Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 10, Issue, 04, pp.67502-67507, April, 2018

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

METABOLISM AND ENZYME ACTIVITY IN YOUNG PLANTS OF SOURSOP SUBMITTED TO WATER DEFICIT

[3,*]Karollyne Renata Souza Silva, [2]Tamires Borges de Oliveira, [6]Milena Pantoja de Moraes, [3]DielleThainá de França Teixeira, [5]Raimundo Thiago Lima da Silva, [2]Wander Luiz da Silva Ataide, [3]Jéssica Taynara da Silva Martins, [4]Vitor Resende do Nascimento, [1]Ismael de Jesus Matos Viégas, [1]Jackeline Araújo Mota Siqueira and [1]Candido Ferreira de Oliveira Neto

[1]Institute of Agricultural Science, Universidade Federal Rural da Amazônia, Belém city, Pará State, Brazil
[2]Forest Science Department, Universidade Federal Rural da Amazônia, Belém city, Pará State, Brazil
[3]Agronomist student at Universidade Federal Rural da Amazônia- UFRA
[4]Forestry student at Universidade Federal Rural da Amazônia- UFRA
[5]InstituteofAgricultural Science, Universidade Federal Rural da Amazônia, Capitão Poço city, Pará State, Brazil
[6]Agricultural Engineer Graduated from the, Universidade Federal Rural da Amazônia, Belém city, Pará State, Brazil

ARTICLE INFO

Article History:
Received 19th January, 2018
Received in revised form
05th February, 2018
Accepted 28th March, 2018
Published online 30th April, 2018

Key words:
Carbohydrates, metabolic parameters, Nitrogen compounds, soursop, water Suspension.

ABSTRACT

The aim was to evaluate metabolic and enzymatic activity in young plants of soursop (Annona muricata) subjected to water deficit. The experiment was conducted in a greenhouse. The experimental design was completely randomized with two water conditions: control and water deficit, with 15 repetitions, totaling 30 experimental units. The parameters analyzed were relative water content, nitrate reductase activity, glutamine synthetase, concentrations of nitrate, starch, sucrose and proline. The suspension of irrigation for 40 days provided significant changes in all parameters being sufficient to change and to promote a decrease in metabolic pathways and enzymatic of young plants of soursop, reducing the relative water content, levels of nitrate, nitrate reductase activity and glutamine synthetase, but raised the free ammonium levels, proline, sucrose and starch in the evaluated parts. Therefore, these changes indicate that these plants probably has moderate resistance to water deficit.

Citation: Karollyne Renata Souza Silva, Tamires Borges de Oliveira, Milena Pantoja de Moraes *et al*., 2018. "Metabolism and enzyme activity in young plants of soursop submitted to water Deficit", *International Journal of Current Research*, 10, (04), 67502-67507.

INTRODUCTION

The soursop *(Annona muricata* L.) is from Annonaceae family, is a fruit of high economic value, grown commercially in several countries of tropical and subtropical climate (Freitas, 2012). In Brazil, it is widely cultivated in the states of Alagoas, Bahia, Ceará, Distrito Federal, Minas Gerais, Pará, Paraíba and Pernambuco (Sacramento *et al*., 2009). It has a well developed root system, and adapts to different types of soils, but develops most strongly in sandy loam soil texture with good depth and medium fertility, water retention capacity, regular drainage and with pH in the range 5,5 to 6,5 (Manica, 1994).

Corresponding author: **Karollyne Renata Souza Silva,** Agronomist studentat Universidade Federal Rural da Amazônia-UFRA.

The water shortage stress triggers a wide variety of responses in the plant, such as changes in gene expression and cellular metabolism, decreases in growth rates and productivity as well as the inhibition of various physiological processes (Carneiro, 2011). The soursop of irrigation requirements vary from one location to another, depending on the evapotranspiration demand, water deficit in the region, soil water retention capacity, plant age and phenological phases (Pinto *et al*., 1994). According Caruso *et al*., (2009) water stress can affect the homeostasis and cause serious toxic effects in plants through complex mechanisms and may affect the photosynthetic activity more than other types of stress. As the water deficit response, plants suffer changes in the relationship between cell / water and its physiological and morphological processes influencing its ability to tolerate the adverse environmental conditions (Pimentel, 2005). The osmoregulation may be one of the responses of plants to water

