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Leaf gas exchange and multiple enzymatic and non-enzymatic antioxidant strategies related to drought tolerance in two oil palm hybrids

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Abstract

Key message The drought tolerance in young oil palm plants is related to greater efficiency in preventing oxidative damage by activating enzymatic and non-enzymatic antioxidant strategies simultaneously.

Abstract Drought is a major environmental constraint limiting growth and yield of oil palm trees. In this study, two oil palm hybrids (BRS Manicoré and BRS C 2501) were grown in large containers and subjected to a water deficit during 57 days. Leaf gas exchange analysis was combined with an in-depth assessment of the antioxidant system over the drought imposition. Under drought, leaf water potential at predawn (Ψ_{pd}) decreased similarly in both hybrids. In parallel, there were decreases in the net CO_2 assimilation rate (A), chlorophyll concentrations and Rubisco total activity. Overall, these decreases were more pronounced in BRS C 2501 than in BRS Manicoré. BRS C 2501 plants triggered more markedly its enzymatic antioxidant system earlier ($\Psi_{pd} = -2.1$ MPa) than did BRS Manicoré, but these responses were accompanied by higher concentrations of H_2O_2 and malondialdehyde in BRS C 2510 than in BRS Manicoré. With the progress of drought stress ($\Psi_{pd} = -2.9$ MPa and below), BRS

Manicoré was better able to cope with oxidative stress through a more robust antioxidant system. In addition, significant decreases in drought-induced NAD^+-malate dehydrogenase activities were only observed in stressed BRS C 2501 plants. Regardless of watering regimes, the total carotenoid, ascorbate and glutathione concentrations were higher in BRS Manicoré than in BRS C 2501. In conclusion, BRS Manicoré is better able to tolerate drought than BRS C 2501 by triggering multiple antioxidant strategies involved both in reactive oxygen species scavenging and dissipation of excess energy and/or reducing equivalents particularly under severe drought stress.

Keywords Antioxidant enzymes · Antioxidant compounds · *Elaeis guineensis* · *Elaeis oleifera* · Water deficit

Introduction

In plants growing under non-stressful conditions, the reactive oxygen species (ROS) are commonly produced at low concentrations as byproducts of normal metabolism in different cell compartments. In chloroplasts, the electron transfer from components of both photosystem (PS) II and PSI to oxygen leads to the formation of superoxide anion ($O_2^{.-}$), which triggers overproduction of hydrogen peroxide (H_2O_2) and hydroxyl radical ($^.OH$) (for a review see Demidchik 2015). In addition, the electron transfer from excited triplet-state chlorophyll at the light-harvesting complex of PSII and from its reaction center (P680) to oxygen also leads to the formation of singlet oxygen ($^1O_2$) (Asada 2006). In mitochondria, the electron transfer from components of the mitochondrial electron transport to oxygen leads to production of $O_2^{.-}$, which is reduced to

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produce H_2O_2 (Rhoads et al. 2006). Furthermore, the glycolate oxidase (GLO_x) activity in the photorespiratory pathway generates H_2O_2, as noted in C3 plants (Foyer 2002). Either under biotic or abiotic stresses, ROS concentration in plant cells may ultimately increase two or three times in relation to non-stressful conditions, thus potentially disrupting cellular homeostasis (Polle 2001). In drought-stressed plants, overproduction of ROS may rise from (i) an imbalance between light capture and electron transfer through photosystems due to down-regulation of PSII activity—this stimulates the dissipation of excess light energy in PSII core and antenna, increasing O_2^-, $^1O_2$, H_2O_2 concentrations; (ii) lower stromal NADP/NADPH ratio—this stimulates Mehler reaction, favoring electron transfer directly to molecular oxygen rather than NADP at PS I level, increasing O_2^- concentration; or (iii) increased photorespiration rate, that overproduces H_2O_2 into peroxisomes (for review see Reddy et al. 2004). Under drought conditions, plants must be able to cope with ROS to prevent or avoid oxidative damages particularly to lipids, proteins and nucleic acids; if plants failure to cope with ROS adequately, oxidative damages may result in cell death (Demidchik 2015).

