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RESEARCH ARTICLE

WATER POTENTIAL AND NITROGEN METABOLISM IN SEEDLINGS OF BALSA WOOD (*OCHROMA PYRAMIDALE* (CAV. EX LAM) URB.) SUBJECTED TO WATER DEFICIT STRESS AND FLOODING

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ABSTRACT

This research aimed to evaluate the metabolic responses of nitrogen forward the effects of water stress and flooding in young plants of pau-de-balsa. The experimental design was completely randomized in a factorial arrangement (3x4) in three water conditions: control (irrigated), water deficit and flooding in four periods of evaluation (0, 4, 8 and 12 days), with 5 repetitions. The water potential, nitrate, nitrate reductase and glutamine synthetase of reduced around 10; 5; 12.5 and 2.69 respectively in stressed plants, which did not occur with Free Ammonia which increased 1.72 in its concentration. The Amino Acid Concentrations, Glycine Betaine and Proline increased in plants under water deficit around 1.9; 2.13 and 2.5 respectively. However in flooding condition these variables significantly decreased from the 4th day of the experiment. Protein content decreased in plants under water stress and flooding about 1.9 and 4.37 respectively. Therefore, the analyzes carried out revealed that the pau-de-balsa plants are highly sensitive to flooding and tolerant to water stress.

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INTRODUCTION

Because it is constituted in a large region, and is considered the largest equatorial forest in the world, with many extraction possibilities of timber resources and non-timber, the Amazon region over the years has always aroused the attention of many researchers who have generated a considerable amount of work in various lines of research (Prates and Bacha, 2011). Relations between nitrogen and natural plant communities have been one of these lines of research and the results have supported the idea that the plant species differ in their ability to use organic and inorganic sources of this element especially in adverse conditions of stress (Schimann *et al.,* 2008). The dry condition can be understood as a natural phenomenon from severe water shortages in any system that requires the presence of water, is often attributed to the deviation of the normal amount of precipitation for a long period of time (Kirono *et al.,* 2011).

The deficit in the availability of oxygen (hypoxia and / or anoxia) is the major stress factor in soils under excess water. In plants, the impact of excess water in soil is, in most cases, sense directly by the roots or indirectly by the shoots. In the shoot, the excess water in the soil can induce chlorosis, premature wilting, loss of photosynthetic capacity, water potential and the concentration of nutrients in the leaves and decreased of growth (Visser *et al.,* 2003). However, knowledge of the ecophysiological characteristics of nitrogen use of tree species in forests, especially on nitrogen cycling and the behavior of the species in areas with dry and wet weather is still quite scarce. Taking into account the above approaches, the aim of this study was to evaluate the metabolic nitrogen answers depending on water availability in young plants of pau-de-balsa.

MATERIALS AND METHODS

Local and experimental conduction: The experiment was carried out in a greenhouse at the Federal Rural University of Amazonia, on the campus of Capitão Poço. Latitude 01° 44 '47'

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'and longitude 47 03'34' '. In July 2013, using seedlings ofthe family Malvaceae, genus Ochroma. *Ochroma pyramidale* (Cav. Ex Lam) Urb. with 15 cm on average. The seedlings were kept in pots with 10L of capacity, dimensions of 0.30 m in height and 0.3 m in diameter and is completely filled with dystrophic yellow latosol substrates and cattle manure in the proportion of 3: 1 respectively, on a layer of 0.02 m stone / crushed to facilitate water drainage. In the three months before the experiment were placed 600 mL of complete nutrient solution (Hoagland and Arnon, 1950) and for each month 200mL of solution, there were irrigation daily to be maintained the soil at field capacity was estimated in laboratory agreement with the soil column method presented by (Fernandes and Sykes, 1968).

