



RESEARCH ARTICLE

GROWTH AND ECOPHYSIOLOGICAL ASPECTS IN YOUNG PLANTS OF *Hymenaea courbaril* L SUBMITTED TO WATER STRESS AND FLOODING

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ABSTRACT

The jatobá (*Hymenaea courbaril* L.) is a Neotropical tree found in sandy soils, loamy well-drained upland and high plains, its importance is related to the fact that it is a promising species for reforestation programs and altered area recovery. Work objective was to evaluate the growth and ecophysiological aspect in young plants jatobá submitted water stress and flooding. The study was conducted in a greenhouse at the Federal Rural University of Amazonia in Belém, PA, Brazil. The experimental design was completely randomized with three water conditions: control, drought and flooding. A preliminary experiment was conducted simulating a severe drought and anaerobic stress, with suspension of irrigation and flood a water blade above the ground on the seedlings within 30 days. It was applied to the analysis of variance in the results and when there was a significant difference, the means were compared by Tukey test at 5% significance. The following parameters were evaluated: number of leaves, plant height, stem diameter, dry root mass, shoot, total leaf area, water potential, transpiration, stomatal conductance, carbohydrate and sucrose concentration. The results showed that the treatments promoted significant changes in plants, with decreases in transpiration and stomatal conductance, followed by the significant reduction in growth parameters, due to the reduction of water potential of plants. In addition, water stress (deficit and flooding) signification promoted increased levels of carbohydrates and sucrose in the plant.

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INTRODUCTION

The jatobá (*Hymenaea courbaril* L.) is a Neotropical species of the Leguminosae family. Since this is an opportunistic semideciduous tree, selective xerófito (Nascimento et al., 2014), their occurs from southern Mexico to South America. In Brazil, the species is found in sandy soils, loamy well-drained upland and floodplain high, but rarely in open fields. Among the native species of Brazil, Jatoba has aroused the interest of researchers to present widespread and occur in ecosystems where most plants cannot grow satisfactorily (MATHEUS et al., 2011). However, plants in natural environment condition, are subject to various conditions of stress (deficiencies and flooding) that compromise your metabolism. These in turn induce a series of biochemical changes, physiological, cellular

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and anatomical in the plant to tolerate them or even avoid them (Nascimento et al., 2014). The physiological indices growth assessment is field of study widely analyzed by researchers with the purpose of evaluating the performance of plants grown in natural stress condition or human-induced. Considering these aspects the knowledge of physiological ecology and growth of tree species seedlings, be an important tool for areas with risks or recovery of degraded areas and devastated by man. However it is valid highlight that although jatobá is presented as promising species for reforestation programs and altered area recovery, knowledge about the physiological responses is still incipient (Nascimento et al., 2015). In response to the reduction in water availability, several species promotes significant decrease in the production and allocation of dry matter, stomatal conductance and transpiration. It is clear that the plants are modifying their physiological and ecological processes in order to adapt and survive for new environmental taxes scenarios by global changes (Coates et al., 2011).

Already in flooding condition occurs absence of oxygen in the soil, which reduces the root system and growth of the aerial parts of trees, adversely affecting the physiological processes of plants, including stomatal conductance and photosynthesis, and consequently the synthesis and translocation of assimilates (PARENT *et al.*, 2008). Based on what was above, this study aimed to evaluate growth and ecophysiological aspect in young plants of jatobá submitted to water stress and flooding.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse of Universidade Federal Rural da Amazônia (UFRA), in Belém, PA, Brazil (01°27' S e 48°26' W) the implementation period was from 20/09/2008 to 20/05/2009. The substrate used for the growth of seedlings was a mixture of 3:1: 1 (v:v:v), being [(3) terra preta, consisting of yellow latosol, mean texture, previously dried in air and impurities removed by the sieving process and scavenging respectively (1) and chicken manure (1) earthworm humus]. The seedlings of Jatobá (*Hymenaea courbaril* L.) from AIMEX (Associação das Indústrias Exportadoras de Madeira do Estado do Pará) having 4 months of age, where it was transplanted into the pots with substrate fixed to its acidity and nutrition. The plants were subjected to three water regimes [irrigated (control), drought and flooding], in which a preliminary experiment was done by simulating a severe drought and anaerobic stress, through the suspension of irrigation and flood a water blade above ground on respectively seedlings, the 30-day period (severe stress) for the seedlings 8 months of age after germination. During the trial period the control plants were irrigated daily to replenish water lost by evapotranspiration phenomenon being made individually for each vessel, taking into account the daily weighing forming a set (pot + plant + soil) and also the control of weeds from manual weeding without causing nutritional deficiency, pests and pathogens. Environmental variables such as temperature and relative humidity, using a thermo-hygrometer (mod 5203 Incoterm, RS, Brazil.); and vapor pressure deficit of water between the sheet and atmosphere (DPVFA) second Landsberg (1986). The photosynthetic radiation sheet (RAF), leaf temperature, stomatal conductance and transpiration was acquired through the portable porometer dynamic equilibrium (mod. 1600 Li, liquor, Nebraska, USA) with light and environmental CO₂. It analyzed the final, fully expanded leaves, in which the measurements were made 10:00, coinciding with the removal of samples for biochemical analysis. The water potential was determined in the before dawn (ψ_{am}) between 4:30 and 5:30 h and 10:00 h being equal to the time of determination of gas exchange, using a pressure pump Sholander type (mod. pms Instrument Co, Corvales, USA) as described by DaMatta *et al.*, (2001).

