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Brassinosteroids increase electron transport and photosynthesis in soybean plants under water deficit

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Abstract

Drought frequently results in significant losses in agricultural systems, including the soybean yield. Brassinosteroids exhibit multiple actions on essential processes, including chlorophyll fluorescence and gas exchange. Considering that the electron transport rate (ETR) into photosystems can exercise interference on net photosynthetic rate (P_N), this research aims to determine whether 24-epibrassinolide (EBR) affects electron transport and find out if there is any repercussion on photosynthesis in soybean plants affected by the water deficit. The experiment was performed using a randomized factorial design, with two water conditions (control and water deficit) and three EBR concentrations (0, 50, and 100 nM EBR). The water deficit reduced effective quantum yield of PSII photochemistry, ETR, P_N, and water-use efficiency. However, the exogenous application of 100 nM EBR mitigated these negative effects, increasing these variables. EBR reduced the oxidant compounds (superoxide and hydrogen peroxide) and membrane damages (malondialdehyde and electrolyte leakage) in stressed plants. Our study proved that EBR increased ETR and P_N in control and stressed plants, revealing that ETR had a strong relationship with P_N. These results suggest that soybean plants with higher values of ETR are more efficient in relation to P_N.

Additional key words: chlorophyll fluorescence; drought; gas exchange; *Glycine max*; 24-epibrassinolide.

Introduction

Soybean is an oleaginous plant with a large capacity to produce grains rich in proteins (Bamji and Corbitt 2017). Soybean are important to human and animal nutrition and are a major source of energy in biofuels (Abdulkhani *et al*. 2017). Currently, soybean is the most cultivated and consumed legume in the world (Thilakarathna and Raizada 2017) with approximately 314 million tons produced in the 2015/2016 harvest (FAO 2017). The main producer countries of soybean worldwide are the United States of America and Brazil.

Drought is the main abiotic stress on crops because it is the most recurrent, and thus, it frequently promotes significant losses in agricultural systems, thereby reducing food production worldwide (Zhang *et al*. 2016). Water deficiency is a complex physicochemical process that affects macro- and micromolecules of plant metabolism, which can be more severe at certain stages and provoke irreversible damages, such as protein denaturation and cell death (Bajguz and Hayat 2009, Rajasekar *et al*. 2016).

Low water availability directly affects metabolism, causing physiological, biochemical, and molecular modifications (Shao *et al*. 2008). In this content, the water photolysis oxidizes the H_2O molecule releasing the electrons. Subsequently, these electrons are captured in PSII and transferred to PSI by specific proteins, such as cytochrome b_6/f complex and plastocyanin (Rochaix 2011).

Under water deficit, plants normally exhibit negative

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Abbreviations: BRs – brassinosteroids; Car – carotenoids; Chl – chlorophyll; C_i – intercellular CO_2 concentration; E – transpiration rate; EBR – 24-epibrassinolide; EL – electrolyte leakage; ETR – electron transport rate; ETR/P_N – ratio between the apparent electron-transport rate and net photosynthetic rate; EXC – relative energy excess at the PSII level; F_0 – minimal fluorescence yield of the dark-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_v – variable fluorescence; F_v/F_m – maximal quantum yield of PSII photochemistry; g_s – stomatal conductance to water vapor; LDM – leaf dry matter; MDA – malondialdehyde; NPQ – nonphotochemical quenching; PEG – polyethylene glycol; P_N – net photosynthetic rate; P_N/C_i – instantaneous carboxylation efficiency; q_P – photochemical quenching; RDM – root dry matter; ROS – reactive oxygen species; STM – stem dry matter; TDM – total dry matter; WUE – water-use efficiency; Φ_{PSII} – effective quantum yield of PSII photochemistry.

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interferences on electron transport rate (Rivas *et al.* 2016), suggesting that the photosynthetic electron flow is intrinsically depending of the water availability in plant cells (Rutherford and Boussac 2004). Other problem caused by the water deficit is connected to gas exchange; it reduces the stomatal conductance that limits the CO_2 influx and consequently decreases the photosynthetic rate (Flexas *et al.* 2006, Yuan *et al.* 2016). The water deficit reduces the electron flow and gas exchange, generating oxidative stress due to accumulation of reactive oxygen species (ROS), such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2) (Bajguz and Hayat 2009, Ozkur *et al.* 2009).

Brassinosteroids (BRs) are substances classified as polyhydroxy steroids (Khalid and Aftab 2016) and are frequently tested in the form of 24-epibrassinolide (EBR). This molecule exhibits multiple actions on essential processes, such as improvement in PSII efficiency (Lima and Lobato 2017), gas exchange (Hu *et al.* 2013), beneficial repercussions on antioxidant systems (Yuan *et al.* 2010), and increases in rates of growth and development in plants (Vriet *et al.* 2013, Wei and Li 2016).

During drought conditions, the EBR application in *Capsicum annuum* plants mitigated the negative effects on chlorophyll (Chl) fluorescence, more specifically, on the effective quantum yield of PSII photochemistry (Φ_{PSII}) (Hu *et al.* 2013). Study conducted by Anjum *et al.* (2011) with *Zea mays* plants treated with EBR revealed beneficial effects on gas exchange, increasing the net photosynthetic rate (P_N) and stomatal conductance (g_s). In *Hordeum vulgare* treated with EBR under effects of polyethylene glycol (PEG)-induced drought stress, applications led to decreases in H_2O_2 and malondialdehyde (MDA) accumulations (Gill *et al.* 2017). Li *et al.* (2012) described increases in Chl *a* and Chl *b* after EBR treatment in *Chorispora bungeana* exposed to water deficit.

We hypothesized that the electron flux into photosystems can exercise interference on photosynthesis as the water supply is fundamental to the release of electrons after the water photolysis. The available literature suggests a probable positive effect of the EBR in relation to electron transport. Therefore, the aims of this research were to determine how the EBR acts on electron transport and if there is any repercussion on photosynthesis in soybean plants affected by the water deficit.

Materials and methods

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 the temperature and humidity controlled, under natural light conditions. The minimum, maximum, and median temperatures were 24, 34, and 26.8°C, respectively. The relative humidity during the experimental period varied between 60 and 80%.

