

## Photosynthesis-Involvement in Modulation of Ascorbate and Glutathione in *Euterpe oleracea* Plants Exposed to Drought

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### Abstract

The present study aimed to determine if photosynthesis interferes with the modulation of antioxidant compounds in young *Euterpe oleracea* plants exposed to water deficiencies. A factorial, completely randomised experimental design was employed, and two water conditions (water deficit and control) and four evaluation points (0, 6, 12 and 18 days) were used, resulting in a total of eight measurements. The measured parameters included the water content and temperature of the leaf, gas exchange, electrolyte leakage, and antioxidant content. Compared to the control treatment, the net loss of photosynthesis due to water restriction increased by approximately 100% on the 18th day of drought. The ascorbate levels decreased due to water restriction, presenting significant differences on the 12th and 18th day. In some cases, the water deficit increased the glutathione content; however, significant effects were only observed on the 18th day after irrigation suspension. Water deficits had a negative impact on stomatal conductance, net photosynthesis rate, transpiration rate, and instantaneous carboxylation efficiency. Additionally, increases in the glutathione content, electrolyte leakage, and malondialdehyde content were observed; however, the ascorbate content decreased. Our results confirmed that the rate of photosynthesis interfered with the modulation of ascorbate and glutathione in young *Euterpe oleracea* plants exposed to drought.

**Keywords:** antioxidant compounds, *Euterpe oleracea*, gas exchange, reactive oxygen species, water deficiency

### Introduction

*Euterpe oleracea* (Mart.) is a palm that grows in the Amazon forest, and this plant has nutritional and economical significance due to the commercialisation of energy drinks and the heart of the palm, which is produced from the fruit and stem (Rogez, 2000; Bobbio *et al.*, 2002). Regarding its nutritional proprieties, the fruit contains higher amounts of lipids and minerals such potassium, calcium, and magnesium (Menezes *et al.*, 2008; Yuyama *et al.*, 2011). Additionally, a study conducted by Bobbio *et al.* (2000) revealed that the fruit is a source of anthocyanins. Brazil is the main producer of *E. oleracea* and is a significant consumer and exporter of products derived from this palm (Menezes, 2005).

Water deficiency represents an abiotic stress and is considered one of the most important agricultural limitations due to its negative effects on growth and development (Kirnak *et al.*, 2001) and the corresponding

yield losses (Endres *et al.*, 2010). Water supplies are essential in several metabolic and physiological processes such as the assimilation of nutrients (Wang *et al.*, 2011), gas exchange (Santos *et al.*, 2009), and translocation of organic solutes (Lobato *et al.*, 2008).

Plants exposed to water restrictions frequently exhibit negative responses linked to gas exchange (Yu *et al.*, 2009), and photosynthesis is strongly affected by drought (Isoda, 2005). Stomatal conductance has been used as indicator of water deficits (Oliveira *et al.*, 2005) because the regulation of this mechanism is influenced by the leaf water potential (Garnier and Berger, 1987) and water availability in the soil (Batista, 2011). In contrast, increases (Blum, 2005; Martins *et al.*, 2011) or decreases (Vu and Allen Jr., 2009) in water use efficiency occur during mild and severe stresses, respectively.

Recently, Sack and Holbrook (2006) reported that aquaporins present in bundle-sheath cells (BSCs) play a role in water regulation in the leaf; this finding was confirmed by

Shatil-Cohen *et al.* (2011), who showed that BSCs function as the first line of defence against drought.

Drought is directly related to the overproduction of reactive oxygen species (ROS) (Asada, 2006) such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and superoxide (O<sub>2</sub><sup>-</sup>) (Queiroz *et al.*, 2002), which are highly toxic compounds. ROS promote the oxidation of membranes and damage essential organelles such as chloroplasts (Carvalho, 2008) and mitochondria (Moller, 2001), which results in cell damage or death (Mittler, 2002).

