± 0.2 ^{Ba}	0.035 ± 0.001 ^{Ba}

B, boron; EBR, 24-epibrassinolide; Chl *a*, chlorophyll *a*; Chl *b*, chlorophyll *b*; Total chl, total chlorophyll; Car, carotenoids. Φ_{PSII}, effective quantum yield of PSII photochemistry; q_p, photochemical quenching coefficient; NPQ, nonphotochemical quenching; ETR, electron transport rate; EXC, relative energy excess at the PSII level; ETR/P_N, ratio between the electron transport rate and net photosynthetic rate; P_N, net photosynthetic rate; E, transpiration rate; g_s, stomatal conductance; C_i, intercellular CO₂ concentration; WUE, water-use efficiency; P_N/C_i, carboxylation instantaneous efficiency. Columns with different uppercase letters between B supplies (low, control, and high B supply under equal EBR level) and lowercase letters between EBR level (with and without EBR under equal B supply) indicate significant differences from the Scott-Knott test (*P* < 0.05). Means ± SD, *n* = 5

PSII, with greater protection to the photosynthetic apparatus (Zhang et al. 2013). Additionally, lower *F*₀ values associated with increased *F*_m in plants exposed to EBR application indicate a positive action of this steroid over the light-harvesting complex (Melo et al. 2017). Wang et al. (2009), evaluating maize plants exposed to 550 mg Mn kg⁻¹ soil combined with 0.1 mg L⁻¹ EBR found increases in *F*_v/*F*_m, compared with equal treatment without EBR. Lima and Lobato (2017), investigating cowpea plants sprayed with 100 nM EBR and submitted to water deficit, reported an increase in *F*_m (32%) and reduction in *F*₀ (15%), respectively, similar to results found in this research.

EBR promoted increases in Φ_{PSII}, q_p, and ETR values and reductions in NPQ, EXC, and ETR/P_N in plants exposed to high and low B supplies. Increases in Φ_{PSII} values indicate that EBR application improved energy capture efficiency and increased the proportion of open reaction centers in the PSII (q_p) (Yu et al. 2004). Higher q_p values are associated with increased capture capacity of the PSII electron-acceptor

molecule (plastoquinone) frequently associated with reducing power and ATP consumption and avoiding photoinhibition (Khamsuk et al. 2018). Additionally, reductions in NPQ values in low B + EBR-treated plants demonstrate the protective effect of this steroid on PSII to excess energy, alleviating damage to thylakoid membranes (Wu et al. 2014). Thussaganpanit et al. (2015) studied rice plants under high-temperature conditions, and the application of EBR (1 nM) also promoted increases in Φ_{PSII}, q_p, and ETR, in agreement with our results. Shu et al. (2016), evaluating tomato seedlings under low irradiance (180 μmol m⁻² s⁻¹) and low temperature (15 °C day/7 °C night), observed beneficial effects of exogenous EBR application (0.1 μmol L⁻¹) on the parameters of Φ_{PSII} and q_p, indicating that EBR alleviated the photoinhibition caused by the simulated stress.

An increase in ETR and a decrease in EXC in response to EBR application indicated that this steroid improved the electron transport in PSII. Reductions in ETR/P_N of EBR-treated plants indicate an increase in carboxylation

Fig. 2 Minimal fluorescence yield of the dark-adapted state F_0 (A), maximal fluorescence yield of the dark-adapted state F_m (B), variable fluorescence F_v (C), and maximal quantum yield of PSII photochemistry F_v/F_m (D) in soybean plants sprayed with 24-epibrassinolide (EBR) and exposed to different boron (B) supplies. Columns with different uppercase letters between B supplies (low, control, and high B supply under equal EBR level) and lowercase letters between EBR level (with and without EBR under equal B supply) indicate significant differences from the Scott-Knott test ($P < 0.05$). Means \pm SD, $n = 5$

