

Full Length Research Paper

Gas exchange and carbon metabolism in young plants of muruci (*Byrsonima crassifolia* L.) submitted to water deficit

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Received 4 November, 2015; Accepted 17 February, 2016

The *Byrsonima* genus covers various fruit species known in the Brazilian Amazon as murucizeiro, which is considered as a species with good nutritional quality and features in its composition a variety of volatile compounds. The adaptation of plants to water stress is a complex physiological and biochemical phenomenon. Depending on the intensity and duration of stress, changes range from a rapid change in the flow of ions to improve the osmotic pressure, reduction of gas exchange, stabilization of cell structures by osmotic protection to a more drastic change in plant growth pattern. The aim of this work was to study gas exchange and carbon metabolism in young plants of muruci (*Byrsonima crassifolia* L.) submitted to water suspension. The experimental design was completely randomized with two water conditions: control and drought, with 14 repetitions, totaling 28 experimental units. The parameters analyzed were relative water content, transpiration, stomatal conductance, photosynthetic pigments, concentrations of starch, total soluble carbohydrates and sucrose. The suspension of irrigation for 25 days provided significant differences in all parameters, promoting decreases in the metabolic pathways of plants and reducing the relative water content by 26.92%, transpiration 90%, stomatal conductance 94.79%, photosynthetic pigments (Chlorophyll a (42.1%), Chlorophyll b (50%), Carotenoids (45.1%) and overall (33.3%)) and starch in leaves and roots (73.43 and 63.63%), but increase in the control plants with the total soluble carbohydrates at 63, 87 and 39.5% and sucrose content as 64.73 and 43.99% in the leaves and roots. Therefore, these changes indicated that these plants are susceptible to soils with low water availability.

Key words: Carbohydrates, drought, muruci, photosynthetic pigments, transpiration, sucrose.

INTRODUCTION

The *Byrsonima* genus covers various fruit species popularly known in the Brazilian Amazon as murucizeiro

and in other regions of Brazil as muricizeiro. *Byrsonima crassifolia* (L.) HBK, whose center of origin and diversity

is in the Brazilian Amazon, is widespread in tropical America, constituting the most important species, not only for being the most cultivated but also for having the best quality fruits for consumption (Cavalcante, 2010). It has a fleshy fruit, drupoide type, with a round shape or oblong, from ovarian tricarpellate, with each carpel containing an egg. It is consumed as fresh fruit or used in the preparation of soft drink, ice cream, sweet paste, jam, liqueur and even in savory dishes such as meat stuffing or soups (Carvalho and Nascimento, 2013). The murucizeiro propagation unit is pyrene (core), which contains one to three seeds located in the walls of isolated locules endocarp (Carvalho and Nascimento, 2008).

It is considered a good nutritional food with high quality and has in its composition a variety of volatiles such as ethanol, butyl hexanoate, butanoic acid, hexanoic acid and methyl butyrate, responsible for the distinctive aroma of the fruit (Souza et al., 2012). Besides, it is rich in polyphenols and flavonoids, which gives it great antioxidant capacity and can, therefore, be framed in the group of functional foods (Siguemoto, 2013).

However, although the fruit of murucizeiro have economic, social, cultural importance for small communities harvesting of extractive form for consumption and marketing, little is known about the information on cultivation and its physiological, biochemical and nutritional characteristics. Mainly, these species are subjected to environmental stress conditions that affect their growth and development as a result of the water stress condition in the soil (Almeida et al., 2011).

The adaptation of plants to water stress is a physiological and biochemical complex phenomenon. Depending on the intensity and duration of stress, changes ranging from a rapid change in the flow of ions to improve the osmotic pressure, reduction of gas exchange, stabilization of cell structures by osmotic protectors, to a more drastic change in plant growth pattern can be observed (Alves, 2007).

Water stress can affect multiple morphological and physiological characteristics of plants, photosynthesis being one of the processes more limited by the increase of drought. The intensification of drought caused by environmental changes in anthropogenic and/or natural way can result in limitations to the growth of young plants in the Amazon region since the early stage of development of the field in culture by the plants can be subjected to natural water deficit, especially for presenting a superficial root system (Silva, 2009).

The photosynthetic process can be separated into three stages: the diffusive stage mainly controlled by opening and stomatal closure; photochemical stage, which has a primary function to absorb the incident

formation of ATP and reducing power; and biochemistry stage, responsible for carbon fixation (Kreuzwieser and Gessler, 2010). Therefore, this research aimed to study gas exchange and carbon metabolism in young plants of muruci submitted to water suspension.

