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RESEARCH ARTICLE

OSMOREGULATORS COMPOUNDS IN YOUNG PLANTS OF *VOUACAPOUA AMERICANA* AUBL. SUBMITTED TO WATER DEFICIT

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ABSTRACT

The aim of this work was evaluate the water potential, relative water content, and the osmoregulators in young plants of acapu submitted to water déficit. The experiment was conducted in a greenhouse at Federal Rural University of Amazônia, Belém, Brazil, in a period of 9 months. The experimental design utilized was completely randomized in factorial 2x4 (two water conditions: control and drought and four times of evaluation) with 5 repetitions, totaling 40 experimental units. The imposition of water deficit was obtained by suspension of irrigation in 30 days time, with first time (zero days of drought), time 2 (10 days), time 3 (20 days) and the time 4 (30 days). In these plants, was verified a reduction in water potential and RWC in the leaf, in the end of the experiment. The drought induced the increase in carbohydrate content, as well as in sucrose, proline and glycine betaine concentrations on leaves and also in the roots. The lack of water caused a reduction of starch in both organs studied. The accumulation of these osmorregulators in response to drought provided a decrease in water potential in these plants, reducing the effects of stress on the relative water content in the leaf.

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INTRODUCTION

The species American Vouacapoua Aubl., Commonly called acapu, belongs to the Fabaceae Family and Occurs in the Amazon region reaching about 15-30 m in height. In Brazil, mainly covers the states of Pará and Amapá in rain forest land. The wood has great economic importance and is used mainly in the construction and shipbuilding. It is considered a great species for reforestation of degraded areas (Lorenzi, 2009). According to Nascimento *et al*. (2011) Brazilian tree species have aroused the interest of many researchers in demonstrating adaptive behaviors that enhance their use in areas that do not have favorable conditions for other species, being of great silvicultural and economical importance. The phytomass for the most part, is water. Only in protoplasm water comes to represent about 90% of the cell volume, moreover, it is of fundamental importance to a series of biochemical processes acting as a reagent in various reactions in the plant (hydrolysis), is the donor of electrons in photosynthesis, and is directly related to cell turgor potential maintenance which is critical for the essential metabolic steps to the processes of growth and development of plant (Farias, 2005; Taiz, Zeiger, 2013). The intracellular accumulation of osmotically active compounds in response to stressful conditions of low water availability and salinity is an important osmotic adjustment mechanism developed by plants that tolerate water deficit with low water potential (Turner 1986). As told by Tabaeizadeh (1998) the synthesis of these osmoprotectors is one of the mechanisms of adaptation to water deficit. These solutes are accumulated in the cells in response to water stress and are degraded when water becomes available again. Acoording to Ferreira (2002), some nitrogen metabolites such as amino acid proline, tend to accumulate in plant tissues under water stress, in order to act in the osmotic adjustment of cells.

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Due to climatic factors and environmental degradation, water availability has been a major problem for the development of plants, especially in deforested areas. Given the above this study aimed to evaluate the water potential, the relative water content, and the osmoregulators in young plants of acapu subjected to water deficit.

MATERIALS AND METHODS

Experimental conditions

The experiment was conducted in a greenhouse, during the period between November 2011 and july 2012, at the Federal Rural University of Amazônia, in Belém city, State of Pará, Brazil (01º27'S e 48º26'W).

Plant material

Seedlings of acapu (American Vouacapoua Aubl.), were provided by Embrapa Eastern Amazon, with about three months old, measuring 30 cm in height. The seeds were collected in Gunma Ecological Park located in Santa Barbara - PA. Were made four destructive collections, always at 9:00 am; the plants were separated into aerial and root system.

Substrate, pots and plant nutrition

The seedlings of acapu were transplanted to pots with 30cm in height and 30cm in diameter, with capacity for 28kg of substrate, containing a mixture of 3: 1: 1 (v: v: v), artificial sandy soil, poultry litter and earthworm humus. Before transplanting were done tests to verify the field capacity of the vessels. The setting process for correcting soil pH and supplementation of macro and micronutrients were made based on chemical analysis of the substrate, applying a complete nutrient solution of Hoagland and Arnon (1950).