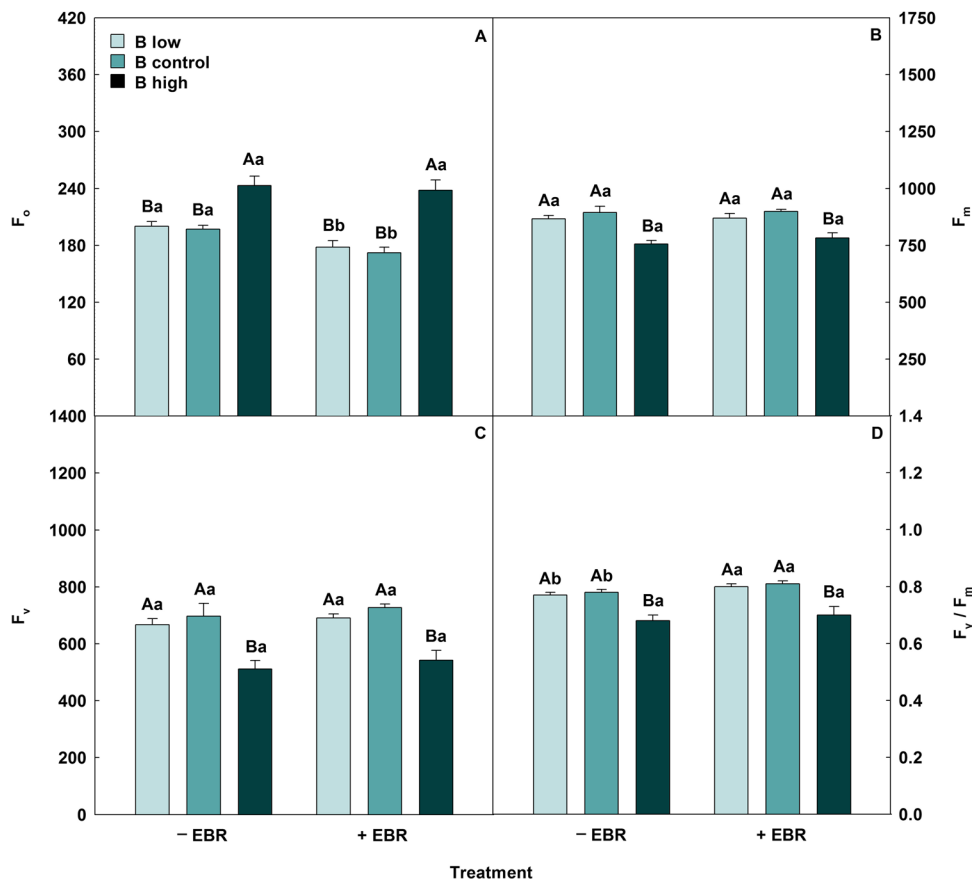


Table 5 Stomatal characteristics in soybean plants sprayed with EBR and exposed to different B supplies

