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EFFECT OF WATER DEFICIT AND ABSCISIC ACID ON PHOTOSYNTHETIC AND ANTIOXIDANT METABOLISM IN SEEDLINGS OF *Calophyllum brasiliense* (CAMBESS.)

REIS, L. C.; FORESTI, A.; SCALON, S. P. Q.; DRESCH, D. M.; PEREIRA, Z. V. Effect of water deficit and abscisic acid on photosynthetic and antioxidant metabolism in seedlings of *Calophyllum brasiliense* (Cambess.). **CERNE**, v. 24, n. 4, p. 387-396, 2018.

HIGHLIGHTS

Intermittent water deficit decreased plant water status and gas exchange.

Application of ABA preserved the photosynthetic apparatus.

Antioxidant activity increased depending on the enzyme, ABA concentration and plant part.

ABSTRACT

The aim of the present study was to evaluate the effect of different concentrations of abscisic acid (ABA) on photosynthetic metabolism and antioxidant enzyme activity in seedlings of *Calophyllum brasiliense* grown under water deficit. The experiment was carried out in a completely randomized design with six treatments: 1) daily irrigation without ABA (I 0 ABA); 2) daily irrigation + ABA 10 μ M (I 10 ABA); 3) daily irrigation + ABA 100 μ M (I 100 ABA); 4) suspension of daily irrigation without ABA (E 0 ABA); 5) suspension of daily irrigation + ABA 10 μ M (E 10 ABA); 6) suspension of daily irrigation + ABA 100 μ M (E 100 ABA). According to results, suspending irrigation decreased gas exchange and water potential, affecting negatively the functioning of the photochemical apparatus, increasing antioxidant enzyme activity. The decrease in stomatal conductance was accompanied by a decrease in photosynthetic and transpiration rates, but seedlings treated with 10 μ M ABA presented a higher photosynthetic rate than the remaining treatments under water deficit. Seedlings under water restriction showed recovery after reirrigation of all studied parameters until the end of the experiment, to values similar to those of control plants. Intermittent water deficit decreased plant water status and gas exchange, and increases the antioxidant enzyme activity. Application of 10 μ M ABA minimizes the effects of water deficit and increases the antioxidant activity of superoxide dismutase (SOD) in the leaves in *C. brasiliense* seedlings and improve plant recovery after resuming irrigation, preserving the integrity and functioning of photosynthetic apparatus.

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INTRODUCTION

Calophyllum brasiliense Cambess. (Clusiaceae) is a tree species with a distribution from Mexico to countries of Central and South America and it is found in almost all Brazilian states with abundant regeneration under shade. It grows preferentially in Dense Ombrophilous Semideciduous Forests, occurring spontaneously in alluvial soils with deficient drainage; periodically flooded, located in swampy soils; and in acid sandy loam soils (pH 4.5 to 6.0). Its wood is classified as noble, being used in the construction industry, furniture and ship building because it does not rot in water. This species has potential for use in the recovery of degraded areas, potentially facilitating the establishment of others species (Carvalho, 2003; Kalil Filho et al., 2007).

Low soil water availability has been observed to result in metabolic changes in different plant species (Taiz and Zeiger, 2013; Costa et al., 2015; Rosa et al., 2017; Nunes et al., 2017; França et al., 2017), changes in photosynthesis and transpiration, damage to the photosynthetic apparatus leading to decreased growth rate, and protection mechanisms such as increased antioxidant activity in response the reactive oxygen species (ROS). According to the review of Watkins et al. (2017), the RODS can be generated by the oxidase enzymes (NADPH oxidase/RBOH), which are induced by hormones, such as ABA. In this way, the activities of these enzymes in the guard cells occur in response to ABA signaling, triggering a complex signaling cascade to close the stomata.

Water deficit may also trigger abscisic acid (ABA) synthesis in the root, which is subsequently transported to different plant parts where it promotes metabolic and morphological changes (Aasamaa and Söber, 2011; Souza et al., 2013). ○ ABA to mitigate the detrimental effects of ROS and regulate these levels in cells, plants have developed an efficient defense system constituted by enzymes such as superoxide dismutases (SODs), catalases (CATs), and peroxidases (PODs) (Carvalho et al., 2012; Rosa et al., 2017; Nunes et al., 2017).

Considering that *C. brasiliense* occurs in swampy environments and alluvial forests, we tested the hypothesis that its seedlings do not tolerate water restriction, however, the application minimizes the effects of the hydric deficit and facilitates the recovery of plants after the normalization of irrigation. We evaluated the effects of different ABA concentrations on photosynthetic metabolism and antioxidant enzyme activity in seedlings of *C. brasiliense* grown under intermittent water deficit.