deficit, which includes an increase in the concentration of solutes. Solutes such as nitrogen organic compounds together with soluble carbohydrates which are converted to starch producing an accumulation of low molecular weight substances inside the cell, decreasing the water potential and allowing the flow of water into the plant continues (Silva, 2013). The study of physiological and biochemical parameters in young plants of *Annona muricata* in different water conditions can help clear up what mechanisms are used by the species to face the low availability of water, contributing not only to a greater understanding of self-ecology of this species, but also to better understanding of acclimatization of species to water deficit. This study aims to evaluate metabolic and enzymatic activity in young plants of *Annona muricata* submitted to water deficit.

MATERIALS AND METHODS

Experimental conditions

The study was conducted at the Universidade Federal Rural da Amazônia (UFRA), Pará state, CapitãoPoço campus, Brazil. This experiment was conducted in a greenhouse under natural light, with temperature of minimum-maximum air with values of 24.5/39.1 and 53.3% and 91%, respectively.

Substrate, pots and plant nutrition

The substrate used was a mixture in the ratio of 3:1:1 (v/v/v), of black soil, chicken manure and earthworm humus, respectively. The used pots were of polyethylene in the dimensions 0.30 m X 0.30 m (height x diameter) and capacity of 20 kg. Were made correction of the levels of macro and micronutrients from the soil. As well, the soil pH, through the results of the chemical analysis of the soil made the soil laboratory of the Embrapa Eastern Amazon, applying 600 mL of complete nutrient solution (Hoagland *et al*., 1950), divided in 3 months, for each month, 200 ml of complete nutrient solution before the experiment.

Used plants

The seedlings came from the association of exporting industries of wood of Pará State (AIMEX), with 05 months after germination. The seedlings were acclimatized in a greenhouse for a period of 03 months for ambiance.

Experimental design and treatments

The experimental design was completely randomized with two water conditions (control and drought stress), with 15 repetitions, totaling 30 experimental units, where each experimental unit consisted of one plant per vase. The water suspension occurred in the 40 days period and the control plants were irrigated daily average of 400 mL of water to compensate 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 Slavick (1979):

$$LRWC = [(FM - DM)/(TM - DM)] \times 100$$

where: FM is fresh matter, TM 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.

Nitrate

For determination of nitrate, 100 mg of leaf dry matter powder was incubated with 5 ml of sterile distilled water at 100°C for 30 min, and the homogenized mixture was centrifuged at 3.000 g for 15 min at 25°C, and the supernatant was removed. The quantification of the nitrate was carried out at 410 nm in accordance with Cataldo *et al*., (1975), with KNO_3 (Sigma Chemical) as standard.

In vivo nitrate reductase activity

Nitrate reductase enzyme (E.C. 1.6.6.1) was extracted from 200 mg of leaf and root samples. and incubated in 5 mL of extraction buffer (KH_2PO4 at 0.1 M, KNO_3 at 50 mM, isopropanol at 1% (v/v) and pH 7.5) for 30 minutes at 30ºC, and all the procedures were carried out in the dark. The quantification of the enzyme activity was in accordance to the method of Hageman and Hucklesby (1971) with absorbance at 540 nm using spectrophotometer (Quimis, model Q798DP).

Free ammonium

Free ammonium was determined with 50 mg of leaf dry matter powder incubated with 5 ml of sterile distilled water at 100°C for 30 min, after the homogenized mixture was centrifuged at 2.000 *g* for 5 min at 20°C and the supernatant was removed. The quantification of free ammonium was carried out at 625 nm in accordance with Weatherburn (1967), with $(NH_4)_2SO_4$ (Sigma Chemical) as standard.

Glutamine synthetase activity

Extraction of the glutamine synthetase enzyme (E.C. 6.3.1.2) was carried out with 200 mg of leaf tissue ground in liquid nitrogen. The samples were then incubated in 5 mL of extraction mix (Tris-HCl buffer pH 7.6 containing 10 mM $MgCl_2$, 10 mM _-mercaptoethanol, 5% (w/v) PVP, and 5 mM EDTA), homogenized, centrifuged at 3.000 *g* for 10 min, and the supernatant was removed. All the procedures were carried out in the interval of 0-4°C. The quantification of the enzyme activity was carried out using the method of Kamachi *et al*., (1991) with absorbance at 540 nm, and gglutamylhydroxamate (Sigma Chemicals) was used as a standard.