Plants conduction

The plants were subjected to three water regimes: irrigated (control), water deficit (total suspension of irrigation at the beginning of the experiment) and flooding (kept in a sheet of 5 cm of water above ground) in an interval of twelve days. During the experimental period the 'control plants' were watered daily to replace water lost, being made individually for each pot, taking into account the daily weighings forming a set (plant +pot + soil). The experimental design was completely randomized in a factorial arrangement (3x4), represented by three water conditions: (control, water stress and flooding) and four evaluation periods (0, 4, 8 and 12 days), with 5 repetitions, totaling 60 experimental units, each unit being composed of a plant / pot. Before dawn water potential: The Ψ am was determined between 4:30 and 5:30 am, by a pressure pump of Scholander type (M670, Pms Instrument Co., Albany, NY, USA) as described by Pinheiro *et al.,* (2007). Determination of the concentrations of nitrate: according to the proposed method used by (Cataldo *et al.,* 1975). The results were expressed in mmol of NO^{3-}. kg^{-1} DM of tissue. Determination of nitrate reductase activity: the method used was the method *in vivo* recommended by (Hageman and Hucklesby, 1971). Expressed in μ moles of NO^{2-}. g. FM^{-1}. h^{-1}. Free ammonium concentrations: was used the method described by (Weatherburn, 1967). The results were expressed inmmol of NH^{4+}/ Kg of DM. Glutamine synthetase activity: was determined by *"in vitro"* according (Kamachi *et al.,* 1991). The results were expressed in mmol of γ-glutamil-hidroximato/kg MS of tissue/hour. Soluble aminoacids concentrations: total amino acid concentrations (TAAC) were determined according to the method described by (Peoples *et al.,*1989). Determination of total soluble protein concentrations: concentrations of total soluble proteins were determined by the method of (Bradford, 1976). The results were expressed in mg protein/g DM. Determination of proline content: proline concentration was determined by a calibration curve proline according to Bates et al. (1973) and the result expressed in mmol g^{-1} proline dry matter (DM). Determining the levels of glycine betaine: it was determined by the method of Grattan andGrieve (1983). The results were expressed in mg glycine - betaine g^{-1}DM.

Analysis of statistical data: The experimental results were submitted to analysis of variance (ANOVA), the averages were compared by Tukey test at 5% significance level. Regression analyzes were performed on variables whose significance was determined by F test ($P < 0.05$). Statistical analyzes were made using the SWNTIA program (EMBRAPA, Campinas-SP, 1995). Regression models that best related to the behavior of the variables were polynomial 2nd order and logarithmic function.

RESULTS

The predawn water potential (Ψam) decreased significantly in plants under drought (-0,36; -1,21; -2,39 e -3,35 MPa) and flooding (-0,30; -1,76; -3,07 e -3,85 MPa.) representing an decrease of water potential in the plant around 10 and 12 times respectively when compared to the control plants (-0,31MPa). The statistical differences between all water conditions occurred from the 4th day of evaluation, staying until the last day as the (Fig. A).

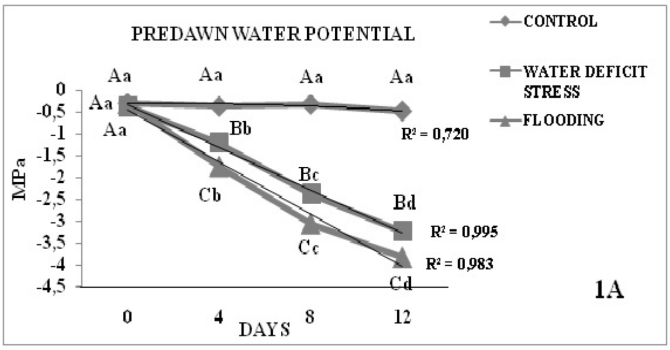

Fig. 1. Predawn water potential in young plants of *Ochroma pyramidale*. The averages followed by the same uppercase or lowercase letter do not differ by Tukey test at 5% probability. The uppercase letters statistically compare between the water conditions and the lowercase letters between the periods of evaluation in each treatment