MATERIAL AND METHODS

The experiment was performed in a protected environment. Seedlings of *Calophyllum brasiliense* were grown under 30% shading and protected from rainfall with a plastic cover. Seven months after emergence, seedlings were transferred to 7 L pots, with two seedlings per pot. All pots were watered to 70% field capacity until seedling characterization at time zero. The soil used in the present study was classified as Red Latosol distroferric clay texture collected from a depth of 80–100 cm, added commercial Carolina® substrate (peat, vermiculite and carbonized rice husk) and sand (2:1:1 v/v). The seedlings with a average of 9, 14 cm height, 21 cm root length and 3 cm stem diameter were submitted to six treatments: 1) daily irrigation without ABA (I 0 ABA); 2) daily irrigation + ABA 10 μM (I 10 ABA); 3) daily irrigation + ABA 100 μM (I 100 ABA); 4) suspension of daily irrigation without ABA (E 0 ABA); 5) suspension of daily irrigation + ABA 10 μM (E 10 ABA); 6) suspension of daily irrigation + ABA 100 μM (E 100 ABA).

For the irrigation treatments, seedlings were divided into two groups. The first group received daily irrigation during the whole experiment (165 days), with the soil maintained at 70% field capacity, according to the methodology of Souza et al. (2000), and was used as control for each ABA treatment. The second group was subjected to water stress until photosynthesis approached zero, in at least one of the treatments, constituting the first zero photosynthesis (1st P0 - 23° day).

After the first P0, all pots were watered daily (soil at 70% field capacity) until plant recovery (REC), when photosynthesis of the seedlings were subjected to water deficit reaching levels statistically similar to irrigated seedlings. After plant recovered, irrigation was again suspended and seedlings were evaluated until photosynthesis again approached zero, constituting the second zero photosynthesis (2nd P0). After the second P0, irrigation was again resumed until plant recovery, as previously described. The end evaluation was performed at 165 days after the beginning of the experiment (END).

On 17° day, when photosynthetic rates were close to 2 $\mu\text{mol m}^{-2} \text{s}^{-1}$, as established in pre-tests, ABA was applied at the different tested concentrations. The application of ABA was held by spraying to the point of dripping.

The remaining measurements were performed at five different times: time zero (T0, outset of the experiment), first zero photosynthesis (23° day), second zero photosynthesis (82° day), recovery (120° day), and end evaluation (165° day).

Leaf water potential (Ψ_w) was measured in individual leaves belonging to the second pair of fully expanded leaves counting from the apex of the seedlings. Measurements were taken immediately after leaf collection between 7:00 and 10:00 a.m., using a Scholander pressure chamber (model 3115 Portable Plant Water Status Console), and expressed in MPa.

Gas exchange measurements were performed every two days, using a LCPro-SD portable photosynthesis analyzer (ADC BioScientific Ltd.). The following parameters were quantified: photosynthetic rate (A , $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), stomatal conductance (g_s , $\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), transpiration (E , $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), internal CO_2 concentration (C_i , $\mu\text{mol}\cdot\text{mol}^{-1}$), carboxylation efficiency (A/C_i , $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}\cdot\mu\text{mol}\cdot\text{mol}^{-1}$), water use efficiency (A/E , $\mu\text{mol}\cdot\text{CO}_2\cdot\text{mmol}\cdot\text{H}_2\text{O}^{-1}$) and intrinsic water use efficiency (A/g_s , $\mu\text{mol}\cdot\text{CO}_2\cdot\text{mol}\cdot\text{H}_2\text{O}^{-1}$).

Antioxidant enzyme activity was measured in leaves and roots from each treatment, previously frozen in liquid nitrogen. From each sample, 1 g of frozen material was weighed, homogenized in 6 mL of 0.2 M potassium phosphate buffer containing 0.3 % (w/v) polyvinylpyrrolidone (PVP), centrifuged at 4000 rpm for 20 minutes at 4°C, and the supernatant used as enzyme extract. Superoxide dismutase (SOD – U of SOD), catalase (CAT – $\text{mmol}\cdot\text{H}_2\text{O}_2\cdot\text{g FM}^{-1}$) and peroxidase (POD – $\mu\text{mol}\cdot\text{g}\cdot\text{FM}^{-1}$) activity were determined according to Broetto (2014).

The gas exchange data was set in a completely randomized design, with six treatments and four replicates per treatment, with one seedling per experimental unit. The data were subjected to a variance analysis (F test), followed by a Scott Knott test when significant differences ($p\leq 0.05$) were found between means.

Water potential and antioxidant enzyme activity data were analyzed using a completely randomized design with a split-split-plot arrangement. Irrigation treatments were applied to the plots (daily irrigation – I; suspension of irrigation – E), three ABA concentrations (0, 10 and 100 μM ABA) were applied to the sub-plots, and the sub-sub-plots consisted of five evaluation times (T0, 1st P0, 2nd P0, REC and END). The data were subjected to a variance analysis (F test), and means were compared by a Bonferroni t-test between plots, and by a Tukey test between sub-plots and sub-sub-plots when significant differences ($p\leq 0.05$) were found. All statistical analyses were performed using the SISVAR software (Ferreira, 2014).

