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Enzymatic and Non-enzymatic Antioxidant System in Young Plants of *Tachigali vulgaris* Submitted to Drought

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Abstract

Tachigali vulgaris is a pioneer species, colonizing marginal lands and roadsides, often initiates secondary succession in open areas by the intense germination of their seeds in the soil. The main components of the antioxidant defense system can be divided into enzyme found primarily intracellularly (superoxide dismutase, catalase, guaiacol peroxidase, ascorbate peroxidase, glutathione reductase, etc.) and small non-enzymatic molecules that can be divided into soluble in water (ascorbic acid, glutathione and bilirubin) and lipid soluble (α-tocopherol, β-carotene and lycopene). Plants were then separated into leaves and roots, wrapped in aluminum foil and stored in Ultrafreezer at -80 °C. To determine the enzymatic activity and biochemical analyzes, the plant material was first frozen in liquid nitrogen and subsequently lyophilized. The experimental design was completely randomized in a factorial 3 × 2 (three times: zero, five and ten days of water suspension, and two water conditions: control and drought stress), with four repetitions. Analysis of variance was applied to the results and when significant difference, the means were compared using the t test adopting the 5% level of probability through the statistical package (7.7 beta Assistat, 2015). Since they were able to drive quickly the enzymatic antioxidant defense system (SOD, CAT and APX), however, failing to reduce oxidative damage resulting in the death of them.

Keywords: SOD, APX, CAT, Tachi branco

1. Introduction

With the advent of climate change and global warming, we are increasingly seeking knowledge about the physiology of plant species under water deficiency conditions, so that they can be recommended for homogeneous and/or intercropped plantations, as well as for recovery of degraded areas. *Tachigali vulgaris* is a pioneer species, colonizing marginal lands and roadsides, often initiates secondary succession in open areas by intense germination of the seeds in the soil. It has been highlighted by the rapid growth and its wood is widely used by rural communities in the Midwest and Northeast, in making fence posts, pillars, packaging and rafters, in construction, as well as source for the production of firewood and charcoal, and even suitable for energy plantations (Franke, 1999).

The species still has few silvicultural characteristics recorded in the literature when compared to the other native species of the Amazon region. But with great energy potential compared to *Eucalyptus* (exotic species) that still presents great local resistance for its implantation, as well as potential for recovery of degraded areas.

The drought, along with factors in over-temperature and high irradiance, causes a reduction in photosynthesis, leading to decrease in the use of the incident radiation, resulting in reducing accumulation of power (NADPH) and reduction in the pool of the final acceptor of the electron transport chain (NADP+). This excess reducing power can lead to super-reduced electron transport chain. In this process can be escaped electrons, which in turn react with molecular oxygen (O_2), thus forming the so-called reactive oxygen species (ROS) (Cavatte et al., 2012).

According to Rendón et al. (2013), the main components of the antioxidant defense system can be divided into enzyme mainly found in the intracellular environment (superoxide dismutase, catalase, guaiacol peroxidase, ascorbate peroxidase, glutathione reductase, etc.) and small non-enzymatic molecules that can be subdivided water soluble (ascorbic acid, glutathione and bilirubin) and lipid soluble (α-tocopherol, β-carotene and lycopene).

The understanding of physiological mechanisms can provide advanced information about some strategies to be used in breeding programs for selection of cultivars tolerant to drought (Silva et al., 2012).

This study aims to evaluate the enzymatic and non-enzymatic antioxidant behavior, the fluid status and electrolyte leakage of Tachi branco plants under drought.

2. Materials and Methods

2.1 Site Description

The experiment was conducted in a greenhouse of Federal Rural University of Amazonia (UFRA/City of Belém/Brazil) during the months of april to august in 2015. The municipality of Belém has a tropical climate. The climate classification is Af according to Koppen Geiger. The average temperature is 26.8 ºC where the hottest month is October (27.2 ºC) and January (26.4 ºC) the warmest month. The average annual rainfall is 2,537 mm, with the driest month being November (89 mm) and the rainy month being March (379 mm).

The relief is flat or slightly undulated consisting of lowland lands and terra firme. The topography is little varied, with elevation of 21 meters above sea level (Cohre, 2006).