MATERIALS AND METHODS

Plant materials

The seedlings were from the Association of Exporting Industries of Wood in the state of Pará (AIMEX), 4 months after germination. The seedlings were acclimatized in a greenhouse for a period of three months for ambiance.

Experimental conditions

The study was conducted at the Federal Rural University of Amazonia (UFRA), state of Pará, campus CapitãoPoço, Brazil (Latitude 01° 44' 47" 'and longitude 47 03'34' '). This experiment was conducted in a greenhouse for 4 months, with temperature of air minimum-maximum with values of 24.5/39.1 and 53.3/91% minimum-maximum humidity, respectively.

Substrate, pots and plant nutrition

The substrate used was a mixture in the proportion of 3:1:1 (v/v/v), black earth, chicken manure and earthworm humus, respectively. The polyethylene vessels were used in the dimensions of 0.30 m x 0.30 m (height x diameter), and capacity of 20 kg. Corrections were made in the concentrations of macro and micronutrients from the soil and the pH soil, through the results of the soil chemical analysis realized in the laboratory of soils in Embrapa Eastern Amazon, applying 600 mL of complete nutrient solution (Hoagland and Arnon, 1950), divided in three months, for every month 200 mL of complete nutrient solution was used before the start of the experiment.

Experimental design and treatments

The experimental design was completely randomized with two water conditions (control and drought), with 14 repetitions, totaling 28 experimental units, where each experimental unit consisted of one plant per pot. The experiment was conducted from April, 2013 to July, 2013 in which the water suspension occurred in the 25 days period and the control plants were irrigated daily in an average of 400 ml of water to compensate for the losses by evapotranspiration.

Leaf relative water content

The leaf relative water content was evaluated using leaf disks with 10 mm of diameter and it was carried out in each plant, in which 40 disks were removed and the calculation was done in agreement with the formula proposed by Slavik (1979):

$$LRWC = [(FM1 - DM)/(FM2 - DM)] \times 100$$

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Where: FM₁ is fresh matter, FM₂ is turgid matter evaluated after 24 h and saturation in deionized water at 4°C in dark, and DM is the dry matter determined after 48 h in oven with forced air circulation at 80°C.

Gas exchange

Stomatal conductance and transpiration were evaluated in fully expanded leaves under light, using a steady state porometer (LI-COR Biosciences, model 1600), with the gas change evaluated during the period between 10:00 and 12:00 h in all the plants.

Chlorophyll contents

The determination of the photosynthetic pigments was carried out with 25 mg of leaf tissue, in which the samples were homogenized in the dark and in the presence of 2 mL of acetone at 80% (nuclear). Subsequently, the homogenized was centrifuged at 5.000 g, by 10 min in the temperature of 5°C, in which the supernatant was removed and the chlorophylls a, b, carotenoids and the total were quantified using spectrophotometer SP – 220 from the QUIPAR Company, in agreement with the methodology of Lichthenthaler (1987).

Concentrations of starch

For determination of starch content, 50 mg of milled material was incubated with 5 mL of ethanol at 80°C for 30 min, centrifuged at 2.000 g for 10 min at 25°C, and the supernatant was removed. In addition, a second extraction was carried out with the same milled material incubated with 5 mL of 30% HClO₄ at 25°C 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 a standard.

Concentrations of total soluble carbohydrates

The total soluble carbohydrates were determined with 50 mg of leaf dry matter, which was incubated with 5 mL of ultra pure water at 100°C by 30 min, subsequently the homogenized was centrifuged at 2.000 g, within 5 min at 20°C and the supernatant was removed. The quantification of the total soluble carbohydrates were carried out at 490 nm according to the method of Dubois et al. (1956), and glucose (Sigma chemicals) was utilized as a standard.

Concentrations of sucrose

The determination of sucrose was carried out with 50 mg of powder (leaf dry matter), which was incubated with 1.5 mL of MCW solution (methanol, chloroform and water), in the proportion 12:5:3 (v:v⁻¹) at 20°C by 30 min and under agitation, subsequently, the homogenized was centrifuged at 10.000 g by 10 min at 20°C and the supernatant was removed. The sucrose quantification was carried out at 620 nm according the method of Van Handel (1968), and sucrose (Sigma chemicals) was used as a standard.

RESULTS

Relative water content (RWC)

The relative water content was significantly affected in

murucizeiro plants under water stress during the 25 days of experiment (Figure 1A), with a decrease of 26.92% in this treatment, with a smaller amount of water assimilation when compared with the control plants and the obtained value of 78% in its relative water content.