RESULTS

Leaf water potential

Seedlings subjected to water deficit and not treated with ABA presented a significant decrease in water potential). Ψ_w was lower for treatments with water

deficit without ABA (E 0 ABA) and with 100 μM ABA (E 100 ABA). Ψ_w for the treatment with water deficit and with 10 μM ABA (E 10 ABA) was 6.93% lower than for irrigated plants with 10 μM ABA (I 10 ABA) and 16.45% higher than for treatment E0 ABA (Figure 1 A). Plants under water stress presented decreases in Ψ_w at the 1st P0 and 2nd P0, but similar Ψ_w values to the control treatment in the end evaluation (END) (Figure 1 B).

Gas exchange analysis

Seedlings from irrigated treatments showed similar photosynthetic rates (A), which varied over time and independently of ABA application (Figure 2 A). For the first cycle of water suspension, all treatments without irrigation showed pronounced decreases in A from 14^o day until the first P0 (23^o day), and no significant differences

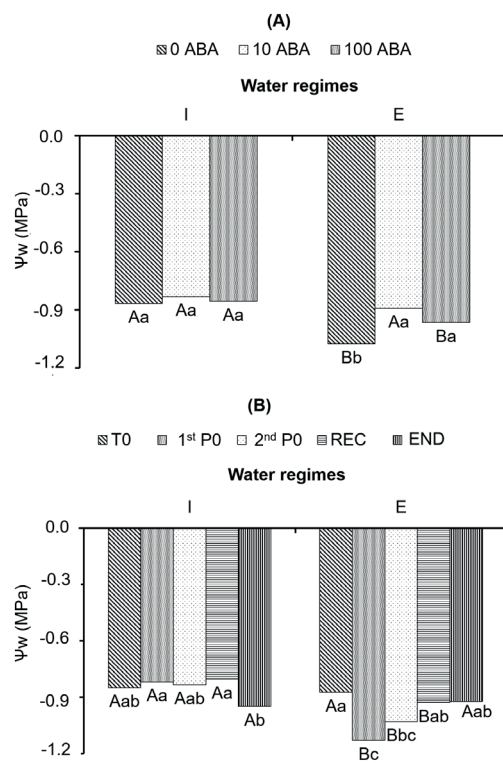


FIGURE 1 Leaf water potential (Ψ_w - MPa) for seedlings of *Calophyllum brasiliense* irrigated (I) or under water deficit (E), and with different ABA treatments (0, 10 and 100 μM), at different times: time zero (T0), first and second zero photosynthesis (1stP0 and 2ndP0), recovery (REC) and end (END). Lower case letters compare different ABA concentrations (A) or time (B) within the same water regime, and uppercase letters compare different water regimes within the same ABA concentration or same time. Averages followed by different lower case letters are significantly different according to Tukey test, and by different uppercase letters according to the Bonferroni t-test, at $p\leq 0.05$.

between ABA treatments were observed. Twenty-eight days after resuming irrigation (51° day from the beginning of the experiment), seedlings that had undergone water deficit presented A values close to the control.

For the second cycle of irrigation suspension, decreases in A were observed 31 days after irrigation suspension, reaching values close to zero for treatment E 0 ABA, but not for treatments E 10 ABA and E 100 ABA. Treatment E 10 ABA presented higher A than the remaining treatments under water deficit, but lower on average than the irrigated treatments (Figure 2 A).

Transpiration (E) decreased under drought conditions when A was close to zero (Figure 2 B). Similar to A, E had the lowest value after 23 days without irrigation, and no significant differences between ABA treatments were observed (Figure 2 A and B).

The decrease in E observed for treatment E 0 ABA resulted in higher water use efficiency (A/E) on 12° day. At the first P0, A/E was higher for treatments E 10 ABA (1.26 $\mu\text{mol}\cdot\text{mmol}^{-1}$) and E 100 ABA (1.00 $\mu\text{mol}\cdot\text{mmol}^{-1}$) than for treatment E 0 ABA (0.52 $\mu\text{mol}\cdot\text{mmol}^{-1}$) (Figure 2 B and C).

The highest values of the ratio A/E was observed in treatments E 0 ABA at 12°, 71° and 75° day (average

