

Full Length Research Paper

Chlorophyll, nitrogen and antioxidant activities in Cumaru (*Dipteryx odorata* (Aubl.) Willd) (Fabaceae) in two water regimes

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The Cumaru (*Dipteryx odorata* (Aubl.) Willd.) is a species used by traditional populations and industries using timber and non-timber forest products. This study aimed to analyze the levels of chlorophyll A, B, total ammonia levels, nitrate, proline, electrolyte leakage and activity of oxidative enzymes in evaluation to tolerance of cumaru plants subjected to drought for 21 days of stress. The experiment was conducted in a greenhouse at the Federal Rural University of Amazonia (UFRA), Belém, Pará, in the period from March to July 2015. The results showed a significant decrease in the relative water content of 50.8 and 55% for chlorophyll b, 45% to total chlorophyll and an increase in proline to the plants under drought. There was no significant difference to chlorophyll a, ammonium and nitrate. Increases in electrolyte leak with 22.74% for roots and 39.55% for leaves were observed. The enzyme catalase (CAT) showed a significant increase from the 14th day of the experiment, while changes in superoxide dismutase (SOD) and ascorbate peroxidase (APX) activities were observed from the 7th day of the experiment. Cumaru plants are not drought tolerant over 21 days; also, young plants of cumaru respond negatively to conditions of low water availability in the soil.

Key words: Drought, oxidative stress, chlorophyll, tolerance, *Dipteryx odorata*.

INTRODUCTION

Cumaru (*Dipteryx odorata* (Aubl.) Willd.) is a species used by traditional populations and industries using

timber and non-timber forest products such as oils for medicinal and cosmetic properties, as well as with the

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reforestation of degraded areas, which in addition to the ecological benefits, increase the supply of wood from reforestation in the region, increasing the income on the farm and reducing the pressure on the remaining natural forest dependent of water resources (Shimizu, 1998).

As water resources become scarce, the commercial exploration of plants tolerant to drought becomes a priority for obtaining high yields (Matos et al., 2012). The impact of drought in the forestry and agricultural activities is an important socioeconomic consequence that affects millions of people around the world (Elliott et al., 2013). Among the various factors affecting the production plant, the water deficit occupies a prominent position, as well as affect the water balance in plants by altering their metabolism, is a phenomenon that occurs in large extensions of arable areas (Nogueira et al., 2001).

Among the many implications of drought on plant development, the restriction on the acquisition of nutrients and water is commonly recognized (Manivannan et al., 2008). Evidence suggests that drought causes oxidative stress in various plants, in which reactive oxygen species (ROS) such as superoxide radical (O_2^-), hydroxyl radical (OH \cdot), hydrogen peroxide (H_2O_2) and singlet oxygen (1O_2), are produced (Jaleel et al., 2007).

To minimize the cytotoxic effects of ROS, the plants causes a complex antioxidant system where specific enzymes act by neutralizing the action of these radicals, starting with the superoxide dismutase (SOD), which inmute radical O_2^- to H_2O_2 . This, in turn, undergoes action of various enzymes such as catalase (CAT), responsible for the conversion of H_2O_2 to H_2O and O_2 , and peroxidase, ascorbate peroxidase (APX) reducing the H_2O_2 to H_2O (Apel and Hirt, 2004). Besides, in water restriction, the plants should be able to handle ROSs particularly to prevent oxidative damage to lipids, proteins and nucleic acids; if there is an inability to adequately handle ROSs, oxidative damage may result in cell death (Demidchik, 2015).

Several methods are adopted by researchers to identify species tolerant to water stress, being more common selection through ecological descriptors associated with physiological and biochemical descriptors. According Pincelli (2010) water deficiency is one of the environmental stresses responsible for the reduction of pigments in the leaves, making the plant life cycle changes. Among these, related to the antioxidant system and osmotic adjustment are supported substantially in identifying promising species, and consequently, the progress of culture of works for improving drought resistance (Azevedo Neto et al., 2009).

The antioxidant enzyme activity is usually enhanced to promote better elimination of ROSs and promote increased cellular protection against oxidative damage (Jaleel et al., 2009). Considering then that collaboration between antioxidant enzymes should provide better protection against the deleterious effects of ROS, minimizing oxidative damage (Blokhina et al., 2003).

Given the above, the study aimed to analyze the content of chlorophyll A and B, ammonium, nitrate and proline as well as the activity of oxidative enzymes in evaluation tolerance of cumaru plants subjected to drought.

MATERIALS AND METHODS

Location and experimental conduction

The experiment was conducted in a greenhouse at the Federal Rural University of Amazonia (UFRA) belonging to the Institute of Agricultural Sciences (IAS), located in Belém, Pará, in the period from March to July 2015. The seedlings of cumaru (*Dipteryx odorata* (Aubl.) Willd.), from seeds were provided by AIMEX (Association of Industries Exporters of Wood in Pará) with four months old, they were placed in plastic pots with a capacity of 3.6 L. The substrate consisted of yellow dystrophic Latosol (EMBRAPA, 2013). Before the start of treatment, all plants were irrigated daily for three months, corresponding to the acclimation time. 5 ml of solution cocktail containing macro and micronutrients (Table 1) was added to all the samples at the start of acclimation, in the form of nutrient solution (Hoagland and Arnon, 1950), modified in Biodiversity Studies Laboratory in Higher Plants (EBPS), UFRA.

The plants were subjected to two water regimes: Irrigated (control) and water deficit, in which the imposition of water deficit was obtained by suspension of irrigation in 21 days, and the time 0 (zero days of drought), time 1 (7 days of drought), time 2 (14 days of drought) and 3 time (21 days of drought). During the period of analysis, control plants were irrigated daily to replace the water lost by evapotranspiration. There was also the weed control manually. It was not detected occurring nutritional deficiency symptoms, as well as the attack of pests and pathogens.

Experimental design and statistical analysis

The experimental design was completely randomized in split plot in time (four times evaluation and two water conditions: Control and drought), with 5 repetitions, totaling 40 experimental units, each experimental unit was composed of a plant/pot. Analysis of variance of the results was applied and when there was a significant difference, the means were compared by Tukey test at 5% significance level. Moreover, the standard deviations were calculated for each treatment, and statistical analyzes performed by Assistant Version 7.7 Beta program.

