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Physiological and biochemical metabolism in Jatoba plants (*Hymenaea courbaril* L.) affected by water stress and flooding

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Abstract

The experiment was conducted in a completely randomized design in three water conditions at greenhouse with treatments as: control, water stress (water deficiency) and flooding (with a blade of water about 5 cm above the ground), and 15 repetitions. The analysis of variance was done and the 5% level of significance of the Tukey's test accomplished to assess the biochemical and physiological parameters of *Hymenaea courbaril* L. The variables were: water potential, nitrate reductase activity, concentration of nitrate, ammonium free, proline, glycine betaine, free protein, free amino acids, glutamine synthetase activity, alcohol dehydrogenase activity and lactate dehydrogenase and contents of chlorophyll a, b, and total carotene. There was a reduction of the enzyme reductase activity of nitrate and glutamine synthetase due to the water deficit and flooding. Moreover, the lack of water in leaf tissue caused an increase in proline, glycine betaine and free amino acids, trying to promote osmotic adjustment. An increase of variation and reduced ammonium, protein, chlorophyll a, b, and carotenoid was also observed. These substances were significantly reduced by the lack of water and also excess water. Thus, the water deficit and flooding promoted a drastic change in behavior and physiological biochemistry of Jatoba plants. The results of this study show that the flooding and water deficiency for 30 days are sufficient to promote changes in biochemical behavior in the Jatobá plants. Thus, these results indicate that the *Hymenaea courbaril* L. plants are less susceptible to flooding than to the water deficiency.

Keyword: forest restoration; Jatoba; nitrogenous compounds; osmoregulators; water conditions.
Abbreviation: FM_fresh mass; DM_dry mass; AA_amino acid; GGH_gamma-glutamyl hydrolase; ATP_adenosine triphosphate; TSP_trisodium phosphate; LDH_lactate dehydrogenase.

Introduction

The Hymenaea genus appeared in Africa about 65 million years, adapting in various neotropical regions, generating many species, among them courbaril (*Hymenaea courbaril* L.), which is of great importance due to its utility in areas of forest restoration in the legal reserve and permanent preservation areas. It is species with attractive fruit for wildlife (Martins, 2013). Species of this genus may occur from Southern Mexico through much of South America, including Brazil, French Guiana, Suriname, Venezuela, Colombia, Peru and Bolivia. In Brazil, Jatoba is present throughout the territory belonging to the Atlantic Forest biome and in the Amazon region (Costa et al., 2011). It has been the target of many studies among the various native tree species in the country (Matheus et al., 2011). Jatoba is a climacteric species of the Fabaceae family, semi-deciduous, heliophyta, easy multiplication with abundant and regular seeds. It is a species with possibility of commercial use (Campos and Uchida, 2002). According to Panizza (1997), this species is indicated as medicinal, producing essential oil, tannins, resinous and pectic materials, starch and sugars. Its barks and leaves are used in the treatment of diarrhea, intestinal cramps, cystitis, coughs, bronchitis and asthma. The plants are subject to various conditions of stress that compromise their metabolism leading to various biochemical, physiological, cellular and anatomical changes (Nogueira et al., 2005). Water stress is an abiotic stress, which is one of the largest limitations for agricultural production due to negative interference with the growth and development of the plant (Endres et al., 2010). The drought stress negatively influences the metabolism of nitrogenous compounds, because the nitrogen is one of the most important nutrients for plant growth (Ferreira et al., 2002). Plants under drought condition survive doing a self-balance between maintaining turgor and the reduction of water loss in order to reduce the deleterious effects of water stress on crop yield (Castro et al., 2007). Temporary or permanent conditions of flooding (water depth of training) or flooding (water saturation) of the soil are also problems that can cause serious damage to agricultural development. In agricultural regions there are many rare events, while some others can be chronic problem constantly faced by producers. Therefore, due to the potential and economic importance of *Hymenaea courbaril* L., The objective of this study was to evaluate the eco-physiological and growth aspects of young Jatoba plants and to determine whether they are tolerant or sensitive when subjected to water deficiency and flooding.

Results and Discussion

Water potential

The plants subjected to significant drought and flooding showed difference at 5% probability for water potential. As shown in Fig 1, the water potential for for control was 0.5 Mpa, and 1.2 and 2.5 for plants under flooded and water deficiency, respectively. The reduction of water potential may have occurred due to the potential predawn (Ψam) present water losses by zero evapotranspiration or negligible, due to lack of light and high humidity. In this situation a balance in the soil-plant-atmosphere is occurred. The decrease in membrane permeability leads to a reduction in the leaf water potential and wilting. Another process that may be involved is the reduction of the metabolic process and decomposition of the roots through the fermentation process, thus promoting a reduction in the absorption of water and minerals for the plant (Taiz and Zaiger, 2013).

The reduction of water potential in plants subjected to flooding is due to the lack of energy to sustain the physiological processes that mainly depends on the access of plant to air required for 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 the absorbent (Kerbauy, 2008).

Nitrate reductase activity and nitrate levels

The ANOVA results showed a significant difference among different treatments for nitrate reductase activity both in roots and leaves. The response to nitrate reductase activity was observed in the roots of plants under irrigation (0.290 µmoles NO_2^- g^{-1} FM h^{-1}), plants under flooding (0.409 µmoles NO_2^- g^{-1} FM h^{-1}) and without irrigation (0.051 µmoles NO_2^- g^{-1} FM h^{-1}) and leaves of plants with irrigation (µmmoles NO_2^- 0.190 g FM^{-1} h^{-1}). Fig 2A shows the nitrate reductase activity in flooded plants (µmmoles NO_2^- 0.306 g FM^{-1} h^{-1}) and without irrigation fluid (µmmoles NO_2^- 0.017 g FM^{-1} h^{-1}), where there was also a significant decrease of 61.05% and 91.05%, respectively.