Experimental design

The experimental design was completely randomized in a factorial 2 x 4 (two water conditions: control and drought, and four times of evaluation), with 5 repetitions, totaling 40 experimental units, each experimental unit was composed of a plant / vase.

Water stress regimes

The plants were submitted to two water regimes: irrigated (control) and drought, in which the imposition of water deficit was obtained by suspension of irrigation in the 30-day period, and the first time (zero days of water deficit), time 2 (10-day drought), time 3 (20 days deficit water) and the time 4 (30 days of drought). During the period of analysis, control plants were irrigated daily to replace the water lost by evapotranspiration. There was also the weed control manually. There was no occurrence of nutritional deficiency and the attack of pests and pathogens.

Leaf water potential and leaf relative water content

Determination of leaf water potential (Ψ_w) was executed in fully expanded leaves and exposed to natural light, during the period between 8:00 and 10:00 h, using a Scholander pressure chamber (PMS Instrument Company, model 600) as described by Scholander *et al*. 1964, and in agreement with recommendations of Turner (1988). The leaf relative water content (LRWC) was carried out with 10 mm disks of diameter, it was calculated as: LRWC = [(FW-DW)/(TW-DW)] x 100, in which FW is fresh weight, TW is the turgid weight measured after 24h of saturation on deionised water at 4ºC in the dark, and DW is the dry weight determined after 48 h in oven at 80ºC (Slavick,1979).

Leaf sample preparation

The leaves were harvested and placed in a 70ºC oven with forced air circulation at 72h. The dried leaves were ground, and the powder was stored in a glass container in the dark at 15ºC until biochemical analysis were performed in the laboratory of Biodiversity Studies in Higher Plants (BSHP).

Total soluble carbohydrates

To determine the quantity of total soluble carbohydrates, 20 mg of leaf and root powder was incubated with 2.0 µL of 80% ethanol at 95°C for 20 min and centrifuged for 5 min at 5.0 g and 20°C. The supernatant was then removed, and the quantification of the total soluble carbohydrates was performed in reactions containing 1.250 µL of 100% H2SO4, 70 µL of 15% phenol, 580 µL H2O, and 100 µL of extract for a total volume of 2.0 µL. Measurements were taken at 490 nm (Dubois *et al*., 1956) using glucose (Sigma chemicals, São Paulo, Brasil) as a standard.

Determination of starch

For determination of total soluble carbohydrates 50 mg of leaf and root powder was incubated with 5 ml of ultra pure water at 100ºC for 30 min, centrifuged at 2,000 *g* for 5 min at 20ºC and the supernatant was removed. For determination of starch 50 mg of powder was incubated with 5 ml of ethanol at 80ºC for 30 min, centrifuged at 2,000 *g* for 10 min at 25ºC, and the supernatant was removed. In addition, a second extraction was carried out with the same powder incubated with 5 ml of 30% $HClO_4$ at 25ºC for 30 min and centrifuged in conditions previously described. The supernatants of the two extractions were mixed. The quantifications of the total soluble carbohydrates and starch were carried out at 490 nm using the method of Dubois *et al*. (1956), using glucose (Sigma Chemicals) as standard.

Sucrose

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

Glycine-betaine

The glycine-betaine was determined with 25 mg of leaf e root dry matter powder, which was incubated with 2 mL of sterile distilled water at 25ºC for 4 h, under agitation. After homogenized, it was centrifuged at 10,000 g for 10 minutes at 25ºC and the supernatant removed. The glycine-betaine quantification was carried out at 365 nm according to Grieve

and Grattan (1983), using glycine-betaine (Sigma Chemicals) as a standard.

Free proline

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

Statistical analyzes

The analysis of variance was applied in the results and when appears significant difference, the means were compared by Tukey test at 5% significance level. In addition, the standard deviations for each treatment were calculated, and the statistical analyzes performed by ASSISTAT program Version 7.7 beta preconized by Silva (2013).

