Emirates Journal of Food and Agriculture. 2017. 29(3): 206-211
doi: 10.9755/ejfa.2016-09-1217
http://www.ejfa.me/

REGULAR ARTICLE

Antioxidant system is insufficient to prevent cell damages in *Euterpe oleracea* exposed to water deficit

Maria Antonia Machado Barbosa[1], Allan Klynger da Silva Lobato[1]*, Thaís Soares Pereira[1], Gélia Dinah Monteiro Viana[1], José Ricardo Santos Barbosa[1], Kelly Nayara Nascimento Coelho[1]

[1]Núcleo de Pesquisa Vegetal Básica e Aplicada, Universidade Federal Rural da Amazônia. Paragominas, Pará, Brazil

ABSTRACT

This study aimed to determine whether antioxidant enzymes are efficient to control the oxidative stress and consequent cell damages on leaves and roots in *Euterpe oleracea* plants exposed to water deficiency. This study was assembled under an experimental design completely randomized with two water conditions (water deficit and control) combined by four evaluation dates (0, 6, 12, and 18 days). Progressive water deficit promoted significant increases in electrolyte leakage and glutathione in both tissue types, and hydrogen peroxide and malondialdehyde were increased in the leaf. Antioxidant enzyme activities showed similar behaviours, with initial increases and subsequent decreases. Our results revealed that the cell damage observed in leaves and roots was induced by multiple effects related to overproduction of oxidant compounds, such as hydrogen peroxide, and by insufficient CAT, APX, and GPX activities in *E. oleracea* plants exposed to progressive water deficit.

Keywords: Antioxidant enzymes; *Euterpe oleracea*; Oxidative damage; Hydrogen peroxide; Water deficiency

INTRODUCTION

Water deficiency is a problem frequently found in field conditions, representing a limiting factor in areas with agricultural potential (Freitas et al., 2007). Interferences on growth plant also are detected on yield (Endres et al., 2010). Water supply is fundamental to keep the metabolic activity (Barbosa et al., 2014), nutrient absorption (Wang et al., 2011), gas exchange (Santos et al., 2009), and translocation of organic substances (Lobato et al., 2008).

The drought is an abiotic stress that induces the accumulation of hydrogen peroxide (H_2O_2) and superoxide (O_2^-) (Queiroz et al., 2002). These compounds promote the oxidation of membranes and cell damages (Mittler, 2002). In other hand, the antioxidant system uses key enzymes, such as catalase (CAT), ascorbate peroxidase (APX), and guaiacol peroxidase (GPX) to avoid the oxidative stress generated during inadequate conditions.

Palms, such as *Euterpe oleracea*, are one of the few plants of the monocot class that reach significant heights (Renninger and Phillips, 2011). Studies with palms frequently use the species *Phoenix dactylifera* and *Elaeis guineensis* as models (Suresh et al., 2012; Gribaa et al., 2013), being described significant cell damages after water deficit (Baslam et al., 2014). In other hand, biochemical approaches linked to antioxidant system and using *E. oleracea* plants can contribute to elucidate the cell damages verified in palm plants during water deficit.

This study aimed to determine whether antioxidant enzyme activities are sufficient to avoid the oxidative stress and consequent cell damage incurred by the leaves and roots of young *Euterpe oleracea* plants exposed to progressive water deficit.

MATERIALS AND METHODS

Growth conditions

The experiment was carried out in greenhouse under controlled temperature and humidity, with minimum, maximum, and median temperatures being 21°C, 34°C, and 25°C, respectively. Relative humidity during the period studded varied between 65% and 93%.