The results showed that the excess and shortage of water promoted the activity of nitrate reductase activity for roots and leaves. In water stress condition of the soil, the water decrease causes the nitrate (NH_3^-) flow to the plant, contributing to the reduction of the enzyme activity (Andrade Netto, 2005). These results suggest that water stress decreases the activity of this enzyme because of the reduced flow of water by the current transpiration and also the nitrate flow to the leaves, since this enzyme is highly dependent on its substrate (Sharner and Boyer, 1976).

There was a significant difference in concentration of nitrate in roots and leaves under different treatments. The concentrations of nitrate in roots of plants under irrigation (0.8 µmoles de NO_3^- g^{-1} DM), plants under flooding (0.3 µmoles de NO_3^- g^{-1} DM) and without irrigation (0.1 µmoles de NO_3^- g^{-1} DM) were shown in Fig 2B. The same differences was reported in the leaves of the plants under irrigation (0.6 µmoles de NO_3^- g^{-1} DM), plants under flooding (0.12 µmoles de NO_3^- g^{-1} DM) and without irrigation (0.04 µmoles de NO_3^- g^{-1} DM), where there was also a decrease of 80% and 93.33%, respectively (Fig 2B).

The results showed a decrease in nitrate concentration in leaves and roots under both treatments. The absorption of NO_3^- by roots was reduced in plants under water suspension. Sharma and Dubey (2005), reported that absorption of NO_3^- and its activity are influenced by the availability of water, by reduction of nitrate reductase activity in leaves. The reduction in nitrate uptake of roots (xylem) will decrease the flow of nitrate, causing inactivity of the enzyme degradative process, which is sufficient to explain the results (Taiz and Zaiger, 2013).

Free ammonium levels and glutamine synthetase activity

The concentration of free ammonium levels in *H. courbaril* leaves and roots showed a significant difference between treatments at 5% probability. The roots of control plants, flooding and water deficit showed values of 22, 29 and 36 mmol of NH_4^+ kg^{-1} DM, respectively, resulting in an increase of 31.8% and 63.6%, compared to control. In leaf, the concentration of free ammonia was 9, 16 and 21 mmol NH_4^+ kg^{-1} DM in control plants, flooding and water deficit, respectively, revealing increases of 77.7% and 133.3%, compared to control plant (Fig 3 A).

The accumulation of free ammonium in *Hymenaea courbaril* L. in water stress situation and flooding could be related to the reduction of the GS enzyme activity. In addition, the accumulation of ion NH_4^+ in the plant may have been caused by photorespiration process of catabolism of nitrogenous compounds such as amino acids (Debouba et al., 2007).

Another factor responsible for increasing ammonium concentration is decreased activity of dehydrogenase by enzyme glutamate (GDH) / NADH which catalyzes and incorporates NH_4^+ in organic medium, in addition to induction of other forming routes by protein breakdown, thereby causing the formation of ammonium.

The evaluation of glutamine synthetase activity in leaves and roots of Jatoba identified a significant difference of probability between treatments at the 5% level. In the roots of control, wetlands and water deficit plants 28, 8.4 and 7 mmol GGH kg^{-1} DM, were observed, respectively, corresponding to reduction of 70% and 75% in the flooded plants and disabled water compared to control plant. In the leaves, the activity of enzyme glutamine synthetase was 30.3, 17.2 and 9.2 mmol GGH kg^{-1} DM in control, wetlands and water deficit plants, respectively, revealing a significant decrease of 43.2%, and 69 6% in the treatments, compared to control plant (Fig 3 B).

The results showed that the activity of this enzyme was reduced in plants due to the low concentration of ATP in the cell medium, given that this enzyme is highly dependent on energy. Another reason for the reduction of glutamine synthetase is the decrease of synthase glutamate and glutamate, chloroplast or the cell plastids (Horchani and Aschi-Smiti, 2010) and reduction of reductase activity in nitrate caused by low ion concentration $NH4^+$ (Alves et al., 2012; Andrade Junior et al., 2016).

Proline levels and glycine-betaine levels

The ANOVA results showed a significant difference between treatments. The proline concentrations were 77% higher in the roots, when plants were subjected to water stress compared to control treatment. Flooding also caused increases of this variable by 68% when compared to the control (Fig 4 A). In leaves, the plants under water deficit also showed higher proline content of 76% compared to control, while subjected to flooding, an increase of 66% compared plants under water control was observed (Fig 4 C).

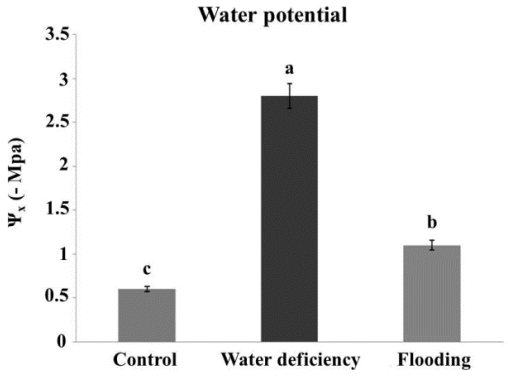

Fig 1. The water potential in leaves of *Hymenaea courbaril* L. subjected to 30 days of drought and flooding. Means followed by the same letter did not differ by Tukey's test at 5% probability. The bars represent the standard deviations of the mean.

