Biochemical analysis of *Eschweilera coriacea* Mori plants submitted to water deficit and rehydration

Accepted 29th May 2023

ABSTRACT

The objective of this study was to evaluate the growth, physiological response, nitrogen metabolism and antioxidant enzymes of young *Eschweilera coriacea* Mori plants in a situation of water deficiency and subsequent rehydration. The experimental design used was a factorial and completely randomized, with 2 water conditions (water suspension and control) and 5 times (0, 7 and 14 days of water suspension, 3 and 6 days of rehydration). Water suspension affected growth, mainly the leaf system, decreasing stomatal conductance and transpiration. The increase in ammonium in plants exposed to stress was accompanied by an increase in soluble amino acids, and in the osmoregulators glycine and proline, an increase in the activity of antioxidant enzymes, followed by a decrease in soluble proteins. Metabolic variables returned to or approached their normal rates when rehydrated. The increase in the variables of nitrogen metabolism and antioxidants, together with the physiological responses of the plant, suggests a regulatory mechanism of metabolic protection of *E. coriacea* against water suspension.

Key words: Amazon, antioxidant enzymes, nitrogen metabolism, osmoregulators, water stress.

INTRODUCTION

During the process of colonization of the Amazon, several occupation and integration plans were created, starting in the 1970s with a development model to integrate the region into the market, emphasizing the critical role of the construction of roads and highways and the disorderly process of occupation of soil, with the felling of thousands of km² of trees (MelloeArtaxo, 2017). The logging in Amazon continues to grow gradually, generating international concern, since this practice generates significant changes in the functioning of the ecosystem, impacting the structure and fertility of soils, contributing to the increase of the greenhouse effect, biogeochemical cycles and, mainly, about the hydrological cycle (Santos, 2017).

Water deficiency is the biggest problem faced in the production of forest species (Nascimento et al., 2019), and this condition is the cause for the non-growth of trees, affecting their development and inducing a series of physiological responses, such as the reduction of leaf water potential, stomatal closure that reduces stomatal conductance and the internal concentration of carbon dioxide (CO₂). In addition, water deficiency affects the entire biochemical process of the plant (Cordeiro et al., 2017), resulting in reduced protein synthesis and accumulation of amino acids, higher concentration of insoluble protein in leaves and roots, resulting in the synthesis of new polypeptides.

Matá-matá (*Eschweilera coriacea* [DC.] S.A.Mori) is a forest species widely used in logging, due to its abundance and good geographic distribution in the Amazon region (Nascimento et al., 2021). Almeida et al. (2019) also reported that this forest species has antioxidants and growth inhibitors for various pathogenic organisms in its
plant extract, giving it significant biotechnological potential. Therefore, new techniques are needed to improve the development and growth of forest species. In this sense, in the search for new production techniques, this present study aims to establish mechanisms of action and response of mata-matá seedlings, in relation to ecophysiological, biochemical and growth aspects associated with water deficit.

MATERIALS AND METHODS

Experiment conditions

The experiment was conducted in a greenhouse at Federal Rural University of Amazon, in Belém. Mata-matá seedlings of the 4-month-old *Eschweilera coriacea* species were used. These were taken to the greenhouse, cultivated in modified Leonard pots containing silica substrate: sand (1:2) and irrigated daily with Hoagland et al. (1950) nutrient solution while still undergoing acclimatization. The experiment period was from April to June 2017 and the biochemical analyzes were carried out at the Laboratory of Biodiversity Studies in Superior Plants (EBPS).

Experimental design and treatments

The factorial and completely randomized design, where 2x5 represent two water conditions (water suspension and control) and 5 the time factor (0, 7 and 14 days of water suspension, 3 and 6 days of rehydration), with 5 replications, generates 50 experimental units. The statistical analysis of the variables was performed with the R Studio software version 1.3.1093 using the ExpDes.pt library and the “fat2.dic” function, which already perform the residual normality analysis using the Shapiro-Wilk test, ANOVA and later Tukey’s Post-Hoc test. Thus, for all analyses, a significance level of 5% was considered.

