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Carbon and nitrogen metabolism in young Tachigali vulgaris plants subjected to water deficit

Metabolismo do carbono e do nitrogênio em plantas jovens de Tachigali vulgaris submetidas à deficiência hídrica

Metabolismo del carbono y nitrógeno en plantas jóvenes de Tachigali vulgaris sometidas a deficiencia de agua

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Abstract

Plants' biochemical responses to water deficit are associated with their ability to synthesize accumulate osmolytes compatible to regulatory properties of water potential. The aim of the current study is to evaluate carbon and nitrogen metabolism in Tachigali vulgaris plants subjected to three water suspension periods. The experiment was carried out in greenhouse and followed a completely randomized design, at 3 x 2 factorial arrangement (three times: zero, five and ten water suspension days; and two water conditions: control and water deficit), with 4 repetitions; results were subjected to analysis of variance and means were compared through t-test at 5% probability level, in statistical package (Assistat 7.7 beta). The following variables recorded decreased values: relative water content RWC in leaf tissue (by 32.14%); nitrate ion in leaves (by 18.67%) and in roots (by 14.40%); nitrate reductase enzyme activity in leaves (by 17.06%) and roots (by 15.77%); starch concentration in leaf tissue (by 44.98%) and roots (by 21.07%). On the other hand, the following variables recorded increased values: free ammonium concentration in leaves (by 64.83%) and roots (by 1.61%); total soluble amino acids in leaf tissue (by 28.03%) and roots (by 8.42%); total soluble carbohydrates in leaves (by 3.12%) and roots (by 11.05%); sucrose in leaves (by 4.77%) and roots (by 24.77%); proline in leaves (by 193.58%) and roots (by 57.26%). Biochemical processes observed in T. vulgaris plants were affected by water deficit, which indicated that this species is capable of adopting mechanisms and strategies in order to survive under stressful conditions.

Keywords: Adjustment; Biochemistry; Synthesis; Osmolytes.

Resumo

As respostas bioquímicas das plantas ao déficit hídrico estão relacionadas à capacidade de síntese e acúmulo de osmólitos compatíveis com propriedades regulatórias do potencial hídrico celular. Objetivou-se avaliar o metabolismo do carbono e do nitrogênio em plantas de Tachigali vulgaris sob três períodos de suspensão hídrica. O experimento foi conduzido em casa de vegetação utilizando-se delineamento inteiramente casualizado em esquema fatorial 3x2 (três tempos: zero, cinco e dez dias de suspensão hídrica, e duas condições hídricas: controle e deficiência hídrica), com 4 repetições, aplicando-se a análise de variância nos resultados e as médias foram comparadas pelo teste Tukey ao nível de 5% de probabilidade com o pacote estatístico (Assistat 7.7 beta). Houve redução para as respectivas variáveis, CRA em 32,14% no tecido foliar; íon nitrato em 18,67% nas folhas e 14,40% nas raízes; atividade da enzima redutase do nitrato em 17,06% nas folhas e 15,77% nas raízes; concentração de amido em 44,98% no tecido foliar e 21,07% na raiz. E aumento para as seguintes variáveis, concentração de amônio livre em 64,83% nas folhas e 1,61% na raiz; aminoácidos solúveis totais em 28,03% para o tecido foliar e 8,42% nas raízes; carboidratos solúveis totais em 3,12% nas folhas e 11,05% nas raízes; sacarose em 4,77% nas folhas e 24,77% nas raízes; prolina 193,58% nas folhas e 57,26% na raiz. Os processos bioquímicos foram fortemente afetados pela deficiência hídrica, expressando mecanismos e estratégias que permitem a sobrevivência da espécie em condições de estresse.

Palavras-chave: Ajustamento; Bioquímica; Síntese; Osmólitos.

Resumen

Las respuestas bioquímicas de las plantas al déficit hídrico están relacionadas con la capacidad de síntesis y acumulación de osmolitos compatibles con propiedades reguladoras del potencial hídrico celular. El objetivo fue evaluar el metabolismo del carbono y nitrógeno en plantas de Tachigali vulgaris bajo tres períodos de suspensión en agua. El experimento se realizó en invernadero con un diseño completamente al azar en un esquema factorial 3x2 (tres tiempos: cero, cinco y diez días de suspensión de agua, y dos condiciones de agua: control y deficiencia de agua), con 4 repeticiones, aplicando el análisis de varianza en los resultados y las medias se compararon mediante la prueba de Tukey al nivel de 5% de probabilidad con el paquete estadístico (Assistat 7.7 beta). Hubo una reducción para las respectivas variables, CRA en 32.14% en el tejido foliar; ion nitrato en 18,67% en las hojas y 14,40% en las raíces; actividad de la enzima nitrato reductasa en 17,06% en hojas y 15,77% en raíces; concentración de almidón en 44,98% en tejido foliar y 21,07% en raíz. Y aumentar para las

siguientes variables, concentración de amonio libre en 64.83% en las hojas y 1.61% en la raíz; aminoácidos solubles totales en 28.03% para el tejido foliar y 8.42% en las raíces; carbohidratos solubles totales en 3.12% en las hojas y 11.05% en las raíces; sacarosa en 4,77% en hojas y 24,77% en raíces; prolina 193,58% en hojas y 57,26% en raíz. Los procesos bioquímicos se vieron fuertemente afectados por la deficiencia de agua, expresando mecanismos y estrategias que permiten a la especie sobrevivir en condiciones de estrés.