As a defense strategy, the plants have different antioxidant enzymes and metabolites involved in the elimination of ROS. Superoxide dismutase (SOD), ascorbate peroxidase (APX), monodehydroascorbate reductase, dehydroascorbate reductase, glutathione reductase (GR), catalase (CAT) and glutathione peroxidase are the most common enzymes scavenging ROS, while ascorbate, glutathione, α-tocopherol and carotenoids are the most important non-enzymatic antioxidants (Mittler 2002; Mittler et al. 2004; Jaleel et al. 2009). Notably, a close relationship between an efficient antioxidant defense system and plant tolerance to biotic and abiotic stresses has been demonstrated in a range of different genotypes (cultivars or hybrids) of dicot and monocot species (Lima et al. 2002; Raza et al. 2007; Bian and Jiang 2009; Carvalho et al. 2013).

The oil palm (*Elaeis guineensis*; Arecaceae) is a perennial palm largely cropped worldwide due to its economical potential for oil production. Both palm oil (or mesocarp oil) and kernel oil are used in the food industry, cosmetics, medicines, soap and more recently in biodiesel production (Homma et al. 2000; Wahid et al. 2005). This palm does not withstand severe or even moderate drought spells and, therefore, crop yields are severely constrained under water-limiting conditions. Nonetheless, moderate (annual water deficiency between 100 and 350 mm) and severe (annual water deficiency up to 350 mm) drought events can occur from July to November in some regions of the Amazonia; indeed soil water deficiency is considered the most important environmental factor limiting oil palm yield in north Brazil, where oil palm plantations are concentrated (Bastos et al. 2001). Furthermore, local experience has shown that plant death, especially in young plantations, may occur in dry years if irrigation is not supplemented. Such drought sensitivity in oil palm plants is in part related to the magnitude of drought-induced effects on physiological variables, affecting the overall plant metabolism. In this context, significant decreases in leaf gas exchange parameters, especially in net CO_2 assimilation rate (A), stomatal conductance to water vapor (g_s), maximum PSII quantum efficiency, effective PSII quantum yield and apparent electron transport rate have been reported in oils palm plants under drought stress (Cha-um et al. 2010, 2012; Suresh et al. 2010, 2012; Méndez et al. 2012). Moreover, the decreases in A during water deficit progress precedes any measurable changes in chlorophyll *a* fluorescence (Suresh et al. 2010), suggesting an imbalance between photochemical and biochemical pathways of photosynthesis, thus potentially leading to the overproduction of ROS in chloroplasts and triggering oxidative damages to cells. Given that magnitude of drought effects varies largely in oil palm hybrids (Méndez et al. 2012; Suresh et al. 2012), it can be hypothesized that tolerance of oil palm plants to water deficit could at least partially be associated with a greater ability to prevent or avoid cellular damages by activating antioxidant enzymatic and/or non-enzymatic strategies. To test this hypothesis, two oil palm hybrids (BRS Manicoré and BRS C 2501) genetically improved to achieve improved fruit productivity and improved tolerance to fatal yellowing in plantings at Brazilian Amazonia (Cunha et al. 2007; Cunha and Lopes 2010) were subjected to a long-term drought (57 days) aiming to evaluate their abilities to prevent cellular damages by the way of different antioxidant enzymes and metabolites. Therefore, the identification of antioxidant mechanisms allowing plants to successfully cope with long-term drought is an important trait for improvements on stress tolerance in this species.

Materials and methods

Plant material, experimental design and sampling procedures

The experiment was setup in a greenhouse located in the Brazilian Amazonia (01°28′03″S, 48°29′18″W). Pre-germinated seeds of the interspecific oil palm BRS Manicoré hybrid (*E. guineensis* Jacq. × *E. oleifera* (Kunth) Cortés; Cunha and Lopes 2010) and intraspecific oil palm BRS C 2501 *tenera* hybrid (*E. guineensis* cv. psifera—La Mé × *E. guineensis* cv. dura—Deli; Cunha et al. 2007) were planted in polyethylene trails for seedling development. Thirty days later, uniform seedlings, in terms of plant

