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Antioxidant Enzymes Activity in the *Elaeis guineensis* Jacq. Submitted to Drought

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Abstract

Oil palm is a very responsive culture in relation to climate change that intensifies or lowers its productivity. Thus, the objective of this study is to evaluate the activity of antioxidant enzymes in two genotypes of *E. guineensis*, both under water deficiency. The experiment conducted in a greenhouse at UFRA used genotypes 2528 and 2501 of *E.* guineensis submitted to water deficiency from the 10th day. The biochemical analysis was evaluated at the 5% level of significance by the Tukey test. The antioxidant variables analyzed were superoxide dismutase, catalase activity, ascorbate peroxidase activity, Malondialdeído (MDA), Glutathione and ascorbic acid content. In view of the obtained results, it was observed increases of the antioxidant enzymes when the genotypes were submitted to the water deficiency, presented significance for the results. Therefore, the study suggests that oil palm had a good use and adaptation when submitted to water deficit and that genotype 2528 was more responsive to maintain its vital biochemical activities.

Keyword: biochemistry, hydric stress, oxidative stress

1. Introduction

Drought is one of the environmental factors of greater agricultural importance, as it causes many physiological, metabolic and morphological alterations causing numerous deleterious effects on plant growth, water relations and photosynthesis (Hasanuzzaman et al., 2014). Concomitant to this, the oil palm responds negatively when subjected to drought affecting the productive processes of the species (Al-Amin et al., 2011).

The oil palm (*Elaeis guineensis* Jacq.) is an oleaginous plant belonging to the Arecaceae family and cultivated mainly in tropical regions such as Latin America, Southeast Asia and Africa (Luis et al., 2010). Because it is a cultivation of manual and long cycle cultivation, it has great productive potential, standing out for the high productivity of oil, used in food, industrial and agro-energy processes (Zimmer, 2010).

Several studies indicate that under conditions of water deficiency the activity of photosynthesis is reduced, regardless of the type of metabolism (Cha-um et al., 2010; Cao et al., 2011; Son et al., 2011; Zlatev & Lindon, 2012; Ashraf & Harris, 2013). Thus, the reduction of palm oil production can be directly related to the inhibition of the photosynthetic rate, which is caused, among other factors, by the low relative water content (Fahramand et al., 2014; Zain et al., 2014).

As a consequence, decreases in CO_2 fixation under stress conditions can cause photochemical and biochemical imbalances of photosynthesis (Asada & Badger, 1984). Resulting in excessive production of reactive oxygen species (ROS) which are unstable molecules capable of causing enzymatic damage affecting proteins, carbohydrates, lipids and nucleic acids (Silva & Gonçalves, 2010).

These molecules can be formed as a result of excitation, producing singlet oxygen ($^1O_2$) or reducing O_2 to the anionic superoxide radical ($O_2^{\bullet-}$), hidroperoxylic radical ($HO_2^{\bullet}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$) (Bhattacharjee, 2010). Despite the importance of O_2 for the performance of cellular functions, under stress conditions there is ROS formation in metabolic events (Karuppanapandian et al., 2011).

However, plants have a complex antioxidant system to contain the deleterious effects of reactive oxygen species, in which specific enzymes act to neutralize the action of these radicals (Miller et al., 2010), among the antioxidant enzymes superoxide dismutase (SOD), ascorbato peroxidase (APX), catalase (CAT) among others, and among the main antioxidant metabolites are ascorbic acid (AsA) and the glutathione (GSH) (Kim & Kwak, 2010).

Therefore, due to the great importance of the oil palm it is necessary to understand its physiological or biochemical functions when submitted to regions with water restriction. Thus, the objective of this study is to evaluate the activity of antioxidant enzymes in two genotypes of *E. guineensis*, both under water deficiency.