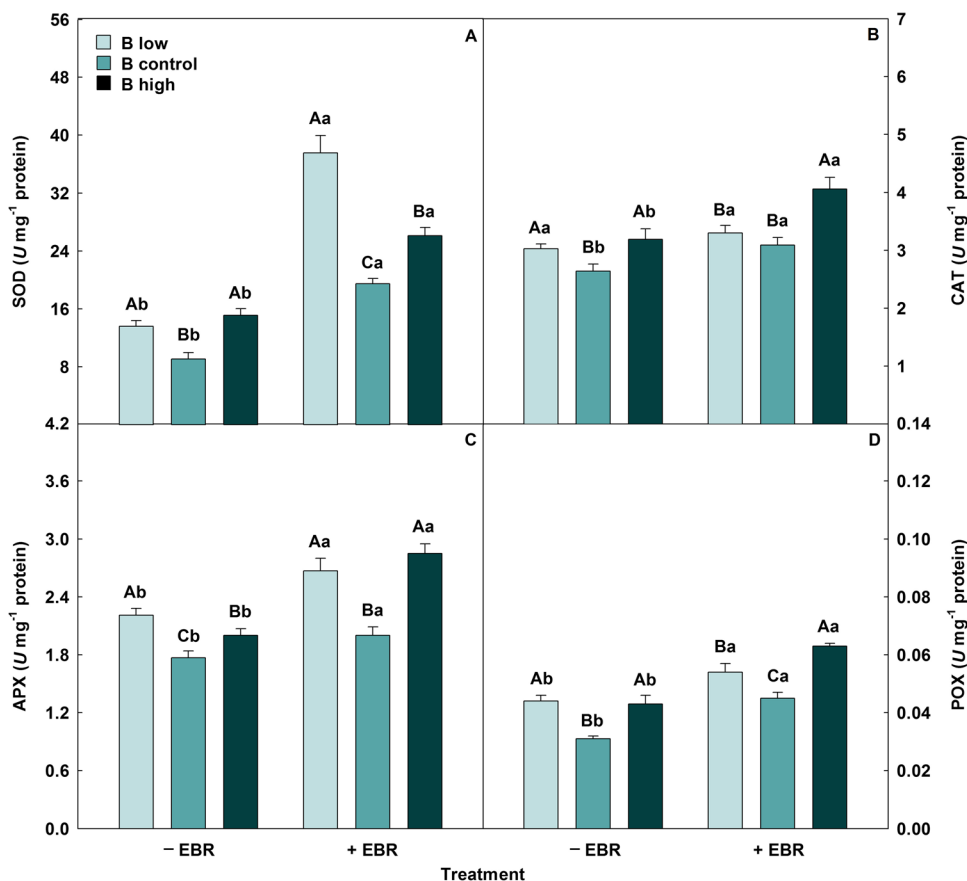
EBR	B supply	SD (stomata per mm ²)	PDS (μm)	EDS (μm)	SF	SI (%)
Adaxial face						
-	Low	71 ± 1 ^{Cb}	8.9 ± 0.5 ^{Ba}	20.9 ± 1.5 ^{Ba}	0.43 ± 0.03 ^{Cb}	3.6 ± 0.2 ^{Cb}
-	Control	92 ± 3 ^{Ab}	8.6 ± 0.5 ^{Ba}	18.1 ± 1.1 ^{Ca}	0.47 ± 0.02 ^{Aa}	4.9 ± 0.2 ^{Ab}
-	High	78 ± 3 ^{Bb}	9.9 ± 0.4 ^{Aa}	22.3 ± 1.5 ^{Aa}	0.44 ± 0.02 ^{Bb}	4.0 ± 0.1 ^{Bb}
+	Low	92 ± 3 ^{Ca}	8.4 ± 0.3 ^{Bb}	18.9 ± 0.8 ^{Ab}	0.44 ± 0.02 ^{Ba}	5.1 ± 0.3 ^{Ba}
+	Control	100 ± 4 ^{Aa}	8.3 ± 0.1 ^{Ba}	17.5 ± 1.1 ^{Ba}	0.47 ± 0.02 ^{Aa}	5.8 ± 0.3 ^{Aa}
+	High	96 ± 3 ^{Ba}	8.8 ± 0.3 ^{Ab}	18.9 ± 0.7 ^{Ab}	0.46 ± 0.02 ^{Aa}	5.1 ± 0.4 ^{Ba}
Abaxial face						
-	Low	257 ± 15 ^{Bb}	11.2 ± 0.7 ^{Aa}	21.3 ± 1.6 ^{Aa}	0.53 ± 0.03 ^{Aa}	10.2 ± 0.7 ^{Bb}
-	Control	292 ± 8 ^{Ab}	9.7 ± 0.5 ^{Ba}	18.1 ± 0.9 ^{Ba}	0.54 ± 0.03 ^{Aa}	12.7 ± 0.8 ^{Ab}
-	High	242 ± 13 ^{Cb}	11.0 ± 0.5 ^{Aa}	20.3 ± 1.4 ^{Aa}	0.54 ± 0.03 ^{Aa}	9.7 ± 0.5 ^{Bb}
+	Low	292 ± 8 ^{Ba}	9.7 ± 0.4 ^{Ab}	18.8 ± 1.0 ^{Ab}	0.51 ± 0.03 ^{Ba}	12.8 ± 0.5 ^{Ba}
+	Control	307 ± 20 ^{Aa}	9.5 ± 0.5 ^{Aa}	17.1 ± 0.8 ^{Ba}	0.55 ± 0.02 ^{Aa}	13.7 ± 1.0 ^{Aa}
+	High	278 ± 15 ^{Ca}	9.6 ± 0.4 ^{Ab}	17.8 ± 0.8 ^{Bb}	0.54 ± 0.03 ^{Aa}	13.1 ± 0.8 ^{Ba}