Collection and plants storage

The plants were gathered in the morning of the day of collection and separated into parts: root, stem and leaves. A sample was taken from each part to determine the percentage of moisture and consequently the dry matter after drying in a forced air circulation oven at 70°C. The height of the plants was determined with the aid of a tape measure and the number of leaves was recorded. Then, the parts were dried in an oven with forced air circulation at 70°C to prepare the flour for the parts.

Stomatal conductance, transpiration and biomass

The determination of stomatal conductance, transpiration and stomatal resistance were obtained due to a porometer. The biomass was measured after drying at 65°C for 48 h, when the dry mass of shoots and roots were determined on an analytical balance.

Determination of the relative water content

The method described by Slavick (1979) was used. Using a stainless steel pourer, 30 leaf discs (10 mm in diameter) were randomly taken from each plant, and the fresh mass of each disc was then measured on an analytical balance. The weighed discs were transferred to a Petri dish containing 35 ml of distilled water and fixed on the bench (25°C) for a period of 6 to 7 h. The discs were then placed on filter paper to remove excess water and then weighed to determine the turgid mass. Subsequently, they were placed in a paper bag, heated in an oven (75°C) for 48 h and then the dry mass of the discs was calculated.

Biochemical metabolism was performed using the following methodologies for the Nitrate reductase: according to the method described by Hageman and Huchesby (1971); Free ammonium: results were expressed in mmol NH₄⁺ / kg DM. For this, we used the method described by Weatherburn (1967); Total soluble amino acids: the concentration of total free amino acids (TFAA) was determined according to the method described by Peoples et al. (1989); Total soluble proteins: the concentrations of total soluble proteins were determined according to the method of Bradford (1976); Glycine betahayne: the method utilized for the determination of glycine betaine was according to Grieve e Grattan (1983); Proline: the proline concentration was determined using a proline calibration curve and the result expressed in µmol proline / g DM. Methodology followed according to Bates et al. (1973).

Ascorbate peroxidase (APX EC 1.11.1.11)

The reaction medium consisted of a mixture containing 50 mM potassium phosphate buffer, pH 6.0, 0.05 mM EDTA, 0.015 M ascorbate, 0.03 M H₂O₂ and 300 µL of the conveniently diluted enzyme extract in a final volume of 1.5 mL. APX activity was estimated by ascorbate oxidation, measured by the decrease in absorbance at 290 nm. The results were calculated using the ascorbate molar extinction coefficient (ε = 0.0028 M⁻¹ cm⁻¹) and expressed in µmolde ascorbato. min⁻¹. mg⁻¹of prot. Catalase (CAT EC 1.11.1.6)

The reaction medium consisted of 86 mM potassium phosphate buffer, pH 7.0, 86 µM EDTA, 20 mM H₂O₂ and
150 μL of the crude extract conveniently diluted, in a final volume of 1.5 mL. The decrease in absorbance at 240 nm caused by the one-minute decomposition of H$_2$O$_2$ was used to measure enzyme activity. The results were calculated using the molar extinction coefficient (Ɛ =36 M$^{-1}$ cm$^{-1}$) and expressed in μmol of H$_2$O$_2$ min$^{-1}$. mg$^{-1}$ of prot.

**Superoxide dismutase (SOD EC 1.15.1.1)**

The reaction medium consisted of 50 mM potassium phosphate buffer, pH 7.8, 0.1 mM EDTA, 19.5 mM methionine, 75 μM blue tetrazolium nitrochloride (NBT), 2 μM riboflavin and 50 μL of the crude extract, conveniently diluted in a final volume of 1.5 mL. The reaction was carried out in a chamber and illuminated with two 20 W fluorescent lamps for 15 min. In order to determine the SOD’s ability to prevent the photoreduction of nitrotetrazolium blue (NBT), the amount of blue formazan produced as a result of the photoreduction of NBT was measured by increasing the absorbance at 560 nm. The results were expressed in UA.mg$^{-1}$prot, where UA (enzyme activity unit) is defined as the amount of enzyme required to cause 50% inhibition of NBT photoreduction within 15 min.