2. Material and Methods

2.1 Plant Material and Treatment

Young plants of *E. guineensis* with 10 months from Embrapa Amazônia Oriental with similar aspects and sizes were selected and placed in 20 L pots filled with soil and bovine manure (3:1 v/v) substrate. The experiment was carried out in a greenhouse at the Federal Rural University of Amazônia, Capitão Poço campus (Latitude 1°44′47″S and Longitude 47°3′34″W), Brazil, and biochemical analyzes in laboratory biodiversity studies of higher plants.

The plants were submitted to two water regimes: irrigated (control) and water deficit (total irrigation suspension at the beginning of the experiment), in a period of 30 days. During the experimental period the plants called control were irrigated daily to replace the lost water, in which the volume of water applied was due to the size of the vessel used.

2.2 Biochemical Analyses of the Samples

The relative water content (RWC) was determined according to Slavick (1974). The activity of superoxide dismutase (SOD) was determined according to Giannopolitis and Ries (1977). Aliquots of 0.1 mL of the protein extract were transferred to test tubes containing reaction medium composed of potassium phosphate buffer 100 mM (TFK; pH 7.8); 0.1 mM of EDTA; 0.1% (v/v) of 2-Mercaptoetanol; 0.1% (v/v) of Triton X-100; 30 mg of Polivinilpirrolidona (PVP); and, 20 mM from ascorbate. The reaction was initiated by addition of 13 mM of methionine (pH 7.8); 2 μM of riboflavin; 0.075 mM of nitrotetrazolium blue (NBT). After 5 min, the readings were performed at 560 nm and the activity of SOD expressed in (enzymatic unit) U mg^{-1} protein.

2.2.1 Extraction: For Leaves and Roots

The extract for the determination of the activity of the SOD, APX and CAT enzymes was obtained from the homogenization in mortar at 4 °C of 0.1 g of lyophilized leaf powder and root with 5 ml of potassium phosphate buffer solution (at 4 °C) at 0.1 mM, pH 7.0, containing 0.1 mM EDTA, followed by homogenization for 4 min. The additions of the phosphate buffer were made in a fragmented form, 50% of the total volume of this solution (2.5 ml) being used in the homogenization for 2 min, after which the other 50% were immediately added, the mixture being homogenized in time equivalent to the previous one. The homogenate was filtered on nylon tissue and transferred to test tubes, and held at 4 °C for two hours, with occasional shaking. The filtered homogenate was centrifuged at 12,000 ×g for 15 min at 4 °C. The supernatant, the crude extract, was stored in a freezer at -80 °C until used in enzyme activity assays.

2.2.2 Determination of the Concentration of Malonic Aldehyde (MDA)

Determination of the concentration of malonic aldehyde (TCA) (0.1% p/v). The homogenate was centrifuged at 10,000 ×g for 15 min at 4 °C and the supernatant was collected and used in the determination of the concentrations of MDA.

2.2.3 Extraction of GSH From Crude Yeast Extracts by ATPS

A total volume of 1 mL crude yeast extracts containing 0.22 g/L glutathione was added into aqueous two-phase systems, and the final concentration of GSH in ATPS was 20 mg/L. The systems containing different salts were prepared by directly dissolving the salt powder into the system. The systems were mixed thoroughly and centrifuged at 3,000 rpm for 30 min to assist phase separation. The centrifuged systems were then allowed to settle for 5 min to separate into two clear phases. Samples from top and bottom phases were then carefully removed and assayed for glutathione concentration (Wu et al., 2004).

For the determination of catalase activity (CAT) the methodology was used according to Havir and Mchale (1987), which the consumption of H_2O_2 was based on the decrease of the absorbance at 240 nm. 20 μL aliquots of the extract were added to 3 mL of reaction medium consisting of 50 mM potassium phosphate buffer (pH7.0) at 30 °C, plus 12.5 mM H_2O_2. The CAT activity was expressed in nmol H_2O_2 g^{-1} DM min^{-1}.