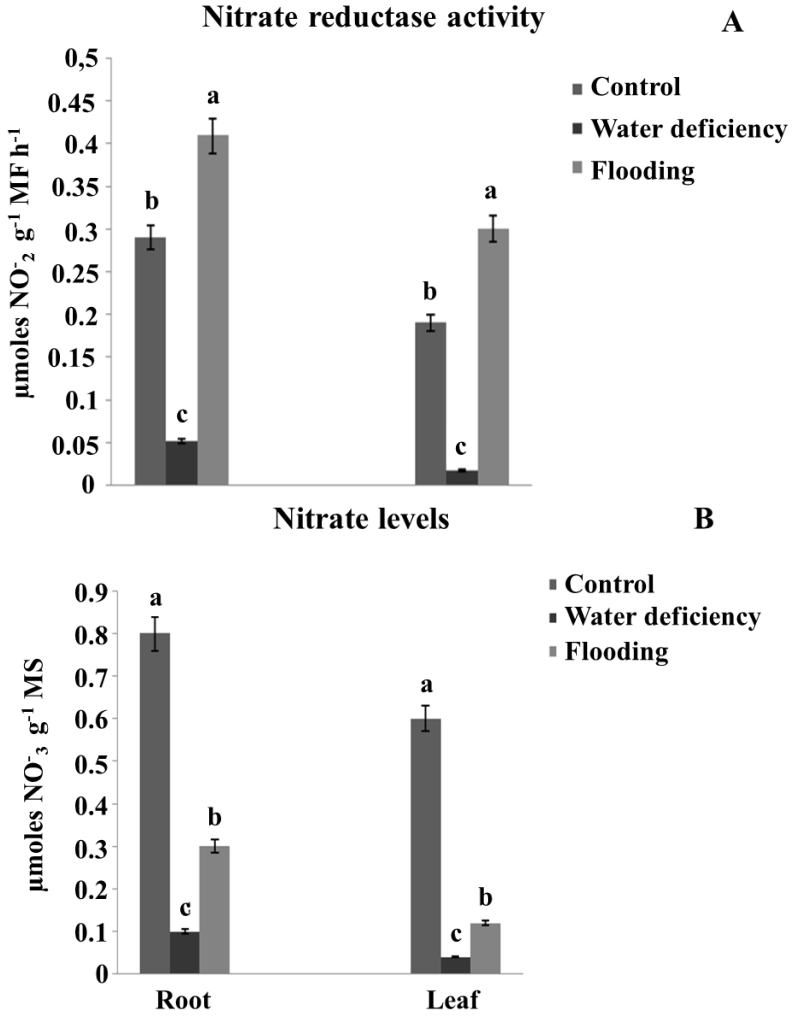

Fig 2. Nitrate reductase activity (A) and nitrate levels (B) in roots and leaves of *Hymenaea courbaril* L. subjected to 30 days of drought and flooding. Means followed by the same letter did not differ by Tukey's test at 5% probability. The bars represent the standard deviations of the mean.

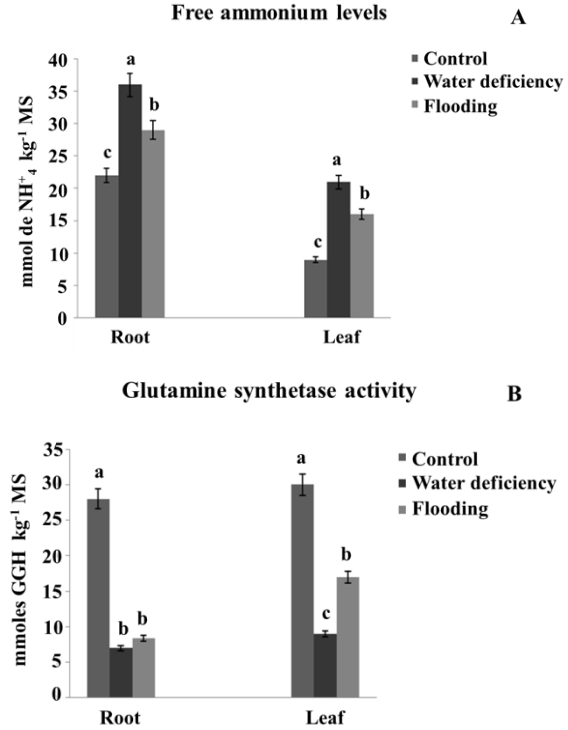

Fig 3. Free ammonium levels (A) and glutamine synthetase activity (B) in roots and leaves of *Hymenaea courbaril* L. subjected to 30 days of drought and flooding. Means followed by the same letter did not differ by Tukey's test at 5% probability. The bars represent the standard deviations of the mean.

