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


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 context, the water photolysis oxidizes the $\text{H}_2\text{O}$ 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
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 CO2 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 (O2−) and hydrogen peroxide (H2O2) (Baguiz 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), 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 (ΦPSII) (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 (Pn) and stomatal conductance (gs). In Hordeum vulgare treated with EBR under effects of polyethylene glycol (PEG)-induced drought stress, applications led to decreases in H2O2 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] (Ahammad 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 KNO3, 7.5 mM Ca(NO3)2·4H2O, 3.25 mM NH4H2PO4, 1.5 mM MgSO4·7H2O, 62.50 μM KCl, 31.25 μM MnSO4·H2O, 2.50 μM MnSO4·H2O, 2.50 μM ZnSO4·7H2O, 0.63 μM CuSO4·5H2O, 0.63 μM NaMoO4·5H2O, and 250.0 μM Na2EDTA·Fe·3H2O. 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 (F0), the maximal fluorescence yield of the dark-adapted state (Fm), the variable fluorescence (Fv) were measured. The maximal quantum yield of PSII photochemistry (Fv/Fm)
The instantaneous carboxylation efficiency (N_{\text{NPQ}}) was calculated as NPQ = (F_{\text{m}} – F_{\text{m}'})/( F_{\text{m}}'). For photochemical quenching coefficient (q_{\text{P}}), the formula q_e = (F_{\text{m}} – F_{\text{i}})/( F_{\text{m}} – F_{\text{0}}) was used, while the nonphotochemical quenching (NPQ) was calculated as NPQ = (F_{\text{m}} – F_{\text{m}'})/( F_{\text{m}}). The electron transport rate (ETR) was calculated as ETR = \Phi_{\text{PSII}} × PPF D × 0.5 × 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_{\text{v}}/F_{\text{m}}) – (\Phi_{\text{at}} the PSII level (EXC) was calculated by the formula

was calculated using the formula F_{\text{i}}/F_{\text{m}} = (F_{\text{m}} – F_{\text{i}})/F_{\text{m}}, the effective quantum yield of PSII photochemistry (\Phi_{\text{PSII}}) was calculated by the formula (F_{\text{m}} – F_{\text{i}})/F_{\text{m}}. For photochemical quenching coefficient (q_{\text{P}}), the formula q_e = (F_{\text{m}} – F_{\text{i}})/( F_{\text{m}} – F_{\text{0}}) was used, while the nonphotochemical quenching (NPQ) was calculated as NPQ = (F_{\text{m}} – F_{\text{m}'})/( F_{\text{m}}).

**Evaluation of gas exchange:** The net photosynthetic rate (P_{\text{N}}), transpiration rate (E), stomatal conductance (g_{\text{s}}), and intercellular CO_{2} concentration (C_{\text{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.

**Leaf water potential:** The leaf water potential (\Psi_{\text{w}}) 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_{\text{i}}/F_{\text{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.

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**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 (Elastner and Heupel 1976) using spectrophotometer (UV-M51, Bel Photonics, Italy). O_{2} concentration was expressed in mmol min^{-1} g^{-1}(FM).

**Extraction of nonenzymatic compounds:** Nonenzymatic compounds (H_{2}O_{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_{2}O_{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_{2}O_{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_{2}O_{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}) × 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 Ψw; 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 F0, but the application of 100 nM EBR induced a significant reduction of 29% when compared to the water deficit without EBR (Fig. 2). For Fm, Fv, and Fv/Fm, 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.](image)

The water deficit reduced ΨPSII, qP, 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/Pn, the stress conditions caused increases in these variables; however, under the EBR treatment, these variables were significantly reduced. The values of NPQ and ETR/Pn 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 Pn, E, gs, WUE, and Pn/Ci, but 100 nM EBR induced increases of 436, 45, 240, 273, and 650%, respectively (Table 2). However, the Ci were higher in the plants under water deficit and values were reduced by EBR utilization. The concentration of 100 nM decreased the value of Ci 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 Pn (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 O2, H2O2, 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 Ψw 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...
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to water deficit and reported that the application of 0.1 mg L\(^{-1}\) brassinolide promoted an increase of 21\% in \(\Psi_W\) 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 \(F_0\).
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 Pn and a reduction in Fv/ Fm 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 Fv/ Fm.