Plants were used whose biomass was measured at the time of each collection of the experiment. The plant after being collected, are brought to the laboratory plant physiology and dried in a forced circulation oven at 65 ° C for 24 hours. Through this process it was determined the weight of the dry mass in analytical balance. Production measures were: shoot dry mass, dry mass of roots, number of leaves, plant height (made by a tape measure), stem diameter (made using a digital calliper), leaf area and total dry mass. The biochemical analysis

were realized in Laboratório de Estudos da Biodiversidade em Plantas Superiores (EBPS), located at Universidade Federal Rural da Amazônia (UFRA), Belém, Pará. For this, were determined the carbohydrates (DUBOIS *et al.*, 1956); sucrose (VAN HANDEL, 1968). The experimental utilized was completely randomized with three water conditions: control, drought and flooding, with 15 repetitions, totalizing 45 experimental units, wick each experimental unit was composed of one (1) plant/vaessel. The analysis of variance was applied in the results and when a significant difference occurs, the means were compared by the Tukey test at 5% of probability. Besides that, the standard deviations were calculated for each treatment, and statistical analyzes performed with Sas- institute (1996) and informed the recommended statistical theories (GOMES; GARCIA, 2002).

RESULTS AND DISCUSION

Water potential

The plants subjected to droughts and flooding shows significant difference at 5% probability for water potential, as can be seen in the figure (01). The potential predawn was of -0.06 MP for the control plant, -2.8 and -1.1 MP MP to plants under flooded and water deficiency, ie, a decrease in 366% of Pw and 83.3 % respectively in relation to the control plant.

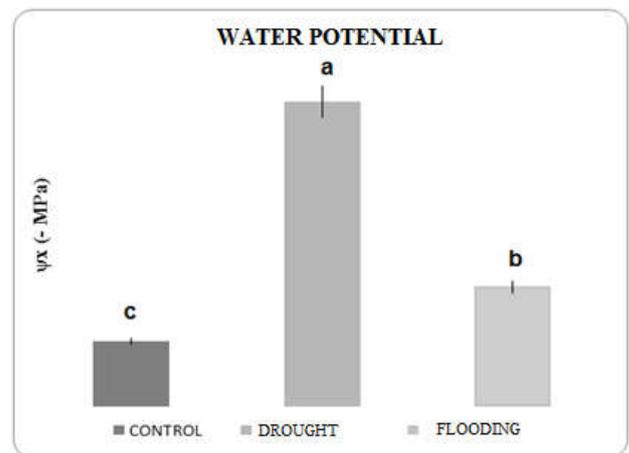


Figure 1. Water potential in leaves of *Hymenaea courbaril* L. submitted to drought and flooding during 30 days. Means followed by the same letter do not differ by Tukey test at 5% probability. The bars represent the standard deviations of the mean

The reduction of water potential may have occurred due to the potential predawn (Ψ_{am}) Present water losses by evapotranspiration nil or negligible, due to lack of light and high humidity, and in this situation a balance in the soil - plant - atmosphere is obtained. The decrease in membrane permeability leads to a reduction in the leaf water potential and wilt. Another process that can be involved is the reduction of the metabolic process and decomposition of the roots through the fermentation process, thus promoting a reduction in water absorption and minerals to the plant (Taiz; Zaiger, 2013). The reduction of water potential of plants to flooding is due to the lack of energy to sustain the physiological processes that depend on the air part to the process of cellular respiration.