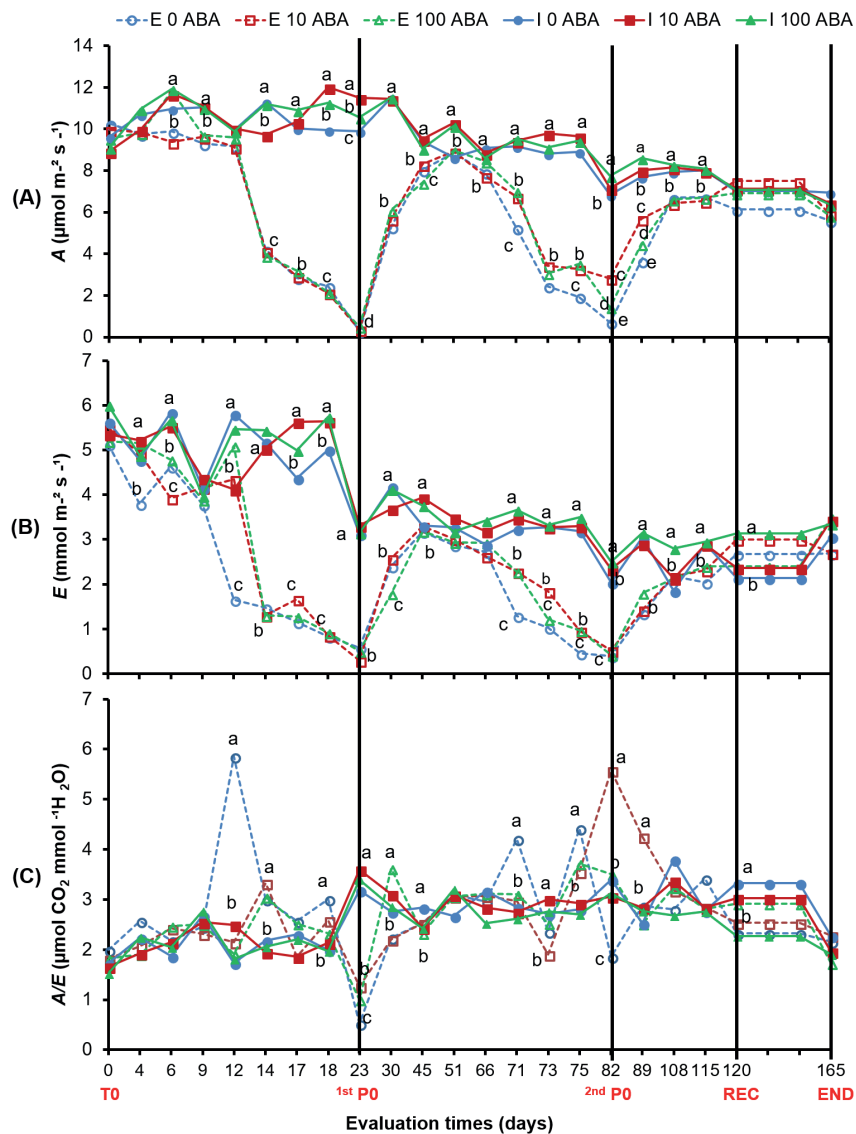


FIGURE 2 (A) Photosynthetic rate (A), (B) transpiration rate (E), and (C) instantaneous water use efficiency (A/E) for seedlings of *Calophyllum brasiliense* irrigated (I) or subjected to water deficit (E) and treated with different ABA concentrations (0, 10 and 100 μM), at different times. Uninterrupted vertical lines indicate the evaluation times: time zero (T0), first and second zero photosynthesis (1st and 2nd P0), recovery (REC) and end (END). Averages followed by different lower case letters are significantly different according to a Scott Knott test at $p \leq 0.05$.

values of 5.85, 4.21 and 4.42 $\mu\text{mol}\cdot\text{mmol}^{-1}$, respectively). This was possibly due to the decrease in E observed on the same days (1.65, 1.30, and 0.46 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively). No significant differences in A/E were observed for irrigated seedlings, which exhibited similar values for the different ABA concentrations.

At the second P0, treatment E 10 ABA showed an increase in A/E , and treatment E 100 ABA presented A/E close to the I 100 ABA (3.51 $\mu\text{mol}\cdot\text{mmol}^{-1}$ and 3.40 $\mu\text{mol}\cdot\text{mmol}^{-1}$, respectively) (Figure 2 C). E was not significantly different between water deficit treatments at the second P0 but was higher for E 10 ABA than for E 0 ABA before the second P0. Similar results were observed for A/E (Figure 2 C).

Internal CO_2 concentration (C_i) markedly decreased at 14° day for plants under water deficit and was 189.85 $\mu\text{mol}\cdot\text{mol}^{-1}$ higher at the first P0 than the averages between 14° and 23° days (Figure 3 A).

Seedlings under water deficit exhibited lower carboxylation efficiency (A/C_i) than irrigated treatments at the first and second P0. However, treatment E 10 ABA presented higher A/C_i (average increased of 0.0114 $\mu\text{mol}\cdot\text{mol}^{-1}$) at the second P0 than the remaining stress treatments, E 0 ABA (average increased of 0.0020 $\mu\text{mol}\cdot\text{mol}^{-1}$) and E 100 ABA (average increased of 0.0045 $\mu\text{mol}\cdot\text{mol}^{-1}$) (Figure 3 B).

Overall, g_s was lower for seedlings under water deficit than for irrigated seedlings from 14° day. After resuming irrigation, g_s again increased to levels close to control plants, showing no significant differences between drought and irrigated treatments on day 51, with average values between 0.16 and 0.19 $\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively. During the second cycle of suspension of irrigation, g_s decreased for all seedlings under water deficit, and then increased the values during the recovery stage (Figure 3C).