Ascorbate and glutathione (GSH) have essential functions in antioxidant metabolism (Wang *et al.*, 2011) because ascorbate (ASC) is used as a substrate (Mehlhorn *et al.*, 1996). Additionally, GSH produces ascorbate and glutathione disulphide (GSSG), which is used to regenerate GSH via glutathione reductase (GR) (Creissen *et al.*, 1999).

The effects of gas exchange and ROS accumulation during water deprivation have been well studied; however, their effects have not been fully investigated in *E. oleracea*. Thus, the present study aimed to determine whether photosynthesis interferes in the modulation of antioxidant compounds in young *E. oleracea* plants exposed to water deficiencies.

## Materials and Methods

### Location and growth conditions

The experiment was performed on the Campus of Paragominas of the Universidade Federal Rural da Amazônia, Paragominas, Brazil (2°55'S and 47°34'W). The study was conducted in a greenhouse without environmental control, and the minimum, maximum, and median temperatures were 22 °C, 33 °C, and 26 °C, respectively.

The relative humidity during the experimental period varied between 65% and 93%, and the photoperiod was set to 12 h of light. During the measurement period (12:00 h), the amount of photosynthetically active radiation varied between 451 and 1,453 μmol m<sup>-2</sup> s<sup>-1</sup>.

### Plants, containers, and substrate

Seeds of *Eutерpe oleracea* (Mart.) 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. After 100 days, seedlings with similar aspects and sizes were selected. Subsequently, 120-day-old seedlings received 0.2 L of Hoagland and Arnon (1950) nutrient solution, which was modified for *E. oleracea*. Nutritional supplementation was applied at regular intervals (every 30 days) until the 12th month.

### Experimental design

The experiment employed a factorial randomised design with two water conditions (water deficit and control) and four evaluation points (0, 6, 12 and 18 days), yielding a total of eight measurements. The experiment was assembled with five replicates for a total of 40 experimental units with one

plant in each unit.

### Water deficit application and harvest

All plants were grown until the 12th month, as described above. Plants receiving the water deficit treatment were submitted to 18 consecutive days without irrigation. Plants subjected to the control treatment were watered daily with distilled water. Parameters associated with gas exchange were measured during each evaluation period. The plants were harvested, and leaves present in the middle region of the plant were removed. The harvested leaves were frozen in liquid nitrogen and stored at -20 °C for subsequent biochemical determination.

### Leaf gas exchange

The stomatal conductance, sub-stomatal CO<sub>2</sub> concentration, leaf temperature, net photosynthetic rate, and transpiration rate were evaluated using an infrared gas analyser (ADC Bioscientific, model LCPro+). The parameters were measured using the adaxial surface of fully expanded leaves, which is localised in the middle of the plant.

The water use efficiency was estimated according to the method of Ma *et al.* (2004), and the instantaneous carboxylation efficiency was calculated using the formula described by Aragão *et al.* (2012). Gas exchange was evaluated in all plants between 9:00 and 12:00 h, and the irradiance was maintained at 800 μmol m<sup>-2</sup> s<sup>-1</sup> during the measurements.

### Leaf relative water content

The leaf relative water content (LRWC) was evaluated in leaf disks with diameters of 10 mm. For each plant, 40 disks 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 was measured according to the method described by Gong *et al.* (1998) with minor modifications. Fresh leaves (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<sub>1</sub>) 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<sub>2</sub>) was measured (Gong *et al.*, 1998). The percentage of electrolyte leakage was calculated using the formula EL (%) = EC<sub>1</sub> / EC<sub>2</sub> × 100.

### Extraction of antioxidant compounds

Antioxidant compounds (malondialdehyde [MDA], ASC, and GSH) were extracted as described by Wu *et al.* (2006). Briefly, an extraction mixture was prepared by

homogenising 500 mg of fresh leaf 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.

**Malondialdehyde quantification**

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<sup>-1</sup> cm<sup>-1</sup>.