The seedlings were purchased from Association of Exporters industries of the state of Pará woods-AIMEX, aged four months. As the batch of seedlings formed by seeds from the city of Alta Floresta, Mato Grosso, where they were transplanted into polyethylene vessels with capacity of substrate 4.5 kg, staying for a period of two months of acclimatization. To meet the nutritional deficiency of the same was applied nutrient solution Hoagland and Arnon (1950), considering that the plants arrived in a greenhouse presenting symptoms of nutritional deficiency. Plants were then separated into leaves and roots, wrapped in aluminum foil and stored in Ultrafreezer at -80 ºC. To determine the enzymatic activity and biochemical analyzes, the plant material was first frozen in liquid nitrogen and subsequently lyophilized. After lyophilization, to be determined shoot dry weight of roots and total. The dried material was ground in mill to obtain a fine powder and duly stored in Falcon tubes to their use in assays.

2.2 Experimental Design

The experimental design was completely randomized in a split plot scheme in time (three times: zero, five and ten days of water suspension, and two water conditions: control and drought stress), with 4 replications, totaling 24 experimental units (a plant/pot). The plants were distributed in three benches in the greenhouse by means of a random lottery, so that there was no bias in their distribution. During the conduction of the experiment the control of invasive plants was carried out in the substrate of the plants, control was done manually.

2.3 Measurement and Methods for Calculating

2.3.1 RWC (Relative Water Content)

The RWC was determined between 05:00 and 06:00 h in each collect. Where were taken the discs for the RWC determination. The method used was that described by Slavick (1979), 30 leaf discs were taken (10 mm diameter) from each plant at random through with a stainless steel-pourer immediately determining the fresh mass (FM1) in analytical balance. Then the discs were transferred to a Petri dish containing 35 ml of distilled water and left on the bench (25 ºC) for a period of 12 hours. Thereafter the discs were placed on filter paper to remove excess water and then weighed to determine the turgid mass (FM2). Thereafter, the disks were placed in paper bags and brought to the oven (70 ºC) for 24 h and was subsequently given the dry mass of the discs (DM). The results were expressed as a percentage, according to the following formula:

$$RWC = (FM1 - DM)/(FM2 - DM) \times 100 \quad (1)$$

where, FM1 = Fresh mass 1; FM2 = Fresh mass 2 (turgid mass); DM = Dry mass.

2.3.2 Leak Electrolyte

Leakage of electrolyte was estimated based on Blum and Ebercon (1981), with minor modifications this being an indirect measure for determining the degree of membrane damage. Were weighed separately, 100 mg of shoots and roots and after triple rinsed with deionized water, the plant material was transferred to vials to which were added 10 ml of deionized water. The vials were allowed to stand at room temperature (25 °C) for 6 hour with

stirring every 20 min. After this period, the supernatant was transferred to new test tubes and with the aid of a conductivity, electrical conductivity was measured (C1) of the solution of test tubes. After this the tubes were again sealed and heated in water bath at 100 °C for 1 h. After cooling to room temperature the vials, the electric conductivity of the extract (C2) was measured again. The readings of the electrical conductivity of the solution were performed with a bench conductivity meter Model DM Digimed 31. The electrolyte leakage was estimated by the equation below:

$$LE\ (\%) = (C1/C2) \times 100 \quad (2)$$

2.3.3 Photosynthetical Active Pigments

The method was described by Lichthenthaler (1987) being placed 100 mg of leaf tissue sample and homogenised in a mortar with 0.1 g of $CaCO_3$, a pinch of sand and 5 ml of 80% acetone in under light. Pouring the extract everything in a table centrifuge, washed with (quantitatively) mortar twice with 5 ml of 80% acetone. The tube was wrapped with aluminum foil, then the tube was centrifuged for 10 minutes at 10 °C and 6000 rpm. The supernatant is carefully poured into a 25 ml volumetric flask also wrapped with aluminum foil and the volume completed with 80% acetone. It was taking an aliquot and held read with absorbance at 470, 646.8 and 663.2 nm. The extract was sheltered from light exposure during the readings. Absorbance values not ranged between 0.2 and 0.8 were diluted. By diluting the volume is always supplemented with 80% acetone. The concentrations of chlorophyll and carotenoids (Mg L^{-1}) were calculated and converted to (FM mmol kg^{-1}) by the following formulas:

$$\text{Chlorophyll a} = Ca = 12.25\ A_{663.2} - 2.79\ A_{646.8} \quad (3)$$

$$\text{Chlorophyll b} = Cb = 21.50\ A_{646.8} - 5{,}10A_{663.2} \quad (4)$$

$$\text{Total Chlorophyll} = C(a+b) = 7.15\ A_{663.2} + 18.71\ A_{646.8} \quad (5)$$

$$\text{Carotenoids (xanthophyll + carotenes)} = (1000A470 - 1.82Ca - 85.02Cb)/198 \quad (6)$$

2.3.4 Obtaining the Enzymatic Extacts

The extract for determining the activity of SOD, CAT and APX was obtained from homogenizing in mortar, at 4 °C, 0.1 g of the lyophilized powder leaf and root with 5 ml of potassium phosphate buffer (4 °C) 0.1 mM, pH 7.0, containing 0.1 mM EDTA, followed by homogenization for 4 min. The phosphate buffer additions were made in a piecemeal way, 50% of the total volume of this solution (2.5 mL) used in the homogenization for 2 min, after this, immediately added the other 50%, the homogenised mixture in time equivalent to the above. The homogenate was filtered through nylon cloth and transferred to test tubes and kept at 4 °C for two hours, performing occasional agitation. The homogenate was filtered centrifuging at 12,000 × g for 15 min at 4 °C. The supernatant crude extract, was stored in a freezer at -22 °C until used in enzyme activity assays.

2.3.5 Superóxido Dismutase (EC 1.15.1.1)

The SOD activity was determined by inhibition of photoreduction of nitroblue tetrazolium chloride (NTC) as Giannopolitis and Ries (1977). The reaction mixture (1.5 ml) was made up of potassium phosphate buffer 50 mM (pH 7.8), EDTA 1 µM L-methionine at 13 mM and NBT at 75 µM and 50 µL of the extract, conveniently diluted with extraction buffer. The reaction was initiated by addition of 2 µM riboflavin, followed by illumination of the reaction medium with two 20 W fluorescent lamps in a closed box. After 15 min, the reaction was interrupted by disconnection of the lights and the readings were performed at 560 nm. One activity unit (AU) was regarded as the amount of enzyme required to inhibit 50% of the photoreduction of NBT for 15 min in comparison to the reaction medium without protein extract. Each extract was assayed in duplicate, SOD activity is expressed in AU mg^{-1} protein.

2.3.6 Catalase (EC 1.15.1.6)

CAT activity was determined by the method of Beers and Sizer, Jr. (1952) with modifications. The reaction mixture (1.5 ml) consisted of potassium phosphate buffer 100 mM (pH 7.0), 0.1 µM EDTA, 20 mM H_2O_2 and 150 µL extract. The reaction was initiated by addition of 150 µL the enzyme extract to the reaction medium; and the enzymatic activity was determined by consumption of H_2O_2, the reduction being monitored by absorbance readings at 240 nm for 5 min in each extract was assayed in duplicate. The molar extinction coefficient of H_2O_2 (36 M^{-1} cm^{-1}) was used to determine the CAT activity was expressed as ol of H_2O_2 min^{-1} g^{-1}.

2.3.7 Ascorbate Peroxidase (EC 1.11.1.11)

The APX activity was determined by the method of Nakano and Asada (1981). The reaction mixture (1.5 ml) was composed of 50 µL potassium phosphate buffer (pH 6.0), 0.1 µM EDTA, 0.5 mM ascorbate, 1 mM H_2O_2,

and 300 µL extract, suitably diluted with extraction buffer. The reaction was initiated by addition of 300 µL the enzyme extract, and APX activity was determined by the H_2O_2-dependent oxidation of ascorbate, the reading being performed in a spectrophotometer at 290 nm for 1 min. The molar extinction coefficient of ascorbate (2.8 mM^{-1} cm) was used to quantitate the enzyme activity, taking into consideration that two moles of ascorbate are necessary to reduce 1.0 moles of H_2O_2 (McKersie & Leshem, 1994). Each extract was assayed in duplicate, APX activity is expressed in micromol H_2O_2 min^{-1} mg^{-1} protein.