Plant transpiration rate and stomatal conductance

The low water content changed the transpiration rates, given that plants subjected to water stress (Figure 1B) showed a significant reduction of 90% in their values, 0.27 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ as compared to the control plants which obtained value of 2.83 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Consequent to this decrease (Figure 1B), stomatal conductance also decreased (Figure 1C), presenting a significant reduction of 94.79% (0.025 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) in the plants subjected to water stress as compared to the control plants (0.48 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

Photosynthetic pigments in leaves

Photosynthetic pigments contents was decreased in plants under water deficit showing significant differences as compared to the control plants (Figure 2), corresponding to a decrease of 42.1% in chlorophyll a (1.1 $\text{mmol}\cdot\text{kg}^{-1}\text{MF}$), 50% in chlorophylls b (0.6 $\text{mmol}\cdot\text{kg}^{-1}\text{MF}$), 45.1% in total chlorophyll (1.7 $\text{mmol}\cdot\text{kg}^{-1}\text{MF}$) and 33.3% in carotenoids (1.2 $\text{mmol}\cdot\text{kg}^{-1}\text{MF}$) as compared to control plants that showed values of 9.0; 1.2; 3.1 and 1.8 $\text{mmol}\cdot\text{kg}^{-1}\text{MF}$, respectively.

Concentrations of starch

The starch concentrations was decreased in plants under water deficit causing significant differences as compared to the control plants (Figure 3A), the values in the starch concentrations in the leaves and roots of murucizeiro plants under water deficit were 0.17 and 0.04 μmol of GLU/gDM representing a decrease of 73.43 and 63.63%, respectively, as compared to leaves of the control plants (0.64 μmol of GLU/gDM) and roots (0.11 μmol of GLU/gDM).

Concentrations of total soluble carbohydrates

The total soluble carbohydrate content was increased in plants under water stress resulting to statistical difference as compared to the control plants (Figure 3B), the values were under water deficit of 11.21 $\text{mmol}\cdot\text{g}^{-1}\text{MS}$ leaves for an increase of 63.87% in control plants (4.05 $\text{mmol}\cdot\text{g}^{-1}\text{MS}$) and the roots of 4.48 $\text{mmol}\cdot\text{g}^{-1}\text{MS}$, an increase of 39.5% in control plants (2.71 $\text{mmol}\cdot\text{g}^{-1}\text{MS}$).