B, boron; EBR, 24-epibrassinolide; SD, stomatal density; PDS, polar diameter of the stomata; EDS, equatorial diameter of the stomata; SF, stomatal functionality; SI, stomatal index. Columns with different uppercase letters between B supplies (low, control, and high B supply under equal EBR level) and lowercase letters between EBR level (with and without EBR under equal B supply) indicate significant differences from the Scott-Knott test ($P < 0.05$). Means \pm SD, $n = 5$

efficiency and probably lower carbon losses by photorespiration (Ahmed et al. 2013a; Pereira et al. 2019). Increases in ETR and Φ_{PSII} were found by Dobrikova et al. (2014),

investigating the effects of three EBR concentrations (0.01, 0.1, and 1 mg L⁻¹) on the membrane structures of thylakoids in pea plants, obtaining increases of 25% and 35% in ETR

Fig. 3 Activities of superoxide dismutase SOD (A), catalase CAT (B), ascorbate peroxidase APX (C), and peroxidase POX (D) in soybean plants sprayed with 24-epibrassinolide (EBR) and exposed to different boron (B) supplies. Columns with different uppercase letters between B supplies (low, control, and high B supply under equal EBR level) and lowercase letters between EBR level (with and without EBR under equal B supply) indicate significant differences from the Scott-Knott test ($P < 0.05$). Means \pm SD, $n = 5$