2.4 Statical Analyses

Analysis of variance was applied to the results and when there was a significant difference, the averages were compared by t test adopting the level of 5% probability, using the statistical package (Assistat 7.7 beta, 2015).

3. Results

3.1 Relative Water Content

The relative water content (Figure 1) showed statistically significant differences from the fifth day of the experiment, with average of 85.31 to 66.07% water content in the leaf tissue of the control treatments and water deficiency respectively, over the decrease soil water. In the last data collection point there was a drastic reduction in RWC of 84.81 for the treatment and 22.67% water deficit, which corresponded to a reduction of 32.14% in the water content of the leaf tissue.

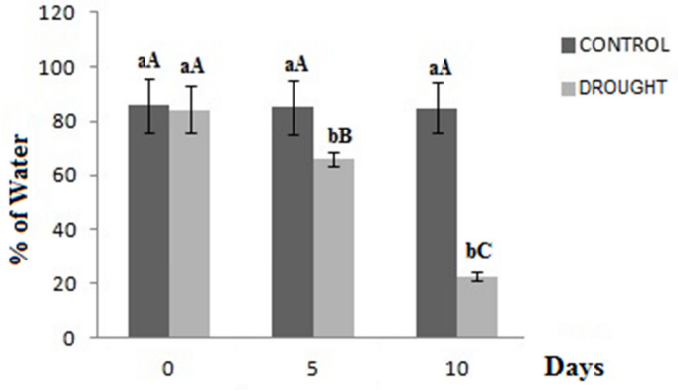

Figure 1. Relative water content in leaves of *Tachigali vulgaris* in funcion of drought, in three evaluation periods Belém (PA), 2015

3.2 Electrolyte Leakage

In (Figure 2) the figures for the percentage of electrolyte leakage of the membrane to the three points of data collection are presented (0, 5 and 10 days of water suspension) for both organs of the sheet as the root. There was statistical difference at 5% probability from the fifth day of observation, with average rising from 17.80 to 64.60% in the control treatments and water stress leaf, respectively. For the tenth day there was an even greater increase, averaging 18.67 in control and 88.55% water deficit, registering an increase of 212.97% in the electrolyte leakage into the leaf tissue. For the body of the root was also recorded a significant increase of 5.64 to 7.67% in the electrolyte leakage for the treatments control and water stress from the fifth day, respectively. The tenth day went on there was an increase in the percentage of extravasated electrolytes, 5.65 to 12.10% in the control treatments and water deficiency, which also represented an increase of about 51.26% in the leakage of electrolytes.

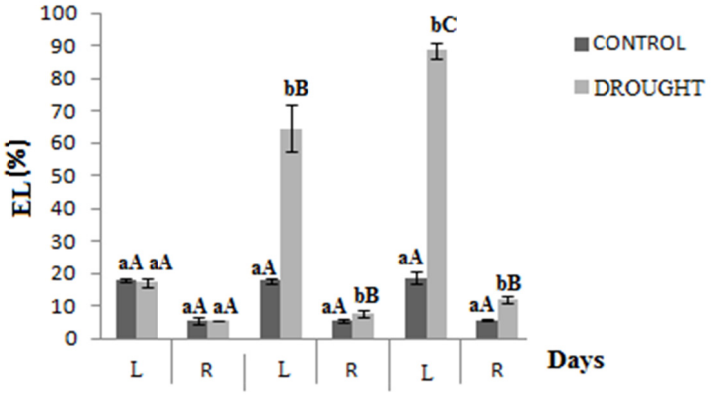

Figure 2. Electrolyte leakage in leaf and roots tissues in *Tachigali vulgaris* plants, due to the drought in three evaluation periods. Belém (PA), 2015

3.3 Photosynthetic Pigments

According to Figure 3 could observe that the statistical difference between treatments started to occur from the fifth day, where the chlorophyll a content (Figura 10A) decreased from 3.78 to 3.23 µmol m^{-2} s^{-1} and in the 10th day from 3.56 to 0.88 µmol m^{-2} s^{-1} in control and drought treatments respectively, which shows a decrease of 29.05%. For the chlorophyll b (Figure 3B) there was no statistical difference for the interaction of treatments, occurring only difference for isolated treatment where for treatment 1 (time) was observed average of 6.70; 5.67 and 5.50 µmol m^{-2} s^{-1} in the days 0, 5 and 10 respectively, and for the treatment 2 (water conditions) the means of 6.33 and 5.58 µmol m^{-2} s^{-1} in control and drought treatments respectively, obtaining with this a reduction of 11.89%.