***Corresponding author:**
Allan Klynger da Silva Lobato, Rodovia PA 256, Paragominas, Pará, Brazil. Núcleo de Pesquisa Vegetal Básica e Aplicada, Universidade Federal Rural da Amazônia. Phone: +55-91-983089845; Fax: +55-91-983089845, E- mail: allanllobato@yahoo.com.br

Received: 21 September 2016; **Revised:** 04 February 2017; **Accepted:** 07 February 2017; **Published Online:** 17 February 2017

Plants, containers, and substrate

Seeds of *Euterpe oleracea* (Mart.) cv. BRS-Pará from EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária/Brazil) were germinated in 8-L pots (0.25 m in height and 0.20 m in diameter) equipped with holes for water drainage. The pots were filled with Plantmax® substrate, and the plants were irrigated daily with 1 L of distilled water. Seedlings received the following macro- and micronutrients from the nutritive solution: 8.75 mM KNO_3, 7.5 mM $Ca(NO_3)_2 \cdot 4H_2O$, 3.25 mM $NH_4H_2PO_4$, 1.5 mM $MgSO_4 \cdot 7\ H_2O$, 62.50 µM KCl, 31.25 µM H_3BO_3, 2.50 µM $MnSO_4 \cdot H_2O$, 2.50 µM $ZnSO_4 \cdot 7H_2O$, 0.63 µM $CuSO_4 \cdot 5H_2O$, 0.63 µM $NaMoO_4 \cdot 5H_2O$, and 250.0 µM $NaEDTAFe \cdot 3H_2O$, being applied 0.4 mL per pot at regular intervals (every 30 days) until the 12th month, being necessary this period to obtain young plants. Subsequently, one-year-old plants with similar aspects and sizes were selected to be used in this study.

Experimental design and application of water deficit

This study used a randomised design in factorial with two water conditions (water deficit and control) and four evaluation dates (0, 6, 12, and 18 days). The experiment was composed by five replicates and 40 experimental units, with one plant in each unit. Plants exposed to water deficit were submitted to 18 days under water restriction, while plants from control treatment were watered daily with distilled water. The leaf and root (middle region of the each tissue) were harvested and frozen in liquid nitrogen and stored at –20°C for subsequent biochemical determinations.

Leaf relative water content

The leaf relative water content (LRWC) was evaluated using leaf discs with diameters of 10 mm. From each plant, 40 discs were removed, and the LRWC was calculated using the formula [(FM-DM)/(TM-DM)] × 100, as proposed by Slavick (1979). Here, FM represents fresh matter, TM represents turgid matter evaluated after 24 h and saturated in deionised water at 4°C in the dark, and DM represents dry matter determined after 48 h in an oven with forced air circulation at 80°C.

Electrolyte leakage

Electrolyte leakage (EL) was measured according to the method described by Gong et al. (1998) with minor modifications. Leaf and root fresh (200 mg) were cut into pieces with a length of 1 cm and were placed in containers containing 8 mL of distilled deionised water. The containers were incubated in a water bath at 40°C for 30 min, and the initial electrical conductivity of the medium (EC_1) was measured. The samples were boiled at 95°C for 20 min to release the electrolytes. After the samples were cooled, the final electrical conductivity (EC_2) was measured (Gong et al., 1998). The percentage of electrolyte leakage was calculated using the formula EL (%) = EC_1/EC_2 x 100.

Extraction of oxidant and antioxidant compounds

Oxidant and antioxidant compounds (H_2O_2, MDA, and GSH) were extracted from leaf and root tissues as described by Wu et al. (2006). Briefly, an extraction mixture was prepared by homogenising 500 mg of fresh matter in 5 mL of 5% (w/v) trichloroacetic acid. Subsequently, the samples were centrifuged at 15,000 x g for 15 min at 3°C, and the supernatant was collected.

Hydrogen peroxide

For H_2O_2 detection, 200 µL of supernatant and 1,800 µL of reaction mixture (2.5 mM potassium phosphate buffer [pH 7.0] and 500 mM potassium iodide) were mixed, and the absorbance was measured at 390 nm (Velikova et al., 2000).