Palabras clave: Ajuste; Bioquímica; Síntesis; Osmolitos.

1. Introduction

Water plays an essential role in soil nutrient absorption by plants. Climate changes caused by natural or man-made factors have led to periods of severe and continuous water deficit, which, in their turn, have negatively affected the environment and plant production (Coates et al., 2011). Thus, it is essential investigating plants' tolerance to water deficit in order to enable crop yield under the aforementioned conditions (Damatta, et al., 2010; Menezes-Silva, et al., 2017).

Tachigali vulgaris - popularly known as Carvoeiro - is a tree species belonging to family Fabaceae, which naturally grows in Northern Brazil; this pioneer species colonizes lands and roadsides, as well as starts its secondary succession in open areas due to intense seed germination in the soil. Some features, such as good biomass production and rapid growth, the ability to bond to nitrogen-fixing bacteria belonging to genus Rizhobium and rapid restoration in degraded areas, are inherent to the study about this species under adverse conditions; therefore, it has been recommended for reforestation programs (Abreu, et al., 2017).

It is essential physiologically and biochemically investigating plants' tolerance to drought to help better understanding the intrinsic mechanisms enabling their adaptation to water deficit. Some mechanisms work alone or together, such as combinations of molecular events that can be activated by plants' perception of stress signals (Bianchi, et al., 2016). One of these events may be associated with nitrogen and carbon metabolism, which substantially depends on water availability in plants, since the activity of some enzymes is extremely sensitive to water deficit. Consequently, plants present explicit quantitative changes in proline content, which is a multifunctional amino acid highly correlated to the osmotic adjustment and protein stabilization observed in leaf and root tissues during water deficit periods.

Therefore, the aim of the current study was to evaluate biochemical parameters

capable of responding to water deficit conditions, as well as of enabling a tolerance condition in terms of carbon and nitrogen metabolism in Tachigali vulgaris plants.

2. Metodologia

2.1. The experiment

The experiment was carried out in greenhouse at the Laboratory for Biodiversity Studies about Superior Plants EBPS/UFRA, from April to August of 2015. Plants were transplanted into polyethylene pots (4.5 kg substrate capacity) and left to acclimate for two months.

Hoagland & Arnon's (1950) nutrient solution was used to supply plants' nutrition deficiencies. Four-month-old seedlings were purchased at Pará State Association of Wood Exporting Industries - AIMEX. The batch of seedlings was formed by seeds deriving from Alta Floresta County, Mato Grosso State.

2.2. Experimental design and statistical analysis

The study followed a completely randomized experimental design (CRD), at split-plot arrangement (three times: zero, five and ten water suspension days; and two water conditions: control and water deficit), with 4 repetitions, thus totaling 24 experimental units (one plant/pot).

Results were subjected to analysis of variance; whenever there was significant difference, means were compared to each other through t-test, at 5% probability level, in statistical package (Assistat 7.7 beta, 2015).

Relative water content (RWC) was determined between 05:00 a.m. and 06:00 a.m. in each collection time, when leaf discs were cut for its immediate determination, based on the method described by Slavick (1979). Next, the discs were placed in paper bags and taken to oven (70 ºC) for 24:00 h in order to have their dry mass (DM) determined.

$$\mathbf{RWC = (FM1 - DM) / (FM2 - DM) \times 100}$$

2.3. Biochemical Variables

2.3.1. Nitrate concentrations

Fifty (50) mg of previously lyophilized leaves and roots were weighed, added to test tubes (filled with 5.0 mL of distilled water) and incubated in water bath at 100ºC, for 30 minutes. Nitrate concentration was measured based on standard curve comprising increasing NO_3^- concentrations (0, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 µmol mL^{-1}). Results were expressed in mmol of NO_3^-. Kg^{-1} DM of tissue, based on the method by Cataldo, et al., (1975).

2.3.2. Nitrate reductase activity (EC 1.6.6.1)

Approximately 200 mg of root and leaf discs (0.5 cm diameter) were weighed. Samples were placed in test tubes (filled with 5 mL of 0.1 M phosphate buffer (pH = 7.5) comprising 1% isopropanol (v/v), KNO_3 (mM)) covered with aluminum foil (dark treatment).

Nitrate reductase activity was estimated based on NO_2^- production in reaction medium; results were expressed in µmols of NO_2^-. g. MF^{-1}. h^{-1}, based on standard curve generated with KNO_2 p.a (Sigma), according to the method *in vivo* recommended by Hageman & Hucklesby (1971).