For the total chlorophyll (Figure 3C) also recorded significant difference at 5% probability from the fifth day of the experiment, reducing of 10.07 to 7.29 µmol m^{-2} s^{-1} and in 10th day of 9.57 to 5.88 µmol m^{-2} s^{-1} in control and drought treatments respectively, which represents a reduction of 21.57%.

Carotenoids (Figure 3D) also showed a significant reduction of 7.01 to 4.84 µmol m^{-2} s^{-1} in the 5th day and of 6.72 to 4.34 µmol m^{-2} s^{-1} in the 10th day for the control and drought treatments respectively, representing a reduction of 22.11%.

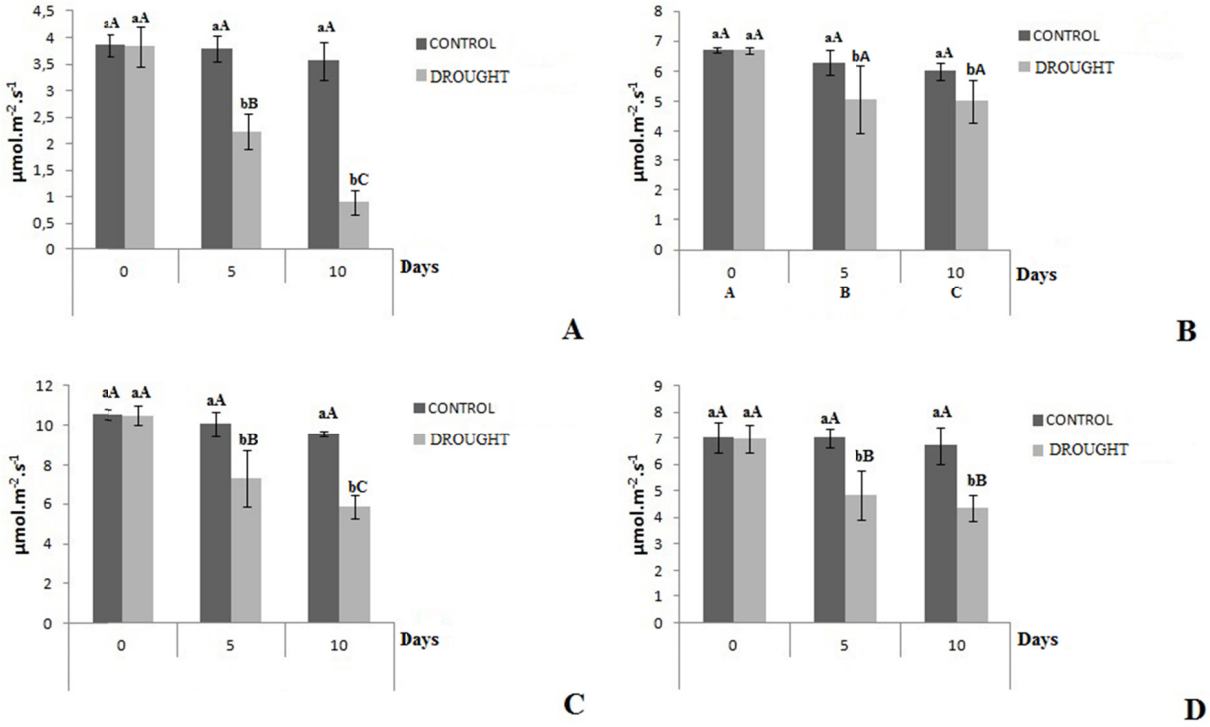

Figure 3. Pigments contents: Chlorophyll a (A), Chlorophyll b (B), Total Chlorophyll (C) and Carotenoids (D) in young plants of *Tachigali vulgaris*, in funcion of drought, in three evaluation periods. Belém (PA), 2015