The exogenous application of EBR promoted increases in ΨP, qE, and ETR. This result linked to ΨP clearly reveals a higher efficiency of the reaction centres aiming the capture of excited light energy (Yu et al. 2004). The increases in qE and ETR promoted by the EBR reveals the positive interference on the activation of the PSII reaction centres, inducing the oxidation of PQ, 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 ΨP and qE, 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/ NP. 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/ NP 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/ NP, respectively, when compared to plants under equal water conditions without EBR.

Plants exposed to the water deficit + EBR treatment presented increases in Pn and Pn/ C. The increase of Pn and decrease of C; is due to EBR increasing the efficiency of Rubisco, an enzyme responsible for the carboxylation of CO2 during the photosynthetic process (Yu et al. 2004). Our study proved that EBR increased ETR and Pn in control and stressed plants, revealing that ETR had a strong relationship with Pn, 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 Pn, and new research is necessary to evaluate the effects of EBR on components of production. Xia et al. (2009) reported a decrease of C; 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 Pn, increasing the CO2 assimilation and efficiency of light use.

The EBR also provided increases in g, and E. The attenuation of the water deficit on Ψw induced beneficial repercussion on these variables (g, 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

Table 2. Gas exchange in soybean plants sprayed with EBR and exposed to water deficit. Pn – net photosynthetic rate; E – transpiration rate; g – stomatal conductance; Ci – intercellular CO2 concentration; WUE – water-use efficiency; Pn/ Ci – 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.

<table>
<thead>
<tr>
<th>Water condition</th>
<th>EBR (nM)</th>
<th>Pn [μmol m−2 s−1]</th>
<th>E [mmol m−2 s−1]</th>
<th>g [mol m−2 s−1]</th>
<th>Ci [μmol mol−1]</th>
<th>WUE [μmol mmol−1]</th>
<th>Pn/ Ci [μmol m−2 s−1 Pa−1]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>15.05 ± 0.33bs</td>
<td>3.04 ± 0.21bs</td>
<td>0.28 ± 0.02bs</td>
<td>262 ± 07bs</td>
<td>4.96 ± 0.14bs</td>
<td>0.060 ± 0.008bs</td>
</tr>
<tr>
<td>Control</td>
<td>50</td>
<td>16.23 ± 0.57bs</td>
<td>3.08 ± 0.29bs</td>
<td>0.31 ± 0.01bs</td>
<td>242 ± 08bs</td>
<td>5.31 ± 0.11bs</td>
<td>0.067 ± 0.006bs</td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
<td>16.58 ± 0.42bs</td>
<td>3.09 ± 0.02bs</td>
<td>0.30 ± 0.02bs</td>
<td>236 ± 04bs</td>
<td>5.36 ± 0.12bs</td>
<td>0.070 ± 0.007bs</td>
</tr>
<tr>
<td>Water deficit</td>
<td>0</td>
<td>0.80 ± 0.03cs</td>
<td>0.92 ± 0.09bs</td>
<td>0.05 ± 0.01bs</td>
<td>350 ± 21bs</td>
<td>0.87 ± 0.05bs</td>
<td>0.002 ± 0.002cs</td>
</tr>
<tr>
<td>Water deficit</td>
<td>50</td>
<td>2.98 ± 0.30bs</td>
<td>1.09 ± 0.10bs</td>
<td>0.08 ± 0.01bs</td>
<td>310 ± 11bs</td>
<td>2.75 ± 0.21bs</td>
<td>0.010 ± 0.001bs</td>
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<tr>
<td>Water deficit</td>
<td>100</td>
<td>4.29 ± 0.21Ab</td>
<td>1.33 ± 0.07Ab</td>
<td>0.17 ± 0.01Ab</td>
<td>269 ± 19bs</td>
<td>3.25 ± 0.20Ab</td>
<td>0.015 ± 0.001Ab</td>
</tr>
</tbody>
</table>

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

Plants submitted to the water deficit + EBR treatment showed reductions in MDA and EL provided by the lower production of O$_2^-$ and H$_2$O$_2$. 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 LHCCI (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 μg 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 ΦPSII, ETR, and Pₘ promoted benefits on growth parameters, previously detected after the EBR utilization. The growth is directly dependent on Pₘ and ΦPSII, the maximization of the plant dry matter is associated with the increases of the assimilation rate, photosynthesis, 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, Pₘ, 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 Pₘ in control and stressed plants, revealing that ETR had a strong relationship with Pₘ. These results suggest that soybean plants with higher values of ETR are more efficient in relation to Pₘ.

References


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