2.3.3. Free Ammonium concentrations

Fifty (50) mg of root and powdered leaf dry mass (DM) were weighed, placed in 15-mL test tubes (filled with 5 mL of distilled water) and subjected to water bath at 100 ºC for 30 min. Free ammonium concentrations were estimated based on the standard curve generated with $(NH_4)_2SO_4$ p.a (Sigma). Results were expressed in mmol NH_4^+/Kg of DM, based on the method described by Weatherburn (1967).

2.3.4. Total soluble Amino Acid concentrations

Fifty (50) mg of leaves and roots were weighed, placed in test tubes filled with 5 mL of deionized water, hermetically closed and incubated in water bath at 100 °C for 30 minutes. Total free amino acid (TFAA) concentration was determined based on the method described by Peoples, et al., (1989).

2.3.5. Starch concentrations

The method by Dubois, et al., (1956) was used to determine starch concentrations; two ethanolic extractions of 50 mg of leaf dry mass were performed: the first one used 5.0 mL of 80% ethanol, at 80 ºC, for 30 min; and the second one used 5.0 mL of 30% $HClO_4$, at 25 ºC, for 30 minutes.

2.3.6. Total Soluble Carbohydrate concentrations

Total soluble carbohydrate (TSC) concentration was determined based on the colorimetric method described by Dubois, et al., (1956), which was modified as follows: Plant samples were homogenized in 5 ml of distilled water and the resulting homogenate was incubated at 100 ºC, for 30 minutes.

2.3.7. Sucrose concentrations

Sucrose concentration was determined based on the method by Van Handel (1968), with some modifications. Samples were macerated in 1.5 mL of MCW (methanol: chloroform: water 12:5:3, v/v/v) and stirred for 20 minutes. The homogenate was centrifuged at 500 g, at room temperature, for 30 minutes, to enable supernatant collection. The extraction process was repeated twice in a row and supernatants were collected in order to have their final volume determined. After cooling, samples' absorbance (ABS) was determined at 620 nm in spectrophotometer (Genesys TM10 series, Thermo Electron Co., Wisconsin, USA).

2.3.8. Proline concentrations

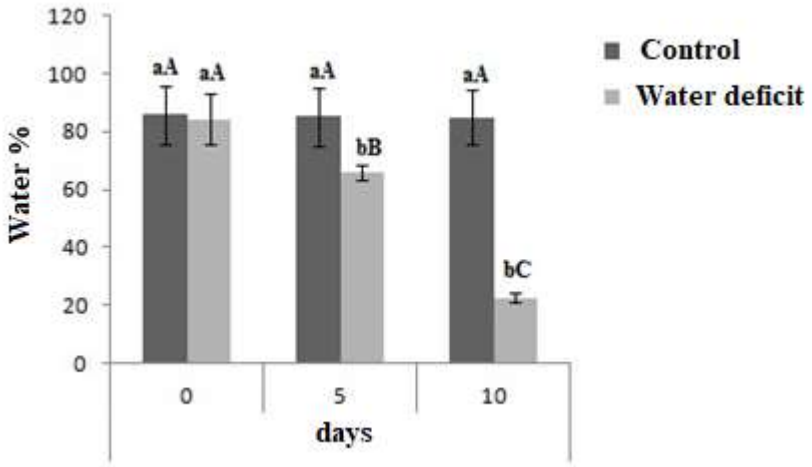
Proline concentration was determined based on Bates, et al., (1973). Hot extraction was performed in water bath at 100 °C, for 30 min; samples were homogenized in 5 mL of distilled water. The supernatant was collected after centrifugation at 700 g, for 20 min; a 1-mL aliquot of it was used for proline quantification, based on the addition of 1 mL of acid ninhydrin and 1 mL of 99.5% glacial acetic acid. Proline concentration was determined by means of a proline calibration curve; results were expressed in mmoL proline g^{-1} of dry matter (DM).

3. Results

3.1. Relative Water content

Relative water content (Figure 1) has shown statistical difference from the fifth experimental day on – control and water deficit treatments recorded water content means in leaf tissue equal to 85.31% and 66.07%, respectively – to the detriment of water decrease in the soil. The last data collection point recorded significant RWC reduction by 84.81% for the control treatment and by 22.67% for the water deficit treatment, which corresponded to water content reduction by 32.14% in leaf tissue.

Figure 1. Relative Water Content (A) in *Tachigali vulgaris* leaves, based on water deficit in three evaluation times. Belém (PA), 2015.



Means followed by the same letter did not statistically differ from each other at 5% probability level. Lowercase letters compare values between water regimes, whereas uppercase letters compare values throughout the experiment. Bars represent the standard deviation from the means. Source: Authors.

