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RESEARCH ARTICLE

BIOCHEMICAL RESPONSES IN YOUNG PLANTS OF CASTANHEIRA-DO-BRASIL (*BERTHOLLETIAEXCELSA*BONPL.) SUBMITTED TO DROUGHT

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ABSTRACT

The *Bertholletia excels* known as castanheira-do-brasil, this species is very exploited of the Amazon, with great importance in generating employment and income in the region. The aim in this research was to evaluate the biochemical responses of the species *Bertholletiaexcelsa* in water stress conditions. The experimental design was completely randomized with two treatments: irrigated and non-irrigated plants, with 9 plants each, considering each plant as a repetition, and these repeated treatments in 3 times (0, 5 and 9 days), totaling 27 plants per treatment. The condition of water deficit for nine days adversely affected the concentration of starch and total soluble proteins. The condition of water deficit for nine days, on the contrary, increased the total soluble carbohydrate concentrations, total soluble amino acids, sucrose and proline.

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INTRODUCTION

The *Bertholletiaexcelsa* known as castanheira-do-brasil is one of the most important species of extractive exploration of the Amazon, with significant participation in the generation of foreign exchange for the region, as well as being a source of jobs and income for thousands of rural and urban workers (Tonini, 2011; Azevedo, 2014). Considered large, can reach 50m of height (Haugaasen *et al.,* 2010). The fruit is ligneous, globular, extremely hard, with variable size and weight that gets the hedgehog name and has seeds rich in fat and good quality proteins. Studies have shown to be one of the species with great potential to be used in reforestation programs in the Amazon region, due to its good growth performance under various environmental conditions and the excellent silvicultural characteristics such as a high survival rate, low percentage of forked trees with long and straight boles (COSTA *et al*., 2009; SCOLES *et al*., 2011). However, the process of deforestation in the region has contributed to the increase of climate change, interfering with agricultural production patterns in global levels, bringing as consequences changes in precipitation conditions with serious impacts on ecosystem services affecting biodiversity, water resources, land resources, agriculture, fisheries, and water supply and the health and quality of life population (IPCC, 2014). Water is a essential resource for the development of the plant, since it participates in all their metabolic processes, and its absence affects many physiological processes, such as decrease in photosynthetic activity together with decreased cell volume (NOGUEIRA *et al*., 2001).As, accumulate in the cytoplasm compatible metabolites of their cells in an attempt to combat water stress, to avoid the damage caused by such effects (MUNNS, 2002). Seedlings of castanheira-do-brasil in young plantations it has shown significant tolerance to drought, requiring a period of up to 58 days of withholding irrigation to minimize gas exchange rates, indicating a high physiological plasticity of the species in the field (Gomes, 2012; Ferreira

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et al., 2012). But the lack of scientific knowledge about the physiological and biochemical changes of this kind because of water stress, either by human or natural action is still superficial. Considering that these changes provide the profile imbalance of organic compounds and nitrogen (Silva *et al*., 2010). Given the above, the aim in this research was to evaluate the biochemical responses of the species *Bertholletiaexcelsa* in water stress conditions. Under stress conditions, the metabolism of amino acids is widely changed, thus the protein synthesis is decreased and proteolysis was increased (Monteiro *et al*., 2014). Therefore, occurs induction of proline biosynthesis promoted by metabolic increment as polyamines, ammonia, arginine, ornithine, glutamine and glutamate (Cvikrová *et al*., 2013).

MATERIAL AND METHODS

Location andcharacterization of the studied area

The experiment was realized in a greenhouse, which belongs to the Institute of Agricultural Sciences at the Federal Rural University of Amazonia (UFRA), Belém, Pará. With plastic cover of 70% of solar light.

Instalationand treatments

Young plants of castanheira-do-brasilwith one year old wereaccomodated in plastic vessels with dimensions of (30cm of height x 25cm of diameter) with a capacity of 10 kg, containing only soil substrate of the type latosol. The acclimation period was 45 days and watered daily until the achievement of field capacity, and the light (maximum = 1.000 μmol m^{-2} s^{-1}) and temperature (30±8ºC). After this period, the plants were subdivided into two groups: irrigated plants (control) and non-irrigated (treatment of water deficit). The plants of the control treatment received irrigation daily throughout the trial period and the plants of the water deficit treatment had its fully suspended irrigation photosynthetic response to reach values close or equal to 0 μmol m^{-2} s^{-1}.

Biochemistry analysis

Biochemical analyzes were performed only on the leaves of plants to 9 days of experiment, being determined the following variables:

Starch concentrations: were performed using the method of Dubois *et al*. (1956). The content of total soluble carbohydrates (CST): was determined by the colorimetric method described by Dubois *et al*. (1956). Levels of sucrose: the method used was Van Handel (1968). proline concentrations: for obtaining the second proline concentrations was Bates *et al*. (1973). Total soluble protein concentrations: The method used to obtain the soluble protein concentration was second to Bradford (1976). Soluble aminoacids concentrations: for obtaining total soluble amino acid concentrations was second Peoples *et al* (1989).

Experimental design and statistical analysis

The experimental design was completely randomized with two treatments: irrigated and non-irrigated plants, with 9 plants each, considering each plant as a repetition, and these repeated treatments in 3 times (0, 5 and 9 days), totaling 27 plants per treatment. The results were statistically analyzed and subjected to analysis of variance and the significant difference between means were determined by F test at 5% probability. The program used was the JMP 4.0 (SAS Institute, 2008).

RESULTS AND DISCUSION

Biochemical variables

Starch concentrations in the leaves of chestnut the Brazil were 64.48 and 63.30 mmol of GLU/g residue for irrigated and non-irrigated plants respectively (Figure 1A). The reduction of the concentrations of starch in the leaves under the water suspension is probably related to decreased photosynthesis and starch degradation enzymes abeam α and β-amilase, forming new sugars such as sucrose (Figure 1C) with osmotic adjustment of order and / or transport to other preferred drains and / or inactivation of key enzyme in the starch synthesis of ADP-glucose pyrophosphorylase (MARTINEZ *et al*., 2004; CRUZ *et al*., 2008; OLIVEIRA-NETO, 2008; SILVA *et al*., 2010). Segundo MARTINEZ *et al*., (2004), increases in carbohydrate content in plants under stress, are associated with decreased cell starch content, besides the decrease of photosynthesis capacity that paralyzes cell growth and reduces the synthesis of sucrose for export. Regarding the total soluble carbohydrate content, the results showed an increase for the plants under water deficit (Figure 1B), carbohydrate values in leaves of the control plants were 1.01 mmol of GLU/g residue, and under drought 1.11 mmol GLU/g residue. The increases observed in plants under stress are associated with the fact that with dehydration provide increases in total soluble carbohydrate which is directly related to the osmotic adjustment process of these plants, reducing its osmotic potential in order to maintain the hydrated plant and consequently slow the dehydration of the tissue (OLIVEIRA NETO, 2008; Souza *et al*, 2013).

The sucrose concentration in the leaf were 69.90 and 100.39 mg sucrose /g DM in control and drought treatments, respectively (Figure 1C). The accumulation of this carbohydrate in leaves of stressed plants induce osmotic adjustment, given that this organic compound binds to water molecules in order to maintain the water level in the leaf organ avoiding the dehydration process (SANCHES 2012). When booking polysaccharides are mobilized, the product of hydrolysis often is sucrose, the main sugar transport in plants. For growth bodies (drains) that can metabolize sucrose, it is necessary degradation (CRUZ *et al*., 2008; OLIVEIRA NETO, 2008; SOUZA *et al*., 2013). The concentrations of total soluble protein in the leaves were 11.95 and 9.21 mg protein /g MS, in control plants under drought stress, respectively. Reducing significantly in plants under drought stress compared to control (Figure 2A). The water deficit promotes a restriction in the water absorption decreased cell turgor pressure, and increasing the synthesis of proteolytic enzymes that break down the plant storage proteins as well as in that compound synthesis process, indicating that deficiency affects all biochemical processes in an attempt to maintain water levels in the sheet as well as cell osmotic balance (LECHINOSKI *et al*., 2007, Souza *et al*, 2014). Also, some proteins involved in the interaction with hydrophilic macromolecules are synthesized cell "again" to stabilize the metabolism and act in the recovery of damages caused by water stress (LECHINOSKI *et al*., 2007, Souza *et al*, 2014).

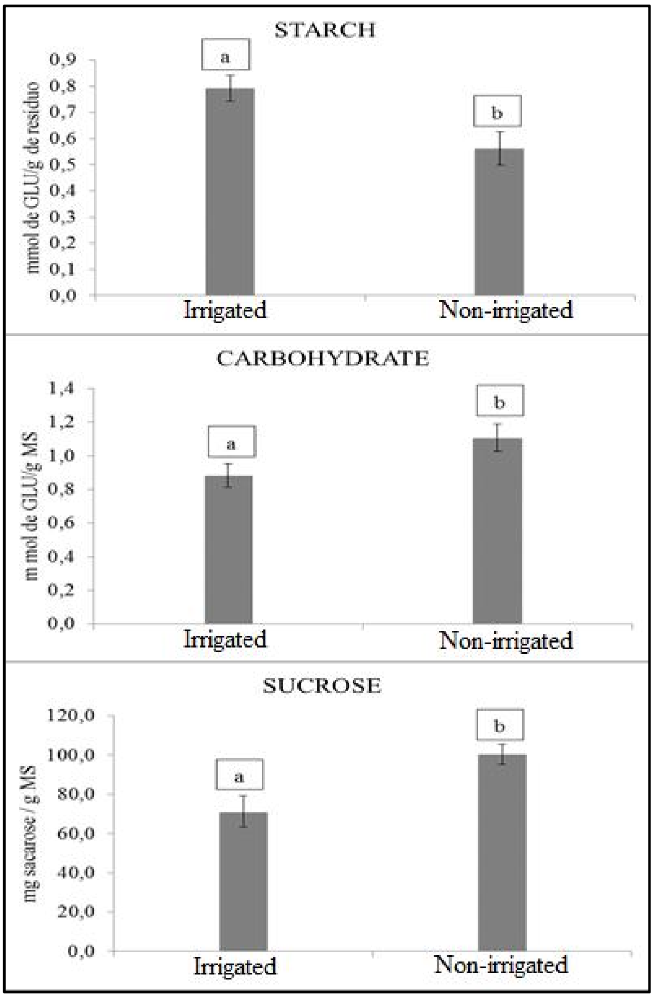

Figure 1. Biochemical analysis of Starch (1A), carbohydrate (1B) and sucrose (1C) of the irrigated and non irrigated plants. Diferent letters in the bars shows statistical differences in a levem of 5% probability

There are several works (LECHINOSKI *et al*., 2007, Santos *et al*, 2010; SOUZA *et al*, 2014) demonstrating that the water deficit under plant has reduced its total soluble protein content. The total soluble aminoacid concentrations showed an increase in plants subjected to drought (Figure 2B). The total soluble amino acid concentrations in the leaves were: 47.77 and 80.01 micromol AA/g MS, in control plants under drought stress, respectively. According to Melo *et al*. (2007), increased free amino acid content may contribute to the tolerance of plants to drought, by an increase in osmotic potential and maintaining the water potential of the cytoplasm in equilibrium with the water potential vacuolar. The amino acid accumulation may be due to restriction of protein synthesis, and the disturbances caused by water stress in phloem tissue, reducing translocation to other organs (Oliveira *et al*., 2013), thus the amino acid buildup can It is considered as a sign of tolerance of plants under different environmental stresses. Similar results were found by Oliveira *et al*. (2013) working with young plants soursop subjected to water deficit; as Carvalho (2005) working with plant paricá (*Schizolobiumamazonicum,* Huber Ducke) andguapuruvu (*Schizolobiumparahyba*). Another possible response to the high levels of amino acids in the leaves are probably due to high activity of nitrogen metabolism, however, in not irrigated, the increase is mainly due to the activity of proteolytic enzymes breaking down proteins into amino acids, in addition to decreasing synthesis there of (OLIVEIRA NETO, 2008). The proline concentrations in leaves were: 1,34 and 2,54 µmol Pro.g^{-1}, in the control plants and under drought, respectively (Figure2C). In plants under drought, increased proline levels may be related to increased activity of proteolytic enzymes promoting greater availability of free amino acids (Figure 2B), to protect plant tissues against this stress by serving as nitrogen reserve, osmo-solute and hydrophobic protective enzymes and cellular structures (Melo *et al*., 2007; OLIVEIRA NETO, 2008; PÉREZ-PÉREZ *et al*., 2009).

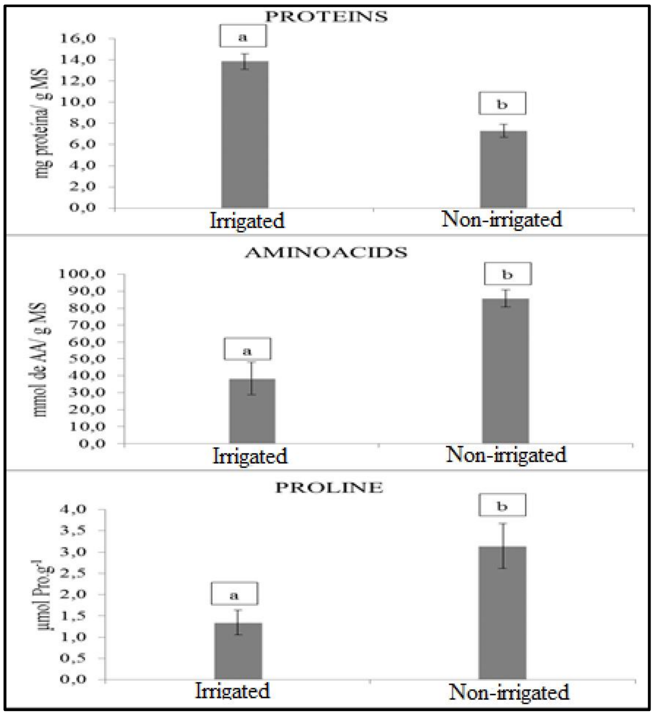

Figure 2. Biochemical analysis of Proteins (1A), carbohydrates (1B) and sucrose (1C) of theirrigated and non irrigated plants. Diferent letters in the bars shows statistical differences in a levem of 5% probability

The ability of cells to accumulate compounds as compatible solutes or osmoprotectors, have as one of its functions to preserve cell integrity, particularly proteins, enzymes and membranes, and to promote the reduction of water potential during periods of osmotic stress provided in a position higher water deficit (Marijuan & BOSCH, 2013). The increase in proline contents can activate various cellular functions: osmotic adjustment reserve of carbon and nitrogen used in growth for recovery after stress, detoxification of excess ammonia, protein stabilizer and membranes and free radical scavengers (ALEMAN, 2011). Amorim *et al*. (2011) found that the water deficit and reduce the production, affects the physiological and biochemical processes of plants. Similar results were found by Castro *et al*. 2007, which evaluated the levels of free proline in *Tectonagrandis* L.f under water stress, in which the proline content in the leaves were increased when plants were subjected to water stress.

Conclusion

- The condition of water deficit for nine days adversely affected the concentration of starch and total soluble proteins.
- The condition of water deficit for nine days, on the contrary, increased the total soluble carbohydrate concentrations, total soluble amino acids, sucrose and proline.

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