Relative water content (RWC)

The RWC was determined at 06:00h a.m in each collect. The method used was that described by Slavick (1979). Results were expressed as a percentage, according to the formula:

$$RWC = (FM1 - DM)/(FM2 - DM) \times 100 (\%)$$

Where, FM1 = Fresh mass 1; FM2 = Fresh mass 2 with saturation; DM = Dry mass.

Determining the ammonium content

50 mg of previously lyophilised leaves and roots were weighed and put in a test tube containing 400 ml of total extract + 2.5 ml of solution A (5 g phenol + 0.025 g of sodium nitroprusside / 500 ml

Table 1. Solution cocktail containing macro and micronutrients.

Composition	Concentration (M)	ml /L
KNO ₃	1	5
Ca (NO ₃) ₂	1	3
NH ₄ NO ₃	1	2
KH ₂ PO ₄	1	0,1
MgSO ₄	1	1
Fe-EDTA		1
1. FeSO ₄ . 7H ₂ O	0.1	
2. Na ₂ (EDTA)	0.08	
Micronutrientes		1
1. H ₃ BO ₃	0.04	
2. MnCl ₂ . 4 H ₂ O	0.009	
3. CuSO ₄ . 5H ₂ O	0.0003	
4. ZnSO ₄ . 7 H ₂ O	0.0007	
5. Na ₂ MoO ₄ . 2 H ₂ O	0.0001	
CoCl ₂ . 6 H ₂ O	0.004	1
Al ₂ (SO ₄) ₃ . 18 H ₂ O [(50 mM) pH= 4.0]	0.3	0,5
Al ₂ (SO ₄) ₃ . 18 H ₂ O [(100 mM) pH= 4.0]	0.3	0,5
Al ₂ (SO ₄) ₃ . 18 H ₂ O [(150 Mm) pH= 4.0]	0.3	0,5

distilled water) and homogenized by vortexing, adding 2.5 ml of Solution B (2.5 g NaOH + 12.6 ml of sodium hypochlorite / 500 ml distilled water), respectively. The free ammonium concentrations of the total extract were estimated from the standard curve constructed with (NH₄)₂SO₄ p.a. (Sigma) according to the method described by Weatherburn (1967).

Determination of nitrate

50 mg each of previously lyophilized leaves and roots was weighed and mixed with extract containing 100 mL + 200 of salicylic acid 5% solution (w / v) in concentrated sulfuric acid. After stirring vigorously in a vortex stirrer was slowly added 4700 uL of 2N NaOH. The concentration of nitrate was obtained from a standard curve with increasing concentrations of NO₃ (0, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 µmol ml⁻¹) according the method described by Cataldo et al. (1975).

Determining the proline content

50 mg were weighed of previously lyophilised leaves and roots by adding in the test tubes the total extract, 1 ml of ninhydrin acid and 1 ml of glacial acetic acid 99.5%. It was determined through a standard calibration curve using proline and proline contents in samples were extrapolated from the curve and expressed in mmol g⁻¹. The dry matter (DM) was determined according to Bates et al. (1973).

Determination of photosynthetic pigments

The determination of photosynthetic pigments was realized according to Lichtenthaler (1987). The concentrations of chlorophyll A, B and total (mg. L⁻¹) were calculated using the formulas:

$$\text{Chlorophyll A} = 12.25 \times L_{(662)} - 2.79 \times L_{(644)} \quad \text{Chlorophyll B} = 21.5 \times L_{(644)} - 5.1 \times L_{(662)}$$

$$\text{Total chlorophyll} = 7.15 \times L_{(662)} + 18.71 \times L_{(644)}$$

Membrane integrity (leak electrolytes)

The degree of membrane integrity was estimated by electrolyte leak according Blum and Ebercon (1981). The electrolyte leak was estimated by the following equation:

$$\text{EL (\%)} = (C_1/C_2) \times 100$$

Enzymatic activity

Superoxide dismutase (SOD)

The SOD activity was determined by inhibition of photoreduction of nitroblue tetrazolium chloride (NTC) according to Giannopolitis and Ries (1977).

Catalase (CAT)

CAT activity was determined by the method of Beers Jr. and Sizer (1952) with modifications.

Ascorbate peroxidase (APX)

The APX activity was determined by the method of Nakano and Asada (1981).

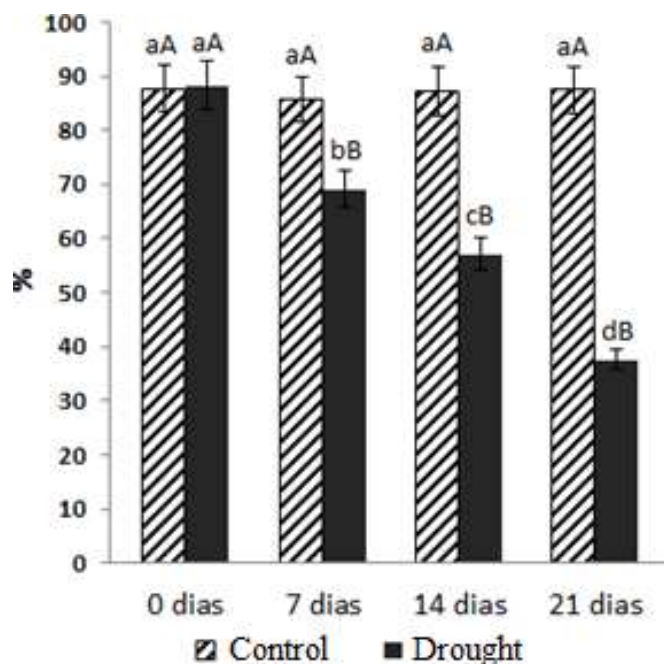


Figure 1. Relative water content in young plants Cumaru subjected to water deficit. Capital letters show statistical differences between water conditions and lower statistical differences between the collections time. Tukey test $p < 0.05$ probability was used for comparison.