3.4 Superoxide Dismutase

The values obtained showed an increase in SOD activity (Figure 4) in leaf tissues, increasing from 40.38 in the control treatment to 48.55 mg^{-1} protein from the fifth day treatment under water stress, thereby conferring difference statistics compared to the control ($p < 0.01$). The enzyme activity further increased the tenth day of water suspension to 54.04 mg^{-1} protein. Getting an increase in enzyme activity in the order of 16.33% in plants under disability when compared to control plants.

For the root tissue was also possible to register statistical difference ($p < 0.01$) between treatments from the fifth day, with valolres increasing from 50.43 in the control treatment to 56.21 mg^{-1} protein to water deficit. Although little further increasing the tenth day of water suspension 57.23 mg^{-1} protein. Registering an increase of 6.94% in the enzyme activity for flat subjected to drought compared to control plants.

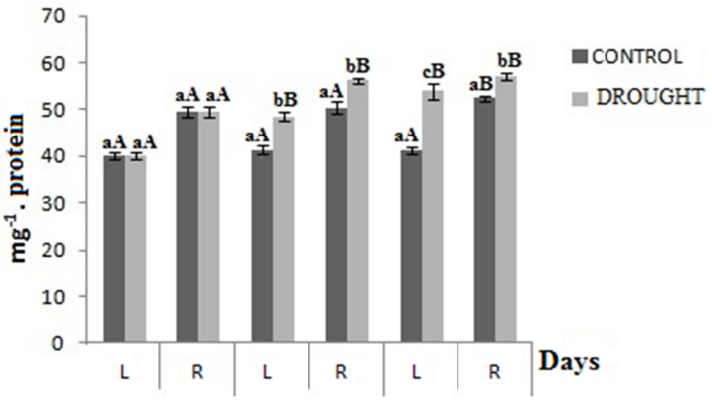

Figure 4. Superoxide dismutase enzyme activity in leaf and root of T. vulgaris plants tissues subjected to drought. The bars represent the standard deviation of the four replicates

3.5 Catalase

The catalase activity in leaf tissue (Figure 5) has differ statistically ($p < 0.01$) the fifth day of water suspension, with values increasing from 0.0492 to 0.0612 in the control treatment H_2O_2 min $^{-1}$ g^{-1} under water stress, increasing slightly further to the tenth day for a 0.0743 µmol of H_2O_2 min^{-1} g^{-1}. What in percentage terms represented an increase of about 23.32% in the enzyme activity in plants under water stress.

Already the roots this increase was slightly lower compared to the leaf tissue, but there is also significant difference to the level of probability set the fifth day of water suspenão, rising from 0.0439 to 0.0459 in the control treatment H_2O_2 min^{-1} g^{-1} under drought, and 0.0557 H_2O_2 min^{-1} g^{-1} your tenth day. With a percentage increase in the order of 14.93% in the enzyme activity in the root tissues.

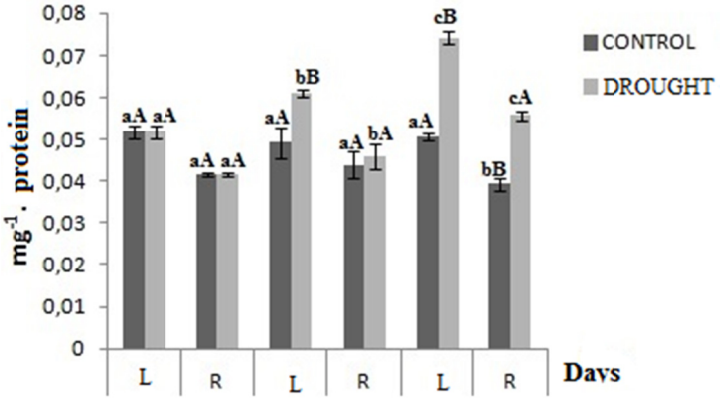

Figure 5. Catalase enzyme activity in leaf and root tissue in young plants of *T. vulgaris* subjected to drought. The bars represent the standard deviation of the four replicates

3.6 Ascorbate Peroxidase

The activity of APX (Figure 6) in the leaf tissue also began to show a statistical difference between treatments ($p > 0.05$), presented a slight increase in its activity in the treatment of 0.0330 to 0.0390 µmol control H_2O_2 min^{-1} mg^{-1} protein under drought to the fifth and reduced the tenth day to 0.0396 µmol H_2O_2 min^{-1} mg^{-1} protein. An increase of 13.49% on enzyme activity for plants under drought.