Plants, containers and acclimation: Seeds of *Glycine max* (L.) Merr. var. M9144RR Monsoy™ were germinated and grown in 1.2-L pots (0.15 m in height, 0.10 m in diameter) filled with a mixed substrate of sand and vermiculite at a ratio of 3:1. The plants were cultivated under semi-hydroponic conditions, and the pots had one hole in the bottom covered with mesh to maintain the substrate and aerate the roots. Solution absorption was by capillary action, with these pots placed into other containers (0.15 m in height, 0.15 m in diameter) containing 500 mL of distilled water for eight days. A modified Hoagland and Arnon (1950) solution was used for nutrients, with the ionic force beginning at 50% and later modified to 100% after two days. After one day, the nutritive solution remained at total ionic force.

Experimental design: The experiment was a factorial design with the factors completely randomized, including two water conditions (control and water deficit) and three concentrations of 24-epibrassinolide (0, 50, and 100 nM EBR). 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.

24-epibrassinolide (EBR) preparation and application: Twelve-day-old plants were sprayed with 10 mL per plant of EBR or Milli-Q water in each application (containing a proportion of ethanol that was equal to that used to prepare the EBR solution) at 5-d intervals until day 27. The 0, 50, and 100 nM EBR (*Sigma-Aldrich*, USA) solutions were prepared by dissolving the solute in ethanol followed by dilution with Milli-Q water [ethanol:water (v/v) = 1:10,000] (Ahammed *et al.* 2013). On day 27 after the experiment was initiated, the plants in the water-deficit treatment were subjected to water restriction.

Plant nutrition and water-deficit treatment: One plant per pot was used to examine plant parameters. The plants received the following macro- and micronutrients contained in the nutrient solution (*Sigma-Aldrich*, USA): 8.75 mM KNO_3, 7.5 mM $Ca(NO_3)_2{\cdot}4H_2O$, 3.25 mM $NH_4H_2PO_4$, 1.5 mM $MgSO_4{\cdot}7\,H_2O$, 62.50 µM KCl, 31.25 µM H_3BO_3, 2.50 µM $MnSO_4{\cdot}H_2O$, 2.50 µM $ZnSO_4{\cdot}7H_2O$, 0.63 µM $CuSO_4{\cdot}5H_2O$, 0.63 µM $NaMoO_4{\cdot}5H_2O$, and 250.0 µM $NaEDTAFe{\cdot}3H_2O$. To simulate the water deficit, the solution was removed completely, the root system was placed in similar pots without water/solution, and the water-deficit treatment was applied within 3 d (day 27 to 30 after the start of the experiment). During the study, the nutrient solutions were changed at 07:00 h at 3-d intervals, with the pH adjusted to 5.5 using HCl or NaOH. On day 30 of the experiment (phenological stage V6), physiological parameters were measured for all plants, and plant tissues were harvested for morphological and biochemical analyses.

Measurement of Chl fluorescence: The minimal fluorescence yield of the dark-adapted state (F_0), the maximal fluorescence yield of the dark-adapted state (F_m), the variable fluorescence (F_v) were measured. The maximal quantum yield of PSII photochemistry (F_v/F_m)

was calculated using the formula $F_v/F_m = (F_m - F_0)/F_m$, the effective quantum yield of PSII photochemistry (Φ_{PSII}) was calculated by the formula $(F_{m'} - F_s)/F_{m'}$. For photochemical quenching coefficient (q_P), the formula $q_P = (F_{m'} - F_s)/(F_{m'} - F_{0'})$ was used, while the nonphotochemical quenching (NPQ) was calculated as $NPQ = (F_m - F_{m'})/(F_{m'})$. The electron transport rate (ETR) was calculated as $ETR = \Phi_{PSII} \times PPFD \times 0.5 \times 0.84$, where 0.5 is the fraction of the excitation energy to PSII and 0.84 the fraction of incoming light absorbed by the leaves. The relative energy excess at the PSII level (EXC) was calculated by the formula $EXC = (F_v/F_m) - (\Phi_{PSII})/(F_v/F_m)$, and the ratio between the electron transport rate and the net photosynthetic rate (ETR/P_N) were determined using a modulated Chl fluorometer (*OS5p*, *OptiSciences*, USA). Chl fluorescence was measured in fully expanded leaves. Preliminary tests determined the location of the leaf, the part of the leaf, and the time required to obtain the greatest F_v/F_m ratio; therefore, the acropetal third of leaves that were in the middle third of the plant and adapted to the dark for 30 min was used for the evaluation. The intensity and duration of the saturation light pulse were 7,500 μmol(photon) m^{-2} s^{-1} and 0.7 s, respectively.

Evaluation of gas exchange: The net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were evaluated using an infrared gas analyser (*LCPro⁺*, *ADC BioScientific*, UK). These parameters were measured at the adaxial surface of fully expanded leaves located in the middle region of the plant. The water-use efficiency (WUE) was estimated by the formula P_N/E, in agreement with Ma *et al*. (2004). The instantaneous carboxylation efficiency (P_N/C_i) was calculated using the formula described by Aragão *et al*. (2012). Gas exchange was evaluated in all plants under constant conditions of CO_2 concentration (360 μmol mol^{-1}), photosynthetically active radiation [800 μmol(photon) m^{-2} s^{-1}], air-flow rate (300 μmol s^{-1}), and temperature (28°C), between 10:00 and 12:00 h.

Leaf water potential: The leaf water potential (Ψ_w) was measured using fully expanded leaves located in the middle region of the plant and exposed to light during the period between 11:30 and 12:00 h, which corresponded to the midday potential. To determinate the Ψ_w, one leaf per plant and five plants per treatment were measured using an analogue plant moisture system (*model 600*, *PMS Instrument Company*, USA). This system is based on the pressure chamber technique (Scholander *et al*. 1964), and the procedure outlined by Turner (1988) was followed.

Superoxide concentration: To determine O_2^-, 1 mL of extract was incubated with 30 mM phosphate buffer (pH 7.6) and 0.51 mM hydroxylamine hydrochloride for 20 min at 25°C. Then, 17 mM sulphanilamide and 7 mM α-naphthylamine were added to the incubation mixture for 20 min at 25 °C. After the reaction, ethyl ether was added in the identical volume and centrifuged at 3,000 × *g* for 5 min. The absorbance was measured at 530 nm (Elstner and Heupel 1976) using spectrophotometer (*UV-M51*, *Bel Photonics*, Italy). O_2^- concentration was expressed in nmol min^{-1} g^{-1}(FM).