The results for the nitrate levels in plants under water stress were: 0.88; 0.41; 0.21 and 0.16 mmol of NO^{3-}/KgDM, while in flooding were: 0,84; 0,25; 0,09 and 0,03 mmoles of $NO^{3-/}$kg DM, representing a significant decrease due to the stress time. In the treatment under water deficit this reduction represented 5 times less when compared to the control plants (0,83 mmoles of$NO^{3-/}$kg-1 MS)and under flooding 27 times less. Statistically differences between treatments are given starting from the 4th day of the experiment, remaining until day 12 (Fig. 2A). Within the treatments under stress differences occur in the first two periods (0 to 4 days).The reductase enzyme activity of nitrate decreased significantly in plants under water stress and flooding when compared to the control plants (Fig. 2B). The values found for plants under drought stress were: 0,26; 0,15; 0,08 and 0,02 μmoles $NO^{2-/}$g. FM-1/h-1 causing a reduction of 12.5 and under flooding values were: 0.25; 0.19; 0.10 and 0.01μmoles NO^{2-}/g. FM-1/h-1, decreasing 25times. These decreases resulted statistical differences between all water treatments from the 4th day. Only on the 8th day of evaluation the treatments under water stress did not differ. For free ammonium concentrations (Fig. 2C) the treatments under water stress increased presented the following results of water deficit: (9,24; 11,89; 12,55 and 15,41 mmol ofNH_4^+/ Kg of DM) and flooding (10,01; 12,08; 16,12 e 18,35 mmol ofNH_4^+/ Kg of DM).