RESULTS

Water potential

The water potential of plants kept under water stress reduced significantly (Fig. 1a). Throughout the experiment, the values for the stressed plants were -0.66; -1.62; -2.56 and -2.86 MPa in the times with 0, 10, 20 and 30 days of water deficit, respectively, representing a progressive fall of 333% at the end of the experiment compared to the plants under control. The values remained almost constant in the control plants until the end of the study period, with a mean of -0.67 MPa.

Relative water content

The relative water content in plants under drought significantly reduced over time when compared to plants kept irrigated (Fig. 1 b). Irrigated plants ranged from 83.98% to 84.39% during the experiment. The values shown by the plants submitted to drought were 83.98%; 74.25%; 66.70% and 52.79% equivalent to 0, 10, 20 and 30 days of water stress, suffering a decrease of 37% at the end of treatment.

Total soluble carbohydrates

The increase in days of drought caused a significant increase in the concentration of total soluble carbohydrates in leaves and roots (Fig. 2a, b). The values obtained for the leaves of stressed plants at time 0, 10, 20 and 30 days respectively were 1.72; 2.23; 3.19; GLU and 4.73 mmol / g of residue, having statistically significant difference between all treatments. Roots in the concentration of total soluble carbohydrates showed the same pattern of leaves, where the values for the times with 0, 10, 20 and 30 days were 0.93; 1.58; 2.43 and 3.56 GLU mmol / g of residue, respectively. In plants under stress increased by 304% and 287% of total soluble carbohydrates in leaves and roots, respectively, after 30 days of water restriction.

Starch

The starch concentration in leaves decreased significantly over water stress (Fig. 2 c). The values of starch obtained between 0 and 30 days of measure were between 0.22 and 0.13 mmol of GLU / g mmol of glicose.g-1 MS in the leaves, with a decrease of 40.90% at the end of the treatment in plants under water deficit compared with plants kept irrigated. Similar behavior was observed in the roots (Fig. 2d), where starch levels also fell significantly throughout the experiment, 0.13 to 0.02 mmol of GLU / mmol g of glucose. g-1 DM, between the first time (day zero) and the time 4 (30 days), respectively, with a decrease of 84.61% after 30 days of water stress, compared to plants under control.

Sucrose

There was a significant increase in the concentration of sucrose in leaves and roots, but this increase was higher in the 20th and 30th day of stress, both in the leaves, as the roots (Fig. 2 e, f). The values at 20 and 30 days for the leaves were respectively 29.83 and 34.15 mg sacarose g^{-1} DM. In the roots, the values were 15.92 and 17.57 mg sucrose g^{-1} DM, for treatments with 20 and 30 days of water stress, respectively. The values remained almost constant in the control plants by the end of the study period, with an average in the leaves of 22.94 mg sucrose g^{-1} DM and roots of 14.02 mg sucrose g^{-1} DM. At the end of the experiment, in the plants subjected to stress, there was an increase of 49% sucrose in the leaves and 25% in the roots.

Glycine-betaine

The concentration of glycine betaine increased significantly in plants under water deficit, in leaves the values were 8.47; 13.22; 17.13 and 22.89 ug glycine betaine .g^{-1} DM for the times at 0, 10, 20 and 30 days of stress, respectively. The same behavior was observed in the roots, presenting the values of 8, 89; 12.17; 16.71 and 19.06 ug glycine betaine. g^{-1} for 0, 10, 20 and 30 days of stress, respectively. In plants subjected to stress there was an increase of 171% in the leaves and roots of 116% after 30 days of water restriction. The glycine betaine concentrations for both the leaves, and to the roots, remained constant in control plants with an average of 8.4 ug glycine betaine. g^{-1} DM for the leaves and 8.85 ug glycine betaine.g^{-1} MD to the roots (Fig. 3a, b).