height and leaf number, were transferred to 50 L pots filled with 40 kg of a superficial soil (0–20 cm) collected from a Yellow Dystrophic Latosol soil typically found in the Amazonian region. The pH of the substrate was adjusted to approx. 6.0 using 30 g dolomitic limestone per pot. Supply fertilization was provided by adding 5 g NPK 20-20-20 (w/w) per pot in intervals of 15 days and 2.5 g magnesium sulfate per pot in intervals of 30 days (Franzini and Silva 2012). All plants were cultivated under full irrigation throughout the following 12 months. The volume of irrigation water was applied to maintain the soil near to field capacity. Throughout the experiment, the climatic conditions at the experimental site were registered using a data logger (HOBO U12-012, Onset Computer Corporation, Bourne, EUA) equipped with specific sensors for air temperature (T_{air}), relative humidity (RH), and light intensity measurements. The averages of diurnal and nocturnal temperatures were 29.2 ± 0.2 and 24.9 ± 0.2 °C, respectively; and averages of diurnal humidity, light duration and intensity were, respectively, of 76.8 ± 0.9 %, 12.8 ± 0.5 h and 1511.6 ± 63.3 µmol photons m^{-2} s^{-1}. Specifically during the morning measurements (between 6:00 and 10:00 h), the averages of T_{air} and RH (registered using a thermohygrometer m5203, Incoterm Ind., Porto Alegre, Brazil) inside greenhouse were 28.8 ± 0.1 °C and 81 ± 0.7 % and vapor pressure deficit (calculated according Landsberg 1986) and photosynthetically active radiation (measured with a quantum sensor attached to infrared gas analyzer chamber) were, respectively, of 0.80 ± 0.03 kPa and 937 ± 39 µmol photons m^{-2} s^{-1}.

The definitive experiment was setup as a randomized design consisting of a $2 \times 2 \times 4$ factorial scheme formed by two oil palm hybrids (BRS Manicoré and BRS C 2501) subjected to two watering regimes [full irrigation (control) and water deficit] evaluated in four different times (0, 21, 34 and 57 days after the water deficit treatment imposition). Each treatment was formed by six replicates, and a single plant per pot was considered as an experimental plot. The irrigation was suppressed completely for water deficit treatment and stress resulted from continued evapotranspiration of each soil plus plant system. The time points of evaluation were chosen to examine stressed plants under different soil water conditions, as characterized by predawn leaf water potentials (Ψ_{pd}) around -0.1 MPa (day 0), -2.0 MPa (day 21), -3.0 MPa (day 34) and -4.0 MPa (day 57), which were measured between 4:30 and 5:30 h using a Scholander-type pressure chamber (m670, PMS Instrument Co., Albany, USA) as described in Pinheiro et al. (2008). These lowest Ψ_{pd} values are believed to represent a severe (non-lethal) internal water deficit and may well reflect the field situations encountered by young oil palm plants in dry years.

All physiological measurements and samplings (see below) were carried out in leaflets from the medium portion of the third leaf from the apices.

Leaf gas exchange

The net CO_2 assimilation rate (A), stomatal conductance to water vapor (g_s) and intercellular to ambient CO_2 concentration ratio (C_i/C_a) were determined using an infrared gas analyzer (LCpro+, ADC BioScientific Ltd., Hoddesdon, UK). The measurements were performed between 7:40 and 8:40 h (solar time) under ambient CO_2 concentration and photosynthetically active radiation (PAR) of 1100 µmol photons m^{-2} s^{-1} (Suresh et al. 2012). The PAR was provided by a light source attached to the gas analyzer chamber (LCM − 014/B, ADC BioScientific Ltd., Hoddesdon, UK).

Biochemical assays

Samplings for biochemical assays were performed between 7:40 and 8:40 h. The collected samples were flash frozen in liquid nitrogen and kept under these conditions until assays.

Chlorophylls and total carotenoids

Pigments were extracted in 80 % (v/v) aqueous acetone plus 0.01 g $CaCO_3$ according to Costa et al. (2010). Chlorophyll (Chl) a and b and total carotenoid (Car) concentrations were estimated according to Lichthenthaler (1987).

Enzymatic assays

Ribulose 1,5 bisphosphate carboxylase/oxygenase (Rubisco, EC 4.1.1.39) and NAD^+-Malate dehydrogenase (NAD^+-MDH, EC 1.1.37) were obtained in 0.8 mL "Stitt" buffer containing 500 mM Hepes pH 7.5, 100 mM MgCl2, 10 mM EDTA, 10 mM EGTA pH 8.0, 10 mM Benzamide, 10 mM E-aminocaproic acid, 2.5 % (w/v) BSA (Geigenberger and Stitt 1993). Glycolate oxidase (GLO_x, EC 1.1.3.15) was extracted in 3 mL of 50 mM Tris–HCl pH 7.8, 5 mM dithiothreitol (DTT), 0.01 % (v/v) Triton X–100 (Booker et al. 1997). Superoxide dismutase (SOD, EC 1.15.1.1) was extracted in 3 mL 100 mM potassium phosphate pH 7.8, 0.1 mM EDTA, 10 mM 2-mercaptoethanol, 0.1 % (v/v) Triton X-100; 1 mM DTT (Giannopolitis and Ries 1977). Ascorbate peroxidase (APX, EC 1.11.1.11) and catalase (CAT, EC 1.11.1.6) were extracted in 50 mM potassium phosphate pH 7.0, 2 mM EDTA, 0.1 % (v/v) Triton X–100, 20 mM ascorbate (Nakano and

Asada 1981; Havir and McHale 1987). GR (EC 1.6.4.2) was obtained in 100 mM Tris-CHl pH 7.5, 50 μM EDTA, 10 mM isoascorbate, 9 mM 2-mercaptoethanol, 3 mM DTT and 0.1 % (v/v) Triton X-100 (Foyer and Halliwell 1976). The supernatants obtained after centrifugation were directly used for enzymatic assays and protein concentration determinations (Bradford 1976).