**Ascorbate quantification**

The reaction for ASC detection contained 200 µL of supernatant and 1,800 µL of reaction mixture and was performed according to a method described by Cakmak and Marschner (1992) with minor modifications. The reaction mixture was prepared in 100 mM phosphate buffer (pH 7.6) composed of 400 µL of 10% trichloroacetic acid, 400 µL of 44% ortho-phosphoric acid, 400 µL of 4% 2,2'-dipyridyl in 70% ethyl alcohol, and 200 µL of 3% FeCl<sub>3</sub>. The mixture was incubated at 40 °C for 40 min, and the resulting colour intensity was measured at 525 nm.

**Glutathione quantification**

For GSH detection, 200 µL of supernatant, 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).

**Data analysis**

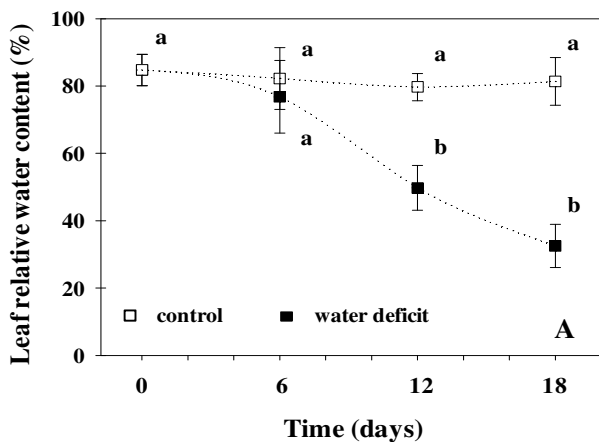


Fig. 1. Leaf relative water content in young *Euterpe oleracea* plants subjected to water deficit. Same letters do not show significant differences at F test (P < 0.05) to each time. Squares represent the mean values of 5 repetitions, and bars represent the standard deviations.

The data were subjected to an analysis of variance, and significant differences between the means were determined using the F test at a probability level of 5% (Steel et al., 2006). Standard deviations were calculated for each treatment at all the evaluation points. Correlation analysis was performed using the Pearson parametric method. The statistical analyses were performed using SAS software.

**Results**

**Relative water content, temperature, stomatal conductance, and substomatal CO<sub>2</sub> concentration in leaves**

Exposure to water deficit occasionally reduced the LRWC; however, significant effects were only observed on the 12th and 18th day after stress application (Fig. 1). Compared with the control plants, a more severe reduction (60%) occurred on the 18th day. Water restriction reduced stomatal conductance, which showed significant changes at all points following water stress. A decrease of approximately 90% compared with the control plants was observed on the