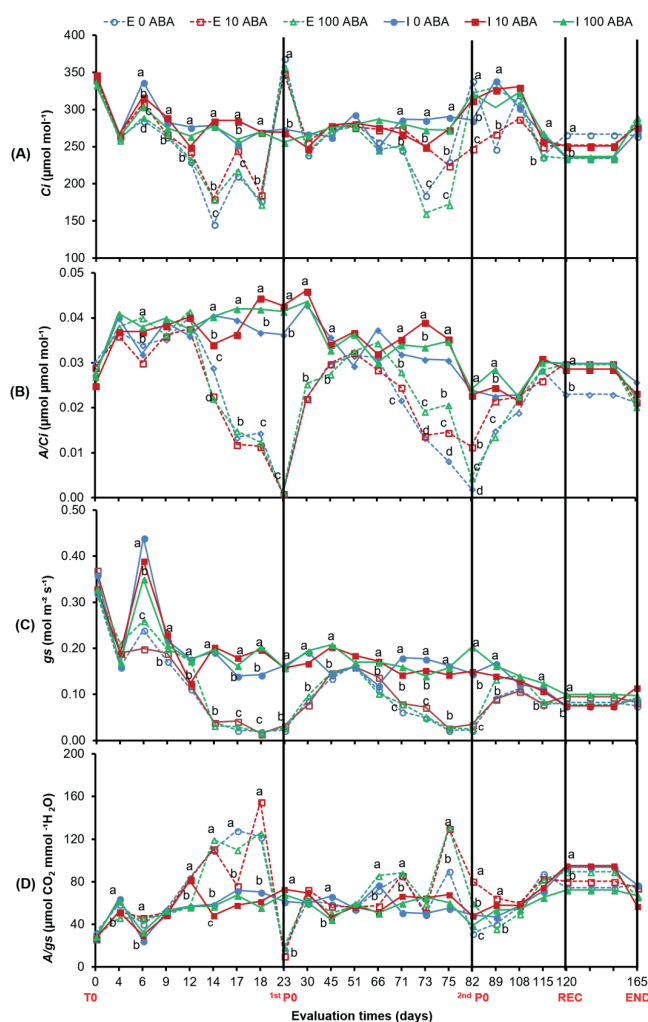


FIGURE 3 (A) Internal CO_2 concentration (C_i), (B) instantaneous CO_2 carboxylation efficiency (A/C_i), (C) stomatal conductance (g_s), and (D) intrinsic water use efficiency (A/g_s) for seedlings of *Calophyllum brasiliense* irrigated (I) or subjected to water deficit (E) and treated with different ABA concentrations (0, 10 and 100 μM) at different times. Uninterrupted vertical lines indicate the evaluation times: time zero (T0), first and second zero photosynthesis (1st and 2nd P0), recovery (REC) and end (END). Averages followed by different letters are significantly different according to the Scott Knott test at $p \leq 0.05$.

Intrinsic water use efficiency (A/g_s) varied during the experiment for the different treatments. A/g_s was higher for seedlings under water deficit than for irrigated seedlings from 14^o day under irrigation suspension until the first P0, but subsequently decreased. The same behavior was observed for the second P0. During reirrigation following the first P0, A/g_s varied among 50.60, 57.71, and 55.02 $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$, for treatments E 0 ABA, E 10 ABA, and E 100 ABA, respectively (Figure 3D).

Antioxidant enzyme activity

Catalase (CAT) activity was higher for the first and second P0 than for the remaining times, but not significantly different between ABA concentrations. Leaf CAT activity was higher for seedlings under drought stress than for irrigated seedlings for the two zero photosynthesis and the recovery cycles, being higher for the first P0 than for the remaining times (Figure 4 A).

CAT activity was similar levels in leaves and roots. After the second P0, CAT activity decreased in seedlings under water deficit, in both roots and leaves, approaching the values observed for T0 (Figure 4 A and 4B).

Leaf peroxidase (POD) activity was higher for seedlings under water deficit and with 100 μM ABA than others treatments (Figure 4C). POD activity was higher at the first and second P0 with all ABA concentrations (Figure 4D).

POD activity was lower in roots than leaves for plants under water deficit, and higher at the first P0 than for the remaining times, being significantly different from the irrigated seedlings (Figure 4E). For irrigated seedlings, POD activity was lower at T0 (0.17 $\mu\text{mol}\cdot\text{mg}^{-1}$ protein min^{-1}) than at the remaining times, for which the overall average was 0.39 $\mu\text{mol}\cdot\text{mg}^{-1}$ ·protein·min⁻¹ (Figure 4F).

Leaf superoxide dismutase (SOD) activity was higher for seedlings under water deficit at the first and second P0 (Figure 5 A). At the first and second P0, SOD activity was highest for seedlings treated with 10 μM ABA (Figure 5 B).