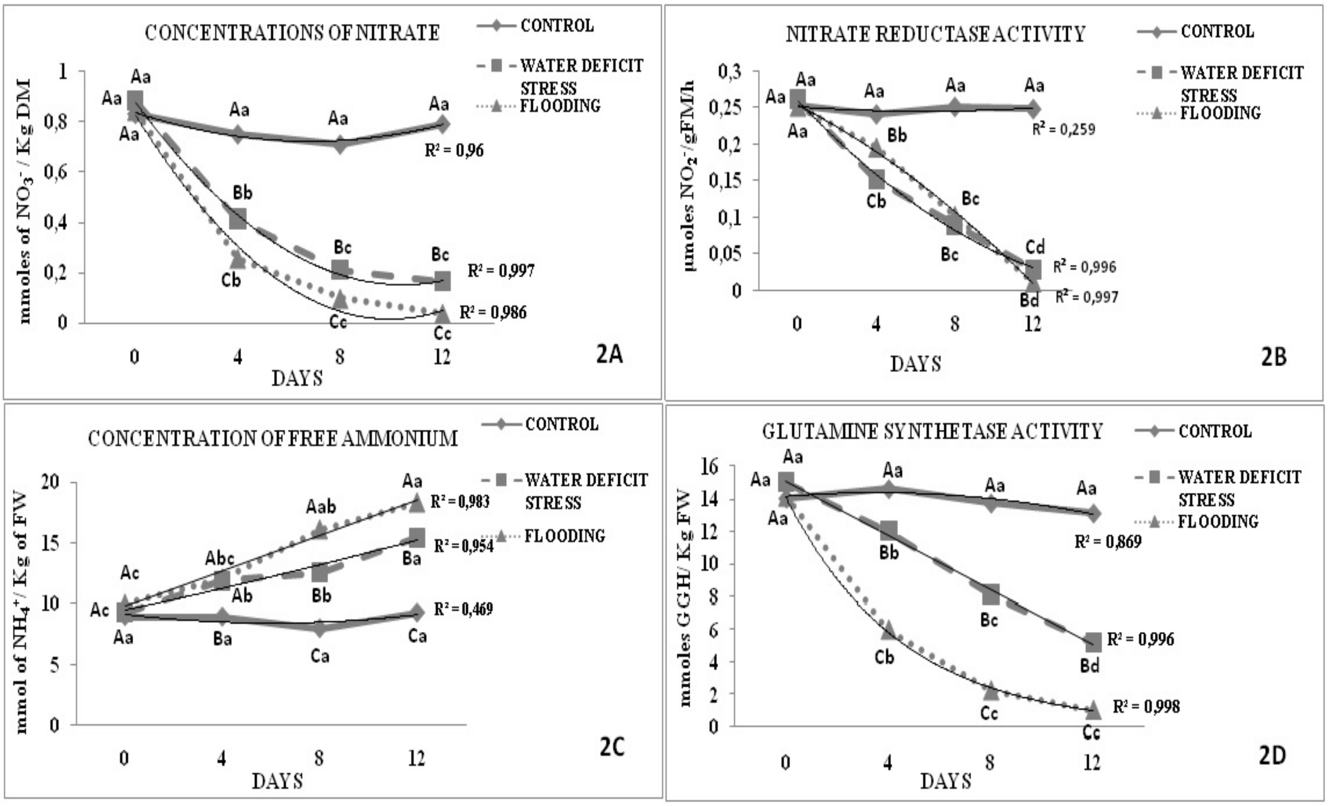

Fig. 2. Nitrate levels (2A), Reductase nitrate activity (2B), Free ammonium levels (2C), Glutamine synthetase activity (2D) in young plants of *Ochroma pyramidale*. The averages followed by the same uppercase or lowercase letter do not differ by Tukey test at 5% probability. The uppercase letters statistically compare between the water conditions and the lowercase letters between the periods of evaluation in each treatment

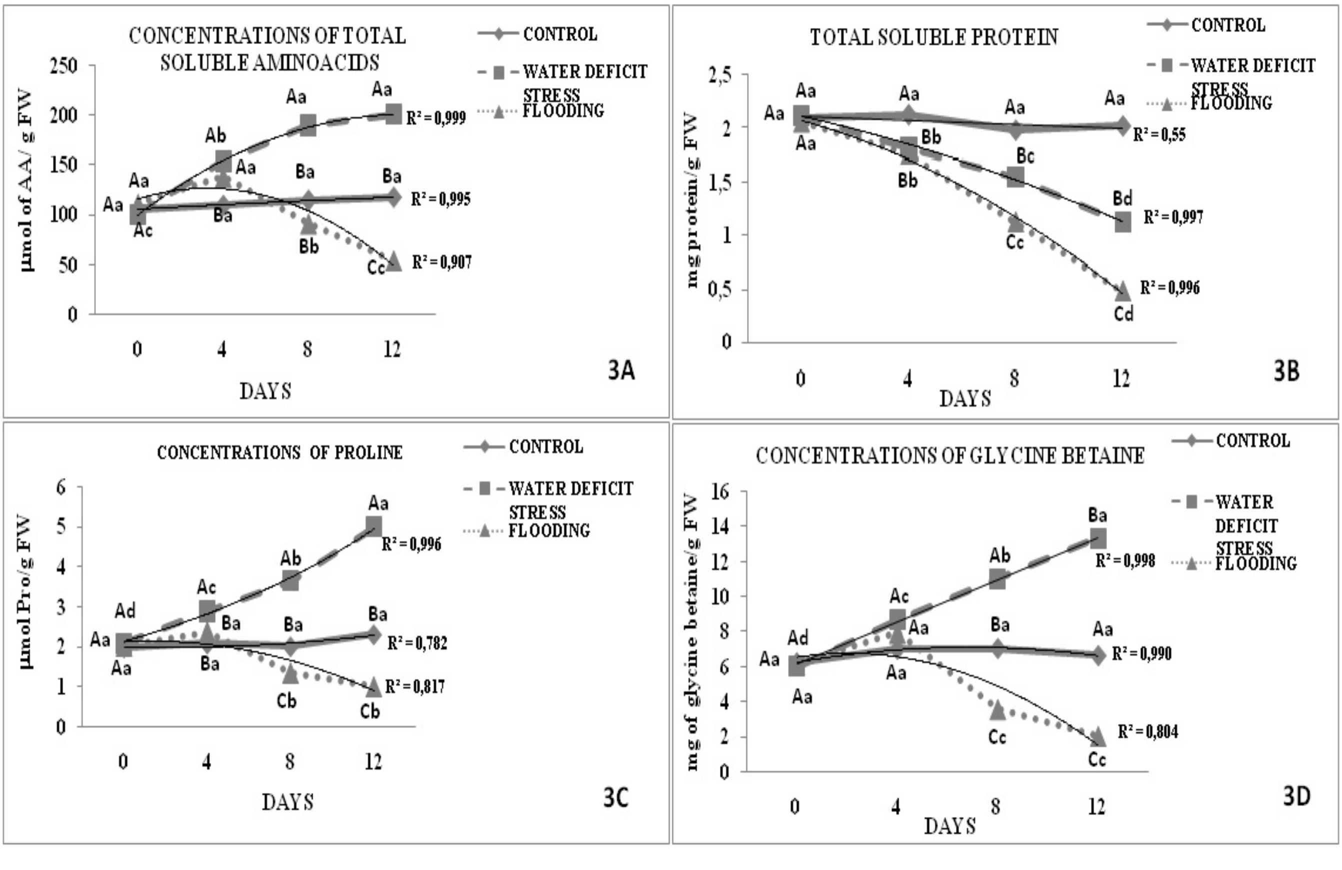

Fig. 3. Total soluble amino acids (3A), total soluble proteins (3B), Proline (3C), Glycine-betaine levels (3D) in young plants of *Ochroma pyramidale*. The averages followed by the same uppercase or lowercase letter do not differ by Tukey test at 5% probability. The uppercase letters statistically compare between the water conditions and the lowercase letters between the periods of evaluation in each treatment

In the case of the control plants results were almost constant. In terms of this increase corresponded approximately 1.72 and 2.05 respectively when compared to most control plants (8,94 mmol ofNH_4^+/ Kg ofDM).Among the treatments under water stress statistical differences occurred starting from 8 days remaining until the last day evaluation. When compared to control plants that difference occurred on the 4th day. The enzymatic activity of glutamine synthetase decreased in plants under water deficit:15,01; 12,01; 8,12 and 5,22 mmoles GGH/ Kg MS and flooding:14,18; 6,02; 2,25and 1,04 mmoles GGH/ Kg DM. Representing a decrease of 2.69 and 13.5 when compared to the control plants (14.05 mmol GGH/kg DM) respectively, the statistical evaluations between treatments differences made themselves from the 4th day in treatments under stress remaining until the 12 days of evaluation. Already within each treatment differences in water stress conditions occurred in all periods of evaluation. In plants under flooding only the two first period (0 to 4 days) (Fig. 2D).

The total soluble amino acid levels increased in plants subjected to water deficit, representing 1.9 times more. In flooding condition there was a decrease from the 4th day evaluation representing a decrease of 1.9 times less when compared to the control plants (105 μmol of AA/ g DM). The results founded for the plants under drought were: 100,1; 153,01; 189,17 and 200,17 μmol of AA/ g DM and under flooding: 111,04; 138; 91,24 e 54,1μmol of AA/ g MS. Under water stress the statistical differences occurred on the 8th day of evaluation. And when these two treatments are compared to control plants that difference occurs on the last day (12) (Fig. 3A). Total soluble protein contents decreased significantly both in water deficit condition as in the flooding. The values found for treatment under water deficit were: 2.11; 1.82; 1.54 and 1.12 mg protein/g DM, and flooding condition were (2.05, 1.76, 1.12 and 0.48 mg protein/g DM). In the first condition there was a decrease in approximately 1.9-fold compared to control plants (2.1 mg protein/g DM), the second one decrease to 4.37 times lower when compared to the control plants. The statistical differences between the water conditions are given on the 8th day of the experiment. In the evaluation periods statistical differences occurred in both water stress conditions when compared to the control plants (Fig. 3B). Proline content in the water deficit condition gradually increased with stress time, representing more than 2.5 times more in flooding condition was a decrease from the 4th day representing 1.96 less when compared to the control plants (1,98 mmol.g-1.DM). The results founded for the water deficit were: (2,1; 2,89; 3,65 and 4,98 mmol.g-1.DM. In flooding condition: 2, 01; 2,36; 1,35 and 1,01 mmol.g-1.DM. The statistical differences between the three water conditions occur from the 8th day. The statistical differences within each treatment show that the water deficiency only occurs in four days. Under flooding occurred in the days 8 and 12(Fig. 3C). As well as proline, glycine betaine the content also increased in plants under drought and decreased in plants under flooding, representing around 2.13 and 3.10 respectively when compared to the control plants (6.25 mg of glycine betaine/g DM). The results for drought were (6.02, 8.7, 11.01 and 13.33 mg of glycine betaine/g DM) to the plants under flooding (6.12; 7.94; 3.56 and 2.01 mg glycine betaine/g DM). The statistical differences between all water conditions occurred on the 8th day of the experiment, in the case of differences within each water deficiency treatment presented in all periods of evaluation. In flooding condition occurred in the 4:08 day (Fig. 3D).