Malondialdehyde

MDA was determined by mixing 500 µL of supernatant with 1,000 µL of the reaction mixture, which contained 0.5% (w/v) thiobarbituric acid in 20% trichloroacetic acid. The mixture was incubated in boiling water at 95°C for 20 min, and the reaction was terminated by placing the reaction container in an ice bath. The samples were centrifuged at 10,000 x g for 10 min, and the absorbance was measured at 532 nm. The amount of non-specific absorption at 600 nm was subtracted from the absorbance data. The amount of MDA–TBA complex (red pigment) was calculated based on the method of Cakmak and Horst (1991) with minor modifications, using an extinction coefficient of 155 $mM^{-1}\ cm^{-1}$.

Glutathione

For GSH determination, 200 µL of supernatant and 1,800 µL of reaction mixture (containing 100 mM phosphate buffer [pH 7.6] and 0.60 mM 2-nitrobenzoic acid) were combined, and the absorbance was measured at 412 nm (Wu et al., 2006).

Extraction of antioxidant enzymes and soluble proteins

To determinate antioxidant enzymes (CAT, APX, and GPX) and soluble proteins were used leaf and root tissues (Badawi et al., 2004). The extraction mixture was prepared by homogenising 500 mg of fresh matter in 5 mL of extraction mix, which contained 50 mM phosphate buffer (pH 7.6), 1.0 mM ascorbate and 1.0 mM EDTA. Subsequently, the samples were centrifuged at 14,000 x g for 4 min at 3°C, and the supernatant was collected. Quantification of the total soluble proteins was performed using the method described by Bradford (1976). The absorbance was measured at 595 nm, and bovine albumin was used as a standard.

Catalase

For CAT determination, 200 µL of supernatant and 1,800 µL of reaction mixture (containing 50 mM phosphate

buffer [pH 7.0] and 12.5 mM hydrogen peroxide) were combined, and the absorbance was measured at 240 nm (Havir and McHale, 1987).

Ascorbate peroxidase

For APX determination, 1,800 µL of reaction mixture containing 50 mM phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.1 mM EDTA, and 1.0 mM hydrogen peroxide was mixed with 200 µL of supernatant, and the absorbance was measured at 290 nm (Nakano and Asada, 1981).

Guaiacol peroxidase

The GPX determination, 1,780 µL of reaction mixture (containing 50 mM phosphate buffer [pH 7.0] and 0.05% guaiacol) was mixed with 200 µL of supernatant. Subsequently, 20 µL of 10 mM hydrogen peroxide was added. The absorbance was then measured at 470 nm (Cakmak and Marschner, 1992).

Data analysis

The data obtained in this study were subjected to an analysis of variance, being significant differences detected using the F test at a probability level of 5% (Steel et al., 2006). The standard deviations were obtained in all treatments. All statistical procedures were performed with ASSISTAT software.

RESULTS

Plants exposure to water deficit presented reduction in LRWC; however, significant effects were only observed on the 12th and 18th day after stress application (Table 1), if compared with control plants. The water deficit caused increase in EL in the leaf and root, which was significant in both tissues at the 12th and 18th days after water restriction. Compared to the control plants, the variations more intense in leaf and root were of 16% (both) on the 18th day (Table 1). Regarding H_2O_2, similar behaviours were observed in the leaf and root. On the 12th day, the water-deficient leaf showed a 39% increase, compared with the control plants (Table 1), while in the root, the increase was not significant (17% at the final evaluation date) (Table 1).

MDA was clearly increased in the leaf and root, but fluctuations were observed in root tissue. The leaf presented increases that were significant on the 12th and 18th day after water restriction (Table 2). Meanwhile, the root showed an initial decrease and subsequent significant increase during the course of the experiment (Table 2). The water deficit triggered similar behaviours in GSH concentrations in the leaf and root. When evaluated in the leaf, the greatest increase (62%) was observed at the last time point (Table 2). In the root, large increases were detected, with an increase of 112% after 18 days of water deficit (Table 2).