Furthermore, there is a decrease in the absorption of ions responsible for tissue development and growth of the roots, reducing the amount of absorbent hairs (KERBAUY, 2008). The accumulation of soluble amino acids, sucrose and soluble carbohydrates, contribute to the osmotic adjustment, especially in the cytoplasm, reducing the osmotic potential of leaf tissue cells, and hence their water potential (Henrique *et al.*, 2010).

Transpiration

The results show a significant difference at 5% probability between treatments for Transpiration in jatobá. The figures emerged a decrease in transpiration rate in plants submitted to water deficit ($0.3 \text{ mol. m}^{-2} \cdot \text{s}^{-1}$), flooding ($2.81 \text{ mol. m}^{-2} \cdot \text{s}^{-1}$) and control ($3.49 \text{ mol. m}^{-2} \cdot \text{s}^{-1}$), providing a significant decrease in transpiration 91.4% and 19.48% respectively, compared to control plants (figure 2).

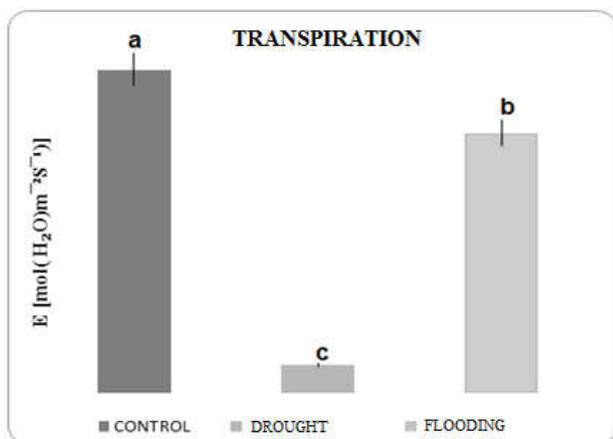


Figure 2. Transpiration in leaves of *Hymenaea courbaril* L. submitted to drought and flooding during 30 days. Means followed by the same letter do not differ by Tukey test at 5% probability The bars represent the standard deviations of the mean

The explanation of decreased transpiration can this related to stomatal closure, as one of the first shoots of responses due to the stress suffered by the plant. Taiz and Zeiger (2013) observed that the absence of oxygen at the root can stimulate the elevation of endogenous levels of abscisic acid in the leaves, especially older ones, causing stomatal closure and reduced transpiration by water loss. Another process can be attributed to reduced water supply, such as reduced hydraulic conductivity of the roots or root death, leading to decrease in the water potential of the leaves. Coelho *et al.* 2013 reported that perspiration significantly affect plants subjected to stress by flooding occurring the reduction of water absorption, either by reducing the length and total surface area of the roots as a consequence of death, such as increased resistance to water flow.

Stomatal conductance

The results obtained in jatobá significant difference at 5% probability between treatments for stomatal conductance. With values of $0.12 \text{ mol.m}^{-2} \cdot \text{s}^{-1}$ (water shortage), $0.28 \text{ mol.m}^{-2} \cdot \text{s}^{-1}$ (flooding) and control plants ($0.49 \text{ mol.m}^{-2} \cdot \text{s}^{-1}$). Thus, flooding

and water stress promoted a significant decrease in stomatal conductance of 75, 5% and 42.8% respectively compared to control plants (Figure 3).

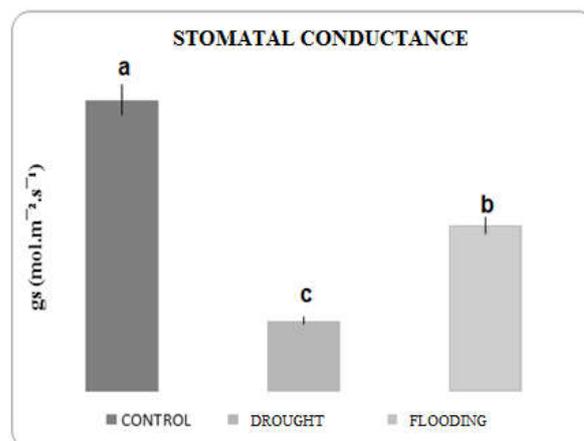


Figure 3. Stomatal conductance in leaves of *Hymenaea courbaril* L. submitted to drought and flooding during 30 days. Means followed by the same letter do not differ by Tukey test at 5% probability The bars represent the standard deviations of the mean

