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The 24-epibrassinolide induces rice tolerance to water stress overcoming losses in grain yield

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Abstract

Water stress leads to large productivity losses in rice cultivation, and plant hormones play a key role in the plant strategies to prevent the harmful effects of water stress in crop development. The objective of this work was to investigate the effects of brassinosteroids (BRs) in rice development and yield under irrigation and under water deficit conditions applied during the pre-anthesis period. Exogenous applications of bioregulators were performed through leaf spray. Two dosages of 24-epibrassinolide (EBL) were applied (0.01 µM and 0.1 µM), in addition to two dosages of ABA (2.27 mM and 4.54 mM) in order to compare the effects, considering its already known beneficial effects in response to drought adaptation. Plants of the cultivar IRGA 424RI grown under greenhouse conditions were submitted to water deficit from V13 stage until reaching a leaf water potential of -2 MPa. Physiological, growth and biochemical parameters were measured. Even though the water stress reduced plant growth, 0.01µ M EBL-treated plants presented unchanged initial dry weight, height and tillering after the stress imposition. The 0.1 µM EBL-treated plants maintained the total dry weight of grains even after water stress imposition, while control and ABA-treated plants showed a 63.9% and 28.1-58.6% reduction, respectively. The 0.01 µM EBL treatment doubled the contents of chlorophyll a, b and total and decreased stomatal conductance under stress in 41.1%. EBL treatments were able to maintain similar peroxidation levels between stressed and non-stressed plants. When analyzed together, these results indicate that EBL shows a promising perspective for improving rice tolerance to controlled water stress.

Keywords: Abscisic acid; Brassinosteroids; Drought; Lipid peroxidation; Water pontential.
Abbreviations: ABA_Abiscisic acid; BRs_brassinosteroids; EBL_24-epibrassinolide.

Introduction

Rice (*Oryza sativa* L.) is a crop of great importance in the world, planted in approximately 11% of the cultivated areas. In 2013, world production was 746 million tons harvested in an area of 165 million hectares (FAO, 2015), with 90% of the production and consumption concentrated in the Asian continent. Thirty percent of the total water requirements of rice are consumed in the vegetative phase, 55% in the reproductive phase and 15% during maturation. Therefore, the requirement of water per month for reasonable production of rice is 180 to 300 mm (Fornasieri Filho and Fornaseri,1993).

Drought is one of the major constraints on crop yield in semi-arid regions of the world (Ozturk, 2002) and affects plant growth and its biomass allocation. Physiological responses to drought include stomatal closure, with decreases in CO_2 assimilation rates; changes in pigment content; rapid reactive oxygen species (ROS) accumulation; alteration in the activity and genetic expression of antioxidant metabolism enzymes; synthesis of non-enzymatic plant antioxidants; accumulation of compatible solutes; and increases in proline concentrations, among others (Reddy et al., 2004; Xu et al.,2010). Despite all of the mechanisms of adaptation, extended water stress can lead to plant death.

Abscisis acid (ABA) plays an important role in the plant response to drought. ABA regulates the stomatal opening (Hartung et al., 1998) and root hydraulic conductivity (Hose et al.,2000). When applied exogenously, ABA causes rapid stomatal closure and reduces water loss via transpiration (Qin and Zeevaart, 1999).

Studies show that ABA applications reduced stomatal conductance in wheat and plays an important role in reducing water use. There was also an increase in antioxidant defenses during soil drying (Du et al., 2013). The role of ABA as a warning sign of drought has also been proven (Jackson, 1997). Under conditions of water stress, the concentration of ABA increases dramatically in shoots of some crops, such as potatoes, tomatoes and grapes (Lemos, 2005).

According to Jiang and Zhang (2002), the accumulation of ABA induced by water stress causes an increase in ROS generation, leading to the induction of the antioxidant defense system. In maize, ABA treatments led to a significant increase in superoxide radical and hydrogen peroxide (H_2O_2) levels, followed by an increased activity of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) (Jiang and Zhang, 2002).