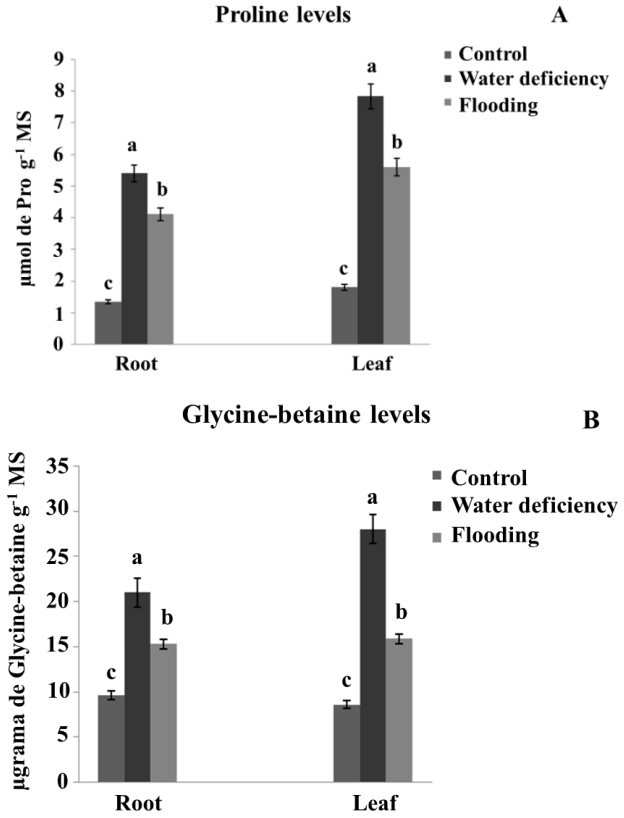

Fig 4. Proline levels (A) and glycine-betaine levels (B) in roots and leaves of *Hymenaea courbaril* L. subjected to 30 days of drought and flooding. Means followed by the same letter do not differ by Tukey test at 5% probability. The bars represent the standard deviations of the mean.

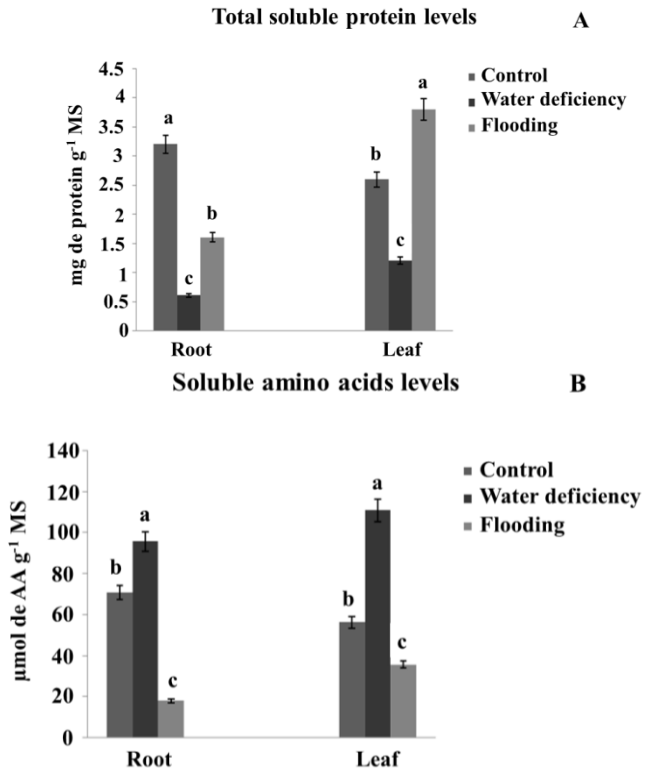

Fig 5. Total soluble protein levels (A) and soluble amino acids levels (B) in roots and leaves of *Hymenaea courbaril* L. subjected to 30 days under drought and flooding. Means followed by the same letter do not differ by Tukey test at 5% probability. The bars represent the standard deviations of the mean.

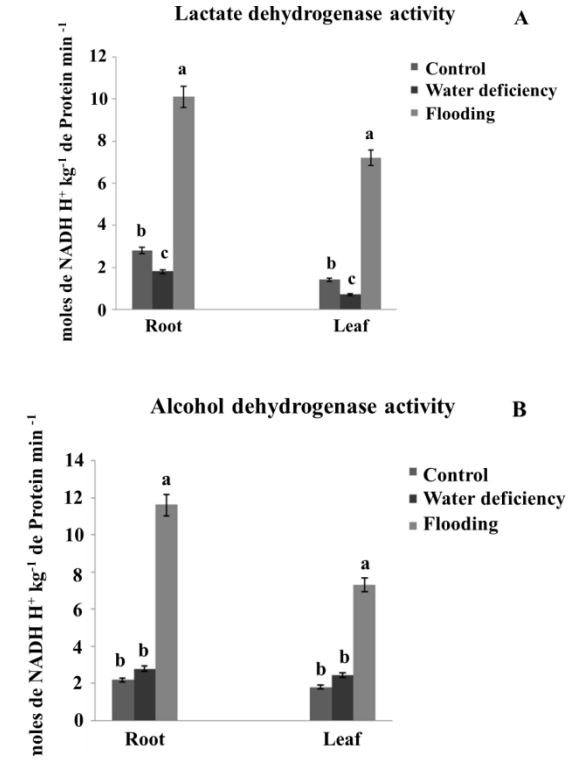

Fig 6. Lactate dehydrogenase activity (A) and alcohol dehydrogenase acticity (B) in roots and leaves of *Hymenaea courbaril* L. subjected to 30 days under drought and flooding. Means followed by the same letter do not differ by Tukey test at 5% probability. The bars represent the standard deviations of the mean.

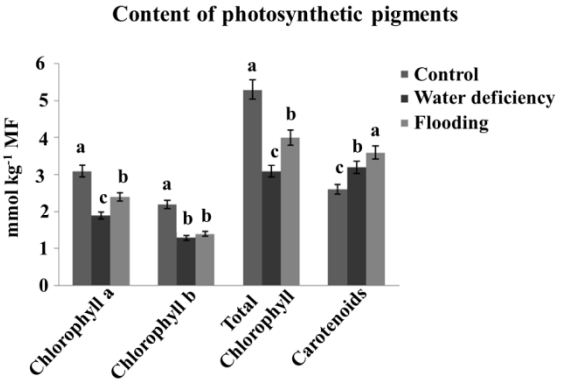

Fig 7. Content of chlorophyll *a*, *b*, total chlorophyll and carotenoids in leaves of *Hymenaea courbaril* L. subjected to 30 days of drought and flooding. Means followed by the same letter do not differ by Tukey test at 5% probability. The bars represent the standard deviations of the mean.