Extraction of nonenzymatic compounds: Nonenzymatic compounds (H_2O_2 and MDA) were extracted as described by (Wu *et al*. 2006) from leaves, fully expanded and located in the middle region of the plant. Briefly, a mixture for extraction of H_2O_2 and MDA was prepared by homogenizing 500 mg of fresh leaf materials in 5 mL of 5% (w/v) trichloroacetic acid. The samples were then centrifuged at 15,000 × *g* for 15 min at 3°C to collect the supernatant.

Hydrogen peroxide concentration: To measure H_2O_2, 200 μL of supernatant and 1,800 μL of reaction mixture (2.5 mM potassium phosphate buffer [pH 7.0] and 500 mM potassium iodide) were mixed, and the absorbance was measured at 390 nm (Velikova *et al*. 2000) using spectrophotometer (*UV-M51*, *Bel Photonics*, Italy). H_2O_2 concentration was expressed in μmol g^{-1}(FM).

Malondialdehyde concentration: MDA was determined by mixing 500 μL of supernatant with 1,000 μL of the reaction mixture containing 0.5% (w/v) thiobarbituric acid in 20% trichloroacetic acid. The mixture was incubated in boiling water at 95°C for 20 min, with the reaction terminated by placing the reaction container in an ice bath. The samples were centrifuged at 10,000 × *g* for 10 min, and the absorbance was measured at 532 nm using a spectrophotometer (*UV-M51*, *Bel Photonics*, Italy). The nonspecific absorption at 600 nm was subtracted from the absorbance data. The MDA–TBA complex (red pigment) amount was calculated based on the method of Cakmak and Horst (1991), with minor modifications and using an extinction coefficient of 155 mM^{-1} cm^{-1}. MDA concentration was expressed in nmol g^{-1}(FM).

Electrolyte leakage was measured according to the method of Gong *et al*. (1998) with minor modifications. Fresh tissue (200 mg) was cut into pieces 1 cm in length and placed in containers with 8 mL of distilled deionized water. The containers were incubated in a water bath at 40°C for 30 min, and the initial electrical conductivity of the medium (EC_1) was measured. Then, the samples were boiled at 95°C for 20 min to release the electrolytes. After cooling, the final electrical conductivity (EC_2) was measured (Gong *et al*. 1998). The percentage of electrolyte leakage was calculated using the formula $EL (\%) = (EC_1/EC_2) \times 100$.

Photosynthetic pigments: Chl and carotenoid (Car) quantifications were performed with 40 mg of fully expanded leaves located in the middle region of the plant. The samples were homogenized in the dark with 8 mL of 90% methanol (Nuclear). The homogenate was centrifuged at 6,000 × *g* for 10 min at 5°C. The supernatant was removed, and Chl *a* and Chl *b*, Car, and total Chl contents were quantified using a spectrophotometer (*UV-M51*, *Bel Photonics*, Italy), according to the methodology of Lichtenthaler and Buschmann (2001).

Morphological parameters: The growth of roots, stems, and leaves was measured based on constant dry mass after drying in a forced-air ventilation oven at 65°C.

Data analysis: The data were subjected to two-way analysis of variance (*ANOVA*), and significant differences between the means were determined using the *Scott-Knott´*s test at a probability level of 5% (Steel *et al*. 2006). Standard deviations were calculated for each treatment. Correlation analysis was performed with the *Pearson´*s parametric method. The statistical analyses were performed with *Assistat* software (Silva and Azevedo 2002).

Results

EBR improves water potential and PSII efficiency in plants exposed to water deficit: The water deficit promoted significant reduction in Ψ_W; however, the water deficit + 100 nM EBR induced an increase of 19% when compared to the same water condition without EBR (Fig. 1). The water deficit caused an increase in values of F_0, but the application of 100 nM EBR induced a significant reduction of 29% when compared to the water deficit without EBR (Fig. 2). For F_m, F_v, and F_v/F_m, these values were reduced under water deficit; however, the concentration of 100 nM of EBR promoted significant increases of 14, 44, and 26%, respectively (Fig. 2).

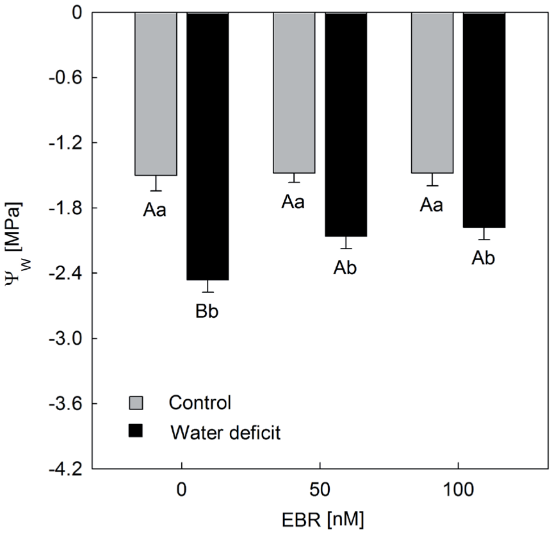

Fig. 1. Leaf water potential in soybean plants sprayed with EBR and exposed to water deficit. *Different uppercase letters* between EBR concentrations (0, 50, and 100 nM EBR under equal water conditions) and *lowercase letters* between water conditions (control and water deficit under equal EBR concentrations) indicate significant differences from the *Scott-Knott´*s test ($P<0.05$). Means ± SD, $n = 5$.

The water deficit reduced Φ_{PSII}, q_P, and ETR. However, the exogenous application of EBR promoted increases of 50, 6, and 44% in these variables, respectively, in plants with 100 nM EBR when compared to plants in the water-deficit treatment without EBR (Table 1). For NPQ, EXC, and ETR/P_N, the stress conditions caused increases in these variables; however, under the EBR treatment, these variables were significantly reduced. The values of NPQ and ETR/P_N suffered declines in the water deficit + 100 nM EBR treatment (22 and 73%, respectively). For EXC, the lowest level was under the water deficit + 50 nM EBR treatment (20%) when compared to equal water conditions and 0 nM EBR.