The total activity of Rubisco was assayed by measuring NADH consumption at 340 nm (Sulpice et al. 2007). The activity of GLO_x was determined by measuring glycolate phenyl hydrazone production at 324 nm (Booker et al. 1997). The activity of NAD^+-MDH was determined by measuring the rate of NADH oxidation at 340 nm (Nunes-Nesi et al. 2005). Total activity of SOD was assayed as the ability of the enzyme to inhibit photochemical reduction of nitroblue tetrazolium at 560 nm (Giannopolitis and Ries 1977). The activity of APX was determined by measuring ascorbate oxidation at 290 nm (Nakano and Asada 1981). CAT activity was assayed following H_2O_2 oxidation at 240 nm (Havir and McHale 1987) and activity of GR was determined by assaying the rate of NADPH consumption at 340 nm (Foyer and Halliwell 1976).

Ascorbate and glutathione pools

Reduced ascorbate (Asc) and dehydroascorbate (DHAsc) were assayed according to Gillespie and Ainsworth (2007) and reduced (GSH) and oxidized (GSSG) glutathione were assayed according to Griffith (1980). From the results, total ascorbate (Asc + DHAsc), total glutathione (GSH + GSSG) and both ascorbate and glutathione redox states (Asc/Asc + DHAsc and GSH/GSH + GSSG, respectively) were calculated (Gondim et al. 2013).

Hydrogen peroxide

Leaf samples were grounded in 50 mM potassium phosphate buffer pH 6.5 containing 1 mM NH_2OH. After centrifugation, an aliquot of the supernatant was used for the H_2O_2 quantification determined by measuring changes in absorbance at 560 nm in a reaction medium containing 100 mM sorbitol, 0.25 mM $FeNH_4(SO_4)$, 25 mM de H_2SO_4 and 0.25 mM xylenol orange (Gay and Gebicki 2000).

Lipid peroxidation

Leaf samples were grounded in 0.1 % (v/v) trichloroacetic acid and the slurries centrifuged at $15,000 \times g$, for 15 min at 4 °C. An aliquot (500 μL) was incubated at 90 °C for 20 min in 1.5 mL 0.5 % (v/v) thiobarbituric acid (TBA). The reaction was stopped under ice bath and the mixture was clarified by centrifugation at $13,000 \times g$,

for 15 min at 4 °C. The specific and non-specific absorbance of the samples was determined at 532 and 600 nm, respectively. The lipid peroxidation was estimated as the content of total TBA reactive substances expressed as equivalents of malondialdehyde (MDA) (Cakmak and Horst 1991).

Statistical analyses

The effects of hybrids (BRS Manicoré and BRS C 2501), watering regimes (control and water deficit), and possible interactions between them over the experimental period (time effect) on Ψ_{pd}, leaf gas exchange and biochemical variables were analyzed using a repeated measures analysis of variance, tested for significance by F test (Lima et al. 2010). All statistical procedures were carried out using the statistical software Systat (v. 12.0.0.1, 2012, Systat Software Inc., Paris, France).

Results

Leaf water potential and leaf gas exchange

Regardless of plant hybrids, Ψ_{pd} of control plants remained at high values ($\Psi_{pd} \sim -0.13$ MPa) throughout the experimental period, while the progressive water deficit caused significant ($P < 0.001$, Table 1) decrease in Ψ_{pd} in both BRS hybrids (Fig. 1a). Although absolute values of Ψ_{pd} registered on day 57 tended to be higher in BRS Manicoré (Fig. 1a), the differences between hybrids were not significant ($P > 0.05$, Table 1), indicating that plant water status was equally affected by drought in both hybrids. Thus, the Ψ_{pd} averaged for water-stressed plants of both hybrids on -2.0 MPa (day 21), -2.9 MPa (day 34) and -4.2 MPa (day 57) (Fig. 1a).