Proline

Proline level was determined with 50 mg of leaf dry matter powder, which was incubated with 5 mL of sterile distilled water at 100 ºC by 30 minutes, after the homogenized was centrifuged to 2.000 g by 5 minutes at 20 ºC. Quantification of proline was carried out at 520 nm according to Bates *et al*., (1973), in which was utilized L-proline (Sigma Chemicals) as standard.

Starch

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 powder coming from leaf dry matter, in which it was incubated with 1.5 mL of solution MCW (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, in agreement with Van Handel (1968), as well as was used sucrose (Sigma chemicals) as standard.

Data analysis

Data were subjected to variance analysis and when significant differences occurred, Tukey's test at 5% level of error probability was applied. The statistical analysis was carried out with the SAS software (SAS Institute, 1996).

RESULTS AN DISCUSION

Relative water content

The relative water content was significantly affected in soursop plants under water deficit throughout the 40 days of the experiment (Fig. 1).

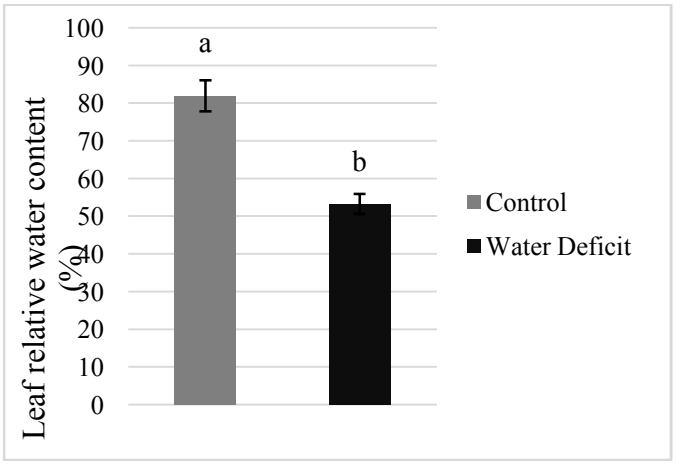

Figure 1. Relative water content in young plant leaves of Annona muricata submitted to water stress for 40 days. The letters a and b show statistically significant differences between treatments compared by Tukey test at 5% probability. The bars represent the standard deviations of the mean

With a reduction of 35% in this treatment, with a smaller amount of water assimilation (53.3%) when compared to control plants that have obtained values of 82% in its content.

Concentration of nitrate, nitrate reductase, free ammonium, glutamine synthetase

A decrease in nitrate concentrations were observed in both the root and in the leaves of plants subjected to water deficit (Fig 2A). The values found in the leaves were 0.42 and 0.09 µmoles of NO_3^-/ Kg DM in control plants and water deficit plats, respectively, with a decrease of 78.57%.