Regarding the times and ABA concentrations, SOD activity was higher at the second P0. For treatments without ABA application (0 ABA), SOD activity was higher at the second P0 than at the remaining times (Figure 5 B).

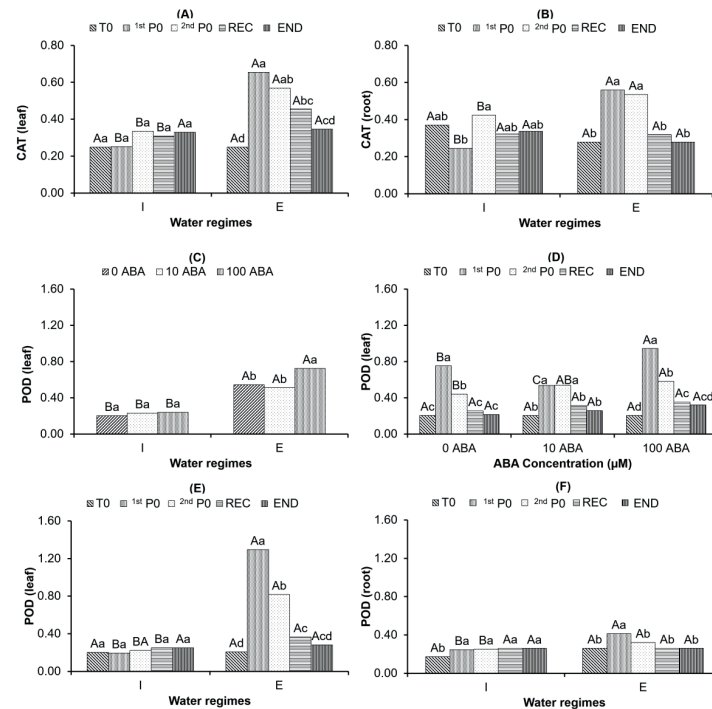


FIGURE 4 Leaf (A, C, D, E) and root (B, F) of catalase (CAT – $\text{mmol}\cdot\text{H}_2\text{O}_2\cdot\text{g}\cdot\text{FM}^{-1}$) and and peroxidase (POD - $\mu\text{mol}\cdot\text{g}\cdot\text{FM}^{-1}$) (POD) activity for seedlings of *Calophyllum brasiliense* irrigated (I) or subjected to water deficit (E), at different times: time zero (T0), first and second zero photosynthesis (1stP0 and 2ndP0), recovery (REC), and end (END). Lowercase letters compare different times (Figures A, B, D, E, F) or ABA concentrations (Figure C) within the same water regime, and uppercase letters different water regimes within the same time or ABA concentration. Averages followed by different lowercase letters are significantly different according to Tukey test, and by different uppercase letters are significantly different according to the Bonferroni t-test, at $p\leq 0.05$.

SOD activity was higher in roots than in leaves for both water regimes. Root SOD activity was highest for seedlings under water deficit at the first and second P0 (Figure 5C).