Proline

Proline concentration had significant changes in acapu plants subjected to water stress, both leaves and roots (Fig. 3c, d). The proline concentrations in the leaves of plants under water suspension were 1.78; 3.58; 5.68 and 6.81 µmol Pro.g^{-1}, and roots were 1.51; 2.18; 3.06 and 5.03 µmol Pro.g^{-1} for the first time (0 days); 2 (10 days); 3 (20 days) and 4 (30 days) respectively, with a total increase of 282% and 233% of proline in leaves and roots concurrently within 30 days. The proline content in water kept under control plants remained constant both in leaves and in roots, with means of 1.78 µmol Pro.g^{-1} for the leaves and 1.50 µmol Pro.g^{-1} to the roots.

DISCUSION

Water potential and RWC

The reduction of water potential (Ψw) and relative water content in the leaf (LRWC) occur because of water deficit in the soil. According Pagter *et al*. (2005), the accumulation of solutes on the leaves decreases the leaf water potential (Ψw), which increases the absorption capacity of water of the plant and reduces the effects of water stress on the water content of the plant.

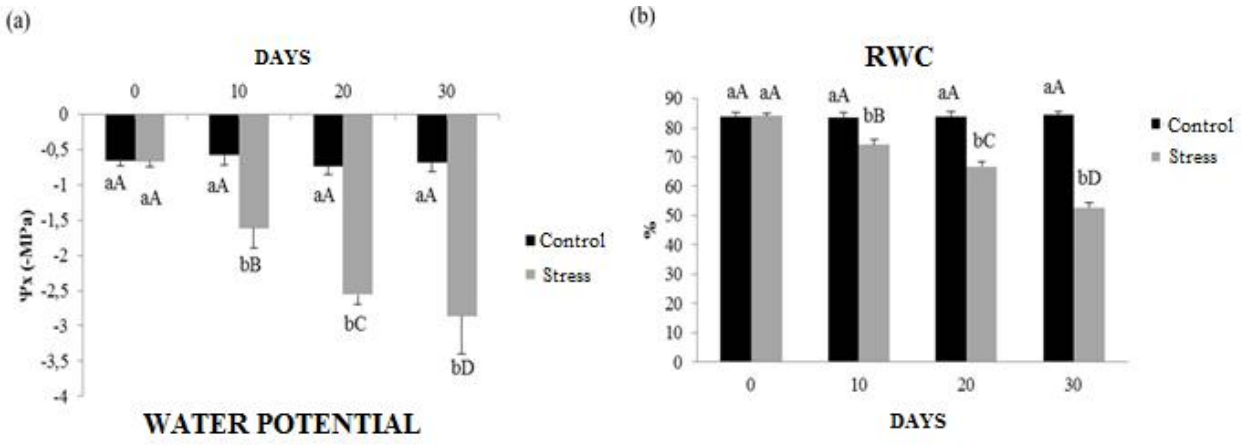

Fig 1. (a) Water potential and (b) Relative water content in plants of acapu submitted to water deficit. Uppercase letters show statistical differences between treatments and lowercase letters show differences between collection days of the same treatment compared by Tukey test at 5% probability