Overall, the modifications related to water deficit include lower water potential in soil and leaves, reflecting the decrease in stomatal conductance (Polizel, 2007). The decrease in stomatal conductance in jatobá sheet, can be explained by the presence of anoxic roots or hypoxic and lack of sufficient energy to sustain physiological processes. the low production of ATP produced in the roots by anaerobic respiration, evidenced by the increase in alcohol dehydrogenase (ADH), lactate dehydrogenase (LDH), may limit the power available to the cellular root processes and thus lead to reduced growth or death of roots and therefore decreased absorption of water and nutrients, which should contribute to stomatal closure and reduction in stomatal conductance. Moreover, the flooding induces a significant reduction in stomatal conductance, with a tendency to become more pronounced with time of treatment, not taking, however, a complete stomatal closure, even in the afternoon, which has usually higher evapotranspiratória demand (JUNIOR *et al.*, 2015).

Number of leaves

The results showed that the plants under water stress (20 sheets) significantly reduced the level of 5% probability approximately 47.36% to the issue of new leaves compared to control plants (38 sheets). In the meantime, there was no significant difference from the plants under flood (36 sheets) as shown in Figure 4. Reducing the emission of new leaves it could be related to the lower rate of cell division, which may have reduced the appearance of new leaves, which apparently became slower in plants under water stress (Nascimento *et al.*, 2011). Thus, the water deficiency affects cell division and growth, causing a reduction in elongation and sugirmento new leaves. Moreover, the reduction can also folial is related to increased activity of the enzymes cellulase and polygalacturonase. For plants on flooding time it was probably not sufficient to promote significant difference or the plant has a greater resistance to this type of stress.

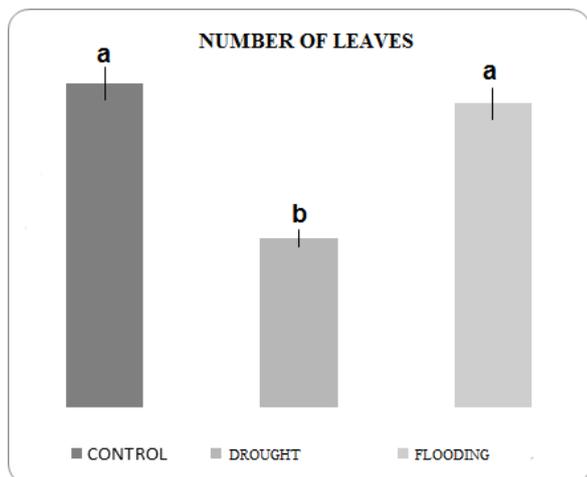


Figure 4. Number of leaves of *Hymenaea courbaril* L. submitted to drought and flooding during 30 days. Means followed by the same letter do not differ by Tukey test at 5% probability. The bars represent the standard deviations of the mean.

Height of plant

The heights of the plants under control and flood showed no significant difference at 5% probability, with 75.83 of values and 70.66 cm respectively. The plants subjected to water deficit the average height was 61.16 cm, representing a decrease of 19.34% in height growth, showing that the water suspension affected significantly at 5% probability plant growth compared control plants (Figure 5).

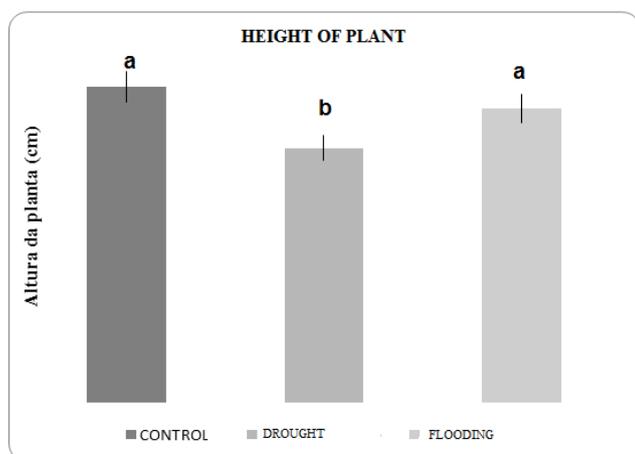


Figure 5. Height in plants of *Hymenaea courbaril* L. submitted to drought and flooding during 30 days. Means followed by the same letter do not differ by Tukey test at 5% probability. The bars represent the standard deviations of the mean.