Table 1: Leaf relative water content, electrolyte leakage in leaf and root, and hydrogen peroxide in leaf and root of young *Euterpe oleracea* plants subjected to water deficit

Leaf relative water content (%)		
Time (days)	Water condition	
	Control	Water deficit
0	80.7±4.6a	82.7±4.1a
6	78.2±6.1a	74.9±3.8a
12	75.7±4.0a	55.7±5.2b
18	77.3±7.0a	48.5±2.3b
Electrolyte leakage in leaf (%)		
Time (days)	Water condition	
	Control	Water deficit
0	83.5±5.6a	83.8±5.2a
6	82.4±5.4a	90.5±3.1a
12	82.6±3.8a	94.2±4.5b
18	81.8±4.5a	95.1±2.0b
Electrolyte leakage in root (%)		
Time (days)	Water condition	
	Control	Water deficit
0	84.0±2.3a	84.1±2.1a
6	84.1±3.4a	90.3±3.4a
12	84.3±3.3a	95.3±4.1b
18	82.3±1.9a	95.6±1.2b
Hydrogen peroxide in leaf (µmol g^{-1} FM)		
Time (days)	Water condition	
	Control	Water deficit
0	5.43±0.51a	5.55±0.42a
6	5.26±0.30a	5.37±0.40a
12	5.51±0.47a	7.66±0.57b
18	5.70±0.22a	7.72±0.33b
Hydrogen peroxide in root (µmol g^{-1} FM)		
Time (days)	Water condition	
	Control	Water deficit
0	1.91±0.18a	1.95±0.13a
6	1.76±0.14a	1.86±0.10a
12	1.86±0.12a	1.96±0.25a
18	1.72±0.13a	2.02±0.23a

Lines with different letters indicate significant differences at F test (P<0.05) to each time values described corresponding to means from five repetitions and standard errors

The CAT activity exhibited a similar tendency in both tissues evaluated, with an increase and subsequent decrease. In leaves, the increase was significant only on the 6th day, with a variation of 145%; however, the level of this enzyme was decreased at other times (Fig. 1 A). Additionally, significant increases were noted in the root at 6 and 12 days, with the highest increase being 309%; however, a reduction was observed at the final time point (Fig. 1 B). Water restriction provoked an initial increase in APX and a subsequent reduction in both the leaf and root (Fig. 1 C and D); significant peaks were observed at the 12th and 6th day, respectively, with reductions observed

Table 2: Malondialdehyde in leaf and root, and total glutathione in leaf and root of young *Euterpe oleracea* plants subjected to water deficit

Malondialdehyde in leaf (nmol g^{-1} FM)		
Time (days)	Water condition	
	Control	Water deficit
0	8.52±0.72a	8.66± 0.61a
6	8.31±0.62a	10.02±0.82b
12	8.66±0.75a	11.15±0.85b
18	8.58±0.81a	11.56±0.72b
Malondialdehyde in root (nmol g^{-1} FM)		
Time (days)	Water condition	
	Control	Water deficit
0	3.23±0.23a	3.21±0.25a
6	3.26±0.31a	2.85±0.22a
12	3.02±0.22a	3.56±0.25b
18	3.03±0.24a	3.68±0.26b
Total glutathione in leaf (µmol g^{-1} FM)		
Time (days)	Water condition	
	Control	Water deficit
0	4.32±0.36a	4.36±0.31a
6	4.13±0.25a	4.83±0.24b
12	4.34±0.29a	5.12±0.27b
18	4.17±0.31a	6.78±0.52b
Total glutathione in root (µmol g^{-1} FM)		
Time (days)	Water condition	
	Control	Water deficit
0	0.41±0.02a	0.43±0.03a
6	0.42±0.03a	0.47±0.03a
12	0.41±0.05a	0.67±0.05b
18	0.40±0.04a	0.85±0.07b

Lines with different letters indicate significant differences at F test (P<0.05) to each time values described corresponding to means from five repetitions and standard errors

at other times. Water deficits induced increases in GPX in leaf and root tissues; the increases were similar in the leaf and root, with 97% and 130% increases observed (Fig. 1 E and F), respectively, at 12 days of water deficit.