In this study, the increase in proline concentrations is expected because this variable acts as indicator of drought tolerance in plants. It is an osmoprotector amino acid, which acts in the protection of membrane structures, enzymes and cell tissues beyond to act as an antioxidant (Fernandes, 2002). Under stress conditions, amino acid metabolism is largely changed and decreased protein synthesis and increased proteolysis (Sodek, 2004). As a consequence, there is induction of proline biosynthesis promoted by metabolic increment as polyamines, ammonia, arginine, ornithine, glutamine and glutamate (Silveira et al., 2002).

The analysis of variance showed a significant difference between treatments in roots: under irrigation 13 µgram Glycine-betaine g^{-1} DM, flooding plants 3,3 µgram of glycine-betaine g^{-1} DM and without irrigation of 21 µgram of glycine-betaine g^{-1} DM were observed. In leaves: under irrigation 19 µgram of glycine betaine g^{-1} DM, flooding plants 8 µgram of glycine-betaine g^{-1} DM and without irrigation 28 µgram of glycine betaine g^{-1} DM were recorded (Fig 4 B).

The accumulation of glycine betaine protected the plant metabolism, because is a compatible osmolyte maintaining the water balance of the plant cell and the environment by stabilizing macromolecules (Carlin and Santos, 2009). This high amount of glycine is due to the probable formation of amino acid through the degradation of proteins and higher ammonium concentrations and coming possibly from photorespiration and desamination processes.

The biggest glycine betaine concentrations in plants under water stress promote a contribution to a better absorption and soil water transport to shoot through osmotic adjustment and also a cell membrane protection as well as protection against the mechanism of oxidative stress plants (Ashraf and Haris, 2004).

Total soluble protein levels and soluble amino acid levels

In leaves and roots of *H. courbaril* the contents of soluble protein and soluble amino acid were significantly varied between treatments at 5% probability level. In roots of control plant, flooded and water stress the values of 3.2, 1.6 and 0.6 mg g^{-1} DM protein were observed, respectively (Fig 5A, corresponding to a reduction of 50% and 81.2 % compared to control plant. In leaves of the control plants, flooded and under water stressed the values of 2.6, 3.8 and 1.26 mg protein g^{-1} DM were recorded, respectively, indicating an increase of 46.1% in PST concentration in leaves flooded plants and reduction of 53.8% in leaf tissue of the plant under water stress, compared to control (Fig 5 A).

The results of soluble protein concentrations are attributed to the increased activity of proteolytic enzymes, which acts as breaking agent of plant storage proteins and reduces its synthesis. The water stress affects the entire biochemical process of plant, and as a form of protection against water shortage, the plant changes its metabolic behavior such as the degradation of proteins into amino acids. Among these, proline is directly linked to the osmotic adjustment of leaf tissue cells (Souza et al, 2014).

The restriction of water uptake decreases cell turgor pressure which consecutively causes stoppage of growth. Drought also causes an increase in proteolytic enzymes that break the stored proteins in plants and decrease protein synthesis, showing that this deficiency affects all biochemical processes in an attempt to maintain water levels in the tissues as well as cell osmotic balance (Szegletes et al., 2000).

In flooding condition, the significant increase in TSP concentration in tissues is linked to a strong correlation between the ability of plants to synthesize proteins in conditions of low availability of oxygen and to maintain the energetic charge of the adenylate ensuring a "pool" ATP to meet the energy demand required for plant growth.

The ATP significantly varied in leaves and roots of plants between treatments at 5% probability level. AST analysis in control plants roots showed concentrations of 70.6 µmol AA g^{-1} DM, while the flooded plants showed 17.6 µmol AA g^{-1} DM. These results indicate a 75% reduction in the flooded treatment, compared to control (Fig 5 B).

In the roots of plants subjected to water deficit, 95.6 µmol g^{-1} DM of amino acid was observed, indicating an increase of 35.4% in treatments, compared to control. The values of 55.9 and 35.4 µmol of AA g^{-1} MS in tissues of flooded and controls plants were obtained, respectively, representing a reduction of 36.6%. In leaves of plants under water stress, the value was 110.8 g AA g^{-1} MS 1, indicating an increase of 98.1% in AST concentration, compared to control plants (Fig 5 B).

In organs of flooded plants, there was a reduction in amino acid levels, possibly due to increased protein synthesis, especially of Rubisco in leaves and the low activity of the

enzyme glutamine synthetase, key as gateway to the formation of all amino acids in the roots and leaves.

The high levels of amino acids in the roots and leaves of water stressed plants are probably due to high activity of nitrogen metabolism, in addition to the activities of protease enzymes that acts as breaking agent of proteins into amino acids, decrease in protein synthesis, which disorders the phloem tissue and reduces translocations to other organs. This causes the accumulation of amino acids thus signaling effect to the tolerance of the plants under different environmental stresses.

Lactate dehydrogenase and alcohol dehydrogenase activities

A significant difference in the different treatments was observed in lactate dehydrogenase activity. The alcohol dehydrogenase activity was reduced by 22% and 18% in the roots and leaves of plants under water deficit, respectively, compared to control treatment. When plants subjected to flooding the concentration grew by 77% and 78%, compared to the control plants (Fig 6 A). In roots and leaves there were no significant differences in the different treatments for alcohol dehydrogenase activity.

The alcohol dehydrogenase activity was increased by 10% in the roots and leaves of plants under water deficit, compared to control treatment. It increased by 83% and 76% compared to plants under control (Fig 6 B), when subjected to floods.