To measure the activity of ascorbate peroxidase (APX), the consumption of ascorbate was detected by decreasing the absorbance at 290 nm, according to Nakano and Asada (1981). 0.1 mL aliquots of the extract were transferred to test tubes containing 2.7 mL of 50 mM potassium phosphate buffer (pH 6.0) and 0.8 mM L-ascorbate. The reaction was started by the addition of 2 mM H_2O_2 and the enzymatic activity was expressed in μmol ascorbate g^{-1} DM min^{-1}.

The MDA was determined from 0.3 g of fresh Plant tissue mass in 4.0 mL of Trichloroacetic Acid (TCA 0.1% w/v), 500 μL of the extract was diluted in 1.5 mL of the solution of Tiobarbituric acid (TBA 0.5% made in TCA 20%). Samples were read at 532 nm according to the methodology of Cakmak and Horst (1991), expressing the results in nmol g^{-1} FM.

Glutathione (GSH) was determined by mixing 200 μL of supernatant and 1800 μL of reaction mix containing 100 μL phosphate buffer (pH 7.6) and 0.60 mM 2-nitrobenzoic acid, the absorbance being measured at 412 nm, with unit expressed in μmol g^{-1} FW (Wu et al., 2006). The ascorbic acid content (AsA) was performed according to Arakawa et al. (1981), for which one gram of homogenized fresh tissue was used in 5 ml of 5% (w/v) TCA in an ice bath. After reading at 534 nm. The AsA content was determined using a standard curve with μmol g^{-1} FW unit.

2.3 Statistical Design and Data Analysis

The experiment was carried out in a completely randomized design with 2 × 2 factorial treatments [two water conditions (control and drought) × two genotypes (2501 and 2528)], with five replications per treatment. The results of the analyzes were submitted to Shapiro-Wilks (Shapiro & Wilks, 1965) and Levene (Box, 1953) tests for normality and homoscedasticity, respectively. Once the assumptions for analysis of variance were met, the data were submitted to statistical evaluations.

In order to evaluate the comparative effect of water deficiency among the genotypes, the analysis of variance (ANAVA) and the mean values compared by the Tukey test at 5% of probability using the statistical program SISVAR 5.3 and the graphs reproduced by the software Microsoft Excel 2010.

3. Results

3.1 Relative Water Content

With drought stress being prolonged, the relative water content (RWC) decreased significantly, comparing to the control treatment in the oil palm genotypes, which presented reduction of 19% for 2528 and 22% for the 2501. However, there was no significant difference between the two genotypes (Figure 1).

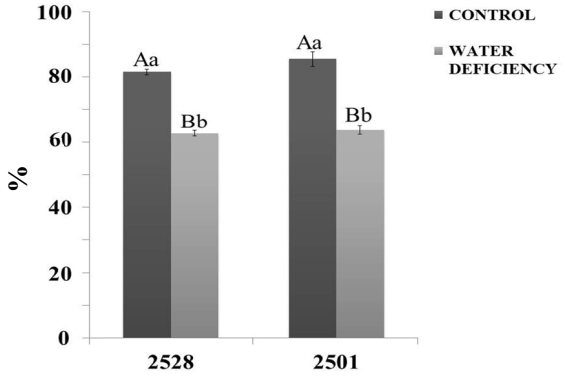

Figure 1. Relative water content in leaves of palm genotypes under drought

Note. Different letters indicate a significant difference by the Tukey test ($p < 0.01$). The bars represent the standard error of the mean.

3.2 Activity of Antioxidant Enzymes

The activity of the antioxidant enzymes increased significantly when the genotypes were submitted to water restriction, for both leaves and roots of the oil palm. The results of this study showed that the activity of SOD showed increased of 74% and 77% to 2528 and, 72% and 75% to 2501 (Figures 2A and 2E), there was no significant difference between the genotypes.