**RESULTS AND DISCUSSION**

The growth variables height (Figure 1A), stem diameter (Figure 1B) and number of leaves (Figure 1C) increased significantly under water stress. In terms of percentage,
height increased by 15% and number of leaves decreased by 29% when rehydrated. The stem diameter experienced a 45% reduction in size. This result contradicts a number of scientific findings about the problem of water deficiency.

Root dry mass production also increased under water stress (48%) when compared with the control plants. In the leaf organ there was also an increase in biomass in the stress condition by 19%. When rehydration of these two organs occurred, the roots returned to their normal condition, decreasing by 31%, and in the aerial part it decreased by about 14% on the 6th day of rehydration (Figure 1D).

The relative water content under water stress condition significantly reduced when compared with the control plants on the 14th day, showing statistical differences, and in terms of percentage, there was a 43% decrease. However, the rehydration process allowed the water content to return to normal levels, providing an increase of 82% (Figure 2A).

Stomatal conductance (Figure 2B) and transpiration (Figure 2C) reduced drastically under water stress condition by 92 and 94%, respectively when compared to control plants, with statistical differences between days and treatments. The rehydration results showed that despite of the days applied, there was no time to return to normal values, but there were substantial increases of about 242 and 194%, respectively.

The reduction in the relative water content under water stress conditions is probably due to the decrease in stomatal opening (Figure 2B) and, consequently, transpiration (Figure 2C) of mata-matá seedlings.

The enzymatic activity of nitrate reductase (RN) in the leaves increased 90.4% in the period of 7 days of water deficit, when the stress evolved to 14 days the enzymatic activity decreased dramatically, showing statistical differences with the control plants of the first period of stress. When the plants were submitted to rehydration the third day showed a 35.2% increase, indicating that this enzyme is water dependent (Figure 3A).

This sudden drop in reductase activity is partly explained by the low process of stomatal conductance (Figure 2B) and transpiration (Figure 2C). Free ammonium concentrations (Figure 3B) increased by 129% in roots and 57.2% in leaves in the 14-day period of water suspension. However, with the 6-day rehydration, the ammonium concentrations in the roots decreased by 45.4%, returning close to normal rates, and with the 3-day rehydration in the leaves, the 33% decrease in ammonium concentrations was enough for the plants return to their normal rates. Total soluble amino acid concentrations in roots and leaves increased by 81.25 and 65%, respectively, in a 14-day water deficit period. A 3-day rehydration was required to normalize the amino acid concentrations in the root, representing a decrease of 34.8%. In the leaves, in order to get close to the normality of amino acid concentrations, rehydration of 6 days was necessary for a decrease of 20.8% (Figure 4A).
Figure 3: Analysis of nitrogen metabolism for concentrations of nitrate reductase and free ammonium enzyme activity in roots and leaves of young mata-matá plants submitted to water deficit for 14 days and rehydration for 6 days. Different letters show statistical difference, compared by Tukey test at 5% significance level. Capital letters represent statistical difference between days and lower case letters represent statistical difference between treatments.

Total soluble protein concentrations in roots and leaves decreased by 37.8 and 32.6%, respectively in the 14-day water deficit period, and rehydration was not able to return to normal concentration but was effective in attenuating the 3-day rehydration stress on roots representing a 22% increase and 6-day rehydration stress on leaves representing a 27.7% increase (Figure 4B).