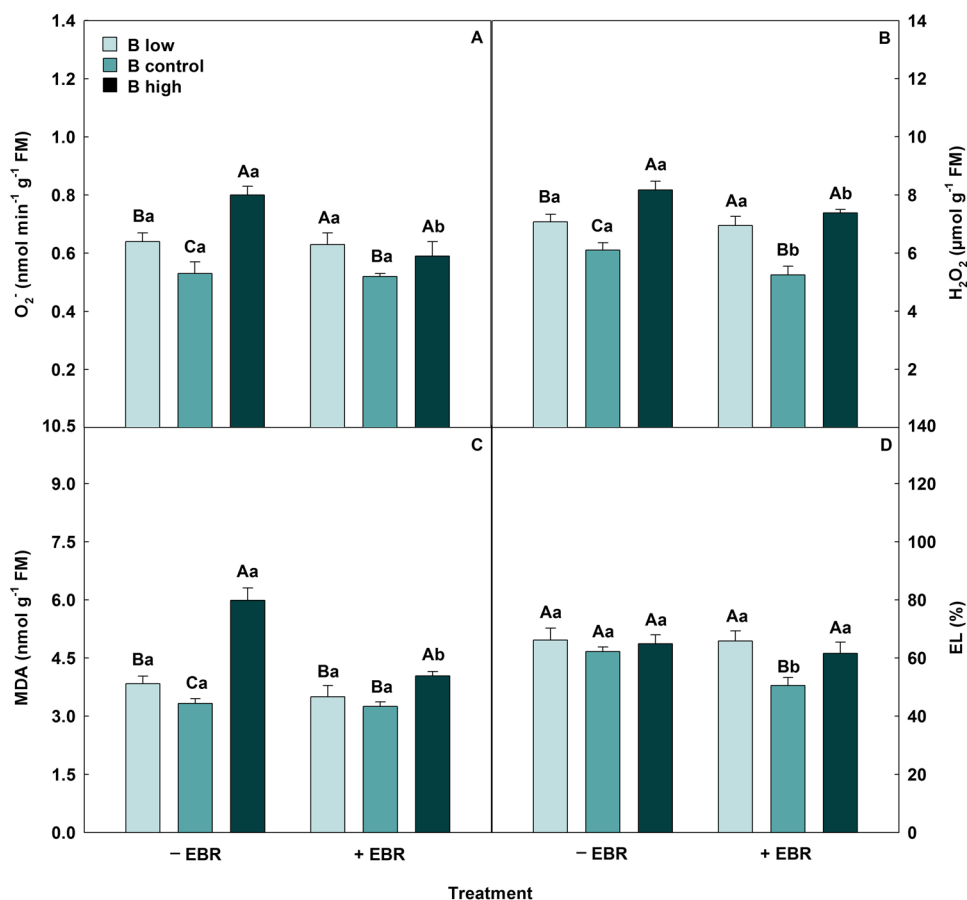


under 0.01 and 0.1 mg L⁻¹ EBR, respectively, when compared to the control treatment.

EBR application mitigated the effects of low and high B treatments on gas exchange. Higher values of P_N , E , g_s , and P_N/C_i under low B conditions and increases in P_N , WUE, and P_N/C_i in high B treatment are associated with positive actions of the EBR on photochemical and diffusional aspects found in this study. Additionally, there is a linear relationship between the ability to capture, utilize, and dissipate light and CO₂ uptake (Wong et al. 2012). We detected increases in F_v/F_m , Φ_{PSII} , and ETR values proving higher photosynthetic efficiency despite low and high B concentrations. Parallely, increases in P_N can be associated with lower C_i values, resulting in higher P_N/C_i , suggesting a higher activity of Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and, consequently, increases in CO₂ uptake in the Calvin-Benson cycle. Increases in g_s values combined with higher SD, SI, and SF and lower PDS and EDS suggested higher photosynthetic capacity in EBR-treated plants due to more uniform CO₂ diffusion to Rubisco sites (Tanaka et al. 2013). Our results are consistent with the findings by Hu et al. (2013), evaluating pepper plants, being found significant increases in P_N , g_s , and E and a decrease in C_i in plants under water deficit and treated with EBR (0.01 mg L⁻¹), compared to plants without steroid.

Plants submitted to low and high B in association with the application of EBR presented a better performance concerning stomatal characteristics than plants not treated with EBR. Increases in stomatal density (SD), stomatal functionality (SF), and stomatal index (SI) on the adaxial face of leaves under the influence of EBR demonstrate that this steroid acts on stomatal development, activating proteins involved in the signaling pathway of the stomatal mechanism (Lin et al. 2013). Changes in stomatal, such as shape, size, and quantity, are efficient strategies of plants to cope with stressful conditions because the increase in the density and reduction in the size of the stomata contribute to a higher diffusion of CO₂ to the carboxylation sites and increases in WUE (Devi and Reddy 2018; Franks and Beerling 2009). Our results show that EBR played an important role against the effects of low and high B, contributing to the increases in stomata quantity and efficiency (SD and SI) while reducing the size (smaller PDS and EDS) and positively reflecting on the P_N values (Zhao et al. 2015). Increases in SD were observed in grape leaves subjected to water deficit and exogenous EBR applications (0.05, 0.10, and 0.20 mg L⁻¹), promoting increases of 43%, 15%, and 53%, respectively, compared to plants without EBR (Wang et al. 2015). Oliveira et al. (2018) observed positive effects of 50 nM EBR against saline

Fig. 4 Superoxide O_2^- (A), hydrogen peroxide H_2O_2 (B), malondialdehyde MDA (C), and electrolyte leakage EL (D) in soybean plants sprayed with 24-epibrassinolide (EBR) and exposed to different boron (B) supplies. Columns with different uppercase letters between B supplies (low, control, and high B supply under equal EBR level) and lowercase letters between EBR level (with and without EBR under equal B supply) indicate significant differences from the Scott-Knott test ($P < 0.05$). Means \pm SD, $n = 5$



stress in young *Eucalyptus urophylla* plants, with increases of 23%, 7%, and 24% in SD, SF, and SI, respectively, and reductions of 8% and 10% in PDS and EDS, in this order, compared to stress treatment without EBR, being similar to our results.