Plants exposed to water deficit + EBR increased their gas exchange: Plants under water deficit presented decreases in P_N, E, g_s, WUE, and P_N/C_i, but 100 nM EBR induced increases of 436, 45, 240, 273, and 650%, respectively (Table 2). However, the C_i were higher in the plants under water deficit and values were reduced by EBR utilization. The concentration of 100 nM decreased the value of C_i by 23% when compared to plants under the water deficit without EBR. The correlation analysis revealed that there is a strong and positive relationship between ETR and P_N ($r = 0.97$; $P<0.01$) (Fig. 3).

EBR reduced the oxidant compounds and membrane damages in stressed plants: The water deficit promoted increases in O_2^-, H_2O_2, MDA, and EL (Fig. 4). However, EBR application significantly minimized these variables with plants exposed to 100 nM EBR exhibiting reductions of 27, 47, 42, and 17%, respectively, when compared to plants after the treatment under water deficit without EBR application.

Minor stress on photosynthetic pigments due to steroid action: The water deficit induced significant decreases in Chl *a*, Chl *b*, total Chl, and Car contents. However, plants sprayed with 100 nM EBR exhibited increases by 45, 106, 56, and 80% for these variables, respectively, when compared to plants under the water deficit + 0 nM EBR (Table 3). The ratio Chl *a*/*b* and ratio total Chl/Car showed increases induced by the water deficit that were minimized after the use of EBR.

EBR attenuated the impact produced by the water deficit on growth: Plants subjected to water deficit showed a reduction in LDM, but the application of EBR promoted the increase of LDM, SDM, and TDM, in plants treated with 100 nM EBR showing increases of 13, 11, and 8%, respectively (Fig. 5). In relation to the water-deficit treatment, the concentration of 50 nM caused an increase of 10% in RDM compared with plants under the water deficit without EBR.

Discussion

The benefit found on Ψ_W in plants subjected to water deficit and treated with EBR suggests that the EBR improves the process of osmotic adjustment (Chaves and Seraphin 2001). This mechanism is fundamental to plants in order to complete important processes such as turgescence maintenance and regulation of stomatal opening in plants under conditions of low water availability (Silveira *et al.* 2009). Zhang *et al.* (2008) studied *Glycine max* exposed

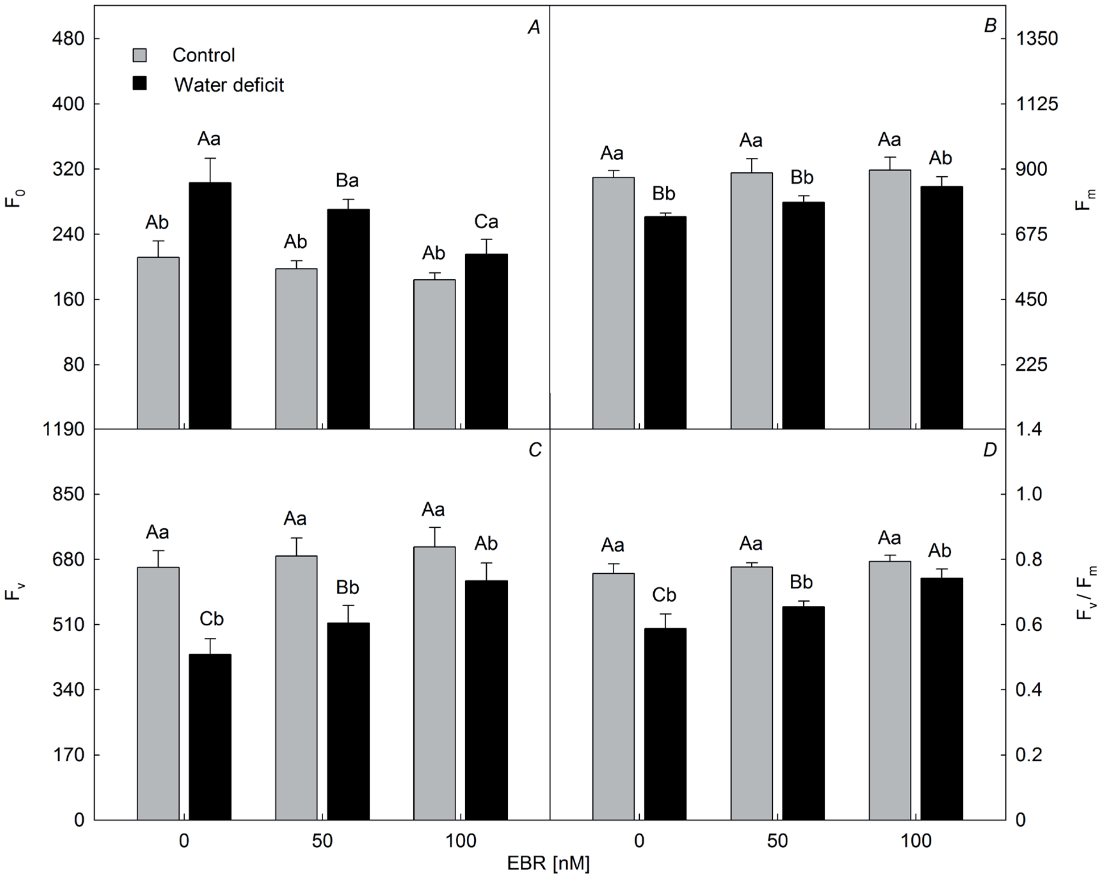

Fig. 2. Minimal fluorescence yield of the dark-adapted state (F_0; *A*), maximal fluorescence yield of the dark-adapted state (F_m; *B*), variable fluorescence (F_v; *C*), and maximal quantum yield of PSII photochemistry (F_v/F_m; *D*) in soybean plants sprayed with EBR and exposed to water deficit. *Different uppercase letters* between EBR concentrations (0, 50, and 100 nM EBR under equal water condition) and *lowercase letters* between water conditions (control and water deficit under equal EBR concentrations) indicate significant differences from the *Scott-Knott´s* test ($P<0.05$). Means ± SD, $n = 5$.

Table 1. Chlorophyll fluorescence in soybean plants splayed with EBR and exposed to water deficit. 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. Columns with *different uppercase letters* between EBR concentrations (0, 50, and 100 nM EBR under equal water condition) and *lowercase letters* between water conditions (control and water deficit under equal EBR concentration) indicate significant differences from the *Scott-Knott´s* test ($P<0.05$). Means ± SD, $n = 5$.