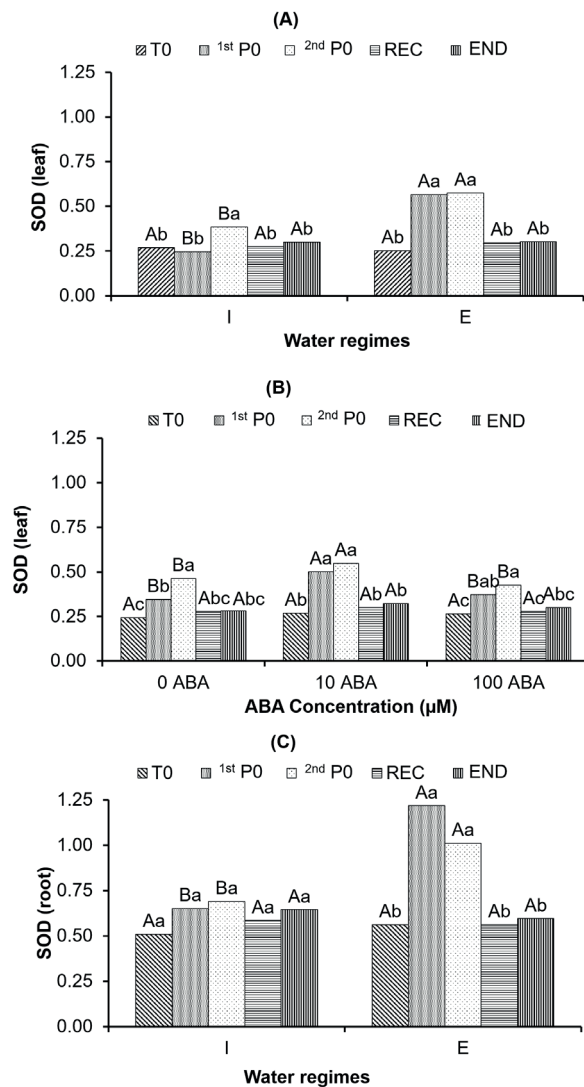


FIGURE 5 Leaf (A, B) and root (C) of superoxide dismutase (SOD – U of SOD), activity for seedlings of *Calophyllum brasiliense* irrigated (I) or subjected to water deficit (E), at different times: time zero (T0), first and second zero photosynthesis (1st P0 and 2nd P0), recovery (REC), and end (END). Lowercase letters compare different times within the same water regime (Figures A, C) or ABA concentrations (Figure B) and uppercase letters different water regimes within the same time or ABA concentration. Averages followed by different lowercase letters are significantly different according to Tukey test, and by different uppercase letters are significantly different according to the Bonferroni t-test at $p \leq 0.05$.

DISCUSSION

A higher Ψ_w was maintained with application of 10 μM ABA to seedlings under water deficit. Water deficit affects leaf water potential, nutritional status, gas exchange, and may also influence other physiological mechanisms and processes related to plant growth (Liberato et al., 2006; Gonçalves et al., 2009).

For example, leaf water potential decreased in *Khaya ivorensis* A. Chev. under water deficit stress, reaching -2.66 MPa (Albuquerque et al., 2013); and young *Carapa guianensis* Aubl. plants under water deficit exhibited a higher than four-fold reduction in Ψ_w 21 days after irrigation suspension, whereas irrigated plants maintained Ψ_w values close to zero (Gonçalves et al., 2009).

Irrigated seedlings exhibited variations in Ψ_w overtime, which may be attributed to variations in relative air humidity and temperature during the experiment (data not shown). At the final measurement, although no significant difference was observed, Ψ_w was slightly lower for irrigated seedlings than for seedlings under water deficit, possibly due to the prolonged ABA activity.

The effect of ABA was also observed on gas exchange, where treatment with 10 μM ABA produced better results than treatment E 0 ABA at the second P0, at 65 days after ABA application.

Seedlings took 43 days to recover from water deficit after resuming irrigation. Significant differences were observed between water deficit treatments at the second P0, where treatment with 10 μM ABA resulted in a higher photosynthetic rate (A). Furthermore, seedlings under water deficit presented values close to the control 24 days after resuming irrigation, independently of ABA application.

Decrease in water availability has previously been shown to directly change plant photosynthetic capacity, affecting biochemical reactions, functioning of the photosynthetic apparatus, and stomatal closure (Tonello and Teixeira Filho, 2012; Cunha et al., 2013).

Reduced A in plants under water deficit may occur for different reasons, such as stomatal limitation in CO_2 assimilation, damage to the photochemical apparatus, decreased ATP synthesis, and a decrease in RuBisCO activity or its turnover (Flexas and Medrano, 2002).

Plant water status directly affects carbon assimilation capacity, both being regulated by rate of g_s . Under water deficit conditions, plants usually increase stomatal resistance to reduce water loss through transpiration, resulting in decreased A (Yokota et al. 2006; Ferreira and Tonini, 2009). Consequently, maintenance of high photosynthetic rates associated with lower g_s and E are characteristics of plants tolerant of low soil water

availability, which is related to higher A/g_s and A/E , (Ma et al., 2004). This was not observed in *C. brasiliense*, indicating its sensitivity to water deficit.

Similarly to A to g_s reduced until the 14^o days after suspension of irrigation in seedlings under water deficit, remaining constant until the first P0, highlighting the relationship between g_s and A . The variations in E followed the same behavior as g_s , suggesting that the plants reduce water loss by transpiration through stomatal closure, according to water availability (Campelo et al., 2015).

Stomatal closure also occurs due to a decrease in relative air humidity, which directly influences the turgescence of guard-cells (Casson and Hetherington, 2010). Stomatal closure is considered the main cause of reduced photosynthetic and transpiration rates in plants under water deficit conditions (Gonçalves et al., 2009).

Campelo et al. (2015) observed that E was more pronouncedly affected in dry seasons, followed by recovery during the rainy season, in *Swietenia macrophylla* King. and *Calophyllum brasiliense* Cambess. than in others species, such as *Astronium fraxinifolium* Schott., *Handroanthus serratifolius* (Vahl.), *Handroanthus impetiginosa* (Mart.) Matos, and *Simarouba amara* Aubl.

At the second P0, internal CO_2 concentration (C_i) was lower for seedlings grown under water deficit and with 10 μ M ABA than for the remaining treatments, suggesting higher RuBisCO carboxylation efficiency (A/C_i). Seedlings under water deficit without ABA application exhibited increased C_i and decreased A/C_i . The increase in C_i observed on 23^o day when there was a decrease in A , that can be attributed to lower RuBisCO carboxylation efficiency. Similar results were observed in seedlings of *Myracrodruon urundeuva* Allemão (Costa et al., 2015) under water stress, which was also attributed to the RUBISCO carboxylation efficiency or to non-stomatal factors such as electron transport and photophosphorylation problems (Kaiser, 1987).