Also registering statistical difference ($p > 0.05$) enzyme activity in root tissue. Being possible to verify a difference to the values from the fifth day of water suspension, rising from 0.0299 in the control treatment to 0.0329 µmol min^{-1} H_2O_2 mg^{-1} protein, and 0.0368 µmol H_2O_2 min^{-1} mg^{-1} protein on the tenth day of suspension. The enzyme activity was increased by 6.98% for the plants under drought.

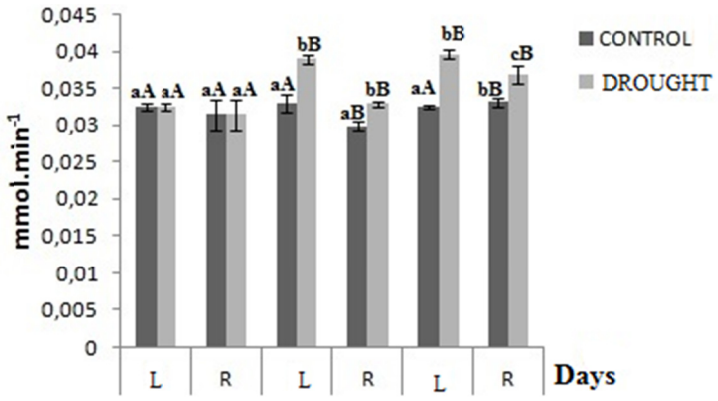

Figure 6. Ascorbate peroxidase enzyme activity in leaf and root tissues in young plants of T. vulgaris subjected to drought. The bars represent the standard deviation of the four replicates

4. Discussion

Reductions in water status of the leaf tissue is a direct consequence of the existing low water availability in the soil matrix, which is heavily occurred from the fifth day after the beginning of the experiment, and aggravating even further the tenth day.

The use of the methodology with rapid hydration of leaf tissue has eliminated the error source of its determinations and the high correlation with the potential of water sheet, indirectly enables its estimate (Calbo et al., 2010).

The degree of damage to the membrane can be indirectly estimated by the electrical conductivity, which in turn measures the electrolyte leak from the cells into the aqueous solution (Bajji et al., 2001). The leak electrolyte expressed by extruding ions was statistically significant ($p < 0.05$) increased in plants under drought. Cell membranes are one of the first structures affected by water and salt stress, causing it to lose its selectivity characteristic into and out of the cell into the extracellular medium.

The results for the electrolyte leakage are consistent as to the degree of damage to the membranes, thus triggering the antioxidant defense system.

The drought is characterized as one of the environmental stresses responsible for the loss of pigments in the leaves, making the plant metabolism is altered, leading to increased reactive oxygen species (ROS's) that cause damage to the membranes, triggered by oxidative processes of lipids and electrolytes loss (Figure 1B), the cell (Lyse et al., 2012). As a result, the leaves stop producing the assimilates satisfactorily, destabilizing the balance source/drain and damaging processes such as growth and development of plants (Coelho, 2014).

In addition to accessory pigments, carotenoids play an essential role in photoprotection, protecting the photosynthetic apparatus against singlet oxygen ($^1O_2$*), highly reactive, damaging many cellular components such as lipids (Taiz & Zeiger, 2013). For the antioxidant defense system starts with an enzymatic cascade, but it also involves non-enzymatic components, among which stand out the ascorbate (AoA), the glutaiona (GSH), the β-carotene and α-tocopherol. These antioxidants can prevent the formation of free radicals, sequestering them or promoting its degradation, preventing the occurrence of cell damage (Serkedjieva, 2011). This result could not be diagnosed in this study, given that there was a reduction of carotenoid levels in the treatments evaluated, try this cell protection enabled to enzymatic antioxidants such as SOD, APX and CAT.