Water condition	EBR [nM]	Φ_{PSII}	q_P	NPQ	ETR [μmol m^{-2} s^{-1}]	EXC [μmol m^{-2} s^{-1}]	ETR/P_N
Control	0	0.55 ± 0.03^{Aa}	0.81 ± 0.05^{Aa}	0.59 ± 0.05^{Ab}	78.9 ± 1.8^{Ba}	0.27 ± 0.03^{Ab}	5.37 ± 0.15^{Ab}
Control	50	0.57 ± 0.03^{Aa}	0.83 ± 0.08^{Aa}	0.54 ± 0.05^{Ab}	84.0 ± 2.7^{Aa}	0.26 ± 0.02^{Ab}	5.18 ± 0.30^{Ab}
Control	100	0.59 ± 0.02^{Aa}	0.88 ± 0.06^{Aa}	0.53 ± 0.04^{Ab}	86.1 ± 3.0^{Aa}	0.26 ± 0.01^{Ab}	5.20 ± 0.23^{Ab}
Water deficit	0	0.28 ± 0.02^{Cb}	0.64 ± 0.06^{Ab}	3.01 ± 0.03^{Aa}	41.4 ± 2.2^{Cb}	0.54 ± 0.02^{Aa}	52.05 ± 2.81^{Aa}
Water deficit	50	0.37 ± 0.01^{Bb}	0.67 ± 0.04^{Ab}	2.50 ± 0.01^{Ba}	54.4 ± 1.6^{Bb}	0.43 ± 0.02^{Ba}	18.39 ± 1.57^{Ba}
Water deficit	100	0.42 ± 0.02^{Ab}	0.68 ± 0.02^{Ab}	2.34 ± 0.11^{Ca}	59.5 ± 2.7^{Ab}	0.46 ± 0.03^{Ba}	13.87 ± 0.81^{Ba}

to water deficit and reported that the application of 0.1 mg L^{-1} brassinolide promoted an increase of 21% in Ψ_W when compared to plants in equal water conditions without the addition of EBR.

Plants exposed to the water deficit + EBR treatment showed increases in F_v and F_v/F_m. The increase in F_v is explained by the maximization of F_m and reduction in F_0, as verified in this study. In addition, the increase of F_v/F_m in plants treated with EBR confirms the attenuation of the photoinhibitory damages promoted by the water deficit on PSII reaction centres (Tukaj *et al*. 2007). Wu *et al*. (2014) evaluated the effects of four concentrations of EBR on seedlings of *Solanum melongena* exposed to elevated temperatures and reported a reduction in F_0

Table 2. Gas exchange in soybean plants sprayed with EBR and exposed to water deficit. P_N – net photosynthetic rate; E – transpiration rate; g_s – stomatal conductance; C_i – intercellular CO_2 concentration; WUE – water-use efficiency; P_N/C_i – carboxylation instantaneous efficiency. Columns with *different uppercase letters* between EBR concentrations (0, 50, and 100 nM EBR under equal water conditions) and *lowercase letters* between water conditions (control and water deficit under equal EBR concentration) indicate significant differences from the *Scott-Knott ′s* test ($P<0.05$). Means ± SD, $n = 5$.

Water condition	EBR (nM)	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 [μmol mmol^{-1}]	P_N/C_i [μmol m^{-2} s^{-1} Pa^{-1}]
Control	0	15.05 ± 0.33^{Ba}	3.04 ± 0.21^{Aa}	0.28 ± 0.02^{Ba}	262 ± 07^{Ba}	4.96 ± 0.14^{Bb}	0.060 ± 0.008^{Ba}
Control	50	16.23 ± 0.57^{Aa}	3.08 ± 0.29^{Aa}	0.31 ± 0.01^{Aa}	242 ± 08^{Aa}	5.31 ± 0.11^{Aa}	0.067 ± 0.006^{Aa}
Control	100	16.58 ± 0.42^{Aa}	3.09 ± 0.02^{Aa}	0.30 ± 0.02^{Aa}	236 ± 04^{Aa}	5.36 ± 0.12^{Aa}	0.070 ± 0.007^{Aa}
Water deficit	0	0.80 ± 0.03^{Cb}	0.92 ± 0.09^{Bb}	0.05 ± 0.01^{Cb}	350 ± 21^{Cb}	0.87 ± 0.05^{Cb}	0.002 ± 0.002^{Cb}
Water deficit	50	2.98 ± 0.30^{Bb}	1.09 ± 0.10^{Bb}	0.08 ± 0.01^{Bb}	310 ± 11^{Bb}	2.75 ± 0.21^{Bb}	0.010 ± 0.001^{Bb}
Water deficit	100	4.29 ± 0.21^{Ab}	1.33 ± 0.07^{Ab}	0.17 ± 0.01^{Ab}	269 ± 19^{Ab}	3.25 ± 0.20^{Ab}	0.015 ± 0.001^{Ab}

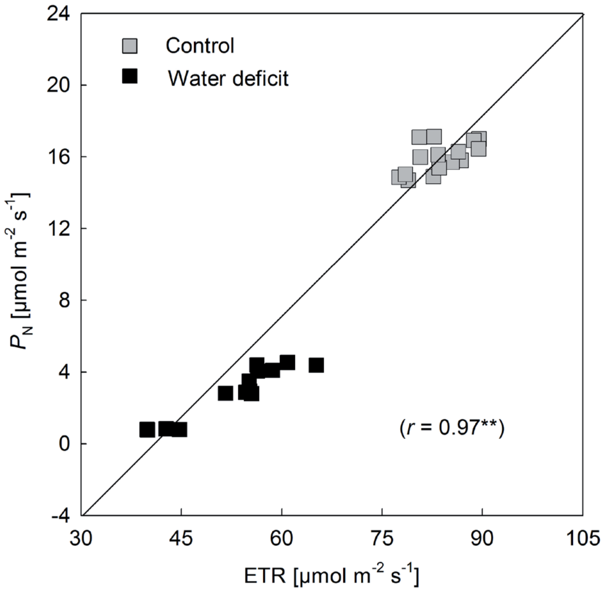

Fig. 3. Relationship between electron transport rate (ETR) and net photosynthetic rate (P_N) in soybean plants sprayed with EBR and exposed to water deficit. Asterisks (**) indicate the significance at 0.01 probability level.

after the use of 0.4 μM EBR. Corroborating our results, Souza *et al.* (2004) studying the Chl fluorescence in *Vigna unguiculata* exposed to water deficit presented an increase in F_0 and a reduction in F_m when compared to the control plants. Wang *et al.* (2015) examined *Vitis vinifera* treated with EBR and submitted to water deficit and reported that the exogenous application of EBR promoted an increase in F_v/F_m.