CAT increased 46% and 43% to 2528 and 44% and 41% to 2501 (Figures 2B and 2F), with a significant difference between the genotypes; it was observed that APX increased its activity in 33% and 28% to 2528 and 30% and 26% to 2501 (Figures 2C and 2G), there was a significant difference only for leaf in the condition of control treatment; and MDA showed increase of 18% and 29% to 2528 and, 15% and 20% to 2501 (Figures 2D and 2H), there was no significant difference between the genotypes for root under control treatment.

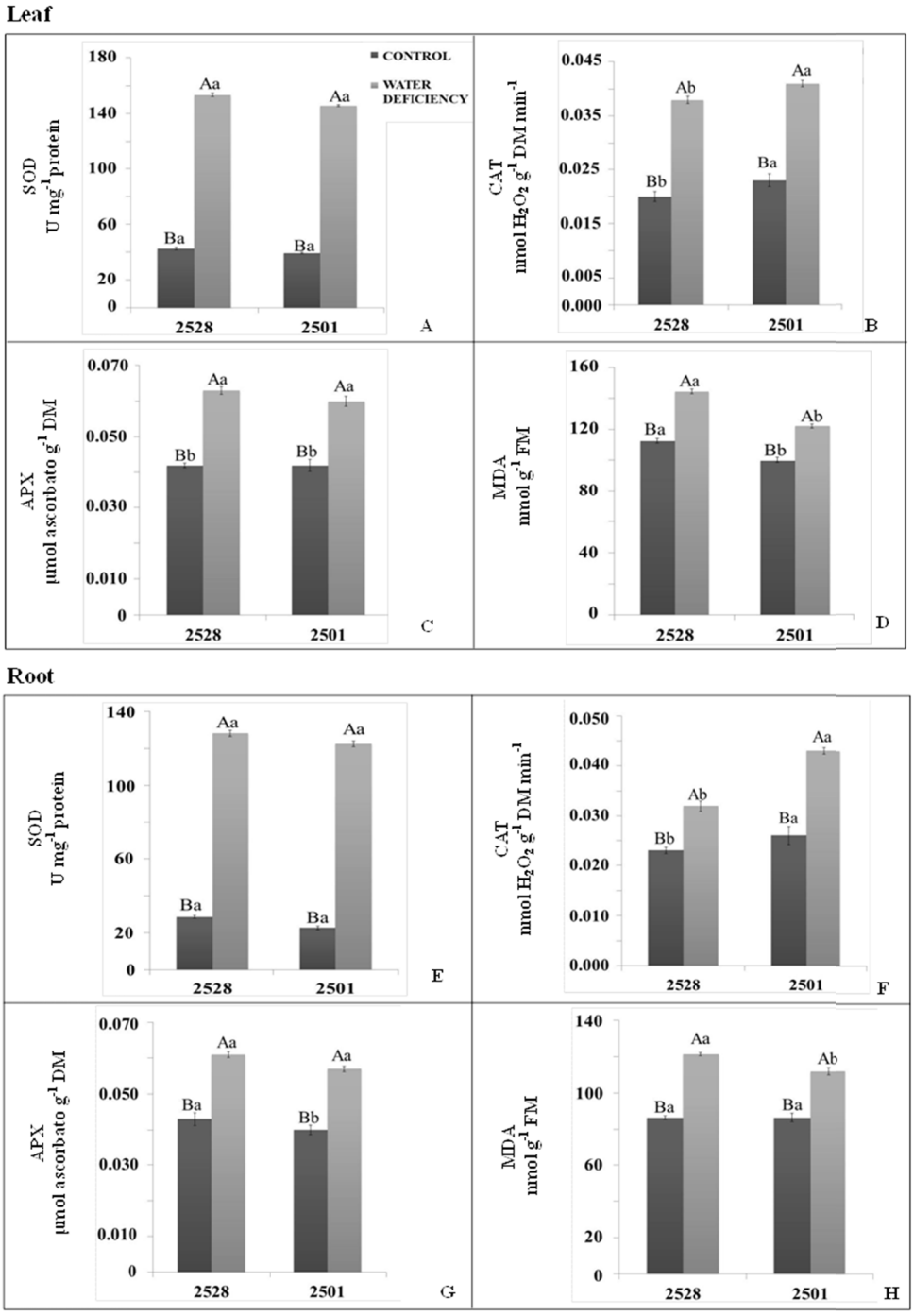

Figure 2. Activity of SOD (A, E), CAT (B, F), APX (C, G) and MDA (D, H) in leaves and roots of oil palm genotypes under drought