RESULTS AND DISCUSSION

Relative water content (RWC)

The relative water contents present in the leaves of cumaru under water stress decreased as the weeks went by, on average control plants showed water percentage between 87.7 and 85.5%, and plants under drought between 88.3 and 37.5%, representing a decrease of 50.8%.

The relative water content present in the leaves represent the water availability in the soil as well as the efficiency of the plant in pick up water in adverse conditions and maintaining water in the system reducing losses. Cumaru seedlings have the high water content in the leaf under normal conditions but had a sharp decrease due to lack of water. The decrease was significant from the 7th day of water suspension and decreasing over the 21 days of stress, as shown in Figure 1.

Photosynthetic pigments

The chlorophyll A contents do not vary significantly throughout the experiment (Tukey test at 5% significance level), while the chlorophyll B and total had a significant reduction in plants under drought compared to control

plants. Mean values for chlorophyll A were $3.31 \text{ mmol.m}^{-2}.\text{s}^{-1}$ for the control plants and $2.8 \text{ mmol.m}^{-2}.\text{s}^{-1}$ for plants under drought. Chlorophyll B shows the mean values of 3.05 and $2.27 \text{ mmol.m}^{-2}.\text{s}^{-1}$, while total chlorophyll was 6.37 and $5.08 \text{ mmol.m}^{-2}.\text{s}^{-1}$ in control plants and plants under drought, respectively (Figure 2). Representing a decrease of 34% to chlorophyll A, 55% for chlorophyll B and 45% for total chlorophyll compared the two water conditions on the 21st day of the experiment. According to Morais et al. (2007), chlorophylls A and B are interconverted in the chlorophyll cycle and form complexes of chlorophyll-protein, that are important in the regulation and organization of the photosystem. Chlorophylls play an important role in photosynthesis, are responsible for capturing light energy, especially chlorophyll A as the main pigment of complex light collectors (LHC) for the photochemical reactions (Taiz and Zeiger, 2013).

Under reduced stomata conductance and consequently lower influx of CO_2 proceeds in reduction of net assimilation rate, which directly affects the biochemistry of photosynthesis and reduces the photochemical energy consumption (Carmo et al., 2014). In these situations there is constant production of reactive oxygen species and other chlorophyll degradation agents (Matos et al., 2012). Chlorophyll degradation occurs according to the level of stress in the plants are submitted and the implication is leaf senescence, occurrence found in this study (Carmo et al., 2014).

In this work the chlorophyll A showed no significant difference, a fact that may be in accordance with the statement of Dinakar et al. (2012), in which the chloroplasts are particularly susceptible to oxidative damage and when it comes to tolerance to drought periods as well as the production of antioxidants, chlorophyll content is maintained after the drying, to prevent the formation of reactive oxygen species (ROS's).

Ammonium, nitrate and proline content

The ammonium and nitrate concentrations had no significant change throughout the experiment in plants under drought and the control plants. Proline already had a significant increase from the 14th day in the leaves and 21th day in roots. The values for ammonium in the last day of collection were 11.2 and $11.5 \text{ mmol of NH}_4^+.\text{Kg}^{-1} \text{ DM}$ in roots and 7.2 and $6.4 \text{ mmol of NH}_4^+.\text{Kg}^{-1} \text{ DM}$ in leaves, control and drought, respectively (Figure 3A). Nitrate was of 0.07 and $0.08 \text{ mmol from NO}_3^-. \text{Kg}^{-1} \text{ DM}$ in roots and 0.06 and $0.06 \text{ mmol of NO}_3^-. \text{Kg}^{-1} \text{ DM}$ in leaves, control and drought, respectively (Figure 3B). Proline was of 3.8 and $20.8 \text{ mmol of Pro. g}^{-1} \text{ DM}$ in roots and 2.3 and $29.8 \text{ mmol of Pro. g}^{-1} \text{ DM}$ in leaves, control and drought, respectively (Figure 3C).

Most plants have a preference for nitrate ion as a nitrogen source, so it is common their levels were lower

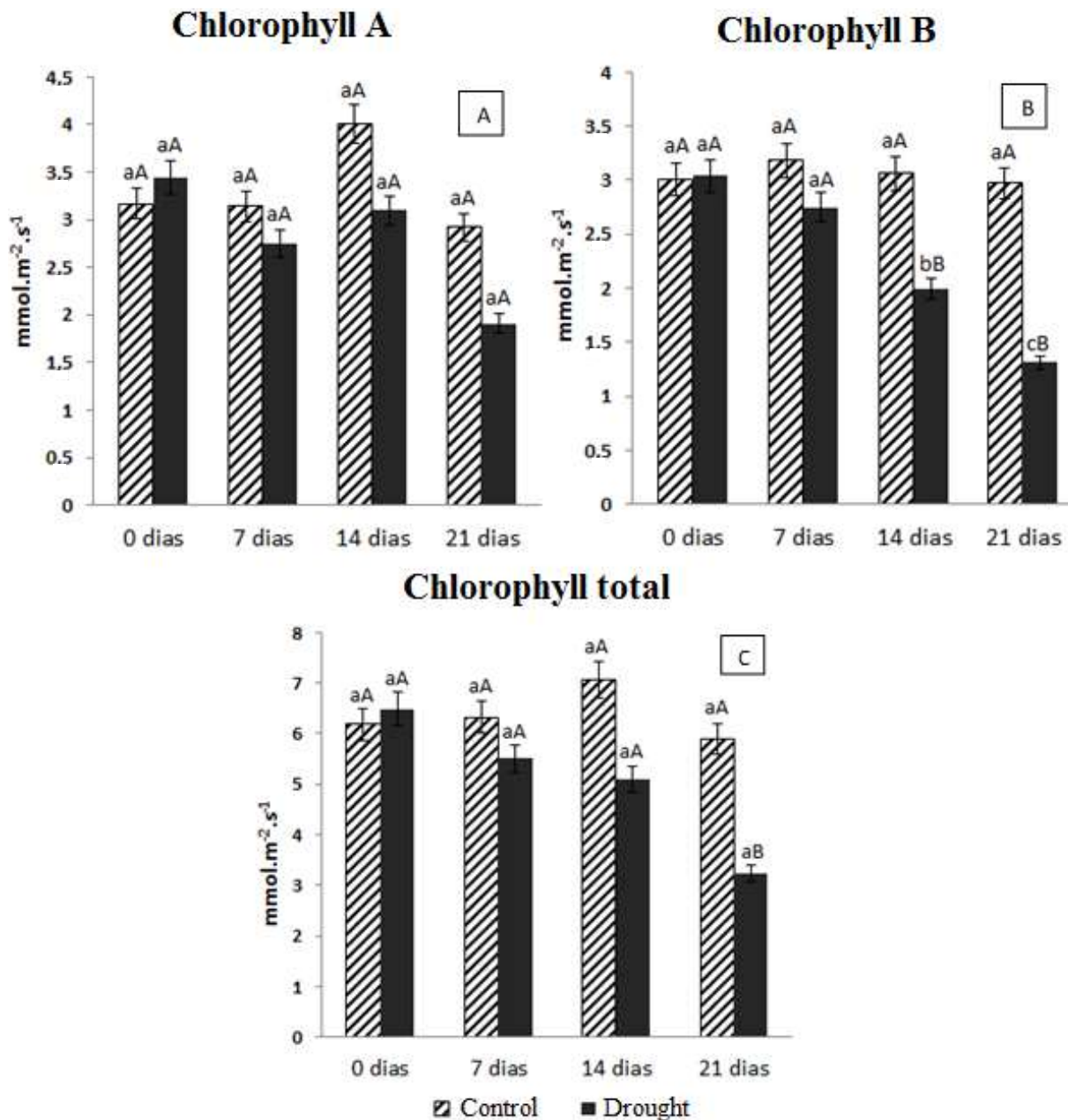


Figure 2. Chlorophyll content A (A), chlorophyll B (B) and chlorophyll total (C) in young plants Cumaru subjected to water deficit. Capital letters show statistical differences between water conditions and lower statistical differences between the collections time. Tukey test $p < 0.05$ probability was used for comparison.

than those found ammonium levels (Martinelli, 2003; Araújo et al., 2004), corroborating with these results.

The ammonium and the nitrate are the main forms of nitrogen available to plants, reduction processes and nitrogen assimilation can be absorbed both in the leaves and in the roots simultaneously or between these bodies becoming an essential process for the plant, since it is through it that it is controlled growth and development of the plant (Shan et al., 2012).

As a result, various forms of N available in the

substrate can affect the morphological, physiological and biochemical plant, possibly in root growth, photosynthetic rates and catalytic activity of several enzymes (Li et al., 2013). In studies comparing the nutrition with nitrate (NO_3^-) or ammonium (NH_4^+) show that these nitrogen sources can induce different metabolic responses (Patterson et al., 2010).

The accumulation of soluble solutes in plant cells provides a type of response to water deficit, called osmotic adjustment, which allows more negative water

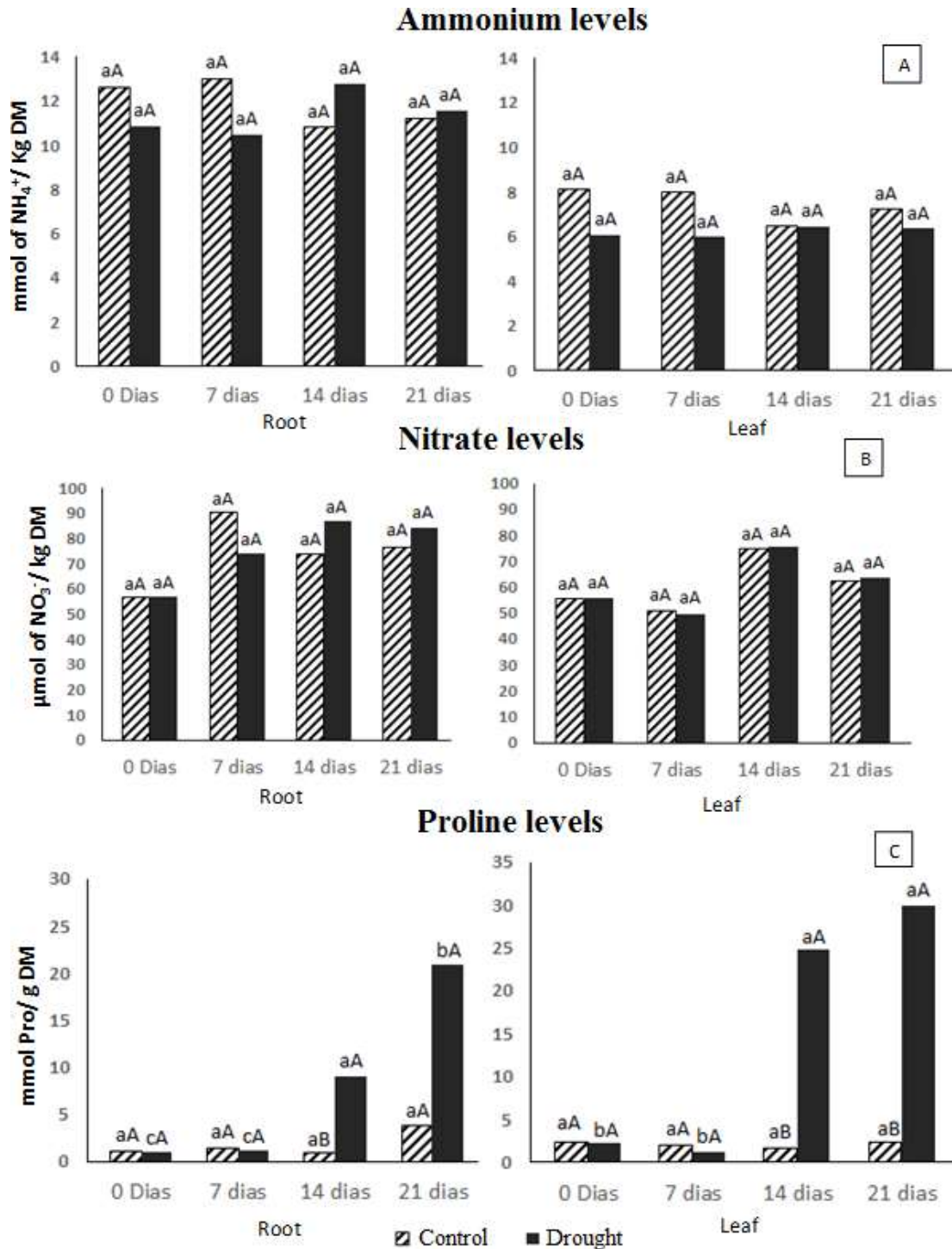


Figure 3. Ammonium levels (A), nitrate (B) and proline (C) in young plants Cumaru subjected to water deficit. Capital letters show statistical differences between water conditions and lower statistical differences between the collections time. Tukey test $p < 0.05$ probability was used for comparison.

potential in leaves, thus helping to keep the movement of water to the leaves (Silva et al., 2014). Proline has been highlighted as a compatible solute occurs in plants in response to environmental stresses that solute

accumulates variety of plant species in response to stresses such as drought, heavy metals, extreme temperatures, salinity and ultraviolet radiation (Siripornadulsil et al., 2002).

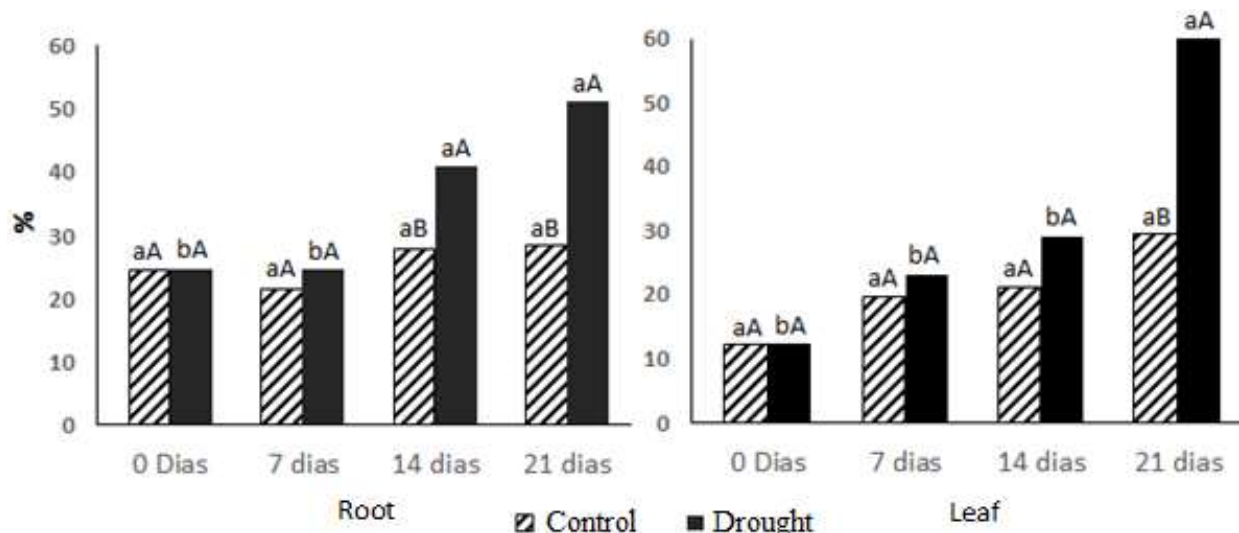


Figure 4. Electrolyte leak in young plants Cumaru subjected to water deficit. Capital letters show statistical differences between water conditions and lower statistical differences between the collections time. Tukey test $p < 0.05$ probability was used for comparison.

It is possible to note a proline increased much more significant in the leaves than the roots that this fact can be given by the need of the plant to have a more negative potential in the leaves so that the water can reach the highest parts of the plant. Proline contents only had increased from 14 days even if there is already a significant reduction in the RWC on the 7th day, this may be because the proline can be a compatible solute (osmoprotectors organic compound and osmoregulator) more secondary role in Cumaru species as it was the case in the study of Pinhão-manso (Sousa et al., 2012), which highlighted the glycine main osmoregulator and osmoprotectors.

Electrolyte Leak

Results show that there was a significant increase in both the leaves and the roots that were under water deficit with values of 12.2 to 29.58% and 12.2 to 60.73% for the leaves (plant control and drought, respectively). As well as 24.56 to 28.55% and 24.6 to 51.29% for the roots (plants control and drought), respectively (Figure 4) with a 39.55% increase in percentage for leaves and roots 22.74% to the 21 day of experiment. Lack of water causes a decrease in liquid photosynthesis and in this case the sharp reduction of water in the cumaru plants probably caused this decrease in liquid photosynthetic rate and to produce more O_2^- and H_2O_2 in chloroplasts (Blokina et al., 2003; Reddy et al., 2004). The increased cellular leak in plants under drought is strongly related to the damage caused by free radicals O_2^- that attacks different parts of the plant as lipids and membrane proteins, nucleic acids and others causing cell death.

Enzymatic activity

Superoxide dismutase (SOD)

Plants subjected to drought showed a significant increase when compared to the control plants over the 21 days of experiment (Figure 5). The values for the roots were 49.86 to 50.85 mg^{-1} .protein, and of 49.79 to 58.59 mg^{-1} .protein (control plants and under drought), respectively. For the leaves the results were similar with values of 41.06 to 40.35 mg^{-1} .protein and of 40.91 to 49.2 mg^{-1} .protein (control plants and under drought), respectively. Plants have enzymatic systems of defense against reactive oxygen species, including SOD, CAT, APX. Activation of genes encoding these enzymes in response to oxidative stress was observed, for examples, tobacco (Bowler et al., 1991), soybean (Lee et al., 1999), and peanut (Sankar et al., 2007). Thus, increased activity of these enzymes is directly related to differential expression of the genes belonging to the antioxidant system, having as one of its functions to prevent H_2O_2 accumulation in cells (Eyidogan and Oz, 2007; Vaidyanathan et al., 2003).

Catalase (CAT)

The enzyme catalase showed significant difference from the 14th day of the experiment (Figure 6), with values for the roots of 0.042 mg^{-1} .protein (control plants) and of 0.042 to 0.054 mg^{-1} .protein (under drought), respectively. The leaves presents values of 0.043 to 0.042 mg^{-1} .protein and of 0.043 to 0.073 mg^{-1} .protein (control plants and under drought), respectively.

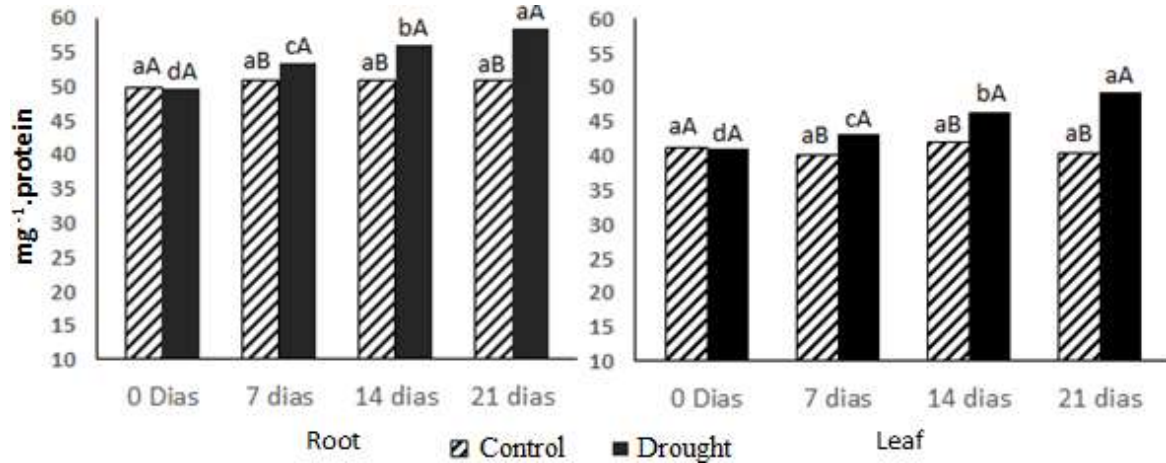


Figure 5. Superoxide dismutase enzyme activity in young plants Cumaru subjected to water deficit. Capital letters show statistical differences between water conditions and lower statistical differences between the collections time. Tukey test $p < 0.05$ probability was used for comparison.

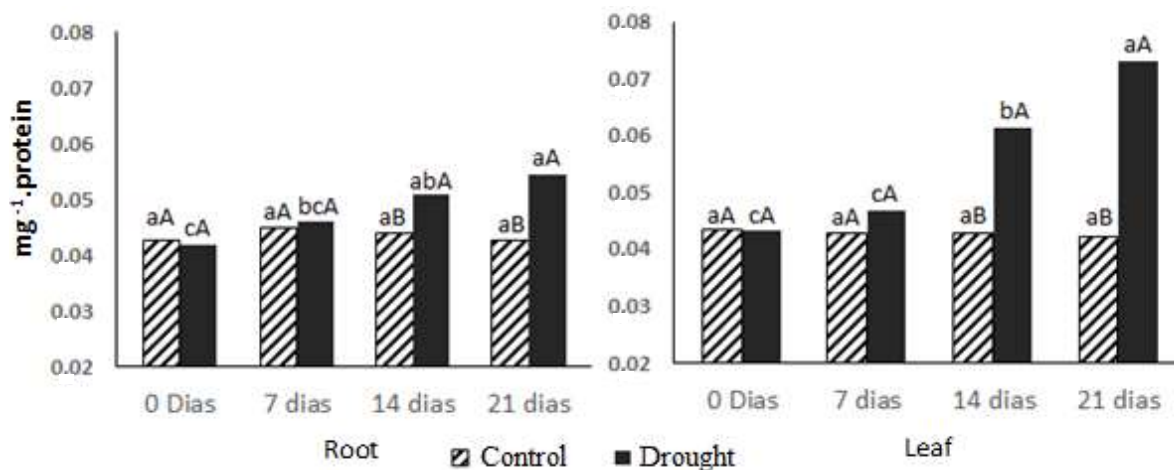


Figure 6. Enzyme catalase activity in young plants Cumaru subjected to water deficit. Capital letters show statistical differences between water conditions and lower statistical differences between the collections time. Tukey test $p < 0.05$ probability was used for comparison.

Akçay et al. (2010), who studied the CAT activity in creeping and erect peanut, found that the enzyme activity increased significantly when subjected to higher stress levels, confirming the results of this work. According to these authors, the CAT is one of the most effective defense enzymes in oxidative processes, since, in the resistant plants enables the integrity of the cell even when the stress is in a more rigorous stage. These results are reported in previous studies of water stress, salinity and other stresses, which reported that there is a reduced production of ROS in tolerant genotypes than in susceptible genotypes (Karabal et al., 2003; Chaitanya et al., 2002; Bhoomika et al., 2013). According to Sankar et al. (2007), as can be seen in his work, where an average increase of up to 230% of activity was obtained at the

earliest material, when subjected to 10 days of water suppression.

Ascorbate peroxidase (APX)

The values of APX enzyme showed significant difference after 7 days of the experiment in plants were subjected to drought, when compared with control plants. The increase for the roots was from 0.0298 to 0.032 mmol.min⁻¹ and of 0.0293 to 0.0376 mmol.min⁻¹ in control plants and under drought, respectively. The leaves show values of 0.0315 to 0.0322 mmol.min⁻¹ and of 0.0309 to 0.0405 mmol.min⁻¹ in control plants and under drought, respectively (Figure 7). These results highlight that

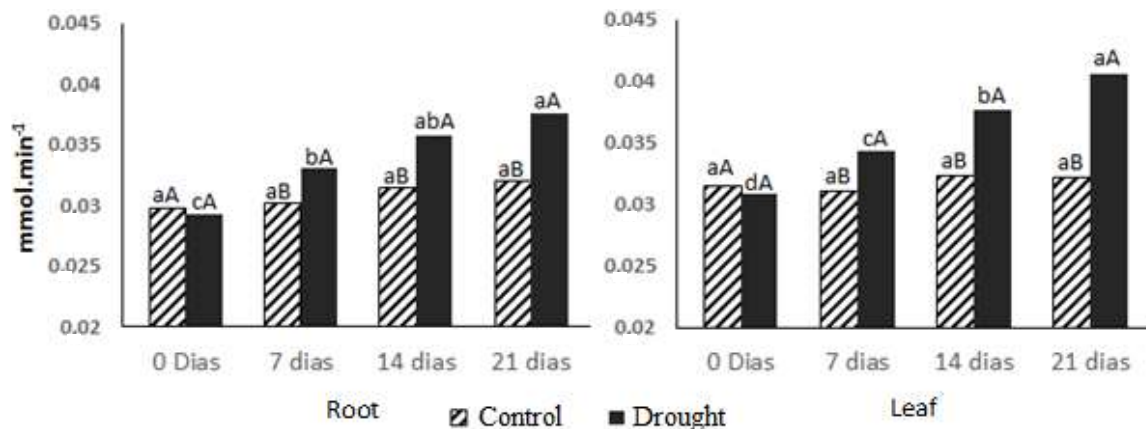


Figure 7. Enzyme peroxidase activity in young plants Cumaru subjected to water deficit. Capital letters show statistical differences between water conditions and lower statistical differences between the collections time. Tukey test $p < 0.05$ probability was used for comparison.

2013).

Thus, the potential oxidative damage due to drought was adequately mitigated by the constitutive activity APX (Cruz et al., 2013). Possibly, this gives rise to the triggering of multiple strategies antioxidants (Silva et al., 2015).

The species studied presents different mechanisms to overcome the drought periods, either by maintaining low RWC values and photosynthetic pigments, or by the increased activity of oxidative enzymes which are variables that can be used as water stress sensitivity indicator.

Young plants of cumaru are not tolerant to more than 21 days of water stress, and respond very negatively to the conditions of low water availability in the soil.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

- Akçay UC, Ercan OM, Yıldız L, Yılmaz C, Oktem HA, Yuçel M (2010). Drought-induced oxidative damage and antioxidant responses in peanut (*Arachis hypogaea* L.) seedlings. *Plant Growth Regul.* 61(01): 21-28.
- Apel K, Hirt H (2004). Reactive oxygen species: Metabolism, oxidative stress and signal transduction. *Annu. Rev. Plant Biol.* 55:373-399.
- Araújo AR, Carvalho JLN, Guilherme LRG, Curi N, Marques JJ (2004). Movimentação de nitrato e amônio em colunas de solo. *Ciênc. Agrotec.* 28(3):537-541.
- Azevedo Neto AD, Nogueira RJMC, Melo Filho PA, Santos RC (2009). Physiological and biochemical responses of peanut genotypes to water deficit. *J. Plant Interact.* 5(1):1-10.
- Beers Junior RF, Sizer IW (1952). A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J. Biol. Chem.* 195:133-140.
- Bhoomika K, Pyngrope S, Dubey RS (2013). Differential responses of antioxidant enzymes to aluminum toxicity in two rice (*Oryza sativa* L.) cultivars with marked presence and elevated activity of Fe SOD and enhanced activities of Mn SOD and Catalase in aluminum tolerant cultivar. *Plant Growth Regul.* 71:235-252.
- Blokhina O, Virolainen E, Fagerstedt KV (2003). Antioxidants oxidative damage and oxygen deprivation stress: a review. *Ann. Bot.* 91:179-194.
- Blum A, Ebercon A (1981). Cell membrane stability as a measure of drought and heat tolerance in wheat. *Crop Sci.* 21(1):43-47.
- Bowler C, Slooten L, Vandenbranden S, De Rycke R, Botterman J, Sybesma C, Van Montagu M, Inzé D. (1991). Manganese superoxide dismutase can reduce cellular damage mediated by oxygen radicals in transgenic plants. *EMBO J.* 10:1723-1732.
- Carmo MS, Borges LP, Torres Junior HD, Santos PGF, Matos FS. (2014). Efeito da Disponibilidade de Nitrogênio e Déficit Hídrico no Crescimento Inicial de Plantas de Pinhão Manso. *Rev. Agrotec.* 5(2):33-48.
- Chaitanya K, Sundar D, Masilamani S, Reddy AR (2002). Variation in heat stress-induced antioxidant enzyme activities among three mulberry cultivars. *Plant Growth Regul.* 36:175-180
- Costa MA, Pinheiro HA, Shimizu ESC, Fonseca FT, Santos Filho BG, Moraes FKC, Figueiredo DM (2010). Lipid peroxidation, chloroplast pigments and antioxidant strategies in *Carapa guianensis* (Aubl.) subjected to water deficit and short-term rewetting. *Trees* 24:275-283.
- Cruz FJR, Castro GLS, Silva Júnior DD, Festucci-Buselli RA, Pinheiro HA (2013). Exogenous glycine betaine modulates ascorbate peroxidase and catalase activities and prevent lipid peroxidation in mild water-stressed *Carapa guianensis* Aubl. plants. *Photosynthetica* 51(1):102-108.
- Demidchik V (2015). Mechanisms of oxidative stress in plants: from classical chemistry to cell biology. *Environ. Exp. Bot.* 109:212-228.
- Dinakar C, Djilianov D, Bartels D (2012). Photosynthesis in desiccation tolerant plants: energy metabolism and antioxidative stress defense. *Plant Sci.* 182:29-41.
- Elliott J, Glotter M, Best N, Boote KJ, Jones JW, Hatfield JL, Rosenzweig C, Smith L, Foster I (2013). Predicting agricultural impacts of large-scale drought: 2012 and the case for better modeling. Center for Robust Decision Making on Climate & Energy Policy (RDCEP) Working Paper Series, 1-8.
- EMBRAPA (2013). Sistema Brasileiro de Classificação de Solos. 3 ed. rev. ampl. – Brasília, DF,.
- Eyidogan F, Oz MT (2007). Effect of salinity on antioxidant responses of chickpea seedlings. *Acta Physiol. Plant.* 29:485-493.
- Giannopolitis CN, Ries SK (1977). Superoxide dismutases I: occurrence in higher plants. *Plant Physiol.* 59:309-314.
- Hoagland DR, Arnon DI (1950). The water culture method for growing plants without soil. The College of Agriculture University of California

- 347:137-147.
- Jaleel CA, Manivannan P, Sankar B, Kishorekumar A, Gopi R, Somasundaram R, Panneerselvam R (2007). Water deficit stress mitigation by calcium chloride in *Catharanthus roseus*: effects on oxidative stress, proline metabolism and indole alkaloid accumulation. *Colloids Surf. B Biointerfaces* 60:110-116.
- Jaleel CA, Riadh K, Gopi R, Manivannan P, Inès J, Al-juburi HJ, Chang-Xing Z, Hong-BO S, Panneerselvam R (2009) Antioxidant defense responses: physiological plasticity in higher plants under abiotic constraints. *Acta Physiol. Plant.* 31:427-436.
- Lee SC, Kang BG, Oh SE (1999). Induction of ascorbate peroxidase by ethylene and hydrogen peroxide during growth of cultured soybean cells. *Mol. Cells* 9:166-171.
- Li SX, Wang ZH, Stewar BA (2013). Responses of crop plants to ammonium and nitrate N. *Adv. Agron.* 118:205-397.
- Lichtenthaler HK (1987). Chlorophylls and carotenoids: pigment photosynthetic biomembranes. *Methods Enzymol.* 148:362-385.
- Manivannan P, Jaleel CA, Somasundaram R, Panneerselvam R (2008). Osmoregulation and antioxidant metabolism in drought-stressed *Helianthus annuus* L. under triadimefon drenching. *C R Biol.* 331:418-425.
- Martinelli LA (2003). Element interactions in Brazilian landscapes as influenced by human interventions. In: Melillo J, Field CB, Moldan B, Scope 60: Interactions of the major biogeochemical cycles: Global change and human impacts. [S.I.]: Islands Press, Pp. 193-210.
- Matos FS, Oliveria LR, Freitas RG, Evaristo AB, Missio RF, Cano MAO. (2012) Physiological characterization of leaf senescence of *Jatropha curcas* L. populations. *Biomass Bioenergy* 45(10):57-64.
- Morais RR, Gonçalves JFC, Santos Júnior UM, Dunisch O, Santos ALW (2007). Chloroplastid pigment contents and chlorophyll a fluorescence in amazonian tropical three species. *Rev. Árvore* 31(5):959-966.
- Nakano Y, Asada K (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.* 22:867-880.
- Nogueira RJMC, Moraes JAPV, Burity HA, Neto EB (2001). Alterações na resistência à difusão de vapor das folhas e relações hídricas em aceroleiras submetidas a déficit de água. *Rev. Bras. Fisiol. Veg.* 13(1):75-87.
- Patterson K, Cakmak T, Cooper A, Lager I, Rasmusson AG, Escobar MA (2010). Distinct signalling pathways and transcriptome response signatures differentiate ammonium-and nitrate-supplied plants. *Plant Cell Environ.* 33(9):1486-1501.
- Pincelli RP (2010). Tolerância à deficiência hídrica em cultivares de cana-de-açúcar avaliada por meio de variáveis morfofisiológicas. 2010. 78 f. Dissertação (Mestrado em Agronomia/Agricultura) - Faculdade de Ciências Agrônômicas, Universidade Estadual Paulista, Botucatu, 78 p.
- Reddy AR, Chaitanya KV, Vivekanandan M, (2004). Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. *J. Plant Physiol.* 161:1189-1202.
- Sankar B, Jaleel CA, Manivannan P, Kishorekumar A, Somasundaram R, Panneerselvam R (2007). Effect of paclobutrazol on water stress amelioration through antioxidants and free radical scavenging enzymes in *Arachis hypogaea* L. *Colloids Surf. B Biointerfaces* 60:229-235.
- Shan AYKV, Oliveira LEM, Bonome, LTS, Mesquita AC (2012). Assimilação metabólica de nitrogênio em plântulas de seringueira cultivadas com nitrato ou amônio. *Pesqui. Agropecu. Bras.* 47(6):754-762.
- Shimizu J (1998). Espécies não tradicionais para plantios com finalidades produtivas e ambientais: Silvicultura e usos. *Anais Curitiba: Embrapa Florestas*, pp. 64-71.
- Silva MA, Santos CM, Vitorino HS, Rhein AFL (2014) Pigmentos fotossintéticos e índice spad como descritores de intensidade do estresse por deficiência hídrica em cana-de-açúcar. *Biosci. J.* 30(1):173-181.
- Silva PA, Oliveira IV, Rodrigues KCB, Cosme VS, Bastos AJR, Detmann KSC, Cunha RL, Festucci-Buselli RA, Damatta FM, Pinheiro HA (2015). Leaf gas exchange and multiple enzymatic and non-enzymatic antioxidant strategies related to drought tolerance in two oil palm hybrids. *Trees* 30:203.
- Siripornadulsil S, Traina S, Sayre RT (2002). Molecular mechanisms of proline-mediated tolerance to toxic heavy metals in transgenic microalgae. *Plant Cell, Rockville* 14: 2837-2847.
- Slavick, B. (1979). *Methods of studying plant water relations.* Springer Verlag, P 449.
- Soares LAA, Brito MEB, Fernandes PD, Lima GSL, Soares Filho WS, Oliveira ES (2015). Crescimento de combinações copa - porta-enxerto de citros sob estresse hídrico em casa de vegetação. *Rev. Bras. Eng. Agric. Ambiental* 19(3):211-217.
- Sousa AEC, Silveira JAG, Gheyi HR, Neto MCL, Lacerda CF, Soares FAL (2012). Trocas gasosas e conteúdo de carboidratos e compostos nitrogenados em pinhão-mansão irrigado com águas residuária e salina. *Pesq. Agropec. Bras.* 47(10):1428-1435.
- Taiz, L, Zeiger, (2013) *E. Fisiologia vegetal.* 5.ed. Porto Alegre: Artmed, 954 p.
- Vaidyanathan H, Sivakumar P, Chakrabarty R, Thomas G (2003). Scavenging of reactive oxygen species in NaCl-stressed rice (*Oryza sativa* L.) - Differential response in salt-tolerant and sensitive varieties. *Plant Sci.* 165:1411-1418.