The exogenous application of EBR promoted increases in Φ_{PSII}, q_P, and ETR. This result linked to Φ_{PSII} clearly reveals a higher efficiency of the reaction centres aiming the capture of excited light energy (Yu *et al.* 2004). The increases in q_P and ETR promoted by the EBR reveals the positive interference on the activation of the PSII reaction centres, inducing the oxidation of Q_A, the primary quinone molecule responsible for receiving and transferring electrons between PSII and PSI (Maxwell and Johnson 2000, Singh and Prasad 2014, Jia *et al.* 2015). Li *et al.* (2015) found benefits for PSII after EBR application in seedlings of *Capsicum annuum* subjected to oxidative stress by low temperature, in which plants exposed to low temperature + 0.1 μM EBR exhibited significant increases of 13.2 and 5.6% in Φ_{PSII} and q_P, respectively, compared to the treatment under low temperature without EBR.

In plants under water deficit, the application of EBR promoted reductions in EXC, NPQ, and ETR/P_N. The decrease in EXC was due to a reduction in NPQ because the EBR reduced the loss of photons mainly in the form of heat, through the optimization in the use of light energy into photochemical processes (Zhang *et al.* 2015). The reduction in ETR/P_N suggests lesser distribution of electrons to alternative drains, such as photorespiration and the Mehler reaction (Fang *et al.* 2011, Silva *et al.* 2012). Similarly, to results described in this study, Lima and Lobato (2017) working with *Vigna unguiculata* under water deficit observed that the application of 100 nM EBR induced significant reductions of 19, 30, and 12% in the variables EXC, NPQ, and ETR/P_N, respectively, when compared to plants under equal water conditions without EBR.

Plants exposed to the water deficit + EBR treatment presented increases in P_N and P_N/C_i. The increase of P_N and decrease of C_i is due to EBR increasing the efficiency of Rubisco, an enzyme responsible for the carboxylation of CO_2 during the photosynthetic process (Yu *et al.* 2004). Our study proved that EBR increased ETR and P_N in control and stressed plants, revealing that ETR had a strong relationship with P_N as evidenced by a strong correlation ($r = 0.97$). These results suggest that soybean plants with higher values of ETR are more efficient in relation to P_N, and new research is necessary to evaluate the effects of EBR on components of production. Xia *et al.* (2009) reported a decrease of C_i in plants submitted to EBR application in a study on the roles of EBR and brassinazole linked to synthesis and activation of enzymes of the photosynthetic apparatus in *Cucumis sativus*. Hu *et al.* (2013) examined *Capsicum annuum* under conditions of 45% water content in the soil and observed that the application of 0.01 mg L^{-1} EBR attenuated the negative effects on P_N, increasing the CO_2 assimilation and efficiency of light use.

The EBR also provided increases in g_s and E. The attenuation of the water deficit on Ψ_W induced beneficial repercussion on these variables (g_s and E). Plants under water deficit frequently decrease the stomatal opening to lower the water loss through the transpiration process (Martineau *et al.* 2017). Singh and Reddy (2011) evaluated