The understanding of the height of a plant in relation to abiotic stress is quite complex. However, the effect of water loss from the sheet can lead to an increase in resistance of photosystem II (PSII) at temperatures above optimum for stress, leading to a decrease in height of several plant species. The physiological consequence for plants subjected to drought stress is the reduction or interruption of growth rates due to the reduction in cell differentiation, influenced by water restriction (Nascimento *et al.*, 2011). For plants on flooding time it was

probably not sufficient to cause significant differences to that kind of stress.

Stem diameter

The values showed a decrease in stem diameter ranging from 0.8 mm (control plants) to 0.6 mm (plants under drought) and 0.76 mm (flooded plants), or a reduction of the diameter 25 % and 5% respectively compared to control plants (figure 6). Thus, the diameter significantly reduced the level of 5% probability for plants under water deficit and with no statistical difference for the plants in the flood (Figure 6).

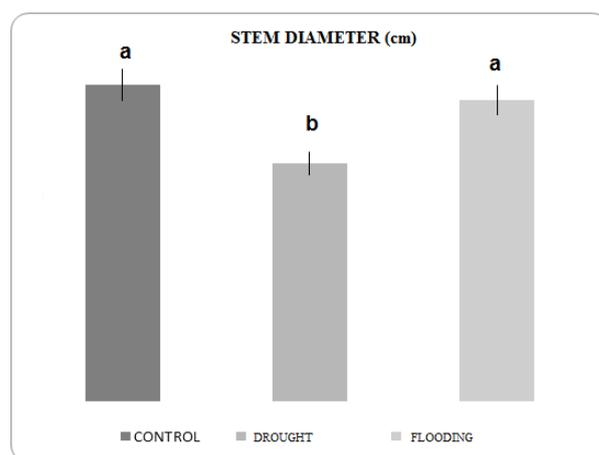


Figure 6. Stem diameter in plants of *Hymenaea courbaril* L. submitted to drought and flooding during 30 days. Means followed by the same letter do not differ by Tukey test at 5% probability. The bars represent the standard deviations of the mean.

The possible response of the reduction in stem diameter, is probably linked to increased stomatal closure, which negatively influenced the production and accumulation of assimilates. This decrease in the production of assimilates and increases the activity of oxidative enzymes, resulting in increased plant temperature increased respiration and assimilates expenditure and consequently reduced diameter growth. According Lechinoski *et al.* (2007), the lack of water in the soil becomes the limiting diameter growth of plant species when subjected to water stress condition. For plants on flooding time it was not sufficient to promote significant difference or the plant has a greater resistance to this type of stress.

Leaf area

The results revealed a significant difference at 5% probability between treatments, a reduction of 68.76 cm² (control plants) to 57.58 cm² (flooded plants) and 45.38 cm² (plants subjected to drought) that is, a significant reduction in leaf area of 16.25% and 34% respectively compared to the control plant (figure 7). The values emerged that water stress was more harmful than the stress flooding and can be justified by the nature of the stressor, and leaf area with linear effect and with high coefficient of determination and low sale. With the decrease in leaf area, assimilated production is reduced and can be reflected in the productive capacity of the plant.

Ramos Junior et al. (2013) reported that the reduction of leaf area is related to low water availability and at the same time the mechanism of drought tolerance. Already Nascimento et al. (2011), said that the reduction of leaf area can be related to the reduction in emission of new leaves.