The results showed an increase in enzyme activity when plants were subjected to water suspension and flooding, since the absence of O_2 ceases electron transport and oxidative phosphorylation in the mitochondria. Therefore, the tricarboxylic acid cycle cannot operate and ATP can be reduced only by fermentation mechanism. Thus, anoxia promotes fermentation of pyruvate via the increased activation of the enzyme lactate dehydrogenase. The other enzyme involved in anoxic process is inhibiting alcohol dehydrogenase LDH by pyruvate decarboxylation (Taiz and Zaiger, 2004).

Photosynthetic pigments

The results of ANOVA showed a significant difference for the different treatments in relation to the photosynthetic pigments. The amount of chlorophyll *a* were recorded as following in the plants under irrigation (3.1 mmol kg^{-1} FM), flooded plants (2.4 mmol kg^{-1} FM) and without water irrigation (1.9 kg^{-1} FM mmol). For chlorophyll *b* plants under irrigation had (2.2 mmol kg^{-1} FM), flooded plants (1.4 mmol kg^{-1} FM) and without water irrigation (1.3 mmol kg^{-1} FM). The total chlorophyll in plants with irrigation (5.3 mmol kg^{-1} FM), flooded plants (4 mmol kg^{-1} FM) and without water irrigation was (3.1 mmol kg^{-1} FM). The amount of carotenoids in irrigated plants was (2.6 mmol kg^{-1} FM), flooded plants (3.6 mmol kg^{-1} FM) and without irrigation water (3.2 kg^{-1} FM mmol) (Fig 7).

The results indicate that plants subjected to drought had a balance in the contents of chlorophyll *a* and carotenoid, reducing the ratio of chlorophyll *a* / carotenoid. Therefore, the chlorophylls reductions probably promoted less absorption of light energy parallel to the largest free energy dissipation via carotenoids.

The degradation of photosynthetic pigments is involved with the continuous flow of electrons through the photosystems. In parallel, the significant decreases in net photosynthesis should have resulted in increased production of O_2^- and H_2O_2 in chloroplasts (Abdul Jaleel et al., 2007), promoting lipid peroxidation and degradation of chlorophyll and pigments under water suspension.

The degradation of chlorophyll causes parallel maintenance or increases in the concentration of total carotenoids. Therefore, a continuous power dissipation of excess is expected (Abdul Jaleel et al., 2007). Due to flooding, there was a decrease and increase in chlorophyll content and carotenoids, respectively. In some cases mentioned in the literature, immersing the plant for long periods of time affects the photosynthetic and photochemical apparatus reduces the growth of leaves, stems and roots, resulting in death of the plants (Schaffer, 1998).

Materials and Methods

Location and growth conditions

The experiment was conducted in a greenhouse localized at Federal Rural University of Amazonia (UFRA), in Belém, PA, Brazil. The implementation took place from September 2008 to May 2009, in which the experiment was conducted under natural conditions and through a thermo-hygrometer. The temperature of minimum-maximum air and relative humidity was checked, which showed values of 24.5-39.1 and 53.3%-91% respectively, observed during the period of experiment, in which the average photoperiod was 12h and the photosynthetically active radiation ranged from 42 μmol^{-2} s^{-1} to 968 μmol^{-2} s^{-1} (1:00 p.m.).

Plant material conditions

The substrate used for the growth of the seedlings was a mixture of 3: 1: 1 (v: v: v), composing of [(3 parts) black soil, includiing soil, yellowish latosol, medium texture, previously air dried and impurities removed; (1 part) decomposed chicken manure and (1 part) earthworm humus]. Tests were carried out to verify the field capacity of the pots and the liming process for soil pH correction and macro and micronutrient supplementation based on soil chemical analysis.

The seedlings of Jatobá (*Hymenaea courbaril* L.) from the AIMEX (Association of Timber Export Industries of the State of Pará) were four-month-old after germination and were transplanted to the vessels with their substrate corrected for acidity and nutrition. It took a period of three months for the acclimatization and fertilization of macro and micronutrients and the conduction of the treatments started over a month. The plants were subjected to three water regimes: control (adequate irrigation), water stress (without irrigation), and floods (with a blade of water 5 cm above the ground).

Biochemical analysis

The water potential was determined at predawn (ψam), between 4:30 and 5:30 a.m., and 10:00 (xylem water potential, ψx), using a pump pressure of Sholander type (mod. Pms Instrument Co, Corvalles, USA) as described by Da Matta et al. (1993).

The collection of plants was scheduled at 05:30 AM for the determination of nitrate reductase activity (RN), which was performed *in vivo* by selecting in the greenhouse. The fully expanded primary leaves were selected from each of the repetitions, according to the method described by Hageman and Hucklesby (1971). The reading was performed at 540 nm and the result of the activity estimated by producing NO_2^- in

the reaction middle, expressed in μmoles NO_2^- g^{-1} MF h^{-1} from a standard curve obtained by KNO_2 p.a (Sigma).

For the biochemical analysis, the material was taken to the hothouse forced air ventilation of 65 ºC for 48 h to determine the dry mass of root and leaf. The dried material was ground in mill to obtain a fine powder being properly stored in falcon tubes until used in assays.

The Cataldo et al. (1975) method was used for the determination of nitrate. The absorbance was determined at 410 nm and the concentration of nitrate was obtained from a standard curve with increasing concentrations of NO_3^- (0, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 μmol mL^{-1}). The results were expressed in μmoles of NO_3^- g^{-1} MS of tissue.