Glycine concentrations in the plant (Figure 5A) increased in the 14-day water suspension period, with an increase of 96.5% in roots and 63.6% in leaves. With the rehydration of these plants for 6 days, the decrease in glycine-betaine concentrations was 44.6% in the roots and 39.5% in the...
Figure 4: Analysis of nitrogen metabolism for the concentrations of total soluble amino acids and total soluble proteins in roots and leaves of young mata-mata plants submitted to water deficit for 14 days and rehydration for 6 days. Different letters show statistical difference, compared by Tukey test at 5% significance level. Capital letters represent statistical difference between days and lower case letters represent statistical difference between treatments.

leaves.

The proline concentrations in the plant (Figure 5B) increased significantly in the 14-day water deficit period, with an increase of 1,571% in the roots and 305% in the leaves; however the plants were rehydrated and decreased by about 77% in the roots, not being enough to return the control rates. As for the payroll, the reduction was 70.6%, returning close to its equilibrium rates.

The percentage increase in the antioxidant activity of superoxide dismutase (20%), catalase (23%) and ascorbate peroxidase (25%) enzymes showed that water deficiency caused oxidative stress. This increase occurred in the two analyzed organs, the activity of SOD and APX were higher in the roots on the 14th day of stress, showing statistical differences between the days and treatments when compared to the control plants, and catalase was more expressive in the leaves on the 14th day of stress, showing a statistical difference when comparing the control plants and the condition of 7th day of stress (Figure 6). When the
Figure 5: Analysis of nitrogen metabolism for glycine-betaine and proline concentrations in roots and leaves of young mata-mata plants submitted to water deficit for 14 days and rehydration for 6 days. Different letters show statistical difference, compared by Tukey test at 5% significance level. Capital letters represent statistical difference between days and lower case letters represent statistical difference between treatments.

Rehydration process occurred, under the stress condition, there was a decrease of 19% for SOD (Figure 6A), 30% for catalase (Figure 6B) and 7% for APX (Figure 6C), evidencing significant differences when compared to the control plants.

This behavior shows tolerance, to justify this statement, some plants have the ability to increase root growth to absorb water in deep soils, even dynamically modifying the architecture of the root system, altering root growth (Hue Xiong, 2014). As soon as the water is absorbed, the plant adjusts itself osmotically. This adjustment allows the partial opening of the stomata and, consequently, the assimilation of CO₂ and the growth of the plants at low water potentials, stimulating the diffusion of water into the cells. Turgidity may vary depending on the species, with the different stages of growth being largely influenced by time, intensity, duration and frequency of stress, in addition to the various plant-soil-atmospheres. The quick recovery of stressed plants indicates that the water deficit did not cause irreversible damage to the roots’ ability to absorb water and transport it to the shoot.

The decrease in transpiration and stomatal conductance
constitute response mechanisms of a plant subjected to water stress (Rigotti et al., 2019). This results in a series of biochemical and physiological reactions as a way to balance the photosynthetic process and stimulate the production of compounds that help the plant go through the stress process (Kapoor et al., 2020). This closing of the stomata and the conductance guarantee the supply of water for a longer time in their tissues. Also, it promotes a decrease in the plant/atmosphere interface and negatively affects plant production, but it is a defensive measure under the condition of water stress (Taiz et al., 2017). This possibly affected plant photosynthesis since NR is dependent on nicotinamide adenine dinucleotide (NADH) generated in the photochemical step. In addition, water stress decreases the transfer of nitrate from the roots to the leaves, which in turn decreases the activity of the RN enzyme because this enzyme needs nitrate as a substrate to form organic compounds (Hasanuzzaman et al., 2018).
Under this stress condition, these plants can absorb and accumulate more ammonium in the proplastid structures of the roots. This can be considered an alternative route triggered by the NADH-dependent glutamate dehydrogenase. The low activity of the glutamine synthetase (GS) enzyme may be contributing to the increase in ammonium concentration because this enzyme is a precursor for the transformation of ammonium into amino acids (Wu et al., 2016). However, when the rehydration process occurs, ammonium concentrations decrease, causing this accumulated ammonium to be rapidly converted into amino acids (Razgallah et al., 2016), as shown in Figure 4A.