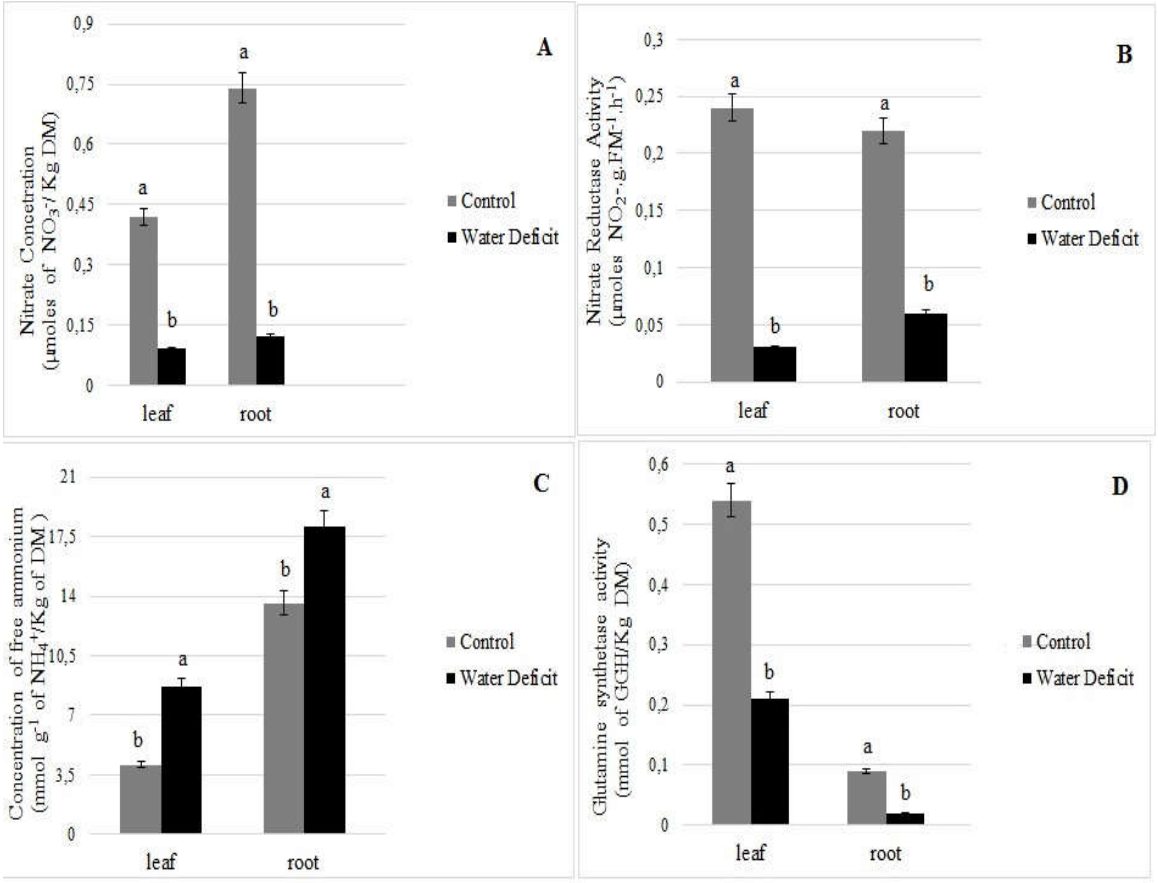

Figure 2. Concentration of nitrate (A) Reductase Activity of nitrate (B) Free Ammonium Concentration (C) Activity of Glutamine Synthase (D) in leaves and roots of young plants of Annona muricata submitted to water stress for 40 days. The letters a and b show statistically significant differences between treatments compared by Tukey test at 5% probability. The bars represent the standard deviations of the mean

To the roots were 0.74 and 0.12 µmoles of NO3-/ Kg DM in control plants and water deficit plats respectively, meaning a decrease of 45.94%. In the nitrate reductase activity (Fig 2B), a

decrease in plants subjected to water deficit was observed, the values found in the leaves were 0.24 and 0,03μmoles NO_2^- .g.$FM^{-1}h^{-1}$ in control plants and water deficit plants, respectively, with a decrease of 87.5%. To the roots were 0.22 and 0.06 μmoles NO_2^-.g.$FM^{-1}h^{-1}$ in the control plants and water deficit plants, respectively, meaning a decrease of 72.72%. In the free ammonium concentrations (Fig 2C), an increase was observed in plants subjected to water deficit, the values found in the leaves were 4.1 to 8.7 mmol $g^{-1}$of NH_4^+/Kg DM in control plants and water deficit plants, respectively, with increase of 24.86%. To the roots were 13.6 to 18.1 mmol $g^{-1}$of NH_4^+/Kg DM in control plants and water deficit plants, respectively, meaning an increase of 52.87%. The activities of glutamine synthetase decreased in plants under water deficit presenting statistical differences when compared to control plants (Fig 2D), the values found in the leaves were 13.9 and 2.5 mmol of GGH/Kg DM in the control plants and water deficit plants, respectively, with a decrease of 82%. To the roots were 18.7 and 4.1 mmol of GGH / kg DM in control plants and water deficit plants, respectively, meaning a decrease of 78.07%.