Note. Different letters indicate a significant difference by the Tukey test ($p < 0.05$). The bars represent the standard error of the mean.

3.3 Content of GSH and AsA

In comparison to control, the drought stress increased the contents of GSH and AsA in leaves and roots of both genotypes, showing a significant increase in glutathione in 53% and 36% for 2528 and 61% and 52% for 2501 (Figures 3A and 3B). There was no significant difference between genotypes only for leaves of the plants under control and for ascorbic acid 51% and 55% for 2528 and 27% and 25 for 2501 (Figures 3C and 3D), with genotype 2528 being highlighted in the roots.

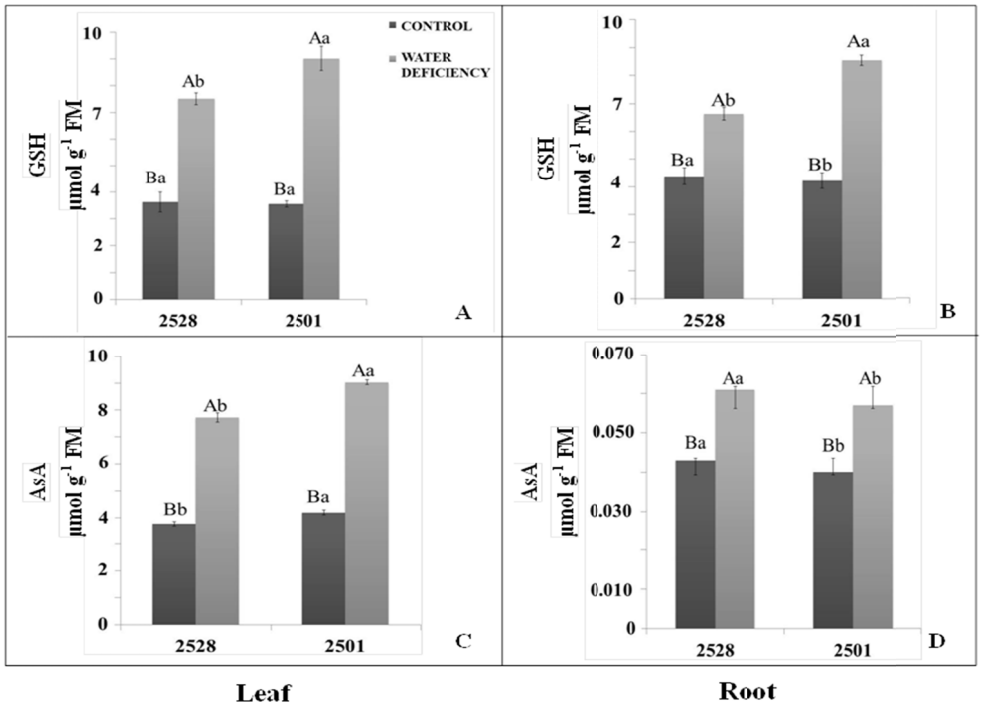

Figure 3. Effect of drought under concentrations of GSH (A, B) and AsA (C, D) in leaves and roots of oil palm genotypes

Note. Different letters indicate a significant difference by the Tukey test ($p < 0.05$). The bars represent the standard error of the mean.

4. Discusion

4.1 Relative Water Content

The reason for the reduction of the RWC can be explained by the stomatal closure due to the maintenance of the plant in the water suspension period as a strategic form in response to the signaling triggered by abscisic acid (Hong-Bo et al., 2008). Water deficiency the two genotypes lose more water than when under control treatment but possibly maintains their water status due to the plant's ability to survive severe water deficits by restricting loss through the leaf epidermis after the stoma has reached a minimum opening (El-Jaafari et al., 2000).

4.2 Activity of Antioxidant Enzymes

The significant increase of the antioxidant enzymes in oil palm under water deficiency probably occurred due to the development of these mechanisms in the plant to prevent and/or neutralize the oxidative stress caused by reactive oxygen species (ROS) (Pintó-Marijuan & Munné-Bosch, 2014).

When plants undergo stress, they develop defense mechanisms that involve non-enzymatic water-soluble antioxidant compounds (glutathione), which provide electrons and act in conjunction with antioxidant enzymes (SOD, APX and CAT), which catalyze redox reactions (Kruk et al., 2014; Miret & Munné-Bosch, 2015). Concomitant to this, the protection resulting from the enzymatic activities may have important influence under the tolerance of the species submitted to drought.

Nevertheless, it was possible to observe that the activity of SOD, known to confer tolerance to oxidative stress and that acts as the first line of defense against ROS, possibly diminished the damage caused in the oil palm

genotypes as a response to metabolize the radicals produced superoxides (Jaleel et al., 2007). SOD, which may be mitochondrial, cytosolic or chloroplastidic, is responsible for the dismutation of O_2 converting it to hydrogen peroxide and oxygen (Grob et al., 2013).

Ascorbate peroxidase and catalase (CAT), which are enzymes that will detoxify the H_2O_2 compounds generated by oxidative stress in order to generate other products that are not toxic to the plant cell (Bhattacharjee, 2010) are required. That is, the combined actions of SOD and CAT are crucial to mitigate the effects of oxidative stress, since the first involves the dismutation of O_2 in H_2O_2 and the latter decomposes H_2O_2 in water and O_2, and may indicate an important role in the control of accumulation of H_2O_2 from oil palm subjected to water deficiency.

The APX enzyme may also have been directly involved in the detoxification components of H_2O_2, due to the stressful condition (Bhatt & Tripathi, 2011). Thus, it is possible to observe that the increase of the enzymatic activities, during the studied period, shows the defense of the plant in the destruction of free radicals as a form of prevention to more severe damages.

With the permanence of the water suspension, there was possibly loss of cell compartmentalization in the two oil palm genotypes, which is accompanied by the increase in the lipid peroxidation caused by ROS formation, being this peroxidation estimated by the concentration of MDA, that is, the accumulation of MDA in a tissue is widely used to estimate cell damage (Sung & Jeng, 1994).

Thus, it is possible to observe that the concentrations of MDA increased significantly when the genotypes were under treatment with water suspension, inferring the increase of the lipid peroxidation. However, with the action of the antioxidant enzymes it was observed a reduction of the oxidative damages in the tissues of the genotypes under water deficiency.

4.3 Content of Glutathione and Ascorbic Acid

The importance of glutathione (GSH) has been studied because it is a tripeptide that is central to the cellular redox state in plants (Noctor et al., 2011). Due to the fundamental role in plant cells including redox signaling and homeostasis, we observed high concentrations of this component in leaves and roots of oil palm when submitted to water deficiency (Koprivova et al., 2010).

Therefore, it is possible that glutathione has acted in favor of the reduction of ROS acting as another plant defense system and being proportional to ascorbic acid (AsA) levels. From this, it is noted that some studies indicate that these two components act together in the ascorbate-glutathione cycle (ASC-GSH), doing the recycling of oxygenated compounds, showing increasing values for both roots and leaves in the two genotypes (Noctor & Foyer, 1998; Serkedjieva, 2011).

5. Conclusion

The study suggests that oil palm had a good use and adaptation when submitted to water deficit and that genotype 2528 was more responsive to maintain its vital biochemical activities.

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