The changes in A, g_s and C_i/C_a over the course of the experiment were essentially similar between BRS hybrids under full irrigation (Fig. 1b–d). After 21 days under water deficit, the A was significantly ($P < 0.001$, Table 1) decreased by 90 % in BRS Manicoré and by 95 % in BRS C 2501 (Fig. 1b) relative to control plants. Decreases in A were accompanied by significant decreases in g_s ($P < 0.001$, Table 1), ranging from 78 % in BRS Manicoré to 88 % in BRS C 2501 (Fig. 1c). Additional decreases in A and g_s were observed in both hybrids on subsequent time points, but it is noteworthy that negative values of A, and g_s values around zero, were registered earlier in BRS C 2501 than in BRS Manicoré (day 34) (Fig. 1b, c). In parallel to decreases in A and g_s, remarkable ($P < 0.001$, Table 1) increases in C_i/C_a were observed in water-stressed plants of both hybrids, reaching the highest values at day 57 (Fig. 1d).

Table 1 F statistics and associated significance levels for the effect of hybrids (H) and watering regimes (WR) and its interaction with times of experimental evaluation (T) on physiological variables in two oil palm hybrids (BRS Manicoré and BRS C 2501)

Variables	Factors					
	H	WR	$H \times$ WR	$T \times H$	$T \times$ WR	$T \times H \times$ WR
Ψ_{pd}	ns	1358.3***	ns	ns	654.7***	ns
A	ns	4684.5***	ns	5.0**	619.3***	ns
g_s	27.8***	3262.9***	ns	8.3***	590.8***	3.5*
C_i/C_a	71.1***	27451.0***	39.0***	14.2***	5299.4***	8.8***
Chl a	724.5***	1090.0***	12.1**	4.3*	396.8***	10.2***
Chl b	183.1***	409.9***	90.3***	9.8***	35.0***	7.8***
Car	401.2***	184.7***	10.4**	12.2***	80.5***	13.8***
Chl $a + b$/Car	123.3***	475.3***	15.9**	2.8*	89.0***	5.7**
Rubisco	26.8***	317.0***	6.9*	ns	37.5***	6.2**
GLOx	20.6***	1786.6***	4.5*	38.8***	653.8***	29.6***
NAD^+-MDH	758.8***	200.3***	400.0***	21.8***	98.9***	33.7***
SOD	83.5***	10847.9***	17.4***	145.2***	3276.2***	146.0***
APX	380.9***	916.5***	33.4***	17.1***	419.7***	23.0***
GR	ns	ns	ns	6.6**	32.3***	6.6**
CAT	132.1***	910.5***	8.8**	28.0***	376.6***	20.1***
Asc + DHAsc	1480.9***	ns	21.2***	7.5***	11.1***	25.9***
Asc/Asc + DHAsc	420.8***	61.6***	ns	11.9***	18.6***	ns
GSH + GSSG	304.4***	107.6***	26.9***	11.3***	35.9***	17.7***
GSH/GSH + GSSG	902.3***	353.5***	25.3***	16.3***	75.5***	17.0***
H_2O_2	138.7***	183.2***	ns	90.2***	105.3***	45.9***
MDA	ns	230.6***	116.1***	49.0***	59.9***	28.9***

ns non-significant

Level of significance * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Chlorophylls and total carotenoids

Under full irrigation, the constitutive concentrations of Chl a, Chl b, Car and Chl $a + b$/Car differed significantly between hybrids with no marked changes between different experimental days (Fig. 2; Table 1). The averages of Chl a and Car were 13 and 22 % higher in BRS Manicoré, while Chl b concentration was 22 % higher in BRS C 2501 (Fig. 2). The Chl $a + b$/Car ratio in well-watered plants of BRS Manicoré was 20 % higher than that in BRS C 2501 (Fig. 2; Table 1). The water deficit triggered significant ($P < 0.001$, Table 1) decreases in Chl a and Chl b concentrations in both hybrids and such decreases were already evident at 21 days after withholding irrigation (Fig. 2a, b). In water-stressed plants, the decreases in Chl a varied from 7 % (day 21) to 37 % (day 57) in BRS Manicoré, and from 8 % (day 21) to 53 % (day 57) in BRS C 2501 (Fig. 2a); the decreases in Chl b varied from 12 % (day 21) to 17 % (day 57) in BRS Manicoré, and from 8 % (day 21) to 53 % (day 57) in BRS C 2501 (Fig. 2b). Taken together, our results suggest that chlorophyll degradation was more prominent in BRS C 2501 compared to BRS Manicoré.