Starch, sucrose and Proline concentration

A decrease was observed in the starch concentrations in the roots and leaves of plants subjected to water deficit (Fig 3A), the values found in the leaves were 0.54 and 0.21 μmol of GLU/gDM in control plants and water deficit, respectively with a reduction of 61.11%. To the roots were observed values of 0.09 and 0.02 μmol of GLU/gDM in control plants and water deficit respectively, meaning a reduction of 77.77%. Was observed an increase in sucrose concentrations both in root and in the leaves of plants subjected to water deficit (Fig 3B).The values found in the leaves were 14 and 19 mg sucrose and g^{-1}DM in control plants and water deficit respectively, meaning an increase of 46.34%. To the roots were found values of 4.4 and 8.2 mg sucrose and g^{-1} DM in control plants and water deficit respectively, being an increase of 26.31%. Proline concentrations have increased in plants under water deficit presenting statistical differences when compared to control plants (Fig 3C), the values found in the leaves were 3.98 and 8.91 μmol of Pro / g DM in control plants and water deficit respectively, with an increase of 55.33%. To the roots were observed values of 2.99 and 6.56 μmol of Pro / g DM in control plants and water deficit respectively, meaning an increase of 54.42%.

Occurred a decrease in relative water content in plants under water deficit. It can be due to the lesser amount of water in the substrate (Fig 1). This reduction is due to the lower availability of water in the soil, interfering on the formation of a concentration gradient, reducing water absorption by the plant. Thereby, the hydraulic conductivity of the roots is limited, occurring inhibition of metabolic activity and reducing the production ATP, compromising root growth and changes in development and physiological and metabolic processes of the plant (Alves, 2010). Maltarolo *et al*., (2015) working with two water regimes in Noni plants observed in their leaf tissue a reduction of 33.7% in relative water content when subjected to 10 days of water suspension. The reductions in the concentrations of nitrate (Fig 2A) and nitrate reductase activity (Fig 2B) suggested that under water stress conditions in the soil may have caused a reduction in soil nitrate uptake by roots which possibly cause decrease of the transport this to top area through the transpiration stream (Alves, 2010). This dependence has been associated with a reduction in the concentration of NO_3^- ion, the substrate of nitrate reductase enzyme, toward the active sites of the enzyme, or even to a direct effect of reduction of tissue water potential, caused by one stress, which affects nitrogen metabolism on the activity or induction of reductase synthesis (Oliveira Neto, 2010). Results found by Pereira *et al*., (2013), when working with two pepper cultivars found that there was a decrease in nitrate concentration in water deficit treatments. Water deficit increased significantly the free ammonium concentrations (Fig 2C), due to the possible reduction of photosynthesis, because the NH_4^+ maintains nitrogen metabolism by the power supply, according to Lobo *et al*., (2011). For Cruz *et al*., (2008), this increase in free ammonium levels may be linked to the increase of the enzyme activity of GS-GOGAT-GDH system.

This, responsible for the assimilation of this ion, promoting an accumulation of ammonium and the reduction of activity of the enzyme glutamine synthetase (Fig 2D), apart from that the retention of significant part of ammonium in the roots is related to their ability to withstand higher ammonium concentrations than the leaves. This work presented line with Carvalho (2012), when working with young plants of Rubber (*Hevea*spp) in a greenhouse, observed increase in ammonium concentration both in the leaves and in the rubber tree roots, as the intensity of water stress time was increasing. For Freitas (2014), the low water content in the tissue of the plant caused by soil water deficiency affected negatively the activity of glutamine synthetase (GS) (Fig 2D). Possibly, the reduction in activity of this enzyme is linked to decrease in photosynthesis, since the reducing power and ATP synthesized during the photochemical step influence in the assimilation of NO_3^-, NH_4^+, and the amino acids inside the chloroplasts. Similar results were found by Nogueira (2015) showed that the reduction in GS activity in roots and leaves of young plants of balsawood (*Ochromapyramidale*) under water deficit. The starch reductions in roots and leaves may be linked to a decrease in photosynthesis and starch degradation through the α and β-amylase enzymes, forming new sugars such as sucrose, increasing concentration of sucrose (Fig 3B), with in order to osmotically adjust and inactivation of key enzyme in starch synthesis (ADP-glucose pyrophosphorylase) (Silva *et al*., 2012). Another answer to this decrease may possibly be associated with an increased abscisic acid in the leaves that provides the increase in root/top area relation and the closing of the stomata, helping the plant to face water deficit (Souza, 2012), reducing their photosynthetic ability and the lowest accumulation of starch in the plant.Results corroborate those found are from Silva *et al*., (2012) when submitted *Jatropha curcas* to water deficit noted the significant increase reaching values of 200% in plants with water scarcity, compared to control plants.