Decrease in photosynthetic rates in plants under water stress have been observed even without changes in internal CO_2 concentrations (Pacheco et al 2011; Costa et al. 2015, Campelo et al. 2015). Pacheco et al. (2011) observed potential effects of ABA on stomatal control six days after suspension of irrigation in seedlings of *Calendula officinalis* L. Cerqueira (2011) studied *Vitis vinifera* L. and observed average of ABA concentrations three times higher for plants under water deficit conditions than for control plants (unpublished data). In the present study, no effect of ABA on g_s was observed for *C. brasiliense* under water stress. However, ABA

application increased intrinsic water use efficiency (A/g_s) before zero photosynthesis was reached, especially on day 75, when treatments E 10 ABA and E 100 ABA presented higher A/g_s than the remaining treatments.

S. macrophylla, *C. brasiliense* and *Handroanthus albus* Vahl. are more sensitive to water deficit than other species, as indicated by more pronounced decrease in gas exchange (Campelo et al., 2015). Higher leaf ABA concentrations may be important for the activation of the enzymatic antioxidant system during early water stress (Yeet et al., 2011). Antioxidant enzyme activity is higher in plants under stress and is related to stress tolerance (Allen et al., 1997; Carvalho et al., 2012).

At the first and second P0, SOD activity was highest for seedlings treated with 10 μ M ABA. This increase in leaves treated with ABA may have favoured the maintenance of the integrity of the photosynthetic apparatus and elevation of the photosynthetic activity ($2.83 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) when compared to other treatments stressed. The POD activity in leaves increased in plants without ABA (0 μ M ABA), however, in plants with 100 μ M ABA the increase was statistically greater. We emphasize that the ABA had no effect on the activity of enzymes in the roots of seedlings of *C. brasiliense*.

This suggests that the ABA effect on enzyme activity varies according to ABA concentration, enzyme and plant part. SOD is considered the first enzymatic defense against oxidative stress because it catalyzes superoxide dismutation, converting it into $H_2O_2 + O_2$ (Barbosa et al., 2014). Following superoxide dismutation into hydrogen peroxide by SOD, the intracellular levels of H_2O_2 need to be regulated. This is performed by enzymes, such as POD and CAT, located in almost all cell parts (Blokhina et al., 2003; Barbosa et al., 2014).

POD prevents and protects from oxidative stress in plants, making it a key enzyme in seedling acclimation (Saraiva et al., 2014). POD activity accelerates the biosynthesis of certain phenolic compounds, leading to lignin biosynthesis, which in turn decreases the tissue water loss and protects plants from water stress (Pacheco et al., 2011). Carvalho et al. (2012) observed increased peroxidase activity resulting from water stress, showing POD potential for ROS sequestration in *Hyptis pectinata* (L.) Poit.

Increase in CAT activity under water deficit conditions was also observed in seedlings of *Copaifera langsdorffii* Desf. (Rosa et al., 2017) and *Schinus terebinthifolius* Raddi. (Nunes et al., 2017). In addition to water deficit, ABA has been shown to stimulate enzyme activity. Souza et al. (2013) studied cultivars of *Zea mays*

L. under water deficit for 10 days, with and without ABA application, observed that ABA application increased CAT activity.

Overall, seedlings of *C. brasiliense* under water deficit exhibited decreased in leaf water potential, stomatal conductance, and photosynthetic and transpiration rates, as well as increased antioxidative enzyme activity. However, photosynthetic rates were higher for seedlings treated with 10 μM ABA than for the remaining water deficit treatments during the periods of zero photosynthesis, presenting higher *A/E*, *A/Ci* and *A/g*s. This may be attributed to ABA promotion of higher tolerance to water deficit in these seedlings.

Chlorophyll *a* fluorescence also suggests that seedlings treated with 10 μM ABA were not stressed at the first and second P0, when compared to stress indicator parameters. However, all studied parameters showed recovery after resuming irrigation, reaching values similar to the control treatments at the last evaluation. This indicates physiological plasticity of *C. brasiliense* seedlings under water deficit which helps to guarantee their survival.

It should be highlighted that all evaluated characteristics were important for the seedlings fit the stress conditions, indicating that the plants had activated protection mechanisms even without exogenous ABA application. As *C. brasiliense* can adapt to a variety of habitats, which occurs preferentially in environments with high water availability (Kalil Filho et al., 2007), this behavior explains the best results observed for irrigated seedlings.

CONCLUSION

Intermittent water deficit decreased plant water status and gas exchange, and increases the antioxidant enzyme activity.

Application of 10 μM ABA minimizes the effects of water deficit and increases the antioxidant activity of SOD in the leaves in *C. brasiliense* seedlings and improve plant recovery after resuming irrigation, preserving the integrity and functioning of photosynthetic apparatus.

The present results do not confirm the tested hypothesis, indicating physiological plasticity in *C. brasiliense* seedlings.

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