3.2. Nitrate concentrations

There was statistical difference in factors interacting in leaf nitrate concentration (Figure 2A) at the herein defined probability level; nitrate concentration values decreased from 0.1843 to 0.1433 µmol of NO_3^-/mg DM in the control and water deficit treatments on the fifth experimental day, as well as from 0.1893 to 0.1283 µmol of NO_3^-/mg DM on the tenth day, respectively – this outcome represents nitrate concentration decrease by 18.67%. Root results were only significant in treatment 2 (water condition); nitrate concentration decreased

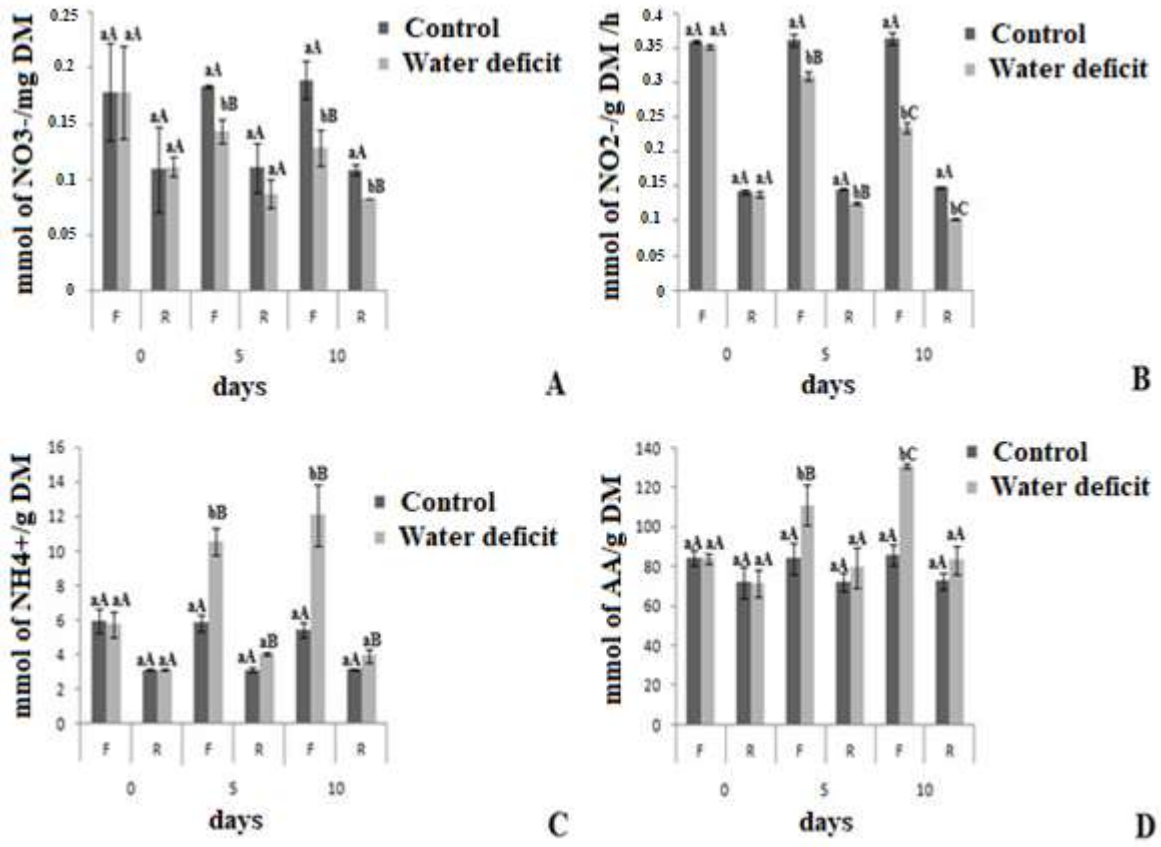
from 0.1097 µmol of NO_3^-/mg DM in the control group to 0.0939 µmol of NO_3^-/mg DM in the water deficit group, which corresponded to root nitrate concentration decrease by 14.40%.

3.3. Nitrate reductase activity

Nitrate reductase activity in leaf tissue (Figure 2B) recorded statistical difference between the water deficit (0.3098 µmol of NO_2^-/g FM/h) and control groups (0.3616 µmol of NO_2^-/g FM/h) at 5% probability level, from the fifth day on. This enzyme recorded the highest activity decrease (Figure 2B) on the tenth day - from 0.3618 µmol of NO_2^-/g FM/h in the control group to 0.2355 µmol of NO_2^-/g FM/h in the water deficit group, which represented enzyme activity decrease by 17.06% throughout the experiment.

Nitrate reductase activity in root tissue also recorded significant difference from the fifth day of water suspension on; values decreased from 0.1461 µmol of NO_2^-/g FM/h in the control group to 0.1261 µmol of NO_2^-/g FM/h in the water deficit group. Nitrate reductase activity decreased from 0.1490 µmol of NO_2^-/g FM/h in the control group to 0.1033 µmol of NO_2^-/g FM/h in the water deficit group in the last collection point; this outcome represented enzyme activity decrease by 15.77%.