The sucrose in plants under water deficit (Fig 3B), possibly increased by these are suffering hydrolysis and therefore releasing hexoses to be used in the adaptation process, can connect to water molecules on the leaf in order to maintain the level of water the leaf organ (Nogueira, 2015).According Ashraf *et al*., (2011), this defense mechanism is due to the accumulation in the cytosol or in the vacuole, of compatible solutes such as sucrose to help maintain the water balance and preservation of the integrity of proteins, enzymes and cell membranes. Similar results were obtained by Lima *et al*., (2015) that when working with *Carapaguianensis* Aubl., also had higher concentrations in plants subjected to water deficit.

Proline accumulation increased due to the low water content in leaf tissue of plant, demonstrating that this amino acid has the function of adjusting the osmotic and / or indicator of stress in the species under study, which is a feature of interest in plants resistant to drought. Occurs thus protecting the membranes from the harmful effects caused by reactive oxygen species (ROS), thus preventing denaturation of proteins, while preserving the structure of enzymes (Sharma *et al*., 2005). The increase in the concentration of this osmolyte in plants under water deficit could be attributed to the activity of enzymes responsible for the synthesis of this amino acid (Maia *et al*., 2007). Pereira *et al*., (2012) working with seedlings of *Arachishypogaea* found an increase in proline content in root tissue.

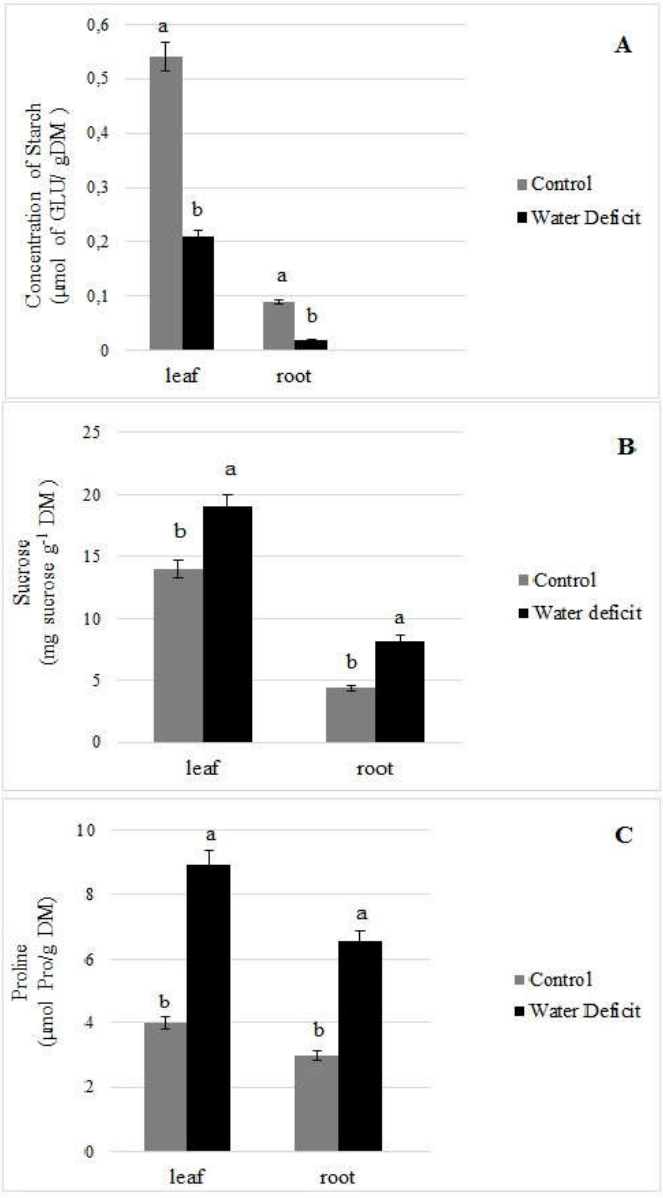

Figure 3. Concentration of Starch (A) Sucrose (B) Proline (C)in leaves and roots of young plants of Annona muricata submitted to water stress for 40 days. The letters a and b show statistically significant differences between treatments compared by Tukey test at 5% probability. The bars represent the standard deviations of the mean

Conclusion

Young plants of soursop subject to 40 days of water deficit showed significant changes in all parameters, reducing the relative water content, levels of nitrate, nitrate reductase activity and glutamine synthetase, but raised the levels of free ammonium, proline, sucrose and starch in the evaluated parties indicating that this plant probably has moderate water deficit resistance

Acknowledgements

The authors are grateful to the Universidade Federal Rural da Amazônia for the financial support of this work and collaborations of researchers participating in the Biodiversity Study Group in Higher Plants.