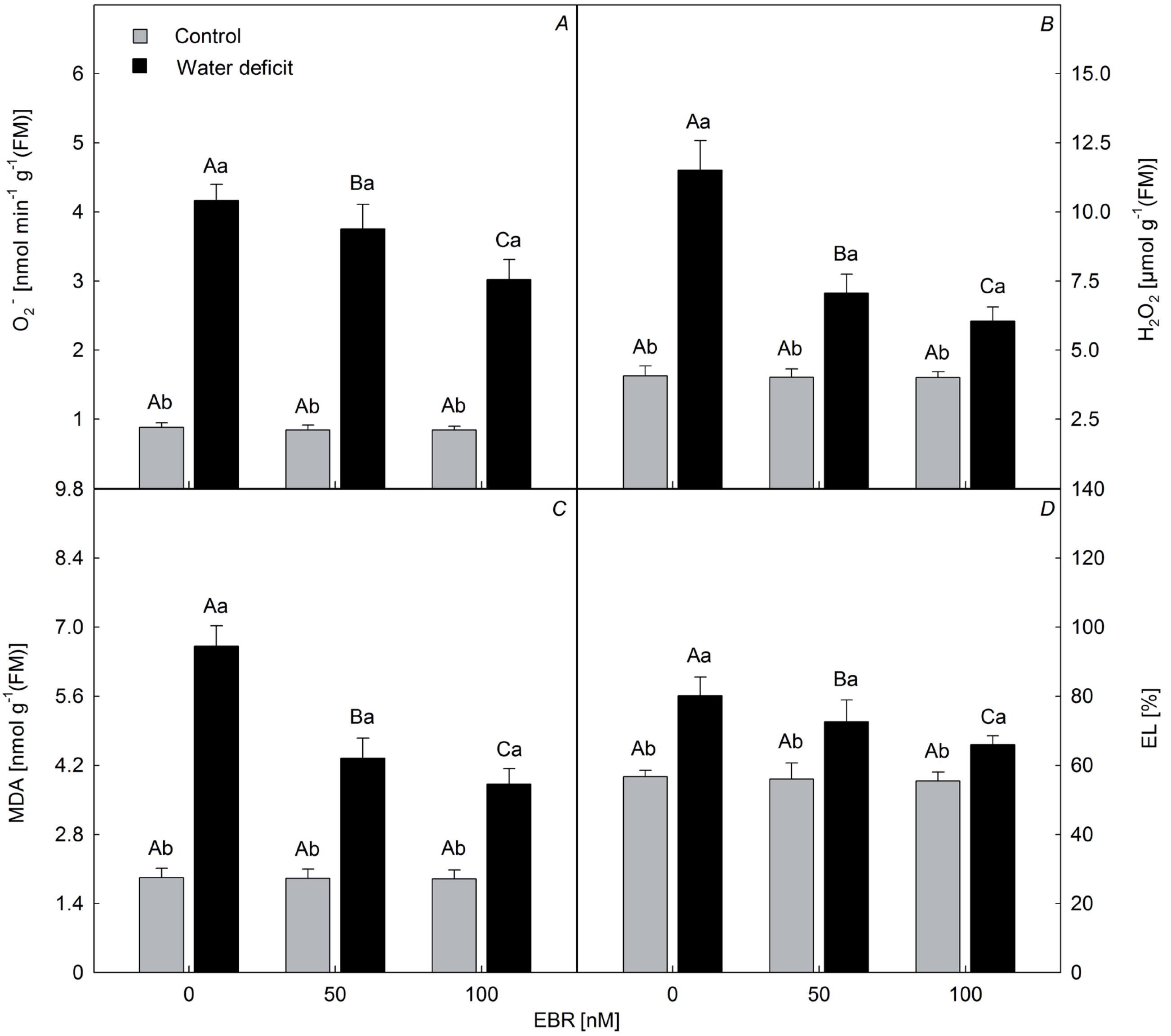

Fig. 4. Superoxide (O_2^-; *A*), hydrogen peroxide (H_2O_2; *B*), malondialdehyde (MDA; *C*), and electrolyte leakage (EL; *D*) in soybean plants sprayed with EBR and exposed to water deficit. Different *uppercase letters* between EBR concentrations (0, 50, and 100 nM EBR under equal water condition) and *lowercase letters* between water conditions (control and water deficit under equal EBR concentrations) indicate significant differences from the *Scott-Knott's* test ($P<0.05$). Means ± SD, $n = 5$.

the dynamics of photosynthesis and the WUE in 15 genotypes of *Vigna unguiculata* under water deficit and described an exponential relationship between g_s and E. In addition, Lima and Lobato (2017) found that g_s and E suffered significant reductions in *Vigna unguiculata* under water deficit; however, the exogenous application of 100 nM of EBR caused increases of 24 and 33% in these variables, respectively.

The increase in P_N positively influenced the WUE in *Glycine max* submitted to the water deficit + EBR treatment. Fariduddin *et al.* (2009), investigating the application of 0.01 μM of 28-homobrassinolide on the gas exchange and the antioxidant system in *Brassica juncea* subjected to water deficit, obtained an increase of 53% in WUE when compared to non-pulverized plants.

The EBR application mitigated the effects caused by the water deficit on O_2^- and H_2O_2 due to the minimization of NPQ and EXC. Excess energy and inadequate electron flow often result in the overproduction of reactive oxygen species (ROS) such as O_2^- and H_2O_2 (Lawlor and Tezara 2009). Yi *et al.* (2016) studying the mechanisms of photosynthetic recovery in leaves of *Gossypium herbaceum* under water deficit and rehydration also obtained increases in O_2^- and H_2O_2 concentrations. Wu *et al.* (2014) investigating the effects of high temperature and five concentrations of EBR on the growth of *Solanum melongena* showed that the use of EBR at 0.1 μM was more effective, reducing O_2^- and H_2O_2 contents by 32 and

Table 3. Photosynthetic pigments in soybean plants splayed with EBR and exposed to water deficit. Chl – chlorophyll; Car – carotenoids. Columns with *different uppercase letters* between EBR concentrations (0, 50, and 100 nM EBR under equal water conditions) and *lowercase letters* between water conditions (control and water deficit under equal EBR concentration) indicate significant differences from the *Scott-Knott* ′s test ($P<0.05$). Means ± SD, $n = 5$.

Water condition	EBR [nM]	Chl *a* [mg g^{-1}(FM)]	Chl *b* [mg g^{-1}(FM)	Total Chl [mg g^{-1}(FM)]	Car [mg g^{-1}(FM)]	Chl *a*/Chl *b*	Total Chl/Car
Control	0	11.16 ± 0.13^{Aa}	6.16 ± 0.38^{Ba}	17.32 ± 0.63^{Aa}	2.92 ± 0.13^{Aa}	1.83 ± 0.17^{Ab}	5.95 ± 0.27^{Ab}
Control	50	11.16 ± 0.79^{Aa}	6.28 ± 0.22^{Ba}	17.44 ± 1.21^{Aa}	3.02 ± 0.12^{Aa}	1.72 ± 0.11^{Ab}	5.78 ± 0.43^{Ab}
Control	100	11.22 ± 0.91^{Aa}	6.98 ± 0.27^{Aa}	18.21 ± 1.01^{Aa}	3.16 ± 0.31^{Aa}	1.62 ± 0.12^{Ab}	5.77 ± 0.34^{Ab}
Water deficit	0	6.64 ± 0.48^{Bb}	2.08 ± 0.10^{Cb}	8.72 ± 0.53^{Cb}	0.95 ± 0.10^{Cb}	3.20 ± 0.09^{Aa}	9.18 ± 0.46^{Aa}
Water deficit	50	8.89 ± 0.58^{Ab}	3.12 ± 0.30^{Bb}	12.00 ± 0.52^{Bb}	1.37 ± 0.10^{Bb}	2.88 ± 0.12^{Ba}	8.81 ± 0.25^{Aa}
Water deficit	100	9.31 ± 0.92^{Ab}	4.29 ± 0.34^{Ab}	13.60 ± 0.60^{Ab}	1.71 ± 0.10^{Ab}	2.19 ± 0.18^{Ca}	7.98 ± 0.38^{Ba}