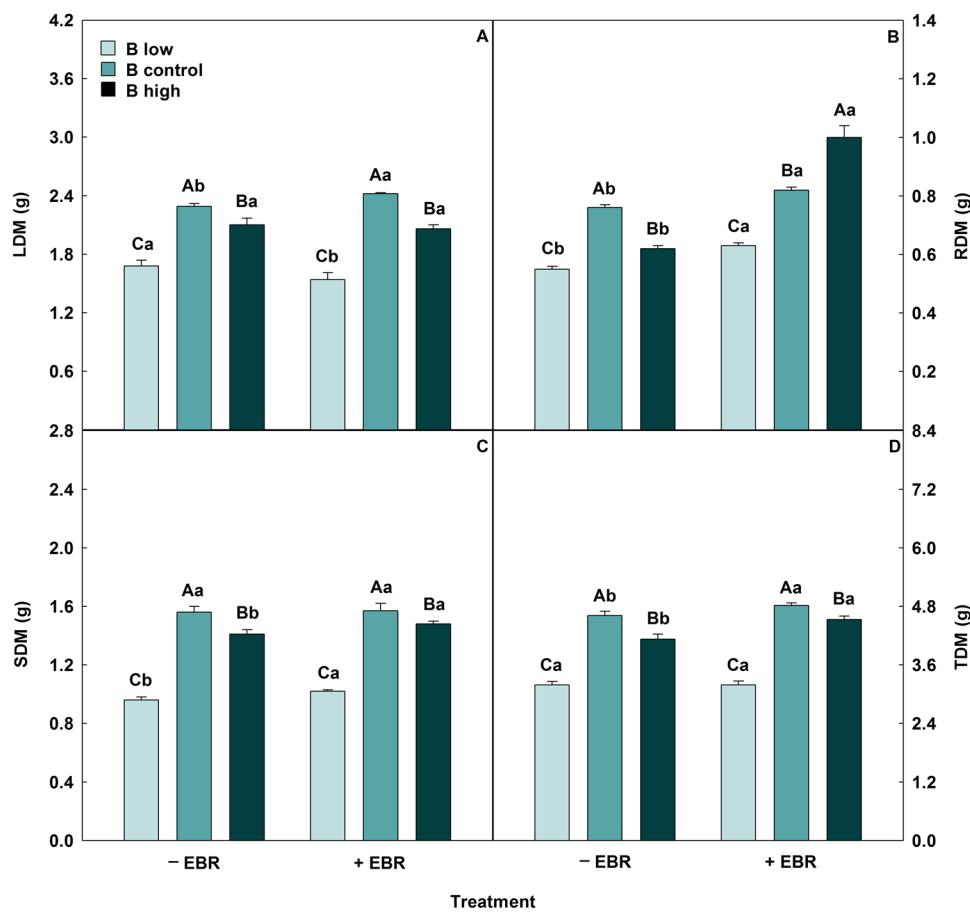
Plants treated with EBR under low and high B supplies had increases in the activities of antioxidant enzymes (SOD, CAT, APX, and POX), indicating beneficial interference of the EBR on the antioxidant defense system. These results are related to the increases in F_v/F_m and ETR because the maximization of the photosynthetic potential reduces the availability of NADPH and ATP (Zhou et al. 2004), which are consumed in the Calvin cycle, facilitating the transport of electrons and reducing the possibility of ROS formation (Ogweno et al. 2008). Li et al. (2015) studying the antioxidant system in pepper seedlings submitted to two thermal regimes (28/18 °C and 15/5 °C) and spray with EBR (0.1 μ M) showed significant increases of 29%, 31%, 25%, and 9% after the EBR application for SOD, POX, CAT, and APX, respectively. Surgun et al. (2016) analyzed the EBR effects on the B-tolerance mechanisms in *Arabidopsis thaliana* plants submitted to three B concentrations (0, 0.80, and 1.60 mM) combined with two EBR levels (0.01, and 1 mM) and described that the application of EBR maximized the

activities linked to antioxidant enzymes (SOD, CAT, APX, and POX).