In rice, exogenous applications of ABA in leaves promoted the expression of some enzymes, such as catalase, suggesting that ABA prevents excessive accumulation of H_2O_2 (Ye et al., 2011). In wheat, beneficial effects of increased activity of antioxidant enzymes and reduced oxidative stress were reflected by the increase in the chlorophyll content, carotenoids and relative water content in wheat (Agarwal et al., 2005).

Brassinosteroids (BRs) are a class of plant steroid hormones that regulate various physiological and developmental processes, such as stretching and cell division, germination, photosynthesis, growth, flowering, fruit development and ripening, grain filling and foliar senescence, among others (Sasse., 2003).

Given the importance of BRs in plant development, it is possible to infer that changes in their concentration in agronomical important crops can guarantee an increase in productivity (Bhardwaj et al., 2014). In fact, transgenic rice plants with increased production of BRs presented higher growth than wild plants, with more tillering and an increase in grain yield greater than 40% (Wu et al., 2008). Rice plants treated with the exogenous brassinolide (BL) during flowering presented increased productivity due to higher grain filling (Saka et al., 2003).

In addition, studies have shown that BRs play an important role in the resistance to various environmental stresses (Kagale et al., 2007; Ahammed et al., 2015). Several studies have shown that BR applications to plants subjected to drought were able to mitigate the effects of water deficit. The application 24-epibrassinolide (EBL) relieved the effects of water stress, stimulating growth and improving the water use efficiency of pea (Xiong et al., 2016). Additionally, EBL application raised the photosynthetic rate, relative water content and antioxidant enzymes in tomato (Yuan et al., 2012) and reduced lipid peroxidation and H_2O_2 production (Yuan et al., 2010). Plants of *Brassica juncea* treated with 28-homobrassinolide (HL) after a long dry period resumed growth and photosynthetic activity after treatment (Fariduddin et al., 2009).

In rice, exogenously applied BRs improved the net CO_2 assimilation, water use efficiency and leaf water status and reduced the production of both malondialdehyde and H_2O_2 (Farooq et al., 2009).

Based on the importance of BRs in plant development and adaptation to different environmental conditions, the objective of this work was to evaluate whether the EBL is able to mitigate the effects of water stress imposed on rice plants during the growing period and provide normal grain production after stress. We also used ABA applications to compare the effects of bioregulators, as the effects of ABA on drought adaptation are already well described.

Results

Effects of water stress in growth, physiological and biochemical parameters

After treatment application, at 37 days after emergence (DAE), water stress was imposed for a 7-day period, until the plant water potential reached around -2 MPa. During this period, the water potential was monitored. The plants were collected later to verify any growth differences. The imposed water deficit reduced the water potential of the plants not treated with bioregulators (control group) (Fig. 1), which clearly wilted when compared to the plants under constant irrigation.

There was a reduction in the initial dry weight of the plants subjected to water stress and not treated with bioregulators (Table 1) as well as reductions in plants height at the end of the cycle, leaf area and tillering (Table 1). Unlike the initial dry weight, the plants were able to recover, and there were no significant differences in the final dry weights. The final dry weight average was 142.5 g. Despite this, total grain weight was affected by water stress (Fig. 2).

After the resumption of irrigation, some plants continued to have a yellowish hue in the leaves. For this, the determination of leaf pigment content was carried out at 53 DAE to identify if the yellowing was a response to the water deficit or to the plant bioregulators. Water stress reduced the chlorophyll a content by half in those plants that did not receive the plant bioregulators (Fig. 3a). The same was observed for total chlorophyll (Fig. 3c). Chlorophyll b and carotenoid contents remained unchanged after stress (Figs. 3b and 3d).

There was a decrease in transpiration in the plants not treated with bioregulators when subjected to water stress, which was associated with a decrease in stomatal conductance (Fig. 4). Regarding the effects of the water deficit on the levels of lipid peroxidation, the water stress led to an increase in the levels in plants not treated with the plant bioregulators (Fig. 5).