Concentrations of sucrose

An increase in sucrose concentrations in the roots and

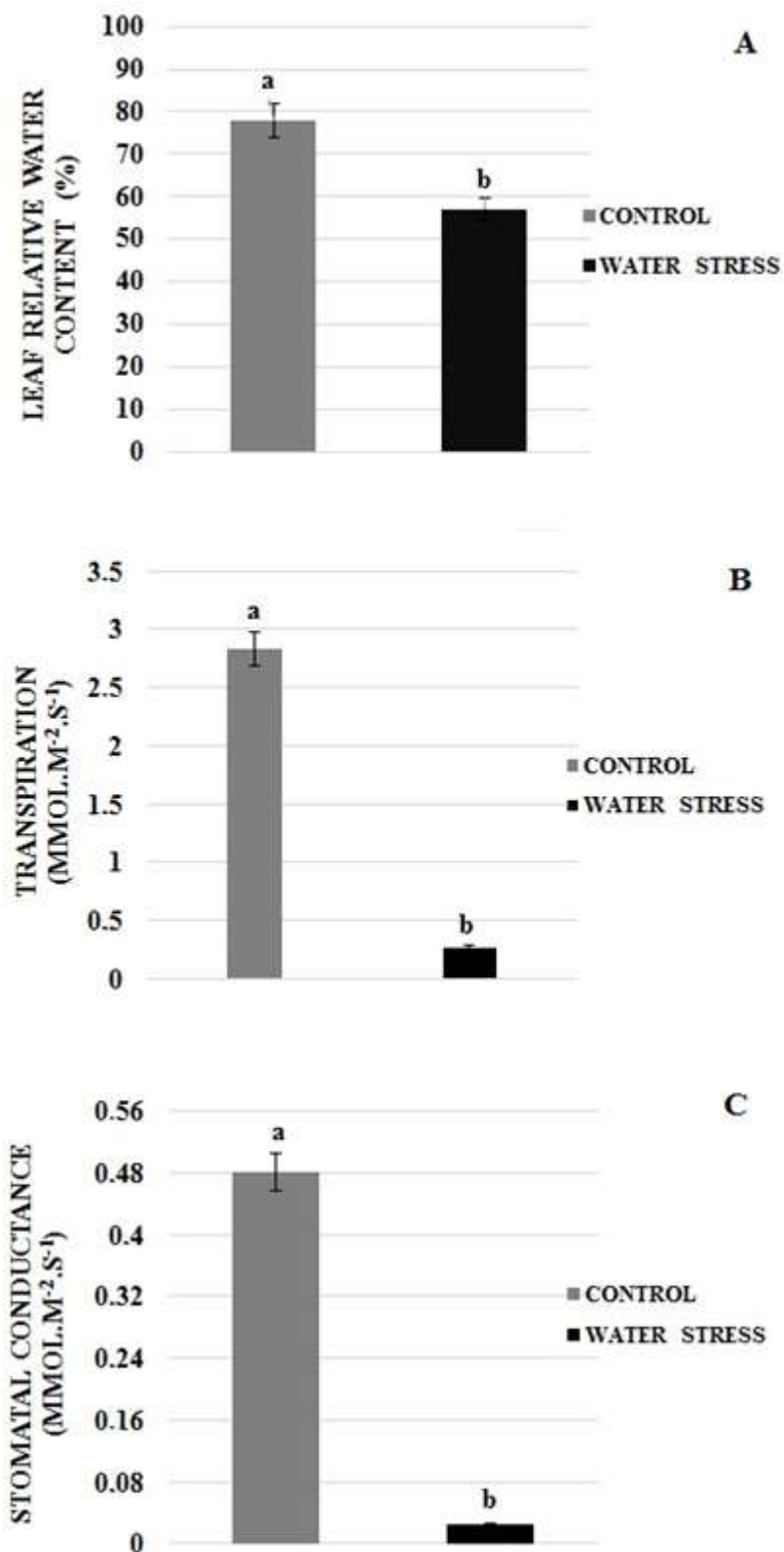


Figure 1. Leaf relative water content (A), transpiration (B) and stomatal conductance (C) in leaves of young plants of murucizeiro submitted during 25 days under water stress. The letters a and b show statistically significant differences between treatments which were compared by Tukey test at 5% probability. The bars represent the standard deviations of the means.

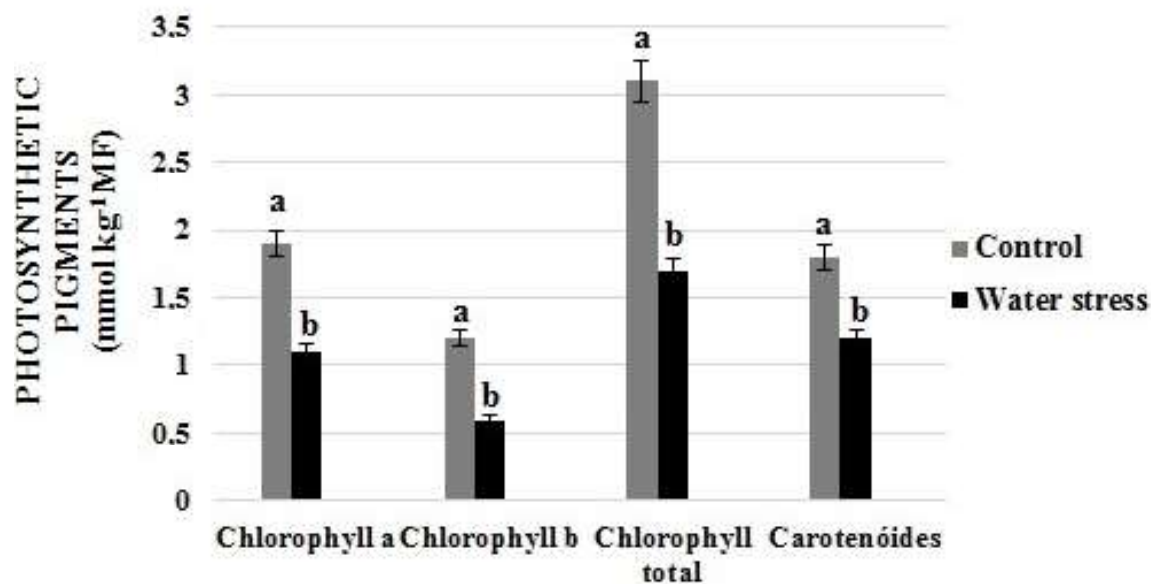


Figure 2. Photosynthetic pigments in leaves of young plants of murucizeiro submitted during 25 days under water stress. The letters a and b show statistically significant differences between treatments which were compared by Tukey test at 5% probability. The bars represent the standard deviations of the means.

leaves of plants subjected to water stress was observed (Figure 3C), the values found in the leaves were 16.98 and 24.45 mg sucrose g⁻¹ MS in control plants and water deficit, respectively, with an increase of 64.73%. For the roots, there were 5.87 and 9.67 mg sucrose g⁻¹ MS in plant control and drought stress, respectively, meaning an increase of 43.99%.