REFERENCES

Alves, G.A.R. 2010. Aspectos ecofisiológicos, bioquímicos e crescimento de plantas jovens de ipê-amarelo (*Tabebuia serratifolia* (Vahl) Nicholson) em condições de déficit hídrico e alagamento. 73 f. Tese (Doutorado em Ciências Agrárias/Agroecosistemas da Amazônia). Universidade Federal Rural da Amazônia/Embrapa. Belém.

Ashraf, M., Akram, N.A., Alqurainy, F. and Foolad, M.R. 2011. Drought tolerance: roles of organic osmolytes, growth regulators, and mineral nutrients. *Advances in Agronomy*, 111:249-296.

Bates, L.S., Waldren, R.P. and Teare, I.D. 1973. Rapid determination of free proline for water-stress studies. Short communication. *Plant and Soil*, V. 39: 205-207.

Carneiro, M.M.L.C. 201. Gas exchange and antioxidant metabolism in sunflower plants in response to water deficit. (Dissertação de Mestrado) - Universidade Federal de Pelotas. 42p.

Caruso, G., Cavaliere, C., Foglia, P., Gubbiotti, R., Guarino, C., Samperi, R. and Lagana, A. 2009. Analysis of drought responsive prtoteins in wheat (Triticumdurum) by 2D-PAGE and MADITOF massspectrometry. *Plant Science, Amsterdam*, 177:570-576.

Carvalho, P.A. 2012. Nitrogen metabolism and antioxidant in young rubber tree (Heveaspp) subjected to low oxygen availability in the presence of nitrate and / or ammonium. (Dissertação de Mestrado) - Universidade Federal de Lavras. 76p.

Cataldo, D.A., Haroon, S.L.E. and Yougs, V.L. 1975. Rpid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Commum Soil Science Plant Analyse*, 6:71-80.

Cruz, J.L., Pelacani, C.R. and Araújo, W.L. 2008. Nitrate and ammonium effect on growth and nitrogen utilization efficiency in mandioca. Ciência Rural, Santa Maria. 38:643-649.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry. 28:305-356.

Freitas, A.L.G.E. 2012. Characterization of production and market of Soursop (*Annona muricata* L.) in the State of Bahia. Dissertação (Mestrado em agronomia), specialization in Plant Science. Universidade Estadual do Sudoeste da Bahia.

Freitas, J.M.N. 2014. Ecophysiological and biochemical behavior of young plants of acapu (*American Vouacapoua*Aubl.) submitted to water deficit. Thesis (Doctorate in Agricultural Sciences) -Universidade Federal Rural da Amazônia.

Hageman, R.H.G., Hucklesby, D.P. 1971. Nitrate reductase from higher plants. In: *Methods in enzimology*, 17:491-503.

Hoagland, D.R. and Arnon, D.I. 1950. The water culture method for growing plants without soil. California Agricultural Experiment Station, Circular. p.347.

Kamachi, K., Yamaya, T., Mae, T. and Ojima, KA. 1991. Role for glutamine synthetase in remobilization of leaf nitrogen during natural senescence in rice leaves. PlantPhysiology. 96:411-417.

Lima, E.G.S., Oliveira, T.B., Conceição, S.S., Ataide, W.L.S., Maltarolo, B.M., Nogueira, G.A.S., Oliveira, Neto CFDE., Costa, R.C.L. and Okumura, R.S. 2015) Biochemical and physiological responses in andiroba (*Carapaguianensis*Aubl.) seedlings subjected to water deficit. *Australian Journal of Crop Science,* (Online). 9:517-522.

Lobo, A.K.M., Martins, M.O., Neto, M.C.L., Bonifácio, A. and Silveira, J.A.G. 2011. Nitrogen compounds and carbohydrates in sorghum under salinity and nitrate and ammonium combinations. *Rev. Ciênc. Agron*, 42:2.