DISCUSSION

The lack of water in the soil possibly caused a increase in elasticity of xilematic vessels in plants under water stress because the more negative the Ψw is, the greater being the force required for the plant to absorb water from the soil and transport to the shoot (LUZ, 2011). In the circumstances of flooding to reduce the water potential (Ψw) is probably related to decreased aerobic respiration and consequently the power outage, as well as the thickening of the cell membrane where this structure in normal conditions would increase the resistance to water flow. (TAIZ and Zaiger, 2013). Results presented by Fu *et al.,* (2010) showed similarity in water potential parameter in xylem of euphratica Populusand *Populus Russkii* plants assigning different water absorption characteristics on the root distribution.The low concentration of nitrate in water stress condition is the reduction in water content as nitrate (NO^{3-}) is the main form of nitrogen absorbed from the soil by plants, followed by ammonium (NH_{4+}). When this substance is in insufficient quantities hampers the nitrate uptake process by the roots and consequently decreasing to transport this to the shoot through current transpiration via the xylem (Shaner and Boyer, 1976), thus reducing the activity of the reductase enzyme nitrate as can be seen in (figure 2B). Studies show that when the soil is under excess water is reduction of oxygen to the root system which would help to reduce the respiration of roots. As a consequence, it moves the aerobic to anaerobic route, resulting in reduced synthesis of ATP directly influences in plant development (PARENT *et al.,* 2008).Results found by Oliveira Neto (2010) working with young plants of Jatoba (*Hymenaea* L.) under water stress and flooding corroborate with the results founded in this study. As well as the results of Andrade Jr., (2013) with young plants of *Parkia gigantocarpa* Ducke under flooding condition. The low water flow in the current transpiration causes a decrease in reductase enzyme activity of nitrate in plants under water stress, bringing as a result of decreased sweating and with it the nitrate flow to the leaves as this enzyme is highly dependent of its substrate (Horchani *et al.,* 2010). The lack of oxygen in the flooded soil contributed to the lack of energy in the form (ATP) in the root system, thereby hindering their development, causing a low absorption of soil nitrate and consequently to the leaves. Another important point is the increase of the alcoholic fermentation, as this process promotes increased DHA which contributes to increasing the pH, a process that inhibits the activity of this enzyme. For Alves, (2010) working with plant Tabebuia serratifolia found similar results in plants under water stress and flooding. Carvalho (2012) working with seringueira (*Hevea spp*) contradict with the results presented here, getting increasing this enzyme in flooding conditions. To Alves (2010) the increase in free ammonium concentrations in plants under water deficit is due to route of glutamate dehydrogenase (GDH), which operates in dependence of NADH, causing glutamate generating ammonium (NH_{4+}) and 2-Oxiglutarato, thereby promoting the accumulation of ammonium and reduction of the enzyme glutamine synthetase activity (Fig. 2D).In the case of plants under flooding the low activity of the enzyme glutamine synthetase must have contributed to the increase of ammonium, as well as the process of photorespiration and catabolism of nitrogen compounds, especially amino acids, another fact that should be highlighted is that probably should have occurred with decreased glutamate dehydrogenase GDH/NADH which would catalyze the incorporation of NH_{4+} in organic form (DEBOUBA *et al.,* 2007).

Carvalho, (2012) researching seedlings (*Hevea sp*) found similarity to the study in question. To Alves (2010) the free ammonium levels in ipe-amarelo plants under water deficit increased significantly over time and decreased significantly in plants under flooding disagreeing with the results presented for plants in excess water. The evidence that the activity of glutamine synthetase reduced in plants under stress conditions are due to low ATP concentrations in the cellular environment, given that this enzyme is highly dependent on energy as ATP, generated by photosystems in the photochemical phase of photosynthesis, as well as the breathing process in roots (Alves *et al.,* 2012). These statements are based on low sweating this plant as seen in Fig. 1B and 1C. This partly explains the low concentration of carbon dioxide (CO_2) in stomatal cells with this damaging energy production in the form of ATP. In work carried out by Carneiro (2014) with rubber plants for 21 days showed that the activity of the enzyme glutamine synthetase (GS) was higher in leaves of plants kept under field capacity. The increase of aminoacids in plants under water stress is due to the activity of the proteolytic enzymes that degrade protein to form aminoacids, this increase probably promotes the decrease of osmotic potential and probable maintenance of cellular turgor, serving nitrogen reserve for possible recovery of plant growth. In plants on flooding there was a decrease in the concentrations of amino acids (Fig. 3A), this is possibly due to low activity of the enzyme glutamine synthetase (Fig. 2D), an enzyme known as gateway to formation of all amino acids in roots and leaves. Besides, the drop in ATP synthesis resulted in lower absorption of nitrate (NO^{3-}), the main form of nitrogen uptake by plants and essential element for the formation of aminoacids. For Moura, (2010), working with *Jatropha curcas* plants, were compared against the total soluble aminoacid contents subjected to water stress to the extent that stress was more intense. According to Andrade Jr., (2013), analyzing the leaf tissue of plants of *Parkia gigantocarpa* under flooding conditions decreased around 93.47%, showing high sensitivity to this environmental condition and similarity to that job. The activity of proteolytic enzymes in plants under drought stress probably break of plant storage proteins, as well as decrease the synthesis "again" of the same, results in the formation of aminoacids among which proline (Oliveira Neto, 2010). According to Bailey-Serres and Chang, (2005) Plant subjected to low oxygen availability, usually reduce protein synthesis, due to the inhibition of certain enzymes, synthesizing only proteins related to flooding, as the ADH and LDH. Work by Nascimento (2009) with jatobá tree seedlings obtained mixed results with respect to the concentration of this organic solute being observed an increase in soluble protein content in plants submitted to 25% of the pot capacity (severe stress caused by lack of water). For Andrade Jr., (2013) using *Parkia gigantocarpa* seedlings under flooding the protein content in the leaves reduced significantly. Increases in the levels of this amino acid in plants under water stress possibly indicate their participation in osmotic adjustment process of these plants. As the increase of the proteolytic activity by promoting increased availability of this amino acid. In the study by Silva (2013) with *Moringa* seedlings oleifera submitted to water stress and flooding showed that the proline content in the leaves decreased significantly in the 10-day evaluation in two parameters, not corroborating the data of this study for plants under water stress. The significant increase in glycine betaine content in the leaves under water stress, are due to osmoregulation, stabilization of structures and enzymes activities of the protein complex, with the purpose of maintaining the integrity of the membranes from the damaging effects of different water stress (Sakamato and Murata, 2002). For *Hymenaea courbari l*and *Hymenaea sstigonocarpa* resulted in substantial increases in glycine betaine levels in water stress condition (LIGHT, 2011). Possible causes for the decrease in glycine betaine levels in plants under flooding condition is possibly due to decreased photosynthesis, even this variable not being assessed in this study, most due to lower leaf transpiration, which prevents the CO_2 input to give initiate the photosynthetic process.

Conclusion

On the physiological and biochemical answer presented by seedlings of *Ochroma pyramidale*, it suggests that may be able to respond positively to a short period of time of water deficit stress (12 days) under experimental conditions in greenhouse.
The leaf proline and glycine-betaine content were higher in plants under water deficit stress, indicating a possible occurrence of osmotic adjust in this specie. The physiological and biochemical answers of plants under flooding condition showed a high sensibility even being subjected a short period of time.

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