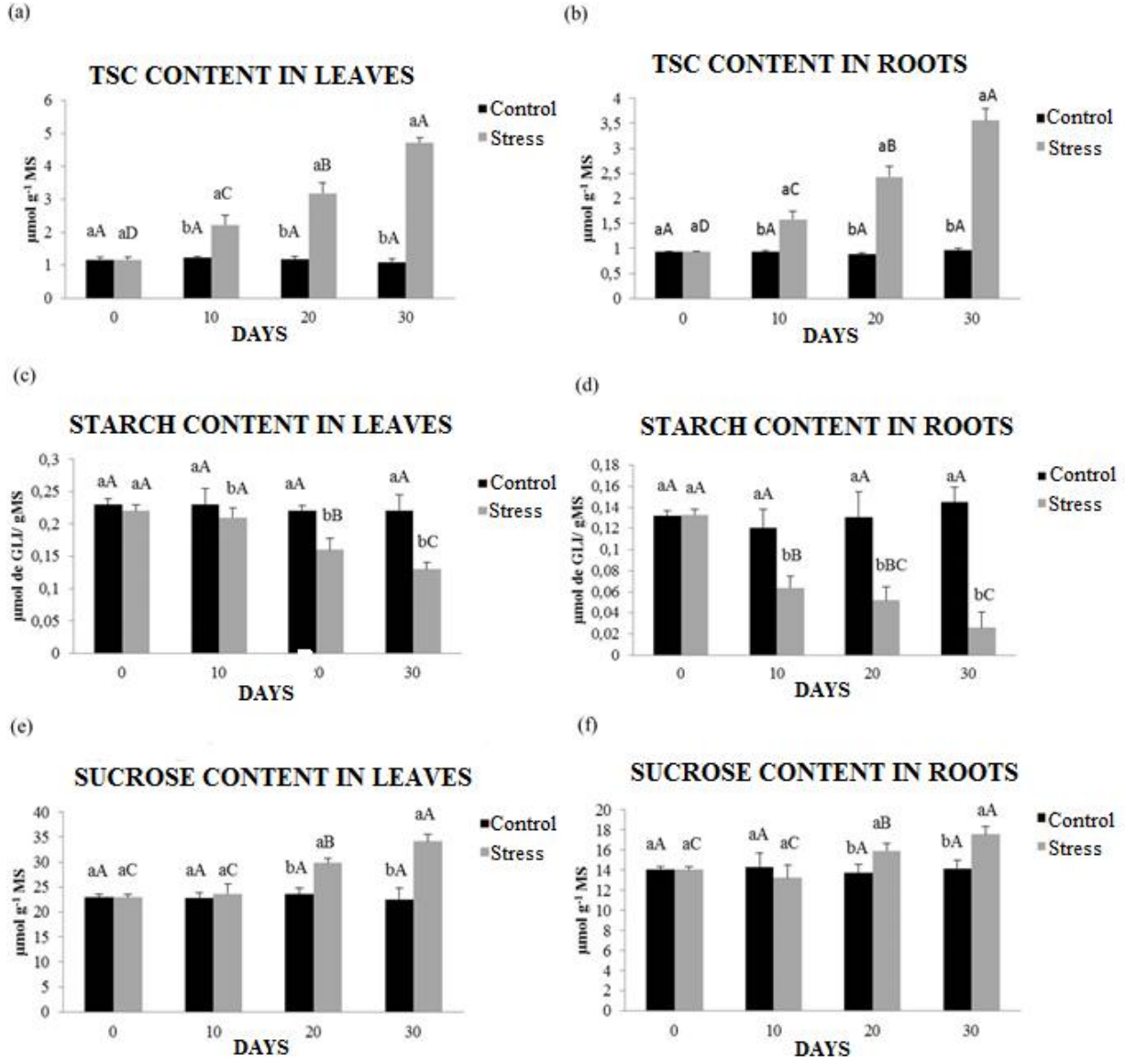

Fig 2. (a) Carbohydrate content in the leaves, (b) Carbohydrate content in the roots, (c) Starch content in the leaves, (d) Starch content in the roots, (e) Sucrose content in the leaves and (f) Sucrose content in the roots of young plants of acapu submitted to water deficit. Uppercase letters show statistical differences between treatments and lowercase letters show differences between collection days of the same treatment compared by Tukey test at 5% probability