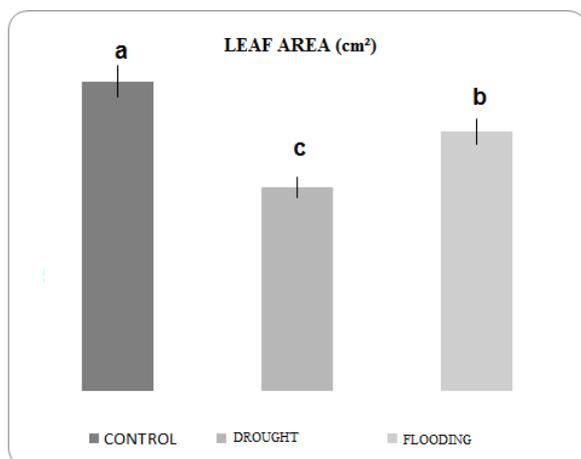


Figure 7. Leaf area in plants of *Hymenaea courbaril* L. submitted to drought and flooding during 30 days. Means followed by the same letter do not differ by Tukey test at 5% probability. The bars represent the standard deviations of the mean.

Dry mass of aerial part and root system

The results revealed that there was a significant decrease in the level of 5% probability in the dry matter of shoot under water stress and flooding (Figure 8). This reduction was a variation of the control plants for plants under drought and under flooding plants 20.93, 8.34g, and 15.92g a decrease of 60.1% and 23.93% respectively in relation to the control plant. The reduction of assimilates caused a likely imbalance between source / drain, and inactivation of hormones responsible for the growth of plant height and stem diameter, significantly affecting the dry mass of shoots. According to Belo (2011) water stress affects plant growth by slowing the growth of roots and shoots, either by inhibition of stretching or initiation of leaf expansion of internodes. Regarding the dry mass of the root system, the results were statistically significant with decreased dry root mass to plants subjected to water stress and flooding (Figure 8B). The figures showed a reduction in dry matter accumulation of 6.71g of plant roots to control 3.22g and 4.9g of plants with water stress and under flooding with significant decrease of 52% and 26.9% respectively compared to the plant control.

As the dry root mass, the results showed that the plants grown without oxygen decreased root growth. This reduction may have been due to the lower amount of energy available for growth processes, since in low oxygen conditions the plants begin to breathe anaerobically, resulting in a reduction in the energy charge of the cell metabolism (low ATP production), in addition, the decreased production and translocation of assimilates to breath. The results suggest also that there was a greater distribution of assimilates to the roots under flooding condition (JUNIOR *et al.*, 2015).

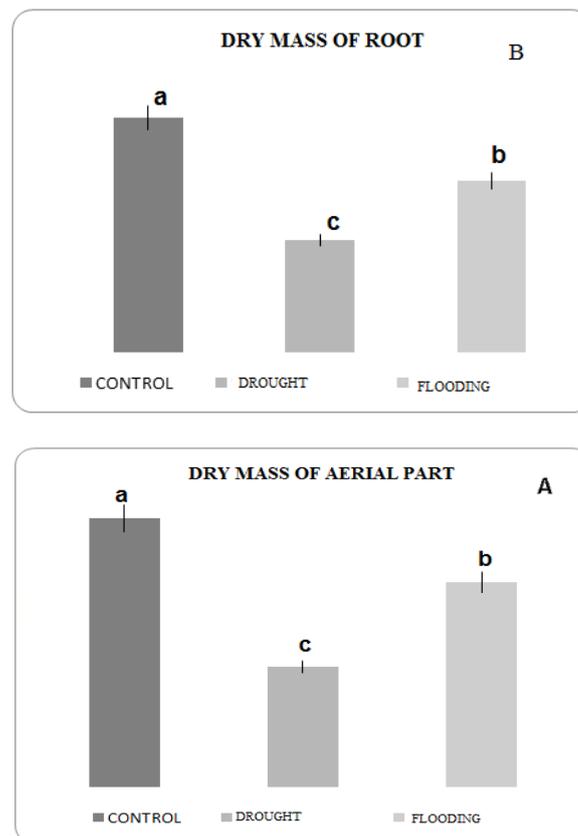


Figure 8. Dry mass of aerial part (A) dry mass of root (B) of *Hymenaea courbaril* L. submitted to drought and flooding during 30 days. Means followed by the same letter do not differ by Tukey test at 5% probability. The bars represent the standard deviations of the mean.

Total dry mass

The results (Figure 9) showed significant differences at 5% probability to total dry mass of the *Jatoba* seedlings. The figures showed that over the thirty (30) days of stress (drought and flooding) caused increment total biomass. control plants, water stress and flooding had total biomass 27.62g, 11.6g and 20.82g with a significant reduction of 58% and 24.61% respectively in relation to the plant control.