DISCUSSION

The decrease in the relative water content in leaf tissues (Figure 1A) is associated with water deficit in soil causing a decrease in the water balance of plants, promoting a reduction in cell turgor, reducing the quantity of water in xylem and increasing the tension in xylematic vessels, and making the plant to exerts a force required to absorb the soil water to be transported to the aerial part. With the absence of water in the soil, the hydraulic conductivity of the roots is reduced, leading to an inhibition of metabolic activity and the reduction in ATP production that ends restricting the power supply to the growth of the roots, causing a reduction in development and physiological processes of the plant (Oliveira, 2010; Molle, 2011). After these processes, according to Silva (2013) as the plants defense strategy, possibly increasing osmotically adjust their carbohydrate levels (Figure 3B) and sucrose (Figure 3C) to maintain the water absorption on soil colloids and continue with their metabolic processes. Similar results were found by Wang (2014) in leaves of rubber tree seedlings clone GT1, which showed a continual decrease

with the gravity of the obtained stress, resulting in nine days of water restriction, approximately 20% as compared to the control treatment.

As the water stress increases in the cells of the leaf mesophyll, there is a dehydration of this tissue reducing its water potential, leading to a decrease in gas exchange and consequently reducing photosynthesis. The decrease of transpiration can be linked to stomatal behavior over stress, which is an important mechanism for the survival of plants under water stress situations (Otto et al., 2013).

Similar results were found by Fu et al. (2010) on study of two species of poplar or aspen (*Populus euphratica* and *Populus russkii*), showing a reduction in gas exchange in different volumes of irrigation in a desert area. Increase in drought on the plant of murucizeiro, may have caused a reduction of photosynthesis which is not only a consequence of chloroplastid low levels, but the reduction in stomatal opening and diffusive restriction of CO₂, and also, the mechanisms of photochemical and biochemical steps (Dias and Brüggemann, 2010). The degradation of pigments possibly leads to a decrease in the photochemical step process. The decrease in availability of internal CO₂ and water loss through transpiration directly influence the chlorophyll fluorescence parameters, mainly associated with the ISP (Martinazzo et al., 2012). Another possible cause is leaf dehydration leading to a disruption of the membranes of the thylakoids, resulting in inactivation of electron transfer reactions, reducing the value of photosynthetic rate (Dias and Brüggemann, 2010). In the work performed by