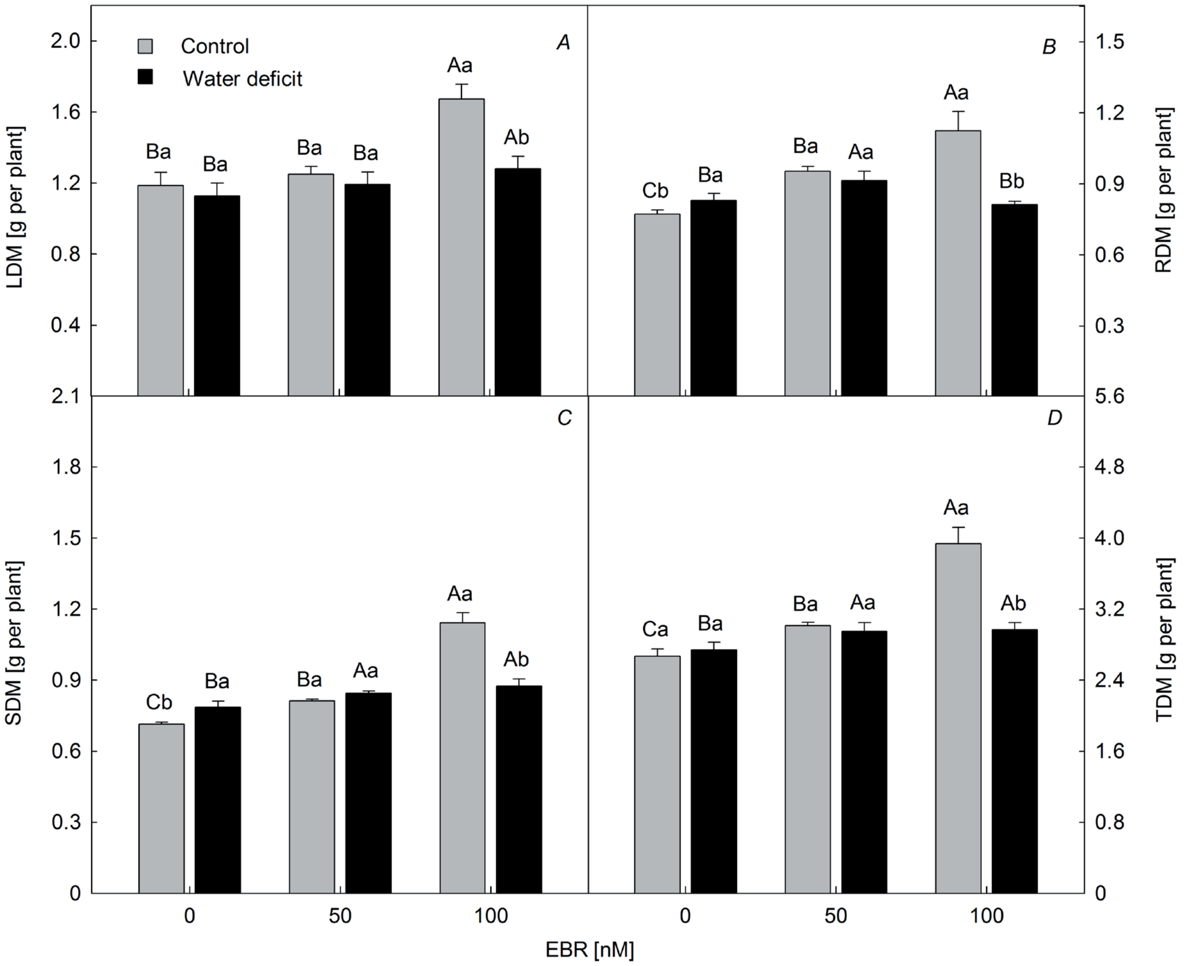

Fig. 5. Leaf dry matter (LDM; *A*), root dry matter (RDM; *B*), stem dry matter (SDM; *C*), and total dry matter (TDM; *D*) in soybean plants sprayed with EBR and exposed to water deficit. *Different uppercase letters* between EBR concentrations (0, 50, and 100 nM EBR under equal water condition) and *lowercase letters* between water conditions (control and water deficit under equal EBR concentrations) indicate significant differences from the *Scott-Knott*′s test (P<0.05). Means ± SD, n = 5.

41%, respectively, compared to non-sprayed plants.

Plants submitted to the water deficit + EBR treatment showed reductions in MDA and EL provided by the lower production of O_2^- and H_2O_2. These compounds are extremely toxic and in excess cause lipid peroxidation, inducing damages to cellular membranes and increases in electrolyte leakage (Gill and Tuteja 2010). Behnamnia *et al*. (2009b) studying the effects of three EBR concentrations in *Lycopersicon esculentum* exposed to five days under water deficit reported that this steroid mitigated the effects of water deficit on MDA. Li *et al*. (2012) evaluating the EBR benefits in *Chorispora bungeana* under water deficit observed that the concentration of 0.1 µM EBR promoted significant reduction in EL.

The EBR mitigated the negative effects on the ratio of Chl *a*/*b* and the ratio of total Chl/Car in plants under conditions of water deficit. Plants sprayed with 100 nM EBR and exposed to the water deficit exhibited an increase (106%) in Chl *b*. These increases verified in Chl *b* contents induced by EBR revealed minor photooxidative stress in chloroplasts because the Chl *b* is a molecule essentially linked to LHCII (Fleta-Soriano and Munné-Bosch 2016). Dobrikova *et al*. (2014), studying *Pisum sativum* exposed to stress by UV-B irradiation and sprayed with 0.1 mg L^{-1} EBR, observed a reduction of 11.6% in the ratio Chl *a*/*b* after 48-h exposure to irradiation UV-B.

Plants treated with EBR presented attenuation of the impact produced by the water deficit on LDM, RDM, STM, and TDM. Responses related to Φ_{PSII}, ETR, and P_N promoted benefits on growth parameters, previously detected after the EBR utilization. The growth is directly dependent on P_N and Φ_{PSII}; the maximization of the plant dry matter is associated with the increases of the assimilation of CO_2 and PSII efficiency (Barbosa *et al*. 2015). Liu *et al*. (2016) examined the effects of the water deficit on the photosynthetic characteristics and dry matter of two genotypes of *Triticum aestivum* at different water regimes, and they reported that moderate or severe water stress promoted a reduction in leaf and stem matters in both cultivars. However, (Behnamnia *et al*. 2009a) evaluated the effects of EBR and water deficits on *Lycopersicon esculentum* and detected an increase of 70% in shoot dry matter of plants under water deficit + 1 μM EBR, corroborating the results of this study.

Our results clearly demonstrated the inferences promoted by the water deficit on photosynthetic apparatus, reducing the effective quantum yield of PSII photochemistry, ETR, P_N, and water-use efficiency. However, the exogenous application of 100 nM EBR mitigated these negative effects, increasing these variables. EBR reduced the oxidant compounds (superoxide and hydrogen peroxide) and membrane damages (malondialdehyde and electrolyte leakage) in stressed plants. Our study proved that EBR increased ETR and P_N in control and stressed plants, revealing that ETR had a strong relationship with P_N. These results suggest that soybean plants with higher values of ETR are more efficient in relation to P_N.

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