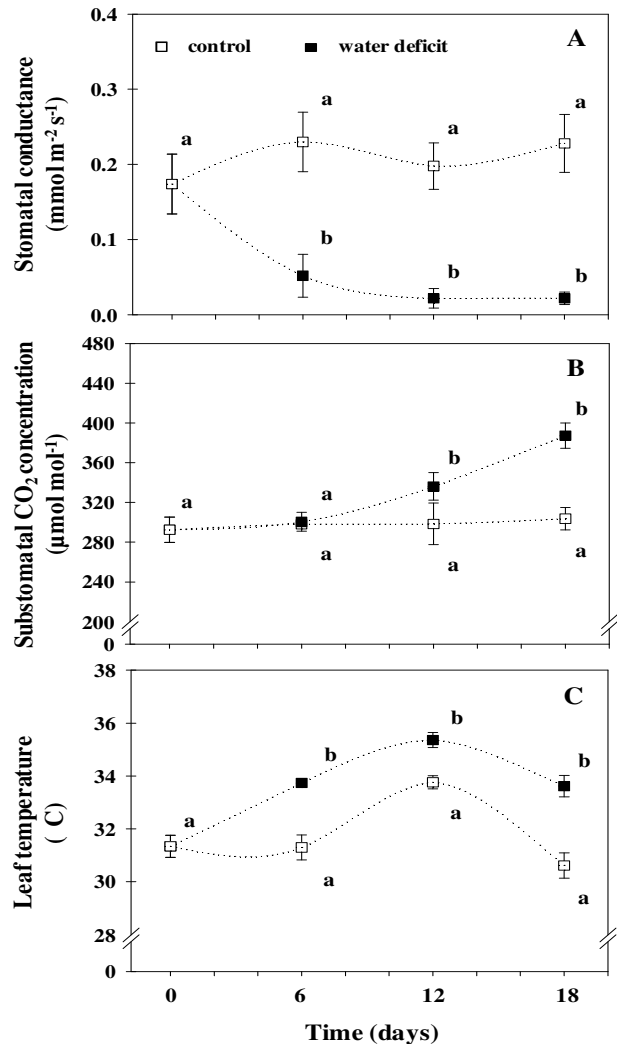


Fig. 2. Stomatal conductance (A), substomatal conductance CO<sub>2</sub> concentration (B), and leaf temperature (C) in young *Euterpe oleracea* plants subjected to water deficit. Same letters do not show significant differences at F test (P < 0.05) to each time. Squares represent the mean values of 5 repetitions, and bars represent the standard deviations.

18th day (Fig. 2 A). A water stress-induced increase in the CO<sub>2</sub> concentration in the sub-stomatal cavity was observed on the 12th and 18th day after the application of drought (Fig. 2 B). The observed 27% increase in the sub-stomatal CO<sub>2</sub> concentration was higher than that of the control treatment on the 18th day. Water deficiency significantly increased the leaf temperature at all time points (Fig. 2 C). The observed increase was greater on the 18th day compared with the control plants.

**Gas exchange**

The net photosynthesis rate decreased due to water restriction, and this effect was significant on the 6th, 12th, and 18th days of water stress (Fig. 3 A). The greatest reduction compared with the control treatment was approximately 100% and occurred on the 18th day. Water stress decreased the transpiration rate, which showed

significant reductions were observed on the 6th day (Fig. 3 D). By the 18th day, the instantaneous carboxylation efficiency had decreased by 100% compared with the control.

**Electrolyte leakage and antioxidant compounds**

Electrolyte leakage increased due to the water deficit, and significant increases were observed on the 12th and 18th days (Fig. 4 A). The increase in electrolyte leakage was more intense on the 12th day and was approximately 14% greater than that of the control plants. The water deficit increased the level of MDA; however, significant increases were only observed on the 18th day after stress application (Fig. 4 B). An increase of 26% compared with the control was observed on the 18th day. The ascorbate levels decreased after water restriction in the present study, and significant differences were noted at all time points (Fig. 4