The total Car concentrations in stressed plants of BRS Manicoré were 43, 27 and 17 % higher ($P < 0.001$, Table 1) than in control plants assessed on days 21, 34 and 57, respectively (Fig. 2c). In stressed plants of BRS C 2501, drought brought about increases by 47 and 38 % ($P < 0.001$, Table 1) in Car on days 21 and 34; in sharp contrast, on day 57 Car concentration was lower (19 %) in drought-stressed plants than in control plants (Fig. 2c). Notably, regardless of hybrids and sampling times drought stress led to lower Chl $a + b$/Car ratios than those found in control plants (Fig. 2d). In BRS Manicoré, these ratios were 35 % (day 21), 37 % (day 34) and 42 % (day 57) lower ($P < 0.001$, Table 1) in stressed plants, while in BRS C 2501, drought caused 40 % (day 21), 50 % (day 34) and 39 % (day 57) decreases in Chl $a + b$/Car ratio ($P < 0.001$, Table 1).

Activities of enzymes related to carbon metabolism

Relative to control plants, water deficit led to significant ($P < 0.001$, Table 1) decreases in Rubisco total activity in both hybrids, varying from 16 % (day 21) to 54 % (day 57) in BRS Manicoré, and from 38 % (day 21) to 63 % (day 57) in BRS C 2501 (Fig. 3a). In contrast, water deficit triggered significant ($P < 0.001$, Table 1) increases in GLO_x activities in both BRS Manicoré (70 and 168 % on days 21 and 34, respectively) and BRS C 2501 (88 and
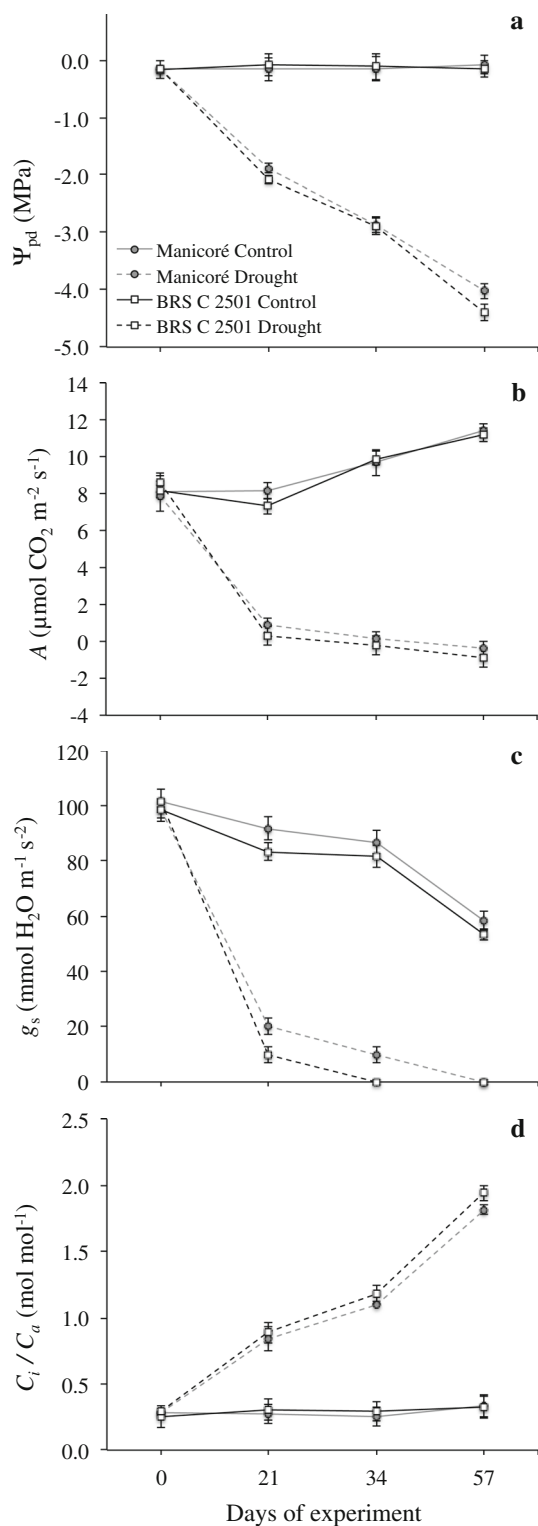
Fig. 1 Changes in leaf water potential at predawn (Ψ_{pd}, Fig. 1a), net CO_2 assimilation rate (A, Fig. 1b), stomatal conductance to water vapor (g_s, Fig. 1c) and intercellular to ambient CO_2 concentration (C_i/C_a, Fig. 1d) in two oil palm hybrids (BRS Manicoré and BRS C 2501) subjected to progressive drought. Data are the mean of six replicates ± standard error (SE)
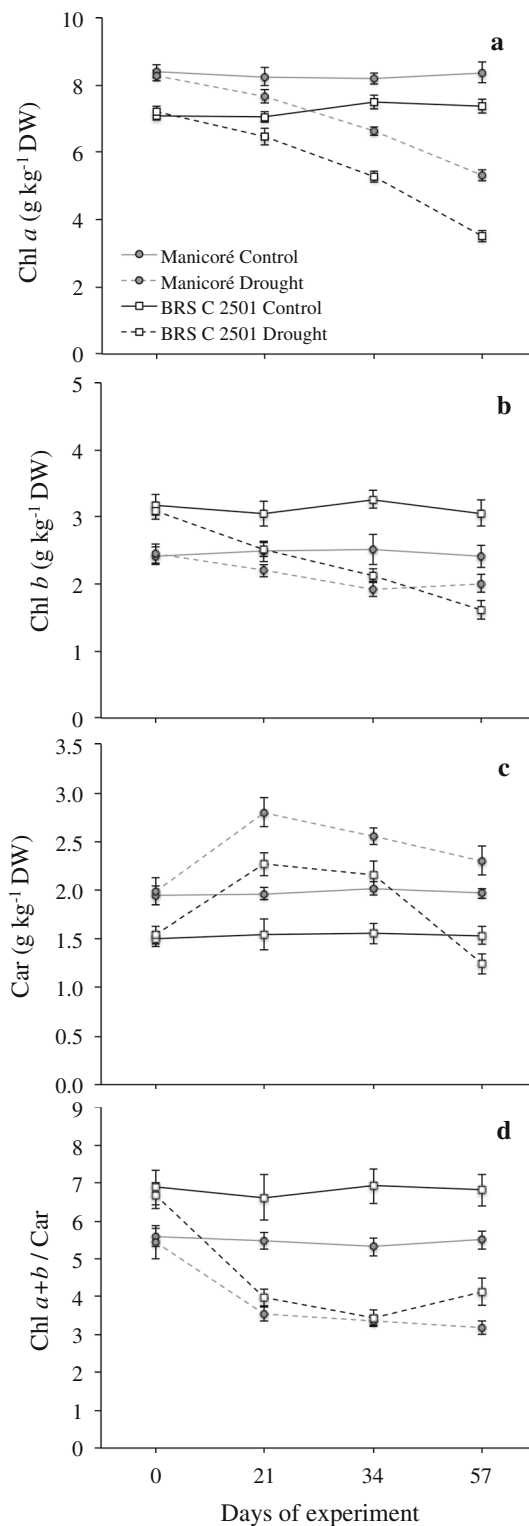
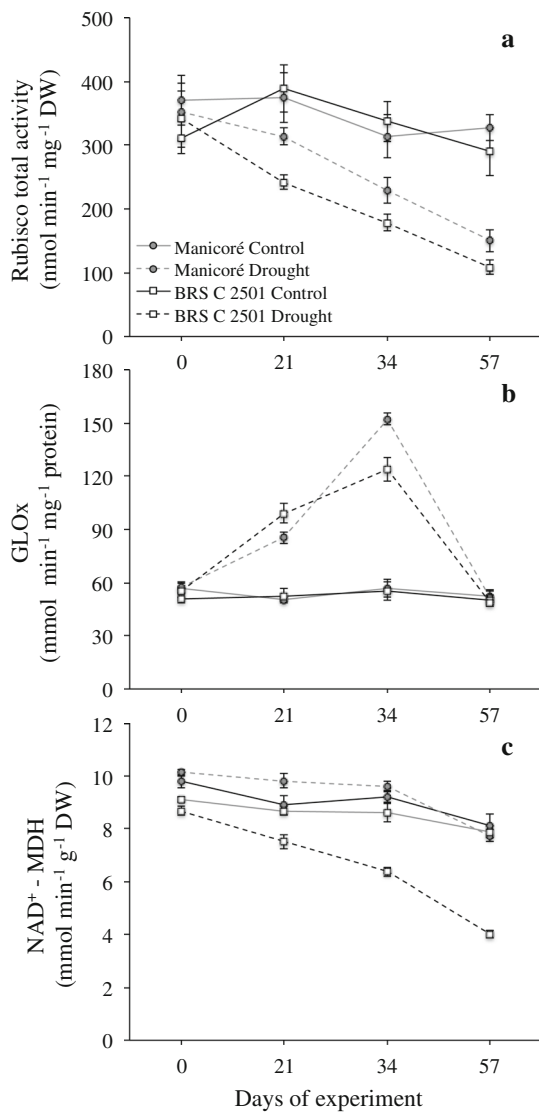**Fig. 2** Concentrations of chlorophyll a (Chl a; **a**), chlorophyll b (Chl b; **b**), total carotenoids (Car; **c**), and total chlorophyll to total carotenoids ratio (Chl $a + b$/Car; **d**) in two oil palm hybrids (BRS Manicoré and BRS C 2501) subjected to progressive drought. Data are the mean of six replicates ± SE