The stress promoted by the high B stress-induced increases in O_2^- , H_2O_2 , MDA, and EL, but these effects were reduced after the application of EBR. EBR spray increased the activities of antioxidant enzymes, as previously detected. The antioxidant enzymes act in the neutralization of the ROS (Dalyan et al. 2018; Zhang et al. 2008), with the SOD enzyme acting at the beginning of the defense process, catalyzing the conversion of O_2^- to H_2O_2 , which is subsequently degraded by the CAT, APX, and POX enzymes (Zhou et al. 2018). Ahammed et al. (2013b), working with tomato plants exposed to EBR and three concentrations of polychlorinated biphenyls (0.4, 2.0, and 10.0 μ g L⁻¹), reported decreases in O_2^- , H_2O_2 , and MDA levels, similar to the results found in this research. Ogweno et al. (2008) investigated the photosynthetic efficiency and oxidative stress in tomato plants under two temperature conditions, normal (25/18 °C) and high (40/30 °C), and three EBR concentrations (0.01, 0.1, 1.0 mg L⁻¹), reporting reductions in H_2O_2 and MDA levels in plants treated with EBR.

Plants subjected to deficiency or toxicity of B when sprayed with EBR had increased related to growth (LDM, RDM, SDM, and TDM). These responses can be attributed

Fig. 5 Leaf dry matter LDM (A), root dry matter RDM (B), stem dry matter SDM (C), and total dry matter TDM (D) in soybean plants sprayed with 24-epibrassinolide (EBR) and exposed to different boron (B) supplies. Columns with different uppercase letters between B supplies (low, control, and high B supply under equal EBR level) and lowercase letters between EBR level (with and without EBR under equal B supply) indicate significant differences from the Scott-Knott test ($P < 0.05$). Means \pm SD, $n = 5$



to the beneficial roles of EBR on anatomical responses, chlorophyll fluorescence, and gas exchange, evidenced by the increases in mesophyll cells (palisade parenchyma and spongy parenchyma), Φ_{PSII} , ETR, and P_N , as presented in this study. The application of EBR also favored increases in photosynthetic pigments and gas exchange, positively influencing the biomass (Xie et al. 2011; Naz et al. (2015); Hayat et al. (2011) studied tomato plants under the three concentrations of EBR (10^{-6} , 10^{-8} , 10^{-10} M) and the three concentrations of HBL (10^{-6} , 10^{-8} , 10^{-10} M) and reported that plants treated with EBR had more intense effects on length, fresh matter, dry matter, and foliar area when compared to plants treated with HBL. A study conducted by Liu et al. (2018) analyzing the B toxicity in *Puccinellia tenuiflora* seedlings submitted to three different B levels described reductions of 69% and 40% in root dry matter and shoot dry matter, respectively, in plants treated with high B.

5 Conclusions

EBR pretreatment promoted improvements in leaf and root structures, also inducing increases in biomass accumulation. EBR alleviated the damages occasioned by the inadequate

B supplies on root tissues, more specifically maximizing the vascular cylinder, metaxylem, and epidermis, improving the nutritional status. This steroid also minimized the harmful effects of B stress on leaf anatomy, stimulating the epidermis on both leaf sides, palisade parenchyma, and spongy parenchyma; both structures are intrinsically related to protection and CO_2 availability to the photosynthetic process. Concomitantly, this steroid had a positive impact on biomass accumulation. These results are explained by beneficial actions on leaf structures and photosynthetic machinery. Therefore, our results demonstrate that the EBR application can improve soybean plants' tolerance under inadequate B supplementation.

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Author Contribution AKSL was the advisor for this project, planned all phases of the research, and critically revised the manuscript. YCP, ANSN, BTSA, BRSS, and MAMB conducted the experiments,

performed physiological, biochemical, anatomical, and morphological determinations, and wrote and edited the manuscript. BLB carried out the nutritional determinations and critically revised the manuscript. AB critically revised the manuscript. All authors read and approved the final version of the manuscript.

Declarations

Conflict of Interest The authors declare no competing interests.

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