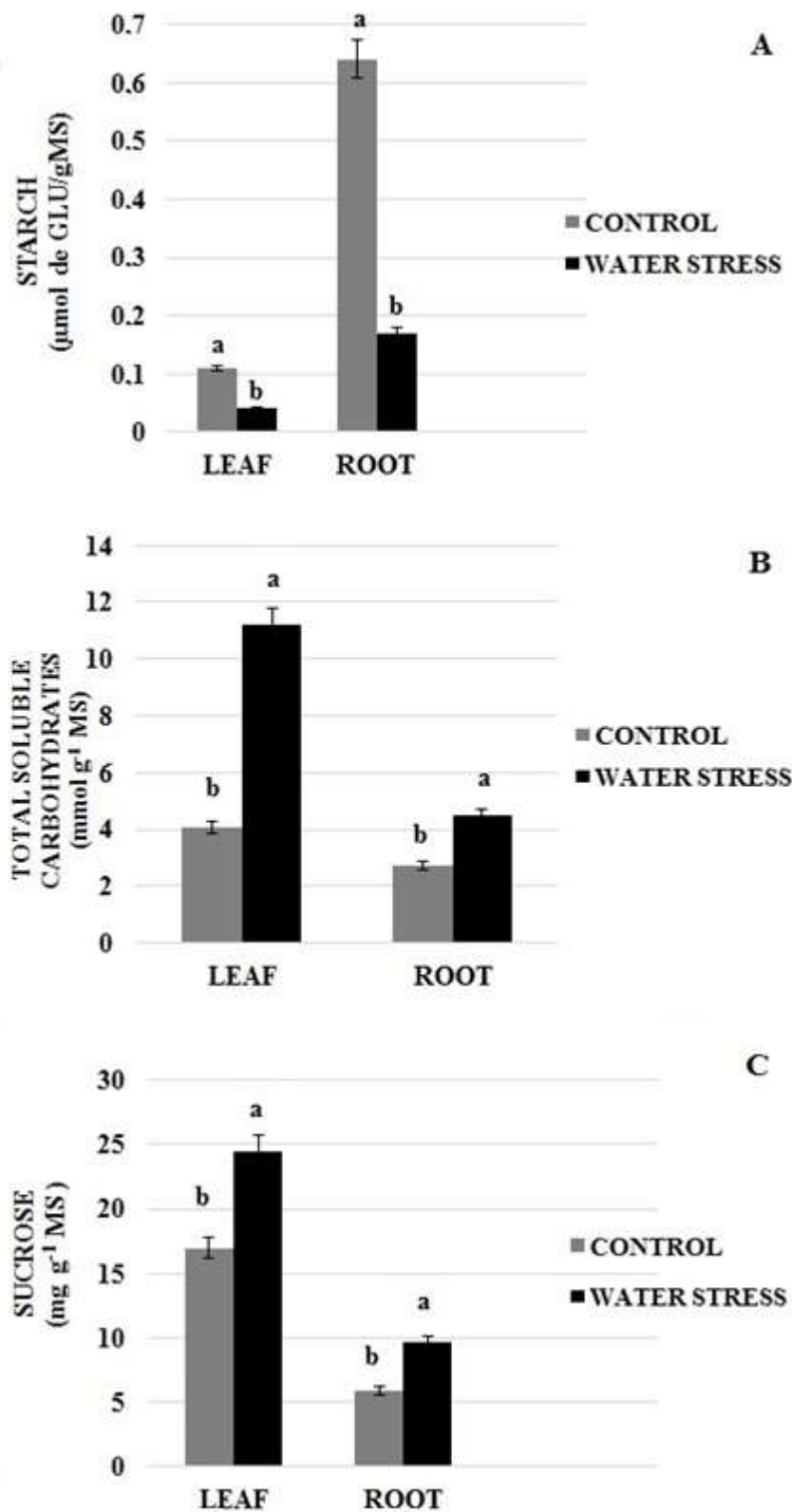


Figure 3. Starch (A), total soluble carbohydrates content (B) and sucrose (C) in leaves of young plants of murucizeiro submitted during 25 days under water stress. The letters a and b show statistically significant differences between treatments which were compared by Tukey test at 5% probability. The bars represent the standard deviations of the means.

Cavalcante (2013) through spectrophotometry analysis, showed that the leaves of *Jatropha curcas* L. under drought, cause a significant reduction in photosynthetic pigments content when compared with irrigated plants.

The decrease in starch levels is related to the function of acting as an osmoprotector, becoming soluble sugars in order to maintain the availability of energy for the plant and especially the influx of water, which occurs at a particular signal and resulting to increase in the synthesis of the amino acid (Silva, 2008). It has been observed in several studies that there is a relationship between the increase in the activity of enzymes responsible for the hydrolysis of starch after stomatal closure and inhibition of photosynthesis; there is an accumulation of sugars in plants subjected to low water availability (Silva et al., 2012). In analysis of physiological changes in coconut, Marinho et al. (2005) found that as a result of water deficit, the starch is degraded in the tissues that accumulate due to the action of the enzyme α and β amylase. The decrease in the quantity of starch is accompanied by some increase in the amount of soluble reducing sugars and it assists in the osmotic adjustment of the plant and consequent reduction in water potential. Similar results were observed by Silva et al. (2010) checking physiological changes and drought tolerance in *Conilon coffee* clones (*Coffea canephora*), which decreased by 70% in starch content. At present, in plant tangerine and acid lime when subjected to water deficit remained constant throughout the stress, and there is a significant reduction when they were irrigated.