Effects of ABA treatments in growth, physiological and biochemical parameters under water stress

The imposed water deficit reduced the water potential even in plants treated with ABA (Fig. 1). Despite this, unlike plants not treated with plant bioregulators, those that received 2.27-mM ABA treatments maintained their initial dry weight when subjected to water stress (Table 1); however, the application reduced the plant dry weight compared to that of untreated plants. For both concentrations, the same was observed in relation to plant height and leaf area (Table 1). The tillering decreased with the imposition of stress in ABA-treated plants. ABA 4.54 mM treatment decreased the tillering in irrigated plants compared to plants untreated with bioregulators (Table 1).

The total dry weight of grains in plants that received the 4.54-mM ABA treatment remained unchanged for both the water deficit ones and those maintained under full irrigation (Fig 2). Unlike plants not treated with plant bioregulators, plants that received both ABA treatments maintained their levels of chlorophyll a and total chlorophyll after the imposition of the water stress (Figs. 3a and 3c). However, the concentration of 4.54 mM decreased the chlorophyll a and total chlorophyll levels in relation to control plants under full irrigation. Carotenoid contents, although unchanged after stress, were elevated by the application of ABA 2.27 mM alone (Fig. 3d). The ABA treatments maintained the rates of transpiration and stomatal conductance after the imposition of stress (Fig. 4). In addition, under full irrigation, there were no changes in stomatal conductance and transpiration in ABA-treated plants in relation to plants not treated with bioregulators.

Regarding lipid peroxidation, 2.27 mM ABA led to reduced levels in stressed plants (Fig. 5). In relation to bioregulators

Table 1. Growth parameters in control plants and stressed plants.

	DW$_i$ (g)[a]		FH (cm)[b]	
Treatment	Control	Stress	Control	Stress
Control	14.5 ± 0.4 Aa*	11.1 ± 0.7 Bb	76.2 ± 1.9 ABa	69.9 ± 3.8 ABb
ABA 2.27 mM	10.6 ± 0.3 Ca	10.5 ± 0.3 Ba	69.0 ± 2.4 CDa	66.3 ± 1.9 Ba
ABA 4.54 mM	13.2 ± 0.9 ABa	11.2 ± 0.2 Bb	64.4 ± 3.3 Da	66.7 ± 3.2 Ba
EBL 0.01 µM	12.0 ± 1.3 BCa	11.0 ± 1.3 Ba	72.7 ± 3.6 BCa	71.9 ± 3.3 Aa
EBL 0.1 µM	13.3 ± 0.3 ABa	13.6 ± 1.0 Aa	78.4 ± 2.0 Aa	68.6 ± 2.9 ABb
CV (%)	7.9		3.6	

	LA (cm^2)[c]		NT (tillers/plant)[d]	
Treatment	Control	Stress	Control	Stress
Control	1281.1 ± 30.6 Aa	761.0 ± 50.7 Bb	19.0 ± 2.5 Aa	12.3 ± 1.3 Ab
ABA 2.27 mM	762.2 ± 124.1 Ba	658.5 ± 42.0 Ba	15.8 ± 1.7 ABa	14.5 ± 2.8 Ab
ABA 4.54 mM	694.1 ± 166.1 Ba	624.2 ± 111.2 Ba	13.5 ± 1.3 Ba	6.0 ± 0.8 Ab
EBL 0.01 µM	1211.6 ± 18.8 Aa	716.9 ± 85.9 Bb	14.5 ± 3.7 ABa	12.5 ± 2.7 Aa
EBL 0.1 µM	1216.4 ± 117.3 Ab	1383.0 ± 113.3 Aa	19.3 ± 1.3 Aa	16.0 ± 3.2 Ab
CV (%)	11.4		17.1	

[a]DW$_i$ - Initial dry weight 15 days after application of treatments; [b]FH - Final height of the plants measured in the period before anthesis; [c]LA – Leaf area of the plants measured in the period before anthesis; [d]NT - Number of tillers measured in the period before anthesis. *The values are mean (n = 10) ± standard error with different letters indicate significant differences by Tukey test (p <0.05). Capital letters represent differences between treatments with bioregulators. Lowercase letters represents differences between hydric regimes.