DISCUSSION

Water deficiency promoted decrease in LRWC, being explained by the lower water quantity absorbed by the plant via substrate and losses by transpiration, causing wilted leaves. The increases in EL in the leaf and root induced after water deficit can be explained due to the higher membrane permeability, which causes damage to membranes and cellular organelles that is frequently irreversible, with a consequent increase in EL (Slama et al., 2011). Gonçalves et al. (2011) evaluating the effects of the water deficit by eight consecutive days on *Jatropha curcas* plants reported non-significant modifications in EL, suggesting that *E. oleracea* is more sensitive that *J. curcas*.

The water deficit caused an increase in H_2O_2 in leaf and root tissues, which was most likely linked to higher superoxide dismutase (SOD) activity and a decrease in catalase activity. These changes are more intense when plants are exposed to water deficiency. H_2O_2 is toxic, and it is overproduced in plant cells exposed to abiotic stress, such as water deficit (Chang et al., 2012). He et al. (2012) described highest H_2O_2 accumulation in leaf, following by stem and root in *Solanum tuberosum* plants exposed to water limitation. The increase in MDA in both tissues was caused by the actions of reactive oxygen species (ROS), such as H_2O_2 and O_2^-, which areproduced during stress (Arcoverde et al., 2011). ROS also promote lipid peroxidation due to membrane denaturation and consequent increases in MDA levels. Sofo et al. (2004) reported also significant increases in leaf and root tissues of *Olea europaea* plants subjected to controlled water deficit for 20 days. In relation to GSH, increases in leaf and root were observed after water deficit, being this fact explained as a consequence induced by oxidative stress; additionally, it can be utilised as an indicator of water deficit (Gill and Tuteja, 2010).

The water restriction caused an initial increase and a subsequent decrease in CAT activity in the leaf and root. The increase in the activity of this enzyme was induced by H_2O_2 accumulation, and the reduction was related to an insufficient capacity to remove oxidant compounds produced during water deficit. This enzyme is involved in antioxidant protection and maintenance of membrane integrity (Carvalho et al., 2012). Marron et al. (2006) showed an increase in CAT activity in leaves of *Populus deltoides* clones. Slight peaks in the APX enzyme were observed with a subsequent decrease in its activity in both tissues. The increases are likely associated with the formation of reactive oxygen species (ROS) during water limitation, while the decrease most likely occurred due to limited ascorbate supply. APX is a key enzyme in the ascorbate-glutathione cycle, in which it attenuates the oxidative stress generated under inadequate conditions (Shvaleva et al., 2006). An increase in APX activity was also found by Pérez-Clemente et al. (2012), who studied *Citrange carrizo* plants under water stress. The limited increase in GPX activity can be explained by the fact that water deficit promotes the formation of free radicals, such as O_2^-, and the consequent accumulation of oxidative compounds because this enzyme catalyses the reaction that uses H_2O_2 and GSH as substrates to produce oxidised glutathione (GSSG) and water (H_2O), exerting a fundamental role in the antioxidant machinery (Sayfzadeh and Rashidi, 2010).

Progressive water deficit promoted significant increases in electrolyte leakage and glutathione in both leaf and root tissues, and hydrogen peroxide and malondialdehyde were increased in the leaf. The activities of antioxidant

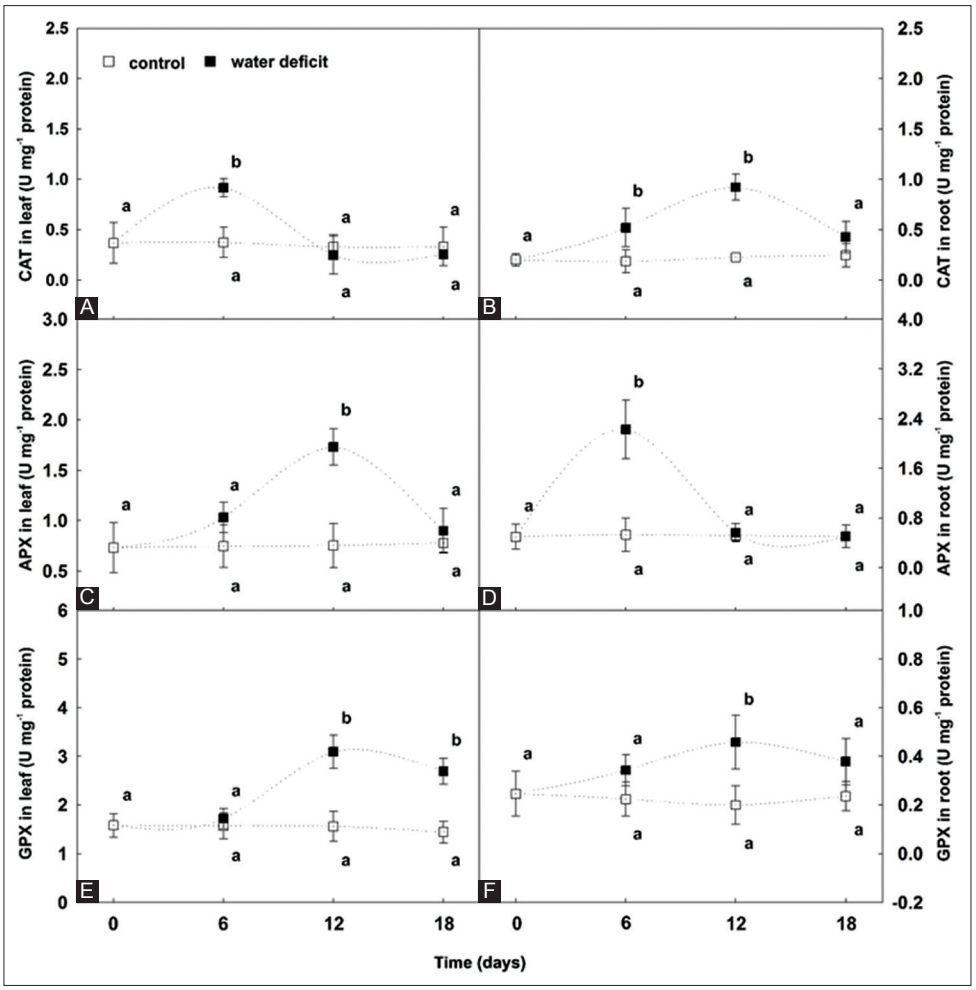

Fig 1. Catalase activities in leaf and root (A – B), ascorbate peroxidase activities in leaf and root (C – D), and guaiacol peroxidase activities in leaf and root (E – F) in young *Euterpe oleracea* plants subjected to water deficit. Different letters indicate significant differences at F test ($P < 0.05$) to each time. Squares represent the mean values of 5 repetitions, and bars represent the standard errors.

enzymes showed similar trends, with initial increases and subsequent decreases. Our results revealed that the cell damage observed in leaves and roots was induced by multiple effects related to the overproduction of oxidant compounds, such as hydrogen peroxide, as well as the insufficient activity of antioxidant enzymes (CAT, APX, and GPX) in young *Euterpe oleracea* plants exposed to progressive water deficit.

ACKNOWLEDGEMENTS

This research had financial supports from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq/Brazil) and Universidade Federal Rural da Amazônia (UFRA/Brazil) to Lobato AKS, and Barbosa MAM, Viana GDM, Barbosa JRS, Coelho KNN, and Pereira TS were supported by undergraduate scholarships also from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq/Brazil).

Author contributions

Lobato A.K.S. was the advisor of this project and planned all phases of this research. Barbosa M.A.M., Pereira T.S., Viana G.D.M., Barbosa J.R.S. and Coelho K. N. N. conducted the experiment in the greenhouse and performed physiological, biochemical and morphological determinations, as well as interpreted the results and wrote the manuscript.

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