Free ammonia was determined using the method by Weatherburn (1967). The concentrations were estimated from the standard curve constructed with $(NH_4)_2SO_4$ p.a (Sigma) and the result expressed in mmol of NH_4^+ kg^{-1} MS. We used the method of Bates et al. (1973) for the determination of proline with spectrophotometric reading at 520 nm. The concentrations were determined from the standard curve with L-proline P.A (Sigma) and results were expressed in μmol Pro g^{-1} DM.

The method used for determination of glycine betaine was second Grieve and Grattan (1983), in which the reading was carried out in a spectrophotometer at 365 nm. For calculation of a standard curve a glycine betaine solution was prepared and the results were expressed in μg Glycine betaine g^{-1} DM. The soluble protein contents were determined by the method described by Bradford (1976), then subjected to reading absorbance at 595 nm. Each extract was assayed in duplicate, and the soluble protein content in mg g^{-1} Protein MS.

For the determination of total soluble amino acid the method of Peoples et al. (1989) with absorbance read at 570 nm in spectrophotometer was used. The results expressed in micromol g AA^{-1} MS. The glutamine synthetase activity was determined by *in vitro* method of Kamachi et al. (1991). The reading was taken in a spectrophotometer at 540 nm and the activity of glutamine synthetase (GS) was determined from the standard curve μ-glutamyl hydroxymate and the results were expressed in mmol GGH kg^{-1} MS.

The incubation mixture for alcohol dehydrogenase comprised mol m^{-3} according to Bertani et al. (1980). The incubation mixture for the lactate dehydrogenase was composed of mol m^{-3} according to Hoffman and Hanson (1986). After three minutes at 25 °C, the enzyme activity was measured at 340 nm. The results were expressed in moles of $NADH^+$ kg^{-1} protein min^{-1}.

The method of Lichthenthaler (1987) was used for determination of chlorophyll *a*, *b*, and total carotenoid. Tey measured in absorbance readings of 470, 646.8 and 663.2 nm and the concentrations of chlorophylls and carotenoids (in mg L^{-1}) calculated and converted to kg^{-1} mmole FM.

Experimental design and statistics

The experimental design was completely randomized with three water conditions: control, water stress and flooding in 15 repetitions, totaling 45 experimental units, in which each experimental unit was composed of a plant/pot. It was applied to analysis of variance in the results and when there was a significant difference, the means were compared by Tukey's test at 5% significance level. In addition, the standard deviations were calculated for each treatment, and the statistical analyzes with SAS-institute (1996) and informed the recommended statistical theories by Gomes and Garcia, 2002.

Conclusion

The results of this study show that flooding and the water deficiency for 30 days are sufficient to trigger changes in biochemical behavior in the Jatobá plants. In general, our results indicate that the *Hymenaea courbaril* L. plants are less susceptible to flooding than to the water deficiency.

Acknowledgements

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References

Abdul JC, Manivannan P, Kishorekumar A, Sankar B, Gopi R, Somasundaram R, Paneerselvam R (2007) Alterations in osmoregulations, antioxidant enzymes and indole alkaloid levels in *Catharanthus roseus* exposed to water deficit. Colloid Surface B. 59: 170-157.

Alves GAR, Santos Filho BG, Lobato AK, Yuen Tan DK, Oliveira Neto CF, Costa RCL, Ávila FW, Marques D, Santos, RG (2012) Water relations, nitrogen compounds and enzyme activities in leaf and root of young yellow lapacho (*Tabebuia serratifolia*) plants subjected to flooding. Plant Omics. 5: 216-222.

Andrade Junior WV, Filho BGS, Neto CFO, Pereira ACC, Silva RTLS, Viégas IJM, Cardoso KPS, Souza LC, Oliveira TB, Okumura RS (2016) Ecophysiological and biochemical behavior in young plants of *Parkia gigantocarpa* Ducke subjected to waterlogging conditions. Afr J Agr Res. 11: 284-297.

Andrade Netto JF (2005) Activity of nitrate reductase enzymes and glutamine synthetase in arabica coffee. 60 f. Dissertation (Master in Agronomy) - Luiz de Queiroz College of Agriculture, University of São Paulo, Piracicaba.

Ashraf M, Haris PJ (2004) Potential biochemical indicators of salinity tolerance in plants. Plant Sci. 166: 3-16.

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil. 39: 205-207.

Bertani A, Brambilla I, Menegus F (1980) Effect of anaerobiosis on rice seedlings: growth, metabolic rate and fate of fermentation products. J Exp Bot. 31: 325- 331.

Bradford MM (1976) The dye binding assay for protein. Anal Biochem. 72:248-254.

Campos MAA, Uchida T (2002) Influência do sombreamento no crescimento de mudas de três espécies amazônicas. Pesq Agropec Bras. 37: 2881-288.

Carlin SD, Santos DMM (2009) Physiological indicators of the interaction between deficit and alcohol in soil in sugarcane. Pesq Agropec Bras. 44: 1106-1113.

Castro DS, Lobato AKS, Mendes FS, Oliveira Neto CF, Cunha RLM, Costa, RCL (2007) Atividade da redutase do nitrato em folhas de teca (*Tectona grandis* L.) sob déficit hídrico. R Bras Bioci. 5: 936-938.

Cataldo DA, Haroon LE, Schrader LE, Youngs VL (1975) Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Commun Soil Sci Plan. 6: 71–80.

Costa W, Souza A, Souza P (2011) Ecology, management, forestry and technology of native species of the atlantic forest; native species of the atlantic forest. Disponível em: < http://www.ciflorestas.com.br/arquivos/d_b_b_4835.pdf > Acesso em: 01 Jan 2017.

DaMatta FM, Rena AB (2001) Tolerance of coffee to drought. In: Zambolim L (ed) Quality coffee production technologies, p. 65-100. Viçosa, Minas Gerais.