Fig. 3 Activities of Rubisco (**a**), glycolate oxidase (GLO$_x$; **b**), and NAD$^+$-Malate dehydrogenase (NAD$^+$-MDH; **c**) in two oil palm hybrids (BRS Manicoré and BRS C 2501) subjected to progressive drought. Data are the mean of six replicates ± SE

125 % on days 21 and 34, respectively) (Fig. 3b). The GLO$_x$ activity was sharply decreased to control levels in stressed plants of both BRS hybrids on day 57 (Fig. 3b).

The constitutive activity of NAD$^+$-MDH was similar between watered plants of both hybrids (around 8.78 mmol min^{-1} g^{-1} DW) and remained nearly constant over experimental period (Fig. 3c). The progressive drought did not cause any significant ($P > 0.05$) effect in NAD$^+$-MDH activity of BRS Manicoré plants, indicating this enzyme remains operating disregarding plant water status (Fig. 3c). By contrast, the progressive drought caused 13, 26 and 49 % decreases in NAD$^+$-MDH activity of BRS C 2501 plants on days 21, 34 and 57, respectively (Fig. 3c).

Activities of antioxidant enzymes

Significantly higher activities of SOD (16 %), APX (32 %), GR (10 %) and CAT (28 %) were found in well-watered plants of BRS Manicoré than in BRS C 2501 (Fig. 4; Table 1). These results highlight constitutive differences in the activity of antioxidant enzymes between these two genotypes and suggest a differential ability between hybrids to cope with ROS metabolism under non-stressful conditions. The drought caused remarkable ($P < 0.001$, Table 1) increases in the activities of SOD, APX, and CAT in both hybrids assessed on days 21 and 34, with a peak of activity on day 34 (Fig. 4). As compared with their respective control plants assessed on day 34, the enzyme activities were higher by 816 % (SOD, Fig. 4a), 84 % (APX, Fig. 4b) and 137 % (CAT, Fig. 4d) in stressed plants of BRS Manicoré, whereas in BRS C 2501 such increases were higher by 799 % (SOD, Fig. 4a), 146 % (APX, Fig. 4b) and 200 % (CAT, Fig. 4d). On day 57, activities of these enzymes decreased sharply in water-stressed plants of both hybrids, although remaining significantly higher than in their respective control counterparts (Fig. 4).

The stressed plants of both BRS hybrids displayed significant ($P < 0.01$, Table 1) increases in GR activity on days 21 and 34, with a peak of activity registered on day 21 (Fig. 4c). Overall, the highest GR activities in stressed plants of BRS Manicoré and BRS C 2501 were 25 and 46 % greater than in their respective control plants. Nonetheless, on day 57, the GR activity decreased in stressed plants, ranging from 54 % in BRS Manicoré to 73 % in BRS C 2501 as compared with their respective control counterparts (Fig. 4c).

Non-enzymatic antioxidant compounds

Regardless of watering regimes, ascorbate metabolism seemed to vary markedly in either hybrid (Fig. 5a). Under control conditions, the total Asc (Asc + DHAsc) averaged over the course of the experiment was 44 % higher in BRS Manicoré than in BRS C 2501 (Fig. 5a). In BRS Manicoré, the total Asc concentrations were 23 % (day 34) and 16 % (day 57) higher in stressed plants than in control plants (Fig. 5a). By contrast, the total Asc in water-stressed plants of BRS C 2501 did not differ from watered plants until day (day 34); however, total Asc in stressed plants assessed on day 57 was 30 % lower in relation to control plants (Fig. 5a). The Asc redox state, represented by the Asc/Asc + DHAsc ratio, increased by 86 % (day 34) and 54 % (day 57) in stressed plants of BRS Manicoré relative to the control individuals (Fig. 5b). By contrast, more discrete increases (20 and 23 % