

Biochemical and physiological responses of andiroba (*Carapa guianensis* Aubl.) seedlings subjected to water deficit**Ellen Gleyce da Silva Lima¹, Tamires Borges de Oliveira¹, Susana Silva Conceição², Wander Luiz da Silva Ataíde¹, Bruno Moitinho Maltarolo¹, Glauco André dos Santos Nogueira¹, Cândido Ferreira de Oliveira Neto^{1,*}, Roberto Cezar Lobo da Costa¹, Ricardo Shigueru Okumura¹**¹Forest Science Department, Universidade Federal Rural da Amazônia, Belém city, Pará State, Brazil²Agronomy Department, Universidade Federal do Ceará, Fortaleza city, Ceará State, Brazil

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Abstract

The adaptive mechanisms of native forest species have promoted considerable interests, since knowledge of plant physiology and biochemistry enhances the use of these species in recovery of degraded areas, especially in the Amazon region. This research aimed to evaluate the physiological and biochemical responses of andiroba (*Carapa guianensis* Aubl.) seedlings submitted to two water regimes. The experiment was conducted in a greenhouse at the Universidade Federal Rural da Amazônia in Belém city, State of Pará, Brazil, in the period from September to November of 2013. A completely randomized design with two water conditions was used as follows: control and water deficit, with 15 replications, totaling 30 experimental units. During the experimental period, the control plants were irrigated daily for replacement of evaporated water. The results showed that suspension of irrigation during 25 days of experiment period promoted a considerable reduction of the water potential (Ψ_{am}) of plants grown under water deficit. The stomatal conductance and transpiration rate showed decrease in non-irrigated plants, with the increase of water deficit period. The water deficit induced an increase in the concentrations of sucrose, free proline and glycine-betaine, as well as in total soluble carbohydrates, both in the leaves and in the roots. The water deficit of 25 days of irrigation suspension served in the regulation of water relations of andiroba plants, restricting transpiration rate, reducing the water potential and stomatal conductance, promoting accumulation of free proline, total soluble carbohydrates, sucrose and glycine betaine concentrations.

Keywords: native forest species, stomatal conductance, water potential.**Abbreviations:** Ψ_{am} _water potential, g_s _stomatal conductance, MPa_megapascal, E _ transpiration rate, MCW_methanol, chloroform and water.**Introduction**

The andiroba (*Carapa guianensis* Aubl) native to the Amazon region, belonging to the Meliaceae family, is considered as multiple use species. However, the most important products are economically timber and oil. Its wood is one of the most studied topics in this species because of excellent structural quality (hardness and durability). Its oil is appreciated by industries producing cosmetics and pharmaceuticals (Mendonça and Ferraz, 2007). Given the economic and ecological importance of andiroba, researchers have developed studies on the physiology and biochemistry of this species to know the effect of abiotic stresses on plant development.

The immediate responses to environmental stresses are the processes of biochemical adjustments correlated with synthesis and / or accumulation of certain compounds with osmotically active properties such as proline (Szabados and Savouré, 2010), glycine betaine (Chen and Murata, 2008) and total soluble carbohydrates (Silva, 2009) among others. Some species reduce the water potential of its cells, especially the leaf through the compatible solute accumulation favoring soil water absorption, or simply close their stomata during periods of intense evaporative demand to maintain the water balance within the cell (Nogueira et al., 2005).

The immediate responses involve biochemical processes of synthesis and/or accumulation of certain compounds with osmotically active properties, such as proline (Szabados and Savouré, 2010), glycine-betaine (Chen and Murata, 2008) and total soluble carbohydrates (Silva, 2009). Some species reduce the water potential of plant cells, especially the leaf, through the accumulation of compatible solutes that promote the absorption of water from the soil, or causing the closure of stomata in times of increased evaporative demand to maintain the water balance inside the plant cell (Nogueira et al., 2005).

According to Melo et al. (2007), identification and understanding the mechanisms of tolerance to water deficit, as well as the development of technologies that promote the plants to tolerate long periods of drought are alternatives for the maintenance of the Brazilian and global forest production. Effects of abiotic stress on the development of plants, presents as subsidies to agricultural and forestry research, and becomes potential indicators of the mechanisms of plant tolerance to abiotic stress conditions. Thus, the aim of this study is to evaluate the physiological and biochemical responses in andiroba seedlings submitted to different water regimes.

Results

Water potential (Ψ_{am})

The suspension of irrigation during the 25 days of experiment promoted a significant reduction in the water potential (Ψ_{am}) of plants grown under water deficit (Fig 1A), reaching values close to -2.00 MPa, while irrigated plants obtained values of -0.08 MPa during the experiment.

Stomatal conductance (g_s)

Reported values of stomatal conductance (g_s) for plants under water deficit were $0.44 \text{ mmol m}^{-2} \text{ s}^{-1}$, representing a decrease of 68.18%, when compared to the irrigated plants ($0.14 \text{ mmol m}^{-2} \text{ s}^{-1}$) according to Fig 1B. It was observed that when the water deficit increased, there was a dehydration of the leaf mesophyll cells and a decrease in water potential in leaves (Fig 1A), causing a reduction in stomatal conductance.

Plant transpiration rate (E)

In the present study, it was observed that there was a reduction in transpiration rate (E) when plants were maintained under the water deficit in the soil, being the values obtained corresponded to $1.10 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ compared to those found in plants maintained irrigated with $3.09 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ (Fig 1C).

Total soluble carbohydrates

It was found that the concentrations of soluble carbohydrates differ statistically between the control treatment and water stress, both at the root and in the leaf (Fig 2A). The concentrations showed elevated levels at 70.96% in the leaves of andiroba seedlings submitted to water deficit ($3.1 \text{ } \mu\text{mol g}^{-1} \text{ FW}$) compared to control treatment with $0.9 \text{ } \mu\text{mol g}^{-1} \text{ FW}$. In roots, the concentration of soluble carbohydrates also showed a significant increase of 76.19% when submitted to water stress ($2.1 \text{ } \mu\text{mol g}^{-1} \text{ FW}$) compared to control treatment of $0.5 \text{ } \mu\text{mol g}^{-1} \text{ FW}$.

Sucrose

The concentrations of sucrose (Fig 2B), showed increased levels of 13% in the leaves of plants submitted to water deficit ($29 \text{ mg g}^{-1} \text{ FW}$) compared to the control treatment, in which the level was $17 \text{ mg g}^{-1} \text{ FW}$. In roots, the sucrose content was significantly lower for both the control treatment ($9 \text{ mg g}^{-1} \text{ FW}$) and the water stress treatment as well ($13 \text{ mg g}^{-1} \text{ FW}$). In both treatments, the levels of osmoregulation showed the highest values in leaves, although under water deficit, these concentrations were higher.

Glycine-betaine

The levels of osmoregulation increased significantly when plants were submitted to water deficit (Fig 2C). In leaves and roots the values were $28.0 \text{ } \mu\text{mol g}^{-1} \text{ FW}$ and $21.0 \text{ } \mu\text{mol g}^{-1} \text{ FW}$, respectively, compared to control treatments that showed $8.6 \text{ } \mu\text{mol g}^{-1} \text{ FW}$ and $9.6 \text{ } \mu\text{mol g}^{-1} \text{ FW}$.

Proline

The changes in proline concentration were observed from 25

days (Fig 2D), in which the control and water deficit treatments were statistically significant. The plants submitted to water deficit showed significant increase in the levels of the osmolyte (approximately 55%) with a corresponding value of $5.83 \text{ } \mu\text{mol g}^{-1} \text{ FW}$ for leaves and $3.40 \text{ } \mu\text{mol g}^{-1} \text{ FW}$ to the roots. For control treatment, the concentrations presented similar values of $1.40 \text{ } \mu\text{mol g}^{-1} \text{ FW}$ and $1.34 \text{ } \mu\text{mol g}^{-1} \text{ FW}$, respectively.

Discussion

Considering a negative water potential, the lower amounts of water available for plant metabolism, promotes a series of physiological, biochemical and morphological responses in plant allowing for water conservation in the plant (Silva, 2009).

Gonçalves et al. (2009), showed that andiroba seedlings satisfactorily tolerated 15 to 30 days of water deficit. The same author observed that these periods of water stress decreased the water potential on leaf with values between -2.0 and -3.0 MPa, similar to the results obtained in this study. Cordeiro (2012), researching of *Tabebuia serratifolia* and *Hymenaea courbaril* L. seedlings, observed significant reduction in water potential during the dry period at conditions of Igarapé-Açú city, Pará state, Brazil. Perhaps, the reduced water potential is directly correlated with the reduction in transpiration rate. According to Souza et al. (2011), plant transpiration is influenced by several factors such as climate, soil, plant age, water availability in soil and forest species evaluated.

Similar results were founded by Albuquerque et al. (2013), studying *Khaya ivorensis* plants under water stress. They observed significant amounts of transpiration reduction (93%), compared to irrigated plants. The decrease in plant transpiration is correlated to stomatal behavior during the water stress, possibly due to reduce in leaf water potential and its relationship with soil water potential (Taiz and Zeiger, 2013). According to Gonçalves et al., (2009), plants submitted to the water stress encounter reductions in their transpiration rate due to total or partial closures of their stomata. This mechanism is considered as a means to retain the water content in plant tissues. The present results is consistent with the results obtained by Lima et al. (2007) and Albuquerque et al. (2013), researching the *Swietenia macrophylla* and *Khaya ivorensis* seedlings, in which they showed reductions in stomatal conductance and transpiration rate, during 20 days of water stress.

The mechanisms of osmotic adjustment showed positive correlations with increased levels of proline (Fig 2D). This osmoregulator (proline) accumulates in the leaves with low water potential to stabilize cell membranes and to maintain the conformation of protein, preventing the dehydration of cytosol (Kishor et al., 2005). Result described by Silva (2009), corroborate with those presented in this study, in which andiroba seedlings submitted to water stress showed significant increases in leaf proline content of approximately 60%.

Sanches and Silva (2013), researching the *Bauhinia forficata*, observed that after fifteen days of water stress, the plants showed significant changes in the levels of proline compared to the control treatment, with the largest changes founded in the leaves ($64 \text{ } \mu\text{mol g}^{-1}$).

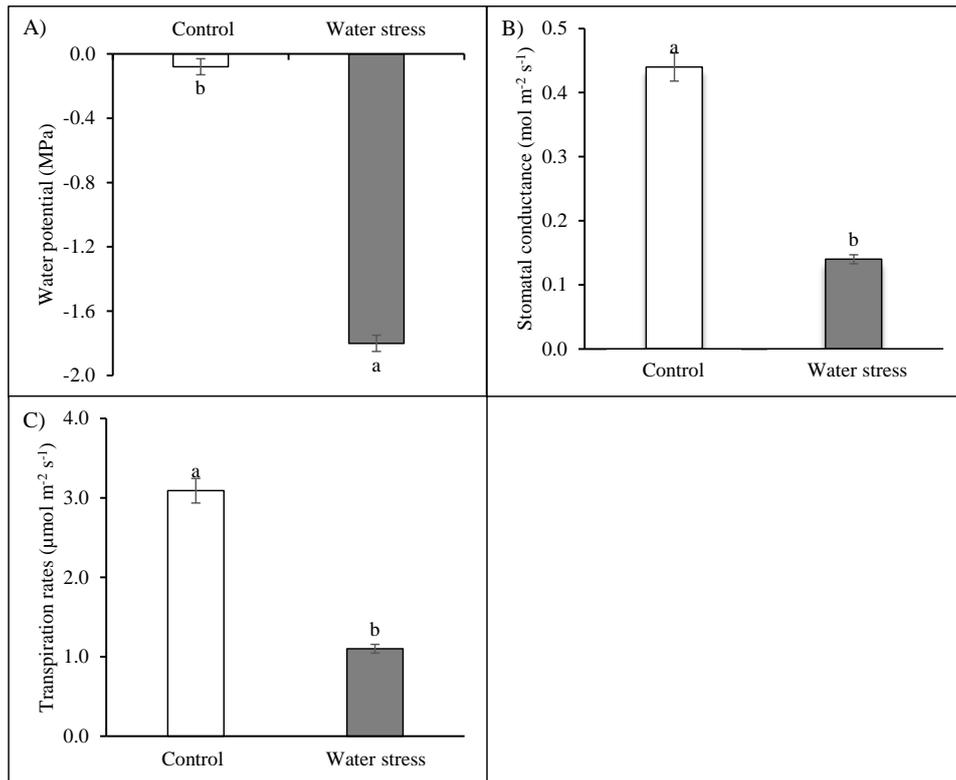


Fig 1. Levels of water potential (A), stomatal conductance (B) and transpiration rates (C) in andiroba (*Carapa guianensis* Aubl.) seedlings submitted for 25 days under water stress. *Averages followed by the same letter do not differ among themselves by the Tukey test at 5% of probability. The bars represent the mean standard error.

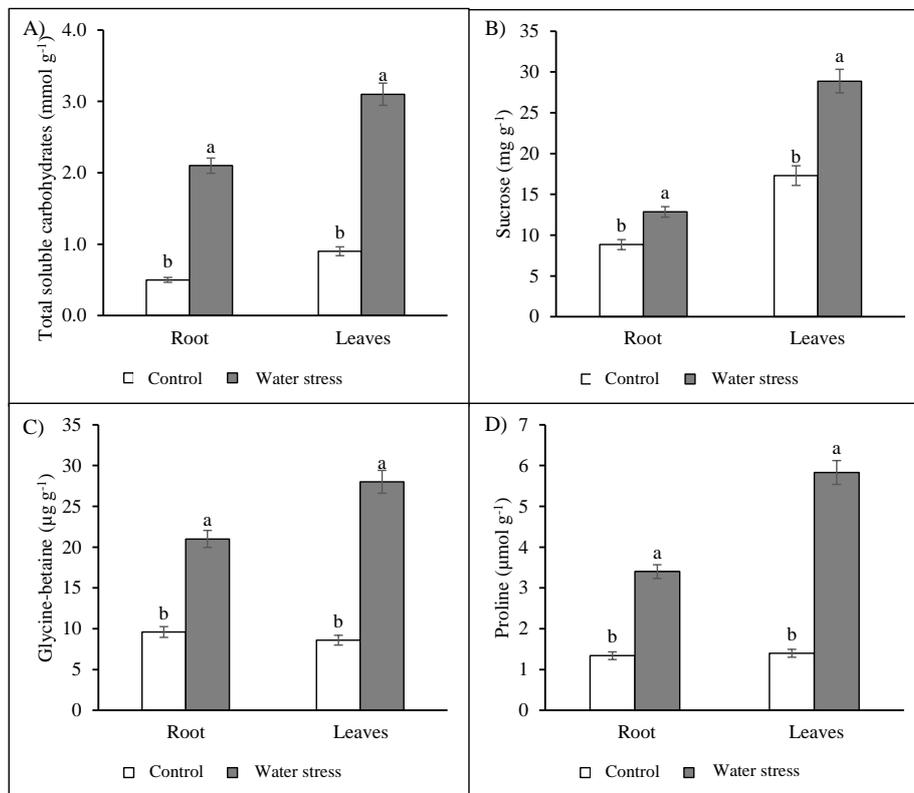


Figure 2. Levels at the root and leaves of total soluble carbohydrates (A), sucrose (B), Glycine-betaine (C), and proline (D) in andiroba (*Carapa guianensis* Aubl.) seedlings submitted for 25 days under water stress. * Averages followed by the same letter do not differ among themselves by the Tukey test at 5% of probability. The bars represent the mean standard error.

The accumulation of proline was caused by re-synthesis, through metabolic pathway that uses glutamic acid as a precursor involving the coordinated action of synthase enzymes and pyrroline-5-carboxylate reductase (Szabados and Saviouré, 2010). The low utilization of proline during stress caused by the reduction in protein synthesis and increased proteolysis, are significantly influencing the additions of osmolyte in plant (Brito et al., 2008). The accumulation of proline is a possible indicator of adaptation to water stress, contributing to the increased tolerance of stressed plants, induced by water deficiency in leaf and root tissues, possibly by promoting an increase in osmotic potential (Krasensky and Jonak, 2012).

Another adaptive response of plants refers to increments of total soluble carbohydrates (Fig 2A), and this occurs due to deficiency of water in soil. Plant promotes an osmotic increase in substances adjusting the adverse conditions, being essential for the maintenance of cellular turgor (Salisbury and Ross, 2012). According to Oliveira Neto (2010), the observed increases are associated with a probable decrease in starch content in the leaf, besides the decrease in photosynthetic capacity paralyzing cell growth by reducing the synthesis of sucrose for export, enabling the increase in the concentration of carbohydrates in cells plant. Other sugars can also function as compatible solutes, promoting the maintenance of water potential by osmotic adjustment and protection of the cells plant during dehydration, forming glassy structures.

Kerbaui (2009) describes an increase in the sucrose-phosphate synthase activity and sucrose synthesis in leaf tissues in response to water stress. Similar results were obtained by Costa et al. (2010), researching the *Costus arabicus* L. They observed that sucrose concentrations were lower in the control treatment compared plants submitted to water stress for 67 days.

According to Meloni et al. (2004), the increase in the concentration of glycine-betaine in leaves (Fig 2C) is to protect the plant metabolism, since this substance has the function of compatible osmolyte, maintaining the water balance between the cell plant and the environment. The glycine-betaine has also the function to protect the membranes of the thylakoids, maintaining the photochemical efficiency of photosynthesis (Ashraf and Foolad, 2007). Cordeiro (2012), researching the *Swietenia macrophylla*, *Hymenaea courbaril* and *Tabebuia serratifolia*, observed increase in concentration of glycine-betaine in leaves by 49.4-53.62% during the water stress. Thus, increasing concentration of glycine-betaine is common in many plant species submitted to water stress.

Materials and Methods

Experimental conditions

The experiment was conducted in a greenhouse, during the period between September and November 2013, at the Universidade Federal Rural da Amazônia in Belém city, State of Pará, Brazil (01°27'S e 48°26'W).

Plant material

The andiroba seedlings were obtained from the Associação das Indústrias Exportadoras de Madeira do Estado do Pará, 1-month-old and 15 cm tall. In the present study, two destructive samplings were carried out, always around 9:00 h, in which the plants were separated from the root, stem and leaf.

Substrate, pots and plant nutrition

The andiroba seedlings were transplanted to pots with a capacity of 12 kg of substrate, containing a mixture composed of black soil, chicken manure and worm castings in the ratio of 3: 1: 1 (v:v:v), respectively. Before the transplanting, testing to field capacity of the pots, and the process of lime to correct the soil pH and macronutrient and micronutrient supplementation were made according to the chemical soil analyze, applying 600 mL the nutrient solution of Hoagland and Arnon (1950).

Experimental design

The experimental design was completely randomized with two water conditions: control and water deficit, with 15 repetitions, totally 30 experimental units, in which each plant being one experimental unit.

Water stress regimes

Plants were subjected to two water regimes [irrigated (control treatment) and water stress], which was made in a preliminary experiment simulating a moderate and severe water stress through interruption of irrigation on the seedlings (4-month-old) during the period of 25 days. During the experimental period, plants were irrigated daily to replace the evaporated water, which made individually for each pot, taking into account the daily weighting (pot + plant + soil).

Leaf water potential and gas exchange

The water potential (Ψ_{am}) was measured between 4:30 pm and 5:30 pm using an pressure pump type Schölander (m670, Pms Instrument Co., Albany, USA) (Pinheiro et al., 2007). The stomatal conductance (g_s) and transpiration rate (E) were evaluated using a steady-state porometer (LICOR AM-300, model 1600, Nebraska, USA), in conditions of light and CO₂ environments, between 09:30 and 10:30 h in all of the plants in the experiment (Costa and Marengo, 2007).

Leaf sample preparation

The leaves were harvested and placed in a 70°C oven with forced air circulation at 96 h. The dried leaves were ground, and the powder was stored in a glass container in the dark at 15°C until biochemical analysis were performed in the laboratory of Biodiversity Studies in Higher Plants (BSHP).

Free proline

Determination of free proline were performed using 50 mg of leaf dry matter powder, and incubated with 5 mL of sterile distilled water at 100°C for 30 min. After incubation, the homogenized was centrifuged at 2,000 g for 5 min at 20°C and supernatant was removed. The quantification of free proline was performed after measuring the absorbance at 520 nm according to Bates et al. (1973) based on L-proline (Sigma Chemicals) as standard.

Total soluble carbohydrates

To determine the quantity of total soluble carbohydrates, 20 mg of leaf powder was incubated with 2.0 μ L of 80% ethanol at 95°C for 20 min and centrifuged for 5 min at 5.0 g and 20°C. The supernatant was then removed, and the quantification of the total soluble carbohydrates was

performed in reactions containing 1.250 µL of 100% H₂SO₄, 70 µL of 15% phenol, 580 µL H₂O, and 100 µL of extract for a total volume of 2.0 µL. Measurements were taken at 490 nm (Dubois et al. 1956) using glucose (Sigma chemicals, São Paulo, Brasil) as a standard.

Sucrose

The determination of sucrose was carried out with 50 mg of leaf powder incubated with 1.5 mL of solution MCW (methanol, chloroform and water) in the proportion of 12:5:3 (v/v) at 20°C by 30 minutes under agitation, centrifuged at 10,000 g for 10 minutes at 20°C and the supernatant was removed. The sucrose quantification was carried out at 620 nm, in agreement with Van Handel (1968), using sucrose (Sigma Chemicals) as standard.

Glycine-betaine

The glycine-betaine was determined with 25 mg of leaf dry matter powder, which was incubated with 2 mL of sterile distilled water at 25°C for 4 h, under agitation. After homogenized, it was centrifuged at 10,000 g for 10 minutes at 25°C and the supernatant removed. The glycine-betaine quantification was carried out at 365 nm according to Grieve and Grattan (1983), using glycine-betaine (Sigma Chemicals) as a standard.

Statistical analyzes

The data were submitted to variance analysis and when significant differences occurred the Tukey's test at 5% level of error probability was applied. The standard errors were calculated in all evaluated points. The statistical analysis was carried out with the SAS software (Statistical Analysis System, 2008).

Conclusions

The 25 days water stress by suspension of irrigation influenced the regulation of water relations in andiroba seedlings, restricting transpiration rates, reducing water potential and stomatal conductance, promoting the accumulation of free proline, total soluble carbohydrates, sucrose and glycine-betaine concentrations.

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