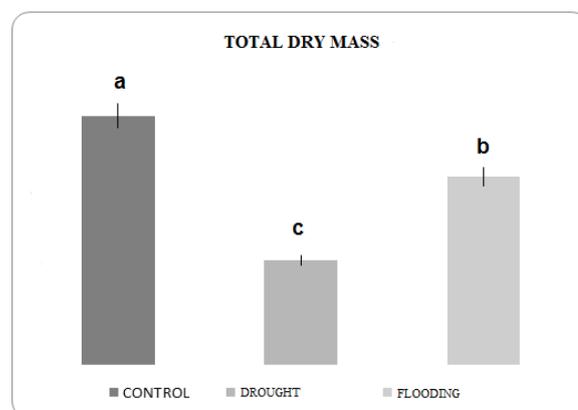


Figure 9. Total dry mass of *Hymenaea courbaril* L. submitted to drought and flooding during 30 days. Means followed by the same letter do not differ by Tukey test at 5% probability. The bars represent the standard deviations of the mean.

The total dry weight of shoot and content of roots jatobá plants to restriction in the availability of molecular oxygen by flooding condition, and water stress. Show significant changes, ie there was a decrease in total biomass accumulation. This was seen in the different flood times and water suspension. So, the time that the plants remained under hypoxia and the availability of water in the soil was no difference in the total dry matter accumulation. The survival of plants depends, among other factors, its growth capacity and distribution of inorganic nutrients. However, the flooding may cause reduction in the leaves of carbohydrate translocation rate to the roots, slowing their growth and their metabolic activities, which begin to demand less of carbohydrates, accumulating the photosynthates the leaves. Thus, the increased availability of respiratory substrate, such as glucose, can be crucial in the survival of the root tissue in anoxic environments (DIAS-FILHO, 2006). For Coelho et al. (2013), increased total dry weight values in control plants are higher than in intact plants under flooding due to stress of flooding have their development affected by physiological processes, and may be a consequence of reduced oxygen concentration on the root system.

Total soluble carbohydrate concentration

The results showed significant difference at 5% probability for different treatments. The roots of control plants (0.83 mmol GLU / g residue), flooded plants (1.82 mmol GLU / g residue) and water deficiency (3.26 mmol GLU / g residue) an increase significant carbohydrate 119.2% and 295% respectively compared to control plants. Since the leaves control plants (GLU 1.32 mmol / g of residue), flooded plants (GLU 2.41 mmol / g residue) and water deficiency (GLU 4.83 mmol / g of residue) with the mean increase of 82.5% and 265.9% respectively compared to control plants as shown in figure 10.

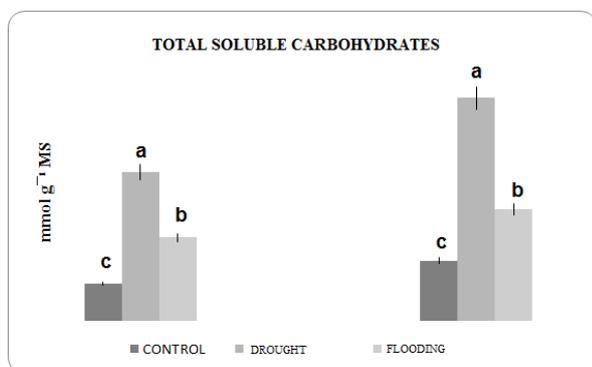


Figure 10. Concentration of total soluble carbohydrates in roots and leaves of *Hymenaea courbaril* L. submitted to drought and flooding during 30 days. Means followed by the same letter do not differ by Tukey test at 5% probability. The bars represent the standard deviations of the mean.

Regarding the total soluble carbohydrate content, the results revealed a significant increase in the level of 5% probability for plants under water stress and under flood. In plants under water stress increased soluble carbohydrate can associated with the physiological adjustment process that occurs in the metabolism of these plants, which causes reduction of water potential and the difference in concentration gradients that