Figure 2. Nitrate (A), Nitrate Reductase (B), Ammonium (C) and Amino Acids (D) concentrations in young *Tachigali vulgaris* plants, based on water deficit in three evaluation times. Belém (PA), 2015.



Means followed by the same letter did not statistically differ from each other at 5%probability level. Lowercase letters compare values between water regimes, whereas uppercase letters compare values throughout the experiment. Bars represent the standard deviation from the means. Source: Authors.

3.4. Free Ammonium concentrations

The control and water deficit groups recorded statistically significant difference in mean free ammonium concentration at 5% probability level, after the fifth experimental day; free ammonium concentration (Figure 2C) in leaf tissues increased from 5.86 to 10.58 µmol of NH_4^+/g DM in the control group and from 5.43 to 12.07 µmol of NH_4^+/g of DM in the water deficit group, at the tenth experimental day, which represented ion increase in the order of 64.83%. Root tissues also recorded statistically significant difference in free ammonium concentration between both treatments after the fifth experimental day; values increased from 3.14 to 4.05 µmol of NH_4^+/g of DM in the control group and from 3.17 to 3.93 µmol NH_4^+/g

DM in the water deficit group, which represented free ammonium concentration increase by 1.61%.

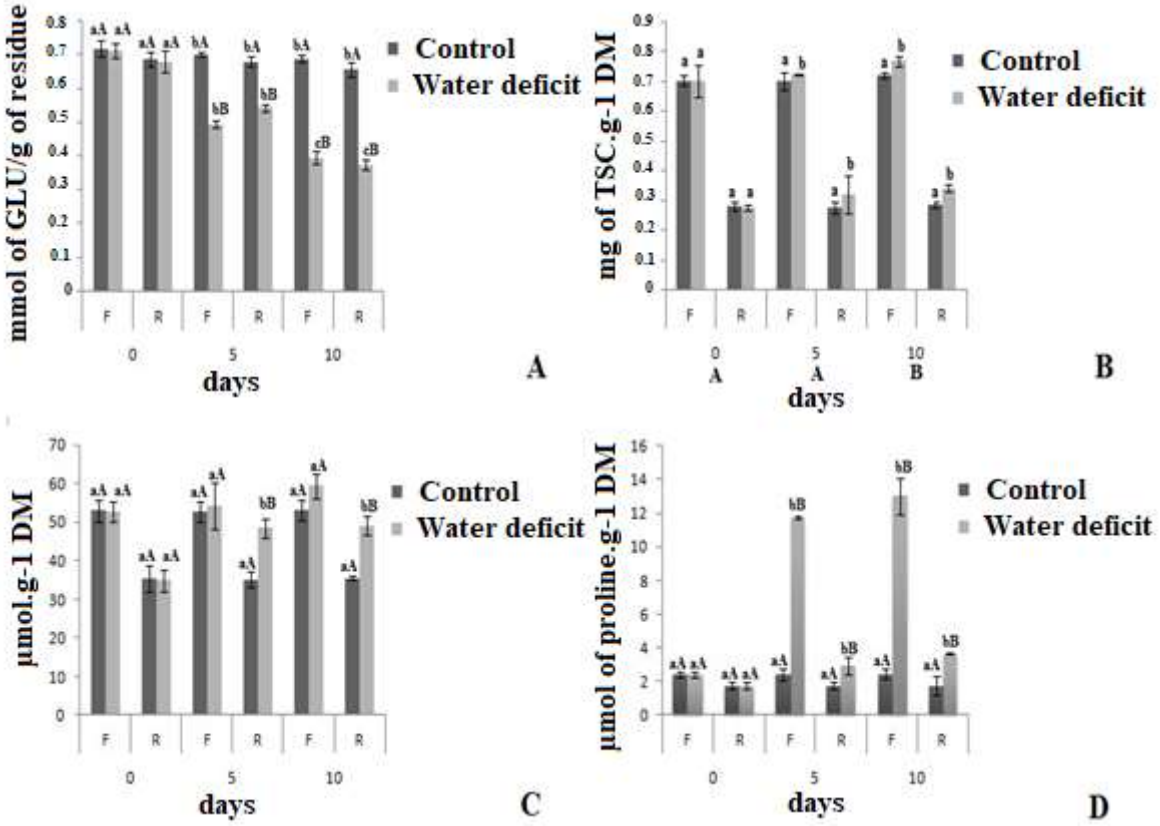
3.5. Total soluble Amino Acid concentrations

Total soluble amino acid concentration in leaf tissues (Figure 2D) recorded statistically significant difference at 5% probability level; values increased from 84.09 to 111.96 µmol of AA/g of DM from the fifth experimental day on and presented further increase to 130.74 µmol of AA/g of DM at the tenth day, which represented total soluble amino acid concentration increase by 28.03%. There was also statistically significant difference in total soluble amino acid concentration in root tissue; values increased from 72.38 to 79.48 µmol of AA/g of DM at the fifth day and from 72.77 to 83.51 µmol of AA/g of DM at the tenth day, in the control and water deficit treatments, respectively, which represented total soluble amino acid concentration increase by 8.42%.