The water availability has strongly influenced the production of pigments in chloroplastid *T. vulgaris*, not showing tolerant behavior to drought through the degradation of the content of these pigments. What probably should have occurred by high production of reactive oxygen species, a fact explained by the antioxidant enzymes activities.

According to Pereira (2010), the role of SOD is possible that two dismutem O_2 molecules to form H_2O_2, which is considered a weak antioxidant. In general, to generate H_2O_2 for CAT and APX can eliminate them.

The high activity of SOD indicates a potential tolerance to damage caused by photo-oxidation, because it is a fundamentally important antioxidant system in protecting the photosynthetic apparatus of photo-oxidative destruction (Silva, 2014).

The results of SOD, which is the first enzyme to act on the enzymatic antioxidant defense system, justify increased APX (in part) and CAT in the same period as the increased production of $O_2^{\bullet}$active SOD and, thus reflects directly the free H_2O_2 concentration thus requiring a stimulus in the activity of enzymes responsible for the degradation of this molecule.

Lima (2013) evaluating the oxidative behavior of *Anacardium occidentale* plants exposed to drought also found similar results to this study, with high activity of superoxide dismutase. This behavior possibly adaptive type, in order to establish metabolic adjustment in the face of stressful conditions imposed in the greenhouse, mainly related to high radiation rates.

Catalase (CAT) is an enzyme whose function is to catalyze the reduction of hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2). Inhibition of CAT leads to an increase in reactive oxygen species, providing the body oxidative stress (Da SilvA et al., 2008).

In plants there are at least three distinct types of Catalases which differ in terms of location and biosynthetic regulation (Scandalios, Guan, & Polidoros, 1997). However, this study was interested only in those found in photosynthetic systems and having functions of H_2O_2 elimination during photorespiration.

Comparison of the response to oxidative stress between C3 and C4 plants indicates that plants with C4 type of carbon metabolism exhibit increased activity of AsA and GSH addition to APX, GR and reductase

desidroascorbato (components of ascorbate-glutathione cycle), while plants C3 type have higher CAT activity. The H_2O_2, which is the protonated form of ion peroxide, is not a free radical, it has no unpaired electron. However, H_2O_2 has a great importance in biological systems for ease of diffusion through the bilipid layer of the cell membrane and for their ability to generate OH radical in the presence of divalent metals.

Silva (2014) noted an intense activity of CAT in native plants of forest species in a semi-deciduous forest in the state of São Paulo during the dry season.

For Molinari et al. (2007) high levels of free proline in tissues relate to the increase of APX enzyme activity. The increased APX appropriate to maintain the H_2O_2 levels and stress signaling, since the APX is an enzyme that has a low Km for this substrate, featuring a greater affinity by H_2O_2 than CAT (Scandalios, 2002). However, on the tenth day of water suspension was recorded a reduction in the activity for APX H_2O_2 0.0280 min-1 mg-1 protein in leaf tissue. According Larré (2011) a reduction in the activity of some enzymes at the end of the evaluation period coincides with the restoring assigned metabolism adaptation of the species to stress, which in this study could not be asserted in order that the plants died at the end of experiment.

Larré (2013) also evaluating plant *Erythrina crista galli* L subjected to water stress obtained significant differences in the evaluation periods and treatments, with no significant interaction for the factors. Where the activity of APX enzyme in the sheet showed values 60% higher when compared to the control for the first evaluation period (10 days). Behavior equal to that obtained in this study, with a reduction in enzyme activity the last assessment period.

5. Conclusion

The drought, during the estudied period, committed the antioxidant activity of T. vulgaris plants, since they were able to quickly trigger the immune system, both enzymatic (SOD, APX and CAT), and non-enzymatic (carotenoids), the latter showed no increase in their activity. However, failing to reduce oxidative damage resulting in the death of them. The rapid death of the plants could be partly explained by the prevailing environmental conditions, mentioning the occurrence of the climatic anomaly (El Niño) in the respective year of study, the most intense one already registered, bringing with it the elevation of the surface temperature and consequently the of the air, causing the plants to be in a state of thermal stress.

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