remained turgor and hence delayed dehydration in plant tissues. The increases observed in plants under stress are associated with the fact that with the dehydration, is decreased starch content in the cell, and decrease in the photosynthetic capacity paralyze cell growth and reduce the synthesis of sucrose export, causing increase the total carbohydrates (Martinez *et al.*, 2007). The carbohydrate accumulation during water deficiency is probably related to mechanisms to prevent loss of water in the plant, through the osmotic adjustment. The osmotic adjustment helps maintain the stomatal opening and functioning of the photosynthetic apparatus, allowing it to operate even in low water potential conditions (HAYAT *et al.*, 2012). The increase of carbohydrates in plants under water stress occurs as a way to tolerate stress so as to maintain the hydrated plant (Souza *et al.*, 2013) where part of the carbohydrate arranged in the cytoplasm are utilized in the activation of drought tolerance mechanisms, regardless of the mechanism in question (lyse *et al.*, 2012). While the starch accumulation in leaves and the highest content of soluble sugars in the roots under the soil flooding, promoted a decrease in demand for carbohydrates because of reduced growth and metabolic activities in the roots. Generally, flooding increases the concentration of total soluble carbohydrates (ROSA *et al.*, 2009). The increase in the concentration of CST in the roots of flooded plants, has been reported as a form of tolerance to stress and can be related to the flow of sucrose and its conversion by the enzyme invertase or reduced demand for carbohydrates due to decreased metabolism of and root growth (Henrique *et al.*, 2010).

Sucrose content

The results showed significant difference at 5% probability for different treatments. The roots of control plants (0,12836mg sucrose g⁻¹ DM), flooded plants (5,654 mg sucrose g⁻¹ DM) and water deficit (18,857 mg sucrose g⁻¹ DM) with a significant increase of sucrose 4304.7% and 14590.7% respectively compared to control plants, as the leaves of the control plants (27,622mg sucrose g⁻¹ DM), flooded plants (15,533mg sucrose g⁻¹ DM) ie significant reduction of sucrose about 43.76 % when compared to control plant, as the plants with hydro deficiency (36,875mg g⁻¹ MS sucrose) showed an increase of 33.49% compared to control plant as shown in figure 11.

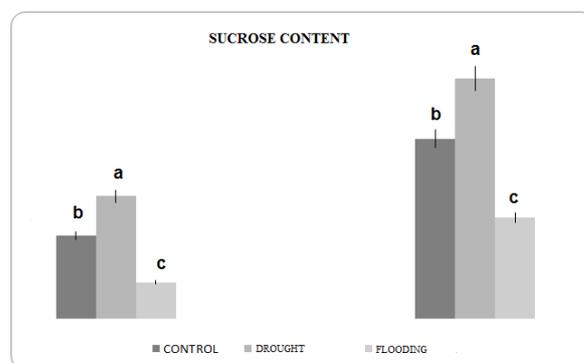


Figure 11. Sucrose content in roots and leaves of *Hymenaea courbaril* L. submitted to drought and flooding during 30 days. Means followed by the same letter do not differ by Tukey test at 5% probability. The bars represent the standard deviations of the mean.

The increase of sucrose possibly occurred by the decrease in photosynthetic and breakdown of starch by α and β - amylase enzyme sugars. This sugar may be broken and processed through biochemical reactions in sucrose. Among these various sugars formed, sucrose is the major sugar formed and transported by the plant. This sugar may have been transported to their drains preferences, which force the main drain at this stage is a panicle. In most plants sucrose is the major sugar export from the synthesis sites (leaves) for consumer regions (stem, leaf buds, roots, and reproductive organs) which is used for growth and / or storage. The hexose liberated from hydrolysis of sucrose may be used in either anabolic or catabolic processes and also the supply of reducing sugars and is widely used for osmotic adjustment process. The sucrose level undergoes progressive increase in plants under water stress because of the increase in total soluble sugar concentrations in plants may be related to the protection of biological membranes that can be degraded by the lack of water in the cytosol and the increase in concentrations of substances ionic, making several inactive enzymes in the cytosol (LIU *et al.*, 2011). Plant already in flooding condition may occur the increase in function in sucrose content of the enzymes hydrolases α and β amylase acting on starch degradation (ANDRADE JUNIOR *et al.*, 2016).

Conclusion

Drought and flooding promoted a significant decrease in water potential, transpiration, stomatal conductance. There was a significant decrease to leaf area, dry weight of shoot and root, total dry weight for the treatments compared to control plants. Stress promoted a greater adjustment of Osmolíticos compounds compared plants controls, with changes in behavior and carbohydrate sucrose. Therefore, the results showed that plants of jatobá are less sensitive to flooding of the plants under water suspension.

Acknowledgements

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