The increase of carbohydrates in plants under water stress (Figure 3B) occurred as a form to tolerate deficiency, which changed its osmotic adjustment process in metabolism, thus reducing their potential osmotic in order to maintain the hydrated plant, preventing dehydration of the tissues (Souza et al., 2013). According to Nogueira (2015), this increase in carbohydrate concentrations induces a greater protective of biomembranes action that can be degraded in this condition. According to Lima (2015), the increase in the concentration of carbohydrate may possibly be linked to increased abscisic acid in the leaves, and at low water conditions in the soil, increase in the relation root/shoot of the plant together with the effect of inducing ABA the closing of the stomata, helping the plant to face water stress by decreasing their photosynthetic capacity, which possibly reflects a lower accumulation of starch and can signal the need for increased levels of sucrose and carbohydrates. These results corroborate those founded by Castro et al. (2007) who observed an increase of 323.15% in the concentration of soluble carbohydrates in plants of teak (*Tectona grandis* L. f.) subjected to 9 days of water deficit and with the results obtained by Rivas et al. (2013) showing increases in concentration of carbohydrates in *Moringa oleifera* leaves under drought for 10 days.

The possible answer to the increase of sucrose in plants under drought is probably the hydrolysis of

sucrose to hexoses release used in the osmotic adjustment processes, which can link to water molecules on the leaf to maintain the water level in leaf organ (Ashraf et al., 2011). Another possible answer is in the activation of enzymes α and β -amylase breaking starch molecules and converting it to sucrose, preventing dehydration and being a source of energy for active cells under water deficit conditions (Gaupels et al., 2011). Silva et al. (2010) observed contrasting results in *Conilon coffee* clones (*Coffea canephora*) in severe water stress condition, where the levels of starch and sucrose decreased.

Conclusion

The suspension of irrigation for 25 days was enough to change and to promote a decrease in the metabolic routes of young plants of muruci, reducing the relative water content, transpiration, stomatal conductance, photosynthetic pigments and starch, however, increasing the total soluble carbohydrate content and sucrose, indicating that this plant can tolerate some periods of water stress. These changes indicate that these plants are susceptible to soils with low water availability.

Conflict of interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors are grateful to the Grupo de Estudos da Biodiversidade em Plantas Superiores of Federal Rural University of Amazon for the collaborations of researchers. This research had financial support from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq/Brazil).

REFERENCES

- Almeida MMB, Sousa PHM, Arriaga AMC, Prado GM, Magalhães CEC, Maia GA, Lemos TLG (2011). Bioactive compounds and antioxidant activity of Fresh exotic fruits from northeastern Brazil. *Food Res. Int.* 7:2155-2159.
- Alves FM (2007). Research center. Available at: <http://www.biologia.ufrj.br/~lgmv/MAF/mtehp.html>.
- Ashraf M, Akram NA, Alqurainy F, Foolad MR (2011). Drought tolerance: roles of organic osmolytes, growth regulators, and mineral nutrients. *Adv. Agron.* 111:249-296.
- Carvalho JEU, Nascimento WMO (2008). Characterization of pyrenes and methods to accelerate of muruci seed germination Açú clone. *Rev. Bras. Frutic.* 30(3):775-781.
- Carvalho JEU, Nascimento WMO (2013). Caracterização biométrica e respostas fisiológicas de diásporos de murucizeiro a tratamentos para superação da dormência. *Rev. Bras. Frutic.* 35:704-712.
- Castro DS, Lobato AKS, Mendes FS, Oliveira Neto CF, Cunha RLM, Cavalcante PB (2010). Edible fruits in the Amazon. Belém: Paraense