This increase can be represented by the process of protein degradation in the stress condition, where the result is the production of amino acids (Liang et al., 2013). These released amino acids are used by the plant to mitigate the deleterious effect of water deficit. According to Zhang et al. (2016), the increase in the concentration of organic solutes such as amino acids, in the cytosol or in the vacuole, occurs to decrease the osmotic potential and, consequently, keep the water potential and cellular turgor close to the optimal level.

The increase in this amino acid is considered a critical determinant of plant stress tolerance (Nawaz and Wang, 2020). It acts in the osmotic adjustment of cells since in large amounts glycine does not influence cellular metabolism and, in contrast, helps to control the entry and exit of water in the cell, cytoplasm and vacuoles. However, if the plant begins to recover, catabolism is activated and will lead to the degradation of glycine. In this study, a response was observed when the plants were rehydrated after 3 and 6 days.

The increase in proline concentrations was possibly due to the osmotic adjustment, which plants resort to as protection when there is low water availability. Osmotic adjustment is an adaptive mechanism that provides the maintenance of turgidity and related processes, such as stomatal opening, growth, cell elongation and the functioning of the photosynthetic apparatus, thus allowing it to operate even under conditions of low water potential. This phenomenon occurs in order to maintain the physiological and metabolic functions of the plant for a certain time while waiting for the environmental conditions to return to the appropriate level (Per et al., 2017). As observed in this study, under the rehydration condition, proline levels decreased and in these situations, proline acts as a supplier of carbon and nitrogen, redistributing these elements for the return of normal plant physiological activity and protecting the plant plasmatic membrane.

Maintaining an optimal level of endogenous enzymatic defense in the cell allows for proper redox reactions and the regulation of several essential processes for plants such as growth and development. This information confirms the results in the growth parameters of mata-matá plants (Figure 1). This level is maintained by the balance between ROS production and ROS elimination. This justifies the increase in antioxidant enzymes in this study.

The enzymatic increase of SOD and APX in roots caused by ROS partly explains the increase in root dry mass in treatments under water stress. According to Naing and Kim (2021), the homeostatic maintenance of ROS plays an important role in root development and, in well-defined amounts, they act as signalers in various situations, such as elongation and differentiation of root cells. Another justification for the increase in the superoxide dismutase enzyme may be that it is found in almost all cellular compartments of plant organs (Wang et al., 2019), facilitating the elimination of reactive oxygen species to maintain cellular redox homeostasis (Huang et al., 2019). The defense system of this enzyme (SOD) consists of the dismutation of superoxide radicals (O2·−) into molecules of hydrogen peroxide (H2O2) and oxygen (O2). The balance between SOD and APX is crucial to determine the effective intracellular amount of O2·− and H2O2, and these enzymes are of paramount importance for the detoxification of these ROS (Borjas-Ventura et al., 2019). Catalase acts in a similar way to SOD, eliminating hydrogen peroxide (H2O2) in water and oxygen, this action is generated during mitochondrial electron transport, fatty acid oxidation and, in photorespiratory oxidation during stress conditions.

CONCLUSION

The water suspension affected plant growth and caused a decrease in the internal water content because the plant increased the diffusive resistance to water vapor with the decrease in stomatal conductance and consequently in transpiration. With rehydration, plant growth and RWC tended to return to normality as physiological activities increased.

The effect of the water suspension was demonstrated by a significant increase in NR activity, an increase in ammonium concentrations, TSA, and in the osmoregulators followed by a decrease in TSP, suggesting a regulatory mechanism, at least in part, in the assimilation and metabolism of nitrogen in E. coriaceae. When rehydration was carried out, the variables returned to their normal rates or rates that were close to them.

Stress promoted an increase in the activity of the enzymes superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) in plants, followed by a return to normality as rehydration was performed.

REFERENCES

Science. 6(2):611-615.