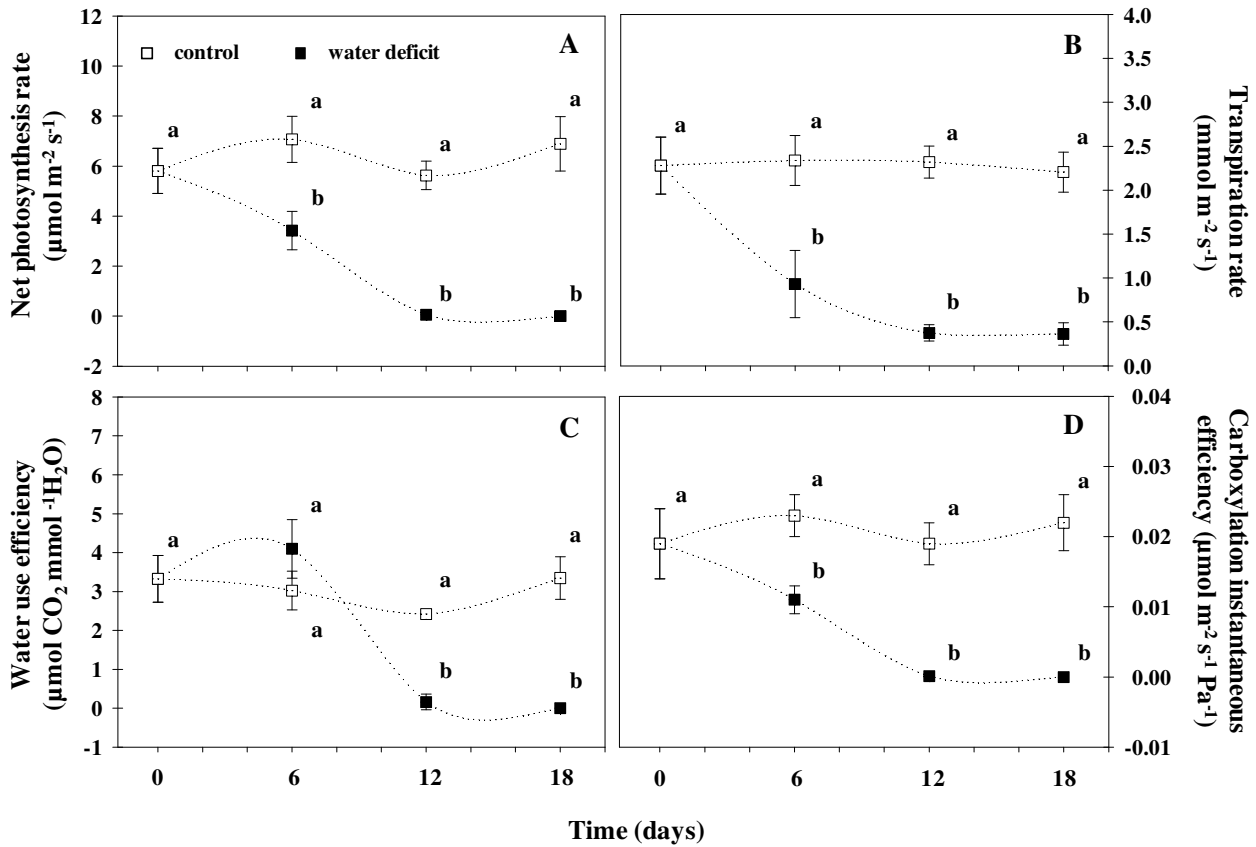


Fig. 3. Net photosynthetic rate (A), transpiration rate (B), water use efficiency (C), and carboxylation instantaneous efficiency (D) in young *Euterpe oleracea* plants subjected to water deficit. Same letters do not show significant differences at F test ( $P < 0.05$ ) to each time. Squares represent the mean values of 5 repetitions, and bars represent the standard deviations.

significant differences from the 6th through the 18th day. The most intense reduction compared with the control was approximately 83% and occurred on the 12th day (Fig. 3 B). Water stress initially increased the water use efficiency, although the effect was not significant early in the experiment.

However, on the 12th and 18th day, the water use efficiency decreased significantly (Fig. 3 C). A more pronounced reduction was observed on the 18th day, when a 100% decrease was observed compared with the control plants. The efficiency of instantaneous carboxylation decreased due to the water deficit, and

C). The greatest reduction (approximately 35%) was observed on the 18th day. Water stress increased the GSH concentration, showing significant effects on the 6th, 12th, and 18th day after irrigation suspension (Fig. 4 D). The greatest increase was observed on the 18th day, when an increase of approximately 62% was observed compared with the control treatment. The correlation analyses revealed two significant relationships between the net photosynthesis rate and the ascorbate content ( $r = 0.91$ ;  $P < 0.01$ ) (Fig. 5 A). Additionally, other relationships related to the net photosynthesis rate and GSH content were observed ( $r = -0.81$ ;  $P < 0.05$ ) (Fig. 5 B).

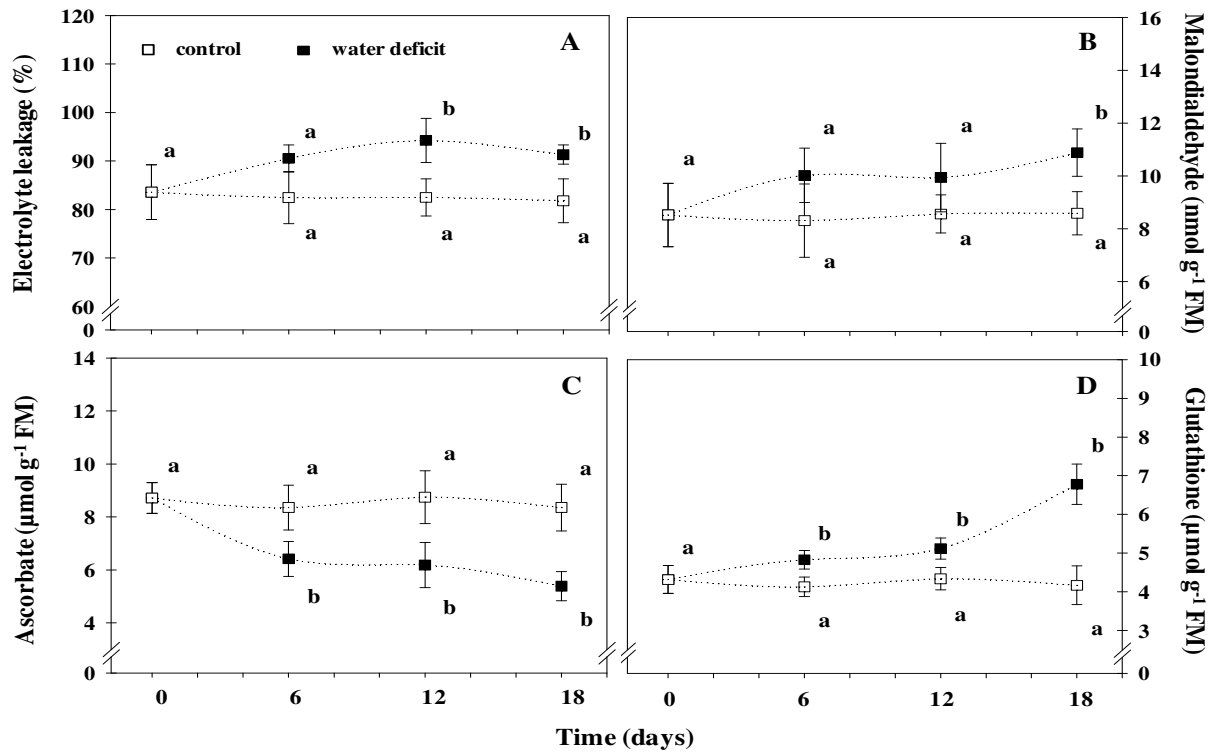


Fig. 4. Electrolyte leakage (A), malondialdehyde (B), ascorbate (C), and glutathione (D) in young *Euterpe oleracea* plants subjected to water deficit. Same letters do not show significant differences at F test ( $P < 0.05$ ) to each time. Squares represent the mean values of 5 repetitions, and bars represent the standard deviations.

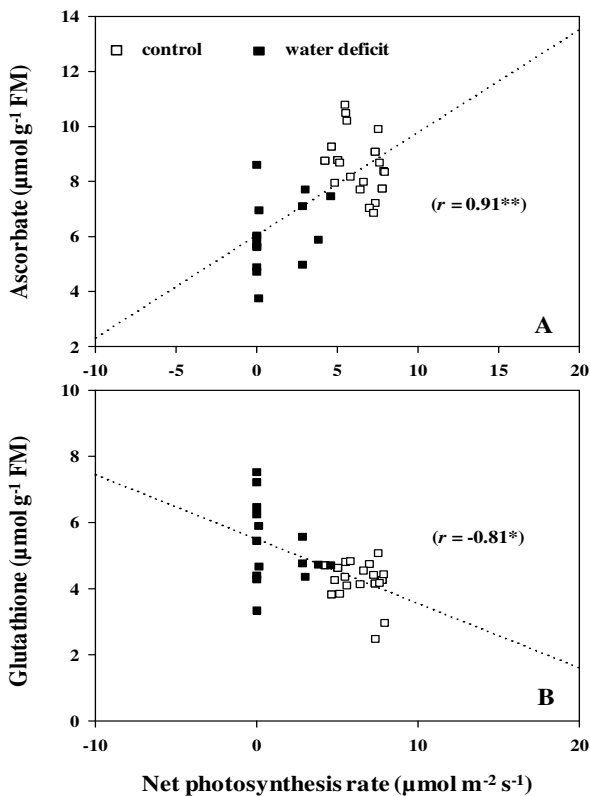


Fig. 5. Relationships between net photosynthesis rate and ascorbate (A), as well as net photosynthesis rate and glutathione (B) in *Euterpe oleracea* plants exposed to drought. Squares represent the values of 5 repetitions and asterisks (\* and \*\*) indicate significance at 0.05 and 0.01 probability level, respectively.

### Discussion

Water stress decreased the LRWC because there was less water available for transpiration, which caused the plant to lose water to the atmosphere, resulting in wilted leaves. In other words, these symptoms occurred because the plant did not obtain water from soil macropores (Freitas *et al.*, 2007). Similar results were obtained by Carvalho *et al.* (1998), who found that water deficits significantly reduced the LRWC of *E. oleracea* plants.

The observed reduction in stomatal conductance in response to water deficit conditions was related to a decrease in the relative water content and the accumulation of abscisic acid in leaves (Egipto, 2011; Costa *et al.*, 2011). Silva *et al.* (2012) suggested that plants use stomatal mechanisms to reduce stomatal conductance and minimise water losses during conditions of drought. Similar results were also obtained by Oliveira *et al.* (2002) with *Bactris gasipaes* plants under water restriction, and these results corroborate the results obtained in the present study.

The observed increase in the substomatal CO<sub>2</sub> concentration of plants exposed to water deficit conditions was attributed to a decrease in the activities of enzymes involved in the process of CO<sub>2</sub> fixation, such as RUBISCO and PEPCASE (Machado *et al.*, 1999). These results also indicate that a reduction in the rate of photosynthesis, as opposed to a change in the stomatal mechanism, is the limiting factor (Mafakheri *et al.*, 2010). Similar results were reported by Repellin *et al.* (1997) with *Cocos nucifera* plants exposed to drought, which confirm the present results.

The observed increase in the leaf temperature during water deficit was due to the low transpiration rate, which reduced the flow of heat from the plant to the atmosphere and reduced the capacity for leaf thermoregulation (Nascimento, 2009). Salinas *et al.* (1996) suggested that an increase in leaf temperature due to reduced heat dissipation could be attributed to decreases in transpiration. An increase in leaf temperature was also observed by Silva *et al.* (2003), who evaluated young *Mimosa caesalpiniiifolia*, *Enterolobium contortisiliquum*, and *Tabebuia aurea* plants exposed to water deficits.

A decrease in the photosynthesis rate was observed during progressive water deficiency. This response was due to lower stomatal conductance combined with the negative effects on instantaneous carboxylation efficiency in plants exposed to water deprivation. Mota (2011) obtained similar results and found that water deficiency significantly reduced the photosynthesis rate of *Acrocomia aculeata* plants.

The observed reduction in the transpiration rate in plants subjected to water restriction was due to the lower turgescence of guard cells, which resulted in stomatal closing and decreased the stomatal conductance (Machado *et al.*, 2009). In agreement with Magalhães Filho *et al.* (2008), stomatal closing is the first reaction of the plant to prevent water losses. Decreases in the transpiration rate were also observed in *E. oleracea* (Calbo and Moraes, 2000) and *Mauritia vinifera* (Calbo and Moraes, 1997) under water deficiency.

With respect to the water use efficiency, significant increases did not occur until the 6<sup>th</sup> day of drought stress due to the efficient response of this species to dehydration, which is mainly a result of a rapid reduction in the transpiration rate and superior water utilisation in the leaf (Taiz and Zeiger, 2009). However, later in the experiment (i.e., on the 12<sup>th</sup> and 18<sup>th</sup> days), the observed decrease in the water use efficiency may have been caused by the dehydration of mesophyll cells, which negatively affected essential processes that depend on the water supply such as photosynthesis and transpiration (Medina *et al.*, 1999). Souza *et al.* (2001) studied *Vitis* plants under water deficiency and reported similar water use efficiency results.

The severe decrease in the instantaneous carboxylation efficiency was attributed to lower values of stomatal conductance. Additionally, the observed decrease in this parameter provoked the accumulation of carbon dioxide in the substomatal cavity (Magalhães Filho *et al.*, 2008). Similar results were obtained by Tezara *et al.* (2002), who investigated the effects of water deficit in *Helianthus annuus* plants.

An increase in electrolyte leakage was occasionally observed due to the water deficit and was attributed to an increase in permeability or membrane rupture (Godoy, 2008). If the integrity of these structures is compromised, organelles can be irreversibly damaged and increases in electrolyte leakage can occur (Fioreze *et al.*, 2011). Gonçalves *et al.* (2011) evaluated *Jatropha curcas* plants under water deficit conditions and found that electrolyte leakage increased. Jiang and Zhang (2001) simulated oxidative stress with different levels of abscisic acid and also reported an increase in electrolyte leakage in *Zea mays* plants.

The observed increase in MDA levels revealed that water stress resulted in lipid peroxidation. This effect is normally produced by free radicals such as hydrogen peroxide, which is one of the main sources of reactive oxygen species (Luis, 2009). Carneiro (2011) also suggested that an increase in MDA levels may be correlated with inadequate activity of antioxidant enzymes. Similar results were obtained by Queiroz *et al.* (2002), who evaluated *Myracrodruon urundeuva* plants subjected to water stress.

The observed decrease in ascorbate levels was likely related to an increase in ascorbate peroxidase (APX) activity because ascorbate (ASC) is used as substrate in this enzymatic reaction (Apel and Hirt, 2004). Moreover, the decreased APX activity was also attributed to monodehydroascorbate reductase (MDHAR), which limits ascorbate production. These results suggest that the reaction catalysed by MDHAR is the limiting step in the antioxidant mechanism of *E. oleracea* plants under drought stress. Similar results were reported by Moran *et al.* (1994), who evaluated *Pisum sativum* plants exposed to water deficits.

The progressive increase in the GSH content was attributed to the minor activity of dehydroascorbate reductase (DHAR), which consumes GSH and catalyses the formation of glutathione disulphide (GSSG) (Gill and Tuteja, 2010). Additionally, GSH plays a role in the plant's defence system against reactive oxygen species that are formed during abiotic stress (May *et al.*, 1998; Potters *et al.*, 2007; Gill and Tuteja, 2010). Similar results were reported by Sofo *et al.* (2005), who studied four *Prunus* hybrids.

## Conclusion

Water deficits have significant negative impacts on stomatal conductance, net photosynthesis rate, transpiration rate, and instantaneous carboxylation efficiency. Additionally, increases in GSH content, electrolyte leakage, and MDA were observed; however, the ascorbate content decreased. Our results confirmed the hypothesis that the photosynthesis rate interferes with the modulation of ascorbate and GSH in young *E. oleracea* plants exposed to drought.

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