- Museum Emílio Goeldi, Adolpho Ducke Collection, 7 ed.
- Cavalcante PGS (2013). Physiological responses of *Jatropha curcas* L. at mycorrhiza, phosphate fertilizer and water stress. Dissertation (Masters in agronomy- plant production). Federal University of Alagoas.
- Costa RCL (2007). Nitrate reductase activity in leaves of teak (*Tectona grandis* L. f.) underwater deficit. Braz. J. Biosci. 5:936-938.
- Dias MC, Brüggemann W (2010). Limitations of photosynthesis in *Phaseolus vulgaris* under drought stress: gas exchange, chlorophyll fluorescence and Calvin cycle enzymes. Photosynthetica 48:96-102.
- Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956). Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:305-356.
- Fu AH, Chen YN, Li WH (2010). Analysis on the change of water potential of *Populus euphratica* Oliv. And *Populus russkii* Jablunder different irrigation volumes in temperate desert zone. Chin. Sci. Bull. 55:965-972.
- Gaupels F, Kuruthukulangarakoola GT, Durner J (2011). Upstream and downstream signals of nitric oxide in pathogen defence. Curr. Opin. Plant Biol. 14:707-714.
- Hoagland DR, Arnon D (1950). The water culture method for growing plants without soil. California Agric. Exp. Station Circular 347 p.
- Kreuzwieser J, Gessler A (2010). Global climate change and tree nutrition: influence of water availability. Tree Physiol. 30:1221-34.
- Lichthenthaler HK (1987). Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. In: Colowick SP, Kaplan NO (Eds.). Methods Enzymol. 148:350-382.
- Lima EG (2015). Ecophysiological and biochemical responses in andiroba leaves (*Carapa guianensis* Aubl.) In three water regimes and suspension of stresses. Dissertation (Master of Forest Science) - Federal Rural University of Amazonia. Belém.
- Marinho FJL, Gheyi HR, Fernandes PD, Ferreira Neto M (2005). Rev. Bras. Engenharia Agrícola e Ambient. 9:370-374.
- Martinazzo EG, Perbonii AT, Oliveira PVde, Bianchii VJ, Bacarin MA (2012). Photosynthetic activity in plum plants subjected to drought and flooding. Rural Sci. 43:35-41.
- Molle FRD (2011). Changes in reserve xyloglucan metabolism in seedling *Hymenaea courbaril* L. (Hayne) Lee & Lang. submitted to water deficit. Thesis (PhD) - Department of Botany Institute of State for the Environment. São Paulo.
- Nogueira GAS (2015). Ecophysiological and biochemical responses of young plants of *Ochroma pyramidale* (Cav. ExLam) Urb submitted to water deficit and flooding. Dissertation (Master of Forest Science) - Federal Rural University of Amazonia. Belém.
- Oliveira NCF de (2010). Growth, ecophysiological and biochemical changes in seedlings of Jatobá (*Hymenaea* L.) submitted to water stress and flooding. Thesis (Doctorate in Agricultural Sciences) - Federal Rural University of Amazonia, Belém-Pará.
- Otto MSG, Vergani AR, Gonçalves AN, Vrechi A, Silva SR, Stape JL (2013). Photosynthesis, stomatal conductance and productivity of Eucalyptus clones under different soil and climatic conditions. Rev. Árvore 37(3):431-439.
- Rivas R, Oliveira MT, Santos MG (2013). Three cycles of water deficit from seed to Young plants of *Moringa oleifera* woody species improves stress tolerance. Plant Physiol. Biochem. 63:200-208.
- Siguemoto ES (2013). Nutritional composition and functional properties of murici (*Crassifolia byrsonima*) and moringa (*Moringa oleifera*) nutrition and public health. (Master's thesis in science). University of São Paulo School of Public Health, São Paulo.
- Silva EC (2008). Physiological responses of umbuzeiro (*Spondias tuberosa* Arruda) to water and salt stress. Thesis (PhD in Botany) - Rural Federal University of Pernambuco, Recife, Pernambuco.
- Silva ECA (2013). Physiological, biochemical and enzymatic responses in *Moringa oleifera* Lam seedlings. Subjected to abiotic stresses. Dissertation (Master of Forest Science) - Rural Federal University of Pernambuco. Recife.
- Silva JRR (2009). Comportamento ecofisiológico de plantas jovens de andiroba (*Carapa guianensis* Aubl.). Dissertação (Mestrado em Agronomia) - Universidade Federal Rural da Amazônia, Belém, Pará.
- Silva RTL, Oliveira Neto CF, Barbosa RRN, Costa RCL, Conceição HEO (2012). Physiological responses the plants the papaya submitted to water deficit. Nucleus 9:113-120.
- Silva VA, Antunes WC, Guimarães BLS, Paiva RMC, Silva VF, Ferrão MAG, Damatta FM, Loureiro ME (2010). Physiological response of Coffee clone Conilon sensitive to water stress tolerant grafted onto rootstock. Pesqui. Agropecu. Bras. 45:457-464.
- Slavik B (1979). Methods of studying plant water relations. New York: Springer Verlag 449 p.
- Souza LC, Siqueira JAM, Silva JLS, Coelho CCR, Neves MG, Oliveira Neto CF (2013). Osmoregulators in sorghum plants under water suspension and different levels of silicon. J. Maize Sorghum 12:1-14.
- Souza VR, Pereira PAP, Queiroz F, Borges SV, Deus JSC (2012). Determination of bioactive compounds, antioxidant activity and chemical composition of Cerrado Brazilian fruits. Food Chem. 134:381-386.
- Van Handel E (1968). Direct micro determination of sucrose. Anal. Biochem. 22:280-283.
- Wang LF (2014). Physiological and molecular responses to drought stress in rubber tree (*Hevea brasiliensis* Muell. Arg.). Plant Physiol. Biochem. 83:243-249.