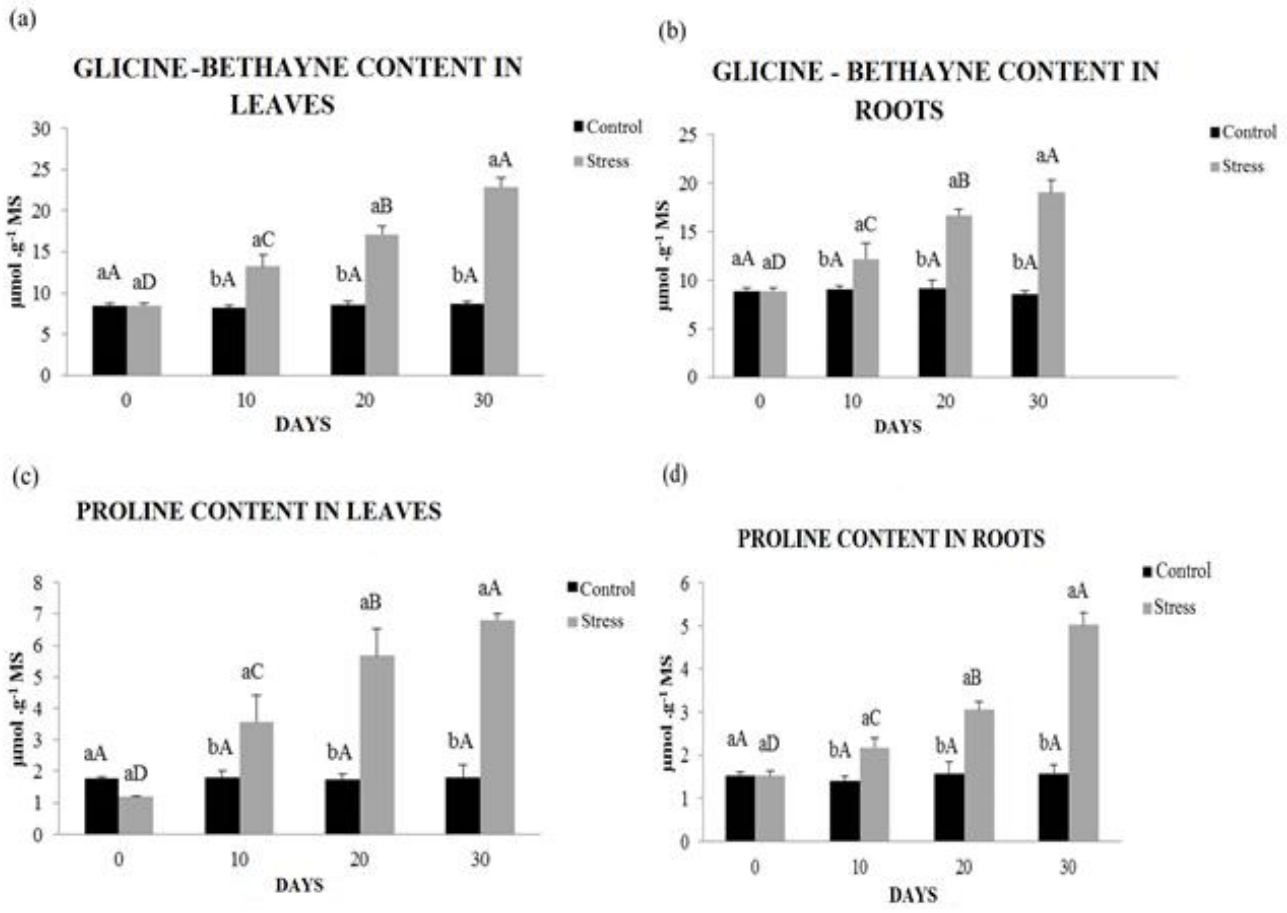

Fig 3. (a) Glicine-bethayne content in the leaves, (b) Glicine-bethayne content in the roots, (c) Proline content in the leaves and (d) Proline content in the roots in young plants of acapu submitted to water deficit. Uppercase letters show statistical differences between treatments and lowercase letters show differences between collection days of the same treatment compared by Tukey test at 5% probability

Rocha and Moraes (1997) working with young plants of Stryphnodendron adstringens grown in a greenhouse under water stress induced by watering suspension, obtained after 30 days of stress values in the water potential of up to -2.7 MPa. Carvalho (2005) found responses similar to those obtained in this work, to observe reduction in RWC of young plants of paricá (Schizolobium amazonicum) and guapuruvu (Schizolobium parahyba) submitted to two cycles of water stress in a greenhouse. Albuquerque *et al*. (2013) working with African mahogany plants in a greenhouse under two water regimes (control and drought) observed 28% reduction in the CRA at 14 days of water stress.

Total soluble carbohydrates

The increase in concentration of total soluble carbohydrates is because this substance is responsible for osmotic adjustment of the plant, being very important for maintenance of cell turgor (Salisbury and Ross, 2012). These results corroborate those found by Castro *et al*. (2007) observed an increase of 323.15% in the concentration of total soluble carbohydrates in plants of teak (Tectona grandis L. f.) submitted to 9 days of water stress. Were *et al*. (2008) working with soybean (Glycine max cv. Sambaíba) in a greenhouse, found similar results, noting an increase of 40% after 6 days of stress in the total soluble carbohydrate concentrations in leaves of plants under drought.

Starch

The starch concentration was significantly decreased, this reduction occurs because there is a decrease in photosynthesis and a increase in the degradation of starch by the β and α amylase enzymes, forming new sugars with the aim of making an osmotic adjustment in the cell. For Melo *et al*. (2007) the reduction in starch concentration, can be indicative of consumption of sugars for maintaining the survival of the plants. These results corroborate with the work of Paula *et al*. (2013) involving Brazilian mahogany trees (Swietenia macrophylla King) in two sampling periods, dry and wet, where there was 30% reduction in average starch content in the leaves analyzed during the dry season compared analyzed during the rainy season.

Sucrose

The accumulation in the concentration of sucrose in leaves and roots was caused, because this sugar is the main sugar exported of local synthesis (source) to the consumer regions (drain), which is used for growth and / or storage. The hexose liberated from hydrolysis of sucrose may be used in anabolic or catabolic processes and also in the supply of reducing sugars, it is widely used for the process of osmotic adjustment of cells (Kingston- Smith *et al*., 1999). Results found by Pereira (2013) also showed an increase in sucrose concentration in leaves and

roots of bean-atanã plants (Parkia gigantocarpa Ducke) under water deficit in soil.

Glycine-betaine

The increase in the concentration of glycine betaine in leaves and roots happens to protect the plant metabolism, since this substance can act as a compatible osmolyte, keeping the "balance" of water between the cell and the environment (Meloni 2004). According to Ashraf and Haris (2004) the concentration of glycine betaine acts as protection against oxidative stress mechanism of plant species, as well as help the plant absorb and carry water from the soil to the aerial part, through the osmotic adjustment of cells. Glycine betaine acts as protector of the thylakoid membranes while maintaining the photochemical efficiency of photosynthesis (Ashraf and Foolad, 2007). Lima *et al*. (2015), working with andiroba (Carapa guianensis), noted an increase of 225% and 119% in the concentration of glycine betaine in leaves and roots, respectively, after 25 days of water deficit.

Proline

The increase in free proline concentration in leaves and roots occurs because proline is a substance responsible for osmotic adjustment of cells subject to stress, avoiding the reduction of cellular turgor, preserving the cellular integrity, and allowing the continuity of essential physiological processes for the growth and development of plants (Kishor *et al*., 2005). According Ferreita *et al*. (2002) the hydrolysis of proteins, besides contributing to the precursor aminoacid (glutamate), can also contribute their own proline, favoring its increase as free aminoacid in the treatments under water stress. A similar result was observed by Albuquerque *et al*. (2013), which found a significant increase in proline concentrations in young plants of African mahogany submitted to water deficit. Paula *et al*. (2013) also found an increase of almost 21% in proline concentration in Brazilian mahogany trees (Swietenia macrophylla King) during the dry season in Santa Barbara-Pará.

Conclusion

The study revealed that the water deficit of 30 days in acapu plants (American Vouacapoua Aubl.), promoted sharpest drop in water potential and relative water content in the leaves, and an increase in concentrations of sucrose, total soluble carbohydrates, proline and glycine betaine in both roots and leaves. The species showed partially tolerant to drought stress as the largest accumulations occurred in osmorregulators longer stress.

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