3.6. Starch concentrations

Starch concentrations in leaf tissues (Figure 3A) recorded statistically significant differences from the fifth experimental day on; values decreased from 0.7003 mmol of GLU/g of residue in the control group to 0.4948 mmol of GLU/g of residue in the water deficit group and further decreased from 0.6868 to 0.3958 mmol GLU/g of residue in leaf tissue at the tenth experimental day, which represented starch concentration decrease by 44.98%. Similar behavior was observed for root tissues; starch concentration decreased from 0.641 to 0.542 mmol of GLU/g of residue at the fifth experimental day and from 0.643 to 0.373 mmol of GLU/g of residue at the tenth day, which represented starch concentration decrease by 21.07%.

Figure 3. Starch (A), Carbohydrate (B), Sucrose (C) and Proline (D) concentrations in young *Tachigali vulgaris* plants, based on water deficit in three evaluation times. Belém (PA), 2015.



Means followed by the same letter did not statistically differ from each other at 5% probability level. Lowercase letters compare values between water regimes, whereas uppercase letters compare values throughout the experiment. Bars represent the standard deviation from the means. Source: Authors.

3.7. Total soluble Carbohydrate concentrations

There was no statistically significant difference in the interaction of factors in total soluble carbohydrate concentration (Figure 3B) in leaf tissues at 5% probability level, except for treatment 2 (water condition), which recorded mean total soluble carbohydrate concentration of 0.7080 mg of TSC.g^{-1} DM in the control group and 0.7301 mg of TSC.g^{-1} DM in the water deficiency group, which represented total soluble carbohydrate concentration increase by 3.12%. Similar behavior was observed for root tissues, which only recorded statistically significant difference at 5% probability level for treatment 2 (water condition), mean total soluble carbohydrate concentration reached 0.2822 mg of TSC.g^{-1} DM in the control and 0.3134 mg of TSC.g^{-1} DM in the water deficit groups, which represented total soluble carbohydrate concentration increase by 11.05%.

3.8. Sucrose concentrations

Sucrose concentration (Figure 3C) in leaves did not statistically differ among the three observation points at 5% probability level; however, there was absolute difference in sucrose concentration values between treatments; mean concentration increased from 52.8 mg of sucrose/mg DM to 54.36 mg of sucrose/mg DM at the fifth experimental day and further increased to 59.4 mg of sucrose/mg DM at the tenth day, which represented quantitative sucrose concentration increase by 4.77%. Sucrose concentration in root tissues increased from 35.12 to 48.42 mg of sucrose/mg DM from the fifth experimental day on and further increased to 49.08 at the tenth day, which represented final sucrose concentration increase by 24.77%.

3.9. Proline concentrations

Proline concentration in leaf tissues (Figure 3D) recorded statistically significant difference between treatments from the fifth experimental day on, when values increase from 2.412 $\mu mol.g^{-1}$ DM in the control group to 11.738 $\mu mol.g^{-1}$ DM in the water deficit group, as well as at the tenth day, when values further increased from 2.426 to 13.006 $\mu mol.g^{-1}$ DM, which represented final proline concentration increase by 193.58% in plants subjected to water deficit. Proline concentration in root tissues has also shown statistically significant difference between treatments at 5% probability level, values increased from 1.761 to 2.915 $\mu mol.g^{-1}$ DM at the fifth experimental day and from 1.764 to 3.647 $\mu mol.g^{-1}$ DM at the tenth day, which represented final proline concentration increase by 57.26%.

4. Discussion

4.1. Relative Water content

The interconnection between relative water content (RWC) and plants' physiological factors results from their strong association with cell volume, which explains the positive correlation between RWC and leaf turgor potential. Plants subjected to stress, manly to water deficit, interferes in many basic metabolic processes such as transpiration - a process according to which water absorbed by plant roots is transported to leaf tissues to enable gas exchange with the atmosphere through stomata opening. Therefore, water stress has negative

influence on RWC and may indicate increased protoplast dehydration (protoplasts define the cell content comprising plasma membrane, cytoplasm, nucleus and vacuole). Consequently, leaf cell size reduces due to decrease in their liquid part, which leads to physiological damages such as leaf turgor loss (Júnior, et al., 2018).

However, the concentration of enzymes and of some compounds in plants can also change, as well as hormonal activity. There may be increase in abscisic acid (ABA) concentrations in leaves and roots under these conditions - one of the roles played by ABA lies on stomatal closure in order to reduce water loss through transpiration. Thus, leaf cells are likely to show reduced water content and, consequently, reduced water potential to prevent water loss into the atmosphere (Kerbauy, 2004).

4.2. Nitrate concentrations

Reduced water potential can lead to osmotic stress and cause nutritional imbalance and ionic homeostasis, which may explain nitrogen (N) absorption and assimilation reduction due to direct interaction between stress and N absorption and use processes taking place inside plants (Coelho, 2018). However, other mechanisms activated during this process - such as differences in nitrate (NO_3^-) and ammonium (NH_4^+) absorption and assimilation dynamics - may be involved in this nitrate content reduction process. For example, the NO_3^- assimilation process requires energy expenditure higher than that of NH_4^+, a factor that is compromised due to little photosynthetic activity and respiratory metabolism to provide energy to plants, which in their turn, demand more energy to circumvent the metabolic damage caused by low water availability in the soil. In addition, NO_3^- absorption happens in an electrochemical gradient generated through the membrane and requires H^+ energetic coupling, whereas NH_4^+ absorption can passively happen through the low affinity system, through aquaporins and cation channels, as well as through high-affinity transporters, without H^+ co-transport.

Nitrate absorption by roots decreases as transpiration rates fall; consequently, nitrate flow (via xylem) to the leaves decreases, as well. Nitrate reductase activity does not fully decrease due to the constant, although reduced, supply of nitrate deriving from the cell vacuole reserve, as seen in the current study. However, nitrate flow through the transpiratory stream can be ten times higher than the nitrate flow provided by storage organelles such as the vacuole.

4.3. Nitrate reductase activity

Water deficit leads to changes in gas exchanges and in nitrate reductase activity, which limits nitrogen assimilation (Leite, 2019). Reduced water supply to plants also reduces its absorption; these factors can induce growth paralysis, protein biosynthesis and nitrate reductase activity decrease. This enzyme uses energy deriving from photosynthesis and is strongly regulated by the supply of carbonic skeletons to incorporate nitrogen into amino acids; plants subjected to low water availability conditions present decreased photosynthesis due to stomatal closure in order to reduce the transpiratory flow and to avoid losing water to the environment.

In addition, nitrate reductase catalyzes a reduction reaction in cytosol, which involves the transfer of two electrons to reduce NO_3^- to nitrite (NO_2^-). However, the formation of the reducing power (NADH or NADPH) used by the enzyme in the reaction is likely affected by water deficit, since such formation happens during the carbon cycle which, in its turn, decreases due to low net CO_2 assimilation, cellular respiration, and transpiratory and stomatal activity under water stress conditions (Taiz, et al., 2017).

4.4. Free Ammonium concentrations

Plants subjected to water deficit can induce ammonium formation through proteolysis, protein breakdown or use other ammonium formation routes. Ammonium accumulation in plant tissues can result from direct nitrate absorption or reduction, as well as from nitrogen compound deamination, photorespiratory cycle or biological fixation (Kant, et al., 2007); besides, it can be converted into glutamine and glutamate by sequential actions of glutamine synthetase and glutamate synthase - which are located in the cytosol and plastids of root chloroplasts – to form amino acids.

Increased photorespiratory activity is another response to free ammonium concentration in plant tissues. Plants increase water absorption through osmotic adjustment in order to reduce their osmotic potential in relation to soil potential. In this case, there is the synthesis of compatible solutes, which are organic and osmotically active compounds. This ability is used to accumulate solutes in cells in order to create a gradient to enable plants to absorb water under water limitation conditions. Compatible solutes mainly comprise amino acids such as proline, quaternary ammonium compounds such as glycine and betaine, and sugars such as mannitol and sorbitol (Taiz, et al., 2017; Bohórquez, 2019).

4.5. Total soluble Amino Acid concentrations

Increased soluble amino acid (AA) concentrations in plants subjected to stress is a mechanism used to balance the osmotic potential between the cytoplasm and the vacuole, to avoid damage to enzymatic systems (Munns & Tester, 2008) and to protect cell structures and functions; besides, this mechanism works as source of metabolic energy. Plants may undergo protein synthesis decrease due to decreased water availability in the soil, which, in its turn, results from increased formation of protease enzymes capable of degrading proteins, breaking peptide bonds and generating amino acids; the accumulation of soluble proteins during water stress is directly related to the maintenance of nitrogen stock to be reused at the end of the stress condition.

Increased AA content may be related to its action as precursor of endogenous hormones, such as cytokinins and abscisic acid, which act to mitigate the damage caused by water deficit or as enzyme activators. The osmotic adjustment is done through the accumulation of these compounds, which work as osmoprotectors and are one of the alternatives used to assure the turgor of, and water content in, cells (Paixão, et al., 2014) by forming protein molecule structuring subunits (Tavares & Vannucchi, 2016).

In addition, water stress leads to changes in the concentration of many metabolites due to interference in transport processes; there are changes in carbohydrates, as well as in amino acid metabolism, due to phloem tissue disorders that decrease their translocation to other plant organs. The accumulation of amino acids and free sugars in the leaf cell vacuole increases the water potential gradient among leaf, root and soil, which enables an efficient signal transduction mechanism and overall results in the necessary stomatal closure. It can also derive from protein synthesis restriction and from starch reserve hydrolysis (Galdino, et al., 2018).

4.6. Starch concentrations

According to Freitas (2014), decreased starch concentration in plant tissues happens to the detriment of decreased photosynthetic activity and to increased starch degradation by α and β amylase enzymes, in order to form new sugars in leaf tissues. This outcome may be associated with the fact that plants grown in soil subjected to water stress conditions invest part of the assimilates deriving from starch leaf degradation, mainly sucrose, to enable their decomposition in the leaf respiration process that releases the energy stored in carbon

compounds for cellular use, in a controlled manner.

In addition, starch is the main carbohydrate stored in plants, since it is the primary energy reserve (Almeida, et al., 2019). Thus, starch hydrolysis is an alternative energy maintenance mechanism used by plants undergoing reduced photosynthetic rates caused by water stress. Moreover, drought may also compromise the production of photoassimilates and carbon skeleton sources essential to enable starch synthesis, as well as reduce the activity of enzymes involved in its biosynthesis route in the endosperm.

4.7. Total soluble Carbohydrate concentrations

Sugar content is highly sensitive to environmental stress and its accumulation is a plant defense mechanism against dehydration; this mechanism adjusts the ionic and osmotic balance, limits cell turgor reduction (Marijuan & Bosch, 2013), helps maintaining stomatal opening and photosynthetic apparatus functioning by allowing it to operate even under low water potential conditions, as well as induces the activity of other components in plants' antioxidant system.

In addition, decreased photosynthetic rates can neutralize cell growth, restrict carbohydrate synthesis for export purposes and enable the accumulation of this metabolite in plant tissues (Hayat, et al., 2012). According to Zhonng, et al., (2018), glutamate dehydrogenase, glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase activity often increases under conditions of low water availability in the soil. Consequently, soluble sugar and amino acids - which have also been characterized by their signaling pathway that modulates the expression of important genes capable of enabling plant tolerance to abiotic stress - significantly increase.

4.8. Sucrose concentrations

Soluble carbohydrate accumulation takes place during water deficit mainly due to hydrolysis of previously stored starch, rather than to new synthesis during stress. It may be related to the protection of biomembranes that can be degraded due to lack of water in the cytosol and to increased ionic substance concentrations, which make several enzymes in the cytosol inactive (Liu, et al., 2011). Furthermore, hexoses released from the decomposition of sucrose metabolized in glycolysis can be used in anabolic and catabolic processes, as well as provide reducing sugars for the osmotic adjustment process; important enzymes, such as

amylases and invertase, among others, may have acted in this process.

Several genes are associated with sucrose maturation and accumulation in plant tissues and they are expressed under water deficit conditions. Sucrose accumulation is significantly influenced by water availability, since water acts as sucrose dilution factor in plants (Oliveira & Braga, 2019). In addition, sucrose deriving from starch degradation in leaves is transported by vascular tissues to other organs; it acts as energy source for plant growth or is stored in the form of reserve polysaccharides.

4.9. Proline concentrations

Plants decrease their osmotic potential due to the accumulation of proline and other solutes in cell vacuoles (osmotic adjustment) and work as nitrogen reserve, mainly for the synthesis of specific enzymes. According to Hemaprabha, et al., (2013), proline accumulated after stress periods can be used as energy, based on nitrogen and carbon redistribution, to recover plants' physiological and biochemical activity.

The P5CS (Pyrroline-5-carboxylate synthase) enzyme, which is responsible for converting glutamate into proline, is activated in the chloroplast of dehydrated plants and, at the same time, the PDH (proline dehydrogenase) enzyme, which is responsible for proline degradation, becomes inactive; this process increases this metabolite's level in cells. Increased amino acid levels, manly proline, may be linked to increased proteolytic enzyme activity, which enables greater free amino acid availability to protect plant tissues against stress. In addition, the accumulation of this class of solutes can be considered as a possible indicator of stress. In biochemical terms, plants change their metabolism in several ways – e.g., by producing osmoregulatory compounds such as proline (amino acid) and glycine betaine (quaternary amine) – in order to adapt to environmental stress (Szabados & Savoure, 2009).

5. Conclusions

Biochemical processes taking place in *T. vulgaris* plants were strongly affected by water deficit and recorded statistically significant differences among the investigated variables in response to water stress. *T. vulgaris* expressed mechanisms and strategies to enable species survival under stressful conditions likely to be seen in its natural environment.

We leave here our suggestion that works following this same lineage of execution can

now be carried out and the metrics obtained in the field, in the most different types of soils, especially in those regions of low precipitation and long periods of drought in the Amazon basin, mainly related to areas degraded and/or altered, a role that the species in question has been well addressed through area and energy recovery projects.

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