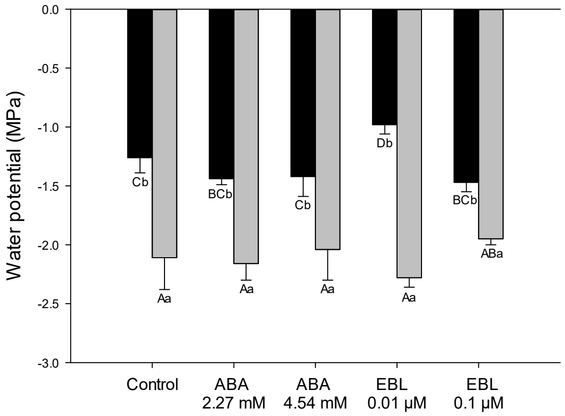

Fig 1. Water potential of plants measured after 5 days of water deficit imposition in control plants (black) and stressed plants (grey). The values of the bars are mean (n = 10) columns with different letters indicate significant differences by Tukey test (p <0.05). Capital letters represent differences between treatments with bioregulators. Lowercase letters represents differences between hydric regimes.

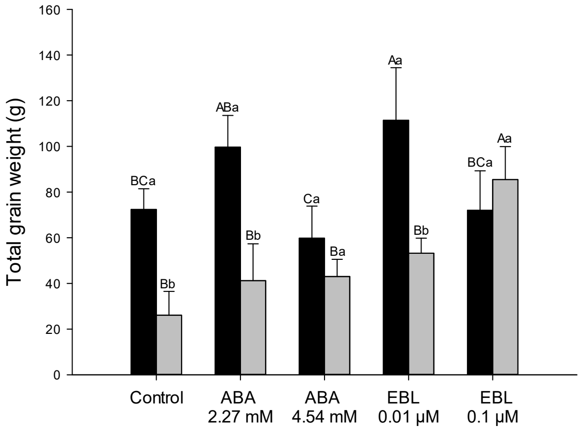

Fig 2. Total grain weight measured after harvesting of spikelets of control plants (black) and stressed plants (grey). The values of the bars are mean (n = 10) columns with different letters indicate significant differences by Tukey test (p <0.05). Capital letters represent differences between treatments with bioregulators. Lowercase letters represents differences between hydric regimes.