Debouba M, Maâroufi-Dghimi H, Suzuki A, Ghorbel MH, Gouia H (2007) Changes in growth and activity of enzymes involved in nitrate reduction and ammonium assimilation in tomato seedlings in response to NaCl stress. Ann Bot-London. 99: 1143-1151.

Endres L, Souza JL, Teodoro I, Marroquim PMG, Santos CM, Brito JED (2010) Change in exchange rate caused by water deficit during the reproductive stage of beans. Rev Bras Eng Agríc Ambient. 14: 11-16.

Fernandes A (2002) Biodiversity of the caatinga. In: Araújo EL, Moura NA, Sampaio EVSB, Gestinari LMS, Carneiro JMT (eds) Biodiversity, conservation and sustainable use of Brazilian flora, p. 298. Recife, Pernambuco.

Ferreira VM, Magalhães PC, Durães FOM, Oliveira LEM, Purcino AAC (2002) Nitrogen metabolism associated with water deficiency and its recovery in maize genotypes. Cienc Rural. 32: 13-17.

Gomes FP, Garcia CH (2002) Estatística aplicada a experimentos agronômicos e florestais exposição com exemplos e orientações para uso de aplicativos. FEALQ, Piracicaba, SP. 309 p.

Grieve CM, Grattan SR (1983) Rapid assay for the determination of water soluble quaternary ammonium compounds. Plant Soil. 70: 303-307.

Hageman RHG, Hucklesby DP (1971) Nitrate reductase from higher plants. Methods Enzymol. 17: 491 – 503.

Hoffman NE, Hanson AD (1986) Purification and properties of hypoxically induced lactate dehydrogenase from barley roots. Plant Physiol. 82: 664-670.

Horchani F, Aschi-Smiti S (2010) Prolonged root hypoxia effects on enzymes involved in nitrogen assimilation pathway in tomato plants. Plant Signal Behav. 5: 1583-1589.

Kamachi K, Yamaya T, Mae T, Ojima K (1991) A role for glutamine synthetase in remobilization of leaf nitrogen during natural senescence in rice leaves. Plant Physiol. 96: 411-417.

Kerbauy GB (2008) Fisiologia vegetal. 2. ed. Rio de Janeiro: Guanabara Koogan. 431p.

Lichthenthaler HK (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. In: Colowick SP, Kaplan (ed) Methods in Enzimology, v.148. Academic Press, San Diego. p.350-382.

Martins SV (2013) Recuperação de áreas degradadas: ações em áreas de preservação permanente, voçorocas, taludes rodoviários e de mineração. 3. ed. Viçosa, v. 1. 264p.

Matheus MT, Amaral JAT, Silva DGG, Neves DM, Pizzol ECS, Sousa FC, Santi GC, Guariz HR, Lima KA, Hoffmann RG (2011) Sintomas de deficiência nutricional em Jatobá. Rev Cient Eletrôn Eng Florest. 17: 89-97.

Nogueira RJMC, Albuquerque MB, Silva EC (2005) Aspectos ecofisiológicos da tolerância à seca em plantas da caatinga. In: Estresses ambientais: danos e benefícios em plantas, p. 22-31. Recife, Pernambuco.

Panizza S (1997) Plantas que curam: cheiro de mato. São Paulo, IBRASA. 279 p.

Peoples MB, Faizah AW, Reakasem BE, Herridge DF (1989) Methods for evaluating nitrogen fixation by nodulated legumes in the field. Aust Cen Intern Agric Res, p. 76.

Schaffer B (1998) Flooding responses and water-use efficiency of subtropical fruit trees in an environmentally sensitive Wetland. Ann Bot-London. 81: 475-481.

Sharma P, Dubey RS (2005) Modulation of nitrate reductase activity in rice seedlings under aluminum toxicity and water stress: role of osmolytes as enzyme protectant. J Plant Physiol. 162: 854-864.

Sharner DL, Boyer JS (1976) Nitrate reductase activity in maize (*Zea mays* L.) leaves. II. Regulation by nitrate flux at low leaf water potential. Plant Physiol. 58: 505-509.

Silveira JAG, Rocha IMA, Viégas RA (2002) Metabolic responses of cowpea and cashew plants exposed to salt and water stress: new aspects on proline accumulation. Disponível em: <sbbq.iq.usp.br/arquivos/regional/2002/cdresumo/Palestras/016.pdf> Acesso em: 24 Jan 2017.

Sodek L (2004) Metabolismo do nitrogênio. In: G.B. Kerbauy (org.), Fisiologia vegetal. Rio de Janeiro: Guanabara Koogan, p. 94-113.

Souza LC, Siqueira JAM, Silva JL, Silva JN, Coelho CCR, Neves MG, Oliveira Neto CF, Lobato AKS (2014) Compostos nitrogenados, proteínas e aminoácidos em milho sob diferentes níveis de sílica e deficiência hídrica. Rev Bras Milho e Sorgo. 13: 117- 128.

Szegletes, Z, Erdei L, Cseuz L (2000) Accumulation of osmoprotectants in wheat cultivars of different drought tolerance. Cereal Res Commun. 28: 403-410

Taiz L, Zeiger E (2004) Fisiologia Vegetal. Trad. Santarém, ER, et al. – 3.ed. –Porto Alegre: Artmed, p.719.

Taiz L, Zeiger E (2013) Fisiologia vegetal. 4. ed. Porto Alegre: Artmed, 819p.

Weatherburn MW (1967) Phenol-hypochlorite reaction for determination of ammonia. Anal Chem. 39: 971-974.