Maia, PSP., Oliveira Neto, CF., Castro, DS., Freitas, JMN., Lobato, AKS. and Costa, RCL. 2007. Relative water content, proline content and total soluble carbohydrates in leaves of two maize cultivars under water stress. *Revista Brasileira de Biociências*, 5:918-920.

Maltarolo, B.M., Teixeira, D.T.F., Ataide, W. L.S., Nogueira, G.A.S. and Oliveira Neto, C. F. 2015. Biometric Evaluation and carbon metabolism in young noni plants subjected to water deficit. *Enciclopédia Biosfera*, 11:251-265.

Manica, Ivo. 1994. Fruit growing - cultivation of Annonaceae: ata - Cherimólia - soursop. Porto Alegre: EVANGRAF. 117p.

Nogueira, G.A.S. 2015. Ecophysiological and biochemical responses of young plants of *Ochromapyramidale* (Cav. Ex Lam) Urb. submitted to water deficit and flooding. Dissertation (Master of Forest Science) – Universidade Federal Rural da Amazônia, 65 f.

Oliveira Neto, C.F. 2010. Growth, ecophysiological and biochemical changes in seedlings of Jatoba (*Hymenaea L.*) submitted to water deficit and flooding. Thesis (PhD in agricultural sciences) - Universidade Federal Rural da Amazônia. Pará. Belém.

Pereira, J.W.L., Melo Filho, P.A., Albuquerque, M.B., Nogueira, R.J.M.C. and Santos, R.C. 2012. Biochemical changes in peanutgenotypesundermoderate water deficit. Revista Ciência Agronômica, Fortaleza. 43:766-773.

Pereira, T.S., Lobato, A.K.S., Tan, D.K.Y., Costa, D.V., Uchoa, E.B., Lobato, M.C., Ferreira, R.N., Pereira, E.S., Avila, F.W., Marques, D.J. and Guedes, E.M.S. 2013. Positive interference of silicon on water relations, nitrogen metabolism, and osmotic adjustment in two pepper cultivars under water deficit. *Aust j crop sci*, 7:1064-1071.

Pimentel, C. 200. Physiological responses to water shortage: diffusive or metabolic limitation? In: NOGUEIRA, R. J. M. C; ARAUJO, E. DE L; WILLADINO, L. G; CAVALCANTE, U. M. T. Environmental stresses: harms and benefits in plants. Recife: UFRPE. 13-21.

Pinto, A.C.Q. and Silva, E.M. 1994. Soursop for export: technical aspects of production. Brasília: EMBRAPA-SPI. 41 p.

Sacramento, C.K., Moura, J.I.L. and Coelho Junior, E. 2009. Graviola. In: SANTOS-SEREJO, J. A. (eds.). Tropical fruitproduction: regional andexoticspecies. Brasília, DF: Embrapa Informação Tecnológica. 95-132.

Sharma, P. and Dubey, R.S. 2005. Modulation of nitrate reductase activity in rice seedlings under aluminium toxicity and water stress: role of osmolytes as enzyme protectant. Journal of Plant Physiology. 162:854-864.

Silva, E.C.A. da. 2013. Physiological, biochemical and enzymatic responses in *Moringaoleifera* Lam seedlings. Subjected to abiotic stresses. 98 f. Dissertation (Master of Forest Science) - Rural Federal University of Pernambuco. Recife.

Silva, E.N., Ribeiro, R.V., Silva, S.L.F., Vieira, S.A., Ponte, L.F.A. and Silveira, J.A.G. 2012. Coordinate changes in photosynthesis, sugar accumulation and antioxidative enzymes improve the performance of *Jatropha curcas* plants under drought stress. biomass and bioenergy. 45:270- 279.

Slavick, B. 1979. Methods of studying plant water relations. New York: Springer Verlang. p.449.

Souza, T.C. 2012. Abscisic acid application (ABA) and physiological and anatomical characterization of two hybrids of diverse maize when drought stress. Thesis – Universidade Federal de Lavras, 133.

Van Handel, E. 1968. Direct micro determination of sucrose. *Analytical Biochemistry*, 22:280-283.

Weatherburn, M.W. 1967. Phenol hipochlorite reaction for determination of ammonia. *Analytical Chemistry*, 39:971-974.

****