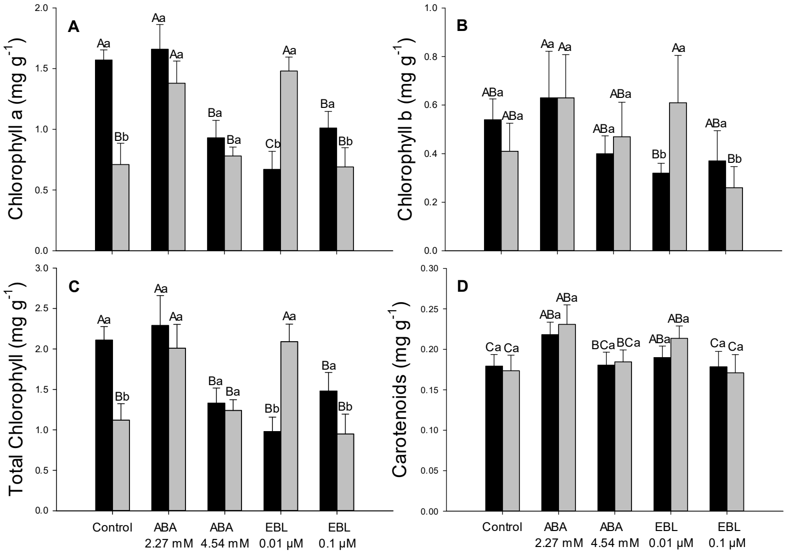

Fig 3. Foliar pigments. Collected 15 days after application of treatments in control plants (black) and stressed plants (grey). A) Chlorophyll a; B) Chlorophyll b; C) Total chlorophyll; D) Carotenoids. The values of the bars are mean (n = 10) columns with different letters indicate significant differences by Tukey test (p <0.05). Capital letters represent differences between treatments with bioregulators. Lowercase letters represents differences between hydric regimes.

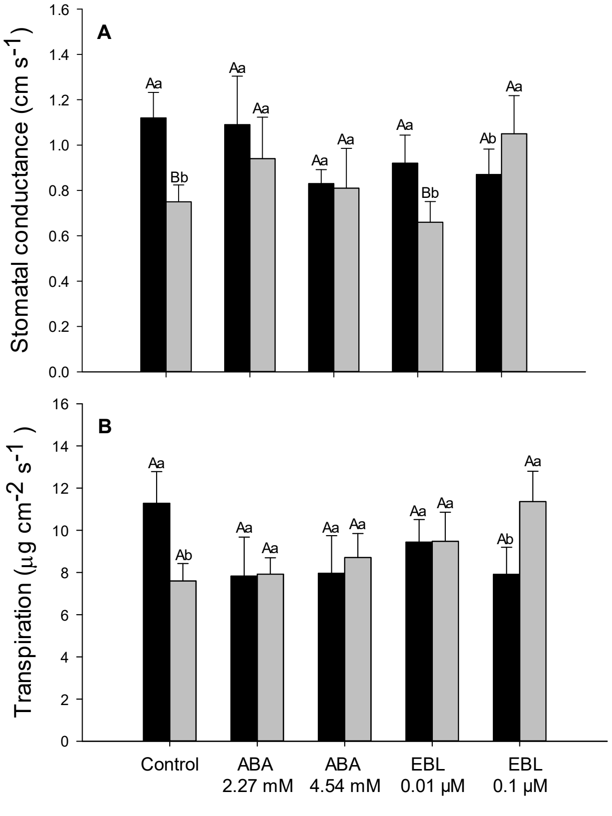

Fig 4. Stomatal conductance (A) and transpiration (B) of plants measured after 5 days of water deficit imposition in control plants (black) and stressed plants (grey). The values of the bars are mean (n = 10) columns with different letters indicate significant differences by Tukey test (p <0.05). Capital letters represent differences between treatments with bioregulators. Lowercase letters represents differences between hydric regimes.

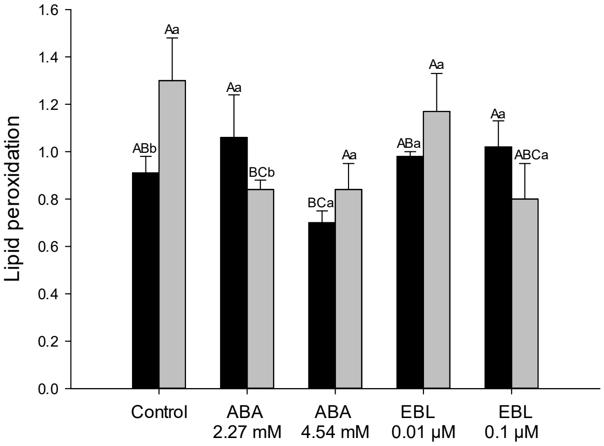

Fig 5. Lipid peroxidation in control plants (black) and stressed plants (grey). The values of the bars are mean (n = 10) columns with different letters indicate significant differences by Tukey test (p <0.05). Capital letters represent differences between treatments with bioregulators. Lowercase letters represents differences between hydric regimes.

treatments, ABA treatment led to unchanged lipid peroxidation levels, even in the presence of water stress compared to control plants.

Effects of EBL treatments in growth, physiological and biochemical parameters under water stress

The application of 0.01 µM EBL increased the water potential of irrigated plants compared with the control group (Fig. 1). Nevertheless, the EBL application did not prevent the reduction of water potential under stress. Despite this, plants treated with EBL maintained their initial dry weight despite the imposition of water stress (Table 1), although 0.01 µM EBL reduced the dry weight compared with that of plants not treated with bioregulators under irrigation. However, 0.01 µM EBL was able to maintain the plant height at the end of the cycle, and the tillering remained unchanged despite the imposition of water stress (Table 1). The concentration of 0.1 µM EBL increased the leaf area in plants subjected to water stress (Table 1).

In terms of productivity, 0.1 µM EBL was able to maintain the dry weight of grains in plants subjected to water stress (Fig. 2). Although this was not observed for 0.01 µM EBL, it is noteworthy that in the absence of stress, such concentrations increased the grain yield compared to plants not treated with bioregulators.

The treatments with EBL reduced chlorophyll a and total chlorophyll contents in the absence of stress compared to plants not treated with bioregulators (Figs. 3a and 3c). However, with the imposition of stress, values for chlorophyll a, chlorophyll b and total chlorophyll content increased about 2-fold with the 0.01 µM EBL treatment (Figs. 3a, 3b and 3c). The 0.01 µM EBL treatment provided an increase in the carotenoids compared with non-treated controls plants, both in absence and presence of stress (Fig. 3d).

There was an increase in transpiration and stomatal conductance in plants treated with 0.1 µM EBL under water stress compared with irrigated plants (Fig. 4). The stomatal conductance in plants treated with 0.01 µM EBL decreased with the imposition of stress. Treatments with EBL applications were able to maintain similar peroxidation levels between stressed and non-stressed plants (Fig. 5).

When considered together, these results indicate that both bioregulators were effective in mitigating the negative effects of water deficit. However, the effects of EBL application were more pronounced, as it was able to maintain grain production even with the imposition of stress.

Discussion

The BRs are a group of naturally hidden steroid compounds with broad biological activity that offer the unique possibility of increasing crop yields, both through changing plant metabolism and plant protection from environmental stresses (Krishna ,2003). BRs are involved in maintaining the water content in tissues, the photosynthetic rate and the biomass production, observed in wheat under water stress (Sairam ,1994). In bean, BR treatment increased yield (based on seed weight per plant) by 45%. Under stress conditions, the application of BRs had very pronounced effects on productivity (Ikekawa and Zhao, 1991).

In the present work, these positive aspects were observed, as the application of EBL allowed the maintenance of dry weight (0.01 and 0.1 µM EBL), height (0.01 µM EBL) and tillering (0.01 µM EBL) as well as the leaf area to increase under water deficit (0.1 µM EBL). The increase in leaf area leads to a greater uptake of sunlight as well as greater photosynthesis, guaranteeing an increase in productivity. This increase in productivity was verified by total production of grains in plants treated with 0.1 µM EBL, which was maintained even under stress.

On the other hand, there was no increase in productivity observed with the application of ABA. It is well known that exogenous application of ABA or increases in endogenous ABA (stress stimulus production) in a plant boosts the growth and grain dry weight production (Yang et al., 2003; Travaglia et al., 2010; Reinoso et al.,2011). In this case, the stimulus (water stress) and the treatment applications occurred during the pre-anthesis period. In this way, the response of gibberellins (GAs) to ABA treatment is negative for the plant because the bioactive response of GA is integral to the development of pollen (Kaneko et al., 2004). All these factors incur in the reduction of total grain yield when comparing irrigated and stressed plants treated with 2.27 mM ABA.

The effect of ABA was observed during the first few days after application. There was a reduction in growth at the higher concentration (ABA 4.54 mM) in which there was a reduction of dry weight in stress condition, as reported by other authors (Achard et al., 2006). Even in the absence of stress, it was possible to observe a reduction of dry weight, height and leaf area in ABA-treated plants compared to control plants.

Greater efficiency in the control of transpiration under water deficit represents a greater water use efficiency. Increasing evidence points to a possible role of BRs in the regulation of stomatal opening and closure. In tomato, EBL induces stomatal opening at concentrations of 0.01 µM and 0.1 µM, while higher concentrations lead to total stomatal closure (Xia et al., 2014).

In fact, in the present study, the effects of EBL on transpiration and stomatal conductance were shown to depend on the EBL concentration. Lower concentration reduced stomatal conductance in the presence of stress, whereas higher concentration increased transpiration and stomatal conductance under stress condition. Ha et al. (2016) demonstrated that BL acts in the stomatal closure in *Arabidopsis thaliana* in an ABA-independent manner and suggested that BRs may act by modulating ABA-mediated stomatal closure both positively and negatively, depending on their concentration. In fact, the process of stomatal opening and closure is not only ABA-dependent. In drought stress conditions, a dose-dependent action has also been reported for other hormones, such as cytokinins and auxins (Daszkowska-Golec and Szarejko, 2013). Therefore, it is possible that the dose-dependent action of BRs on stomatal behavior is due to their crosstalk with other hormones.

Regarding the occurrence of cellular damage, both concentrations of EBL were able to avert the negative effects of water stress. This was evidenced by the reduction of the lipid peroxidation verified in comparison to the control. According to Farooq et al. (2009), the application of 0.01 µM EBL to leaves of rice subjected to water stress is effective at reducing the levels of lipid peroxidation. This reduction is associated with an increase in the activity of antioxidant enzymes, reducing both H_2O_2 and membrane permeability.

Similarly, the 2.27-mM ABA treatment had a marked effect on reducing levels of lipid peroxidation when comparing stressed and non-stressed plants. It is possible that one of the mechanisms of action of ABA in rice during drought is through the induction of the CAT enzyme, which prevents excessive accumulation of H_2O_2, avoiding the occurrence of cellular damage (Ye et al., 2011). However, this effect appears to be dependent on the concentration used, as the 4.54-mM ABA concentration did not have the same effect.

The beneficial effects of EBL application on the improvement of growth parameters as well as productivity observed here are possibly related to the improvement of the antioxidant system. This improvement is reflected in the reduction of membrane damage and in the protection of the photosynthetic apparatus. This fact, coupled with the increase in chlorophyll content provided by the 0.01-µM EBL treatment and the increase in leaf area by the 0.1-µM EBL treatment, ensures the maintenance of photosynthetic rates and growth, reflecting an increase in final yield.

Therefore, we conclude that EBL influences the plant, increasing its growth as well as its leaf area. The reduction in chlorophyll content was transient and possibly influenced by an improvement in its efficiency. In addition, is possible that the activation of antioxidant defenses led to a greater adaptation of the plant to stress, reducing lipid peroxidation and increasing production even in adverse conditions.

Materials and Methods

Plant material, growth conditions and treatments

Rice seeds of cultivar IRGA 424RI were planted in 20-L pots containing soil, sand and compost in the proportion 2:1:1. Ten seeds per pot were sown and after germination, only 5 plants were left in each pot. The plants were grown under greenhouse conditions in the experimental field of the Biological Sciences Department of the University of São Paulo in Piracicaba (22°42’30”S, 47°38’00”W), under a solar irradiance of 1000 µmol m^{-2} s^{-1}, an average temperature of 28.5°C during the growing cycle (nighttime average: 24.5°C; daytime average 32.5°C), an average relative humidity of 76% (nighttime average: 80%; daytime average 55.3°C) and a photoperiod of 12 h. The plants were maintained under constant irrigation throughout the growing period. At 37 DAE, at the V13 stage, when the plants reached the maximum leaf area and growth, the following applications were performed: control (no application), EBL from a stock solution of 2 g L^{-1} (Sigma-Aldrich, Saint Louis, MO, USA) at the final concentrations of 0.01 µM and 0.1 µM and ABA from a stock solution of S-ABA 10% w/v (Valent Biosciences, Libertyville, IL, USA) at the final concentrations of 2.27 mM and 4.54 mM. The plant bioregulators were applied by foliar spraying, using 80 mL of solution in order to wet all of the leaves homogeneously. Water stress was imposed through the suspension of irrigation.

Plants were monitored, and after seven days, when the stress was moderate (evident from leaf rolling), the water potential was measured. When the water potential approached -2 MPa, all the plants were irrigated and later cultivated until grain formation. The water potential was monitored using the vapor pressure equilibrium method using a model HR-33T microvoltmeter (Wescor, Logan, UT, USA) coupled to Wescor C52 chambers.

Stomatal conductance and transpiration

Stomatal conductance and transpiration were monitored during the experiment using a dynamic equilibrium porometer (model LI-1600, LI-COR, Lincoln, NE, USA). It was analyzed at 2 distinct periods: at the end of water stress period (maximum stress) and 5 days after plant rehydration. Measurements were obtained from the newest fully expanded leaves.

Growth parameters

At 53 DAE, plant height and tiller number were measured. Samples for leaf area determination were collected using the

LI-3100 (LI-COR). The plant material was collected after the grain-filling phase (at 128 DAE) to determine the plant and grain dry weights. Dry weights were determined by drying the plant material at 60°C for 72 h in an air circulation oven and subsequent weighing. The grain dry weight was determined using the total grain content produced per plant.

Foliar pigments

At 53 DAE, the newest most-expanded leaves were collected for the determination of chlorophyll a and b contents, total chlorophyll and carotenoids. Extractions of the pigments were performed using an 80% (v/v) aqueous acetone solution. Approximately 0.1 g of leaves was ground in liquid nitrogen until a complete powder was formed. Then, 10 mL of the acetone solution was added and centrifuged for 10 min at 3000 x g. Afterward, 1.5 mL of the supernatant was added to 1.5 mL of acetone solution. The absorbance was then determined at the following wavelengths: 663, 646 and 440 nm. To determine the pigment contents, calculations proposed by Lichtenthaler (1987) were used.

Lipid peroxidation

Leaf tissue (0.1 g) from the newest most-expanded leaf was macerated and added to 0.5 ml of 0.1% trichloroacetic acid (TCA) (w/v). Samples were centrifuged for 10 min (15000 x g at 4°C). Afterward, 0.5 mL of the supernatant was added to 1.5 mL of 0.5% thiobarbituric acid (TBA) diluted in 20% TCA. These solutions were incubated in a water bath at 95°C for 25 min. Then, they were incubated on ice to terminate the reactions. The absorbance of the samples was then measured in a spectrophotometer at wavelengths of 532 and 600 nm (Heath and Packer 1968).

Experimental design

The experimental design was completely randomized with 4 replications in a 2x5 factorial scheme consisting of two levels of moisture (stress and irrigated), 2 concentrations of ABA (2.27 mM and 4.54 mM) and 2 concentrations of EBL (0.1 µM and 0.01 µM), in addition to the control treatment, in which there was no application of plant growth regulator. The results were submitted to analysis of variance and, when significant, were submitted to the Tukey test at the 5% probability level.

Conclusion

Rice plants subjected to stress exhibited impaired growth and production. The EBL treatment promoted an improvement in the plant's ability to adapt to drought conditions, resulting in maintained growth and reduced lipid peroxidation in the plant. In addition, EBL use promoted better osmotic control in the plant, resulting in a higher grain yield, even under stress. EBL is, therefore, a promising perspective for improving cultivation conditions. Under the conditions of this experiment, the EBL application promoted better results for avoiding drought damage during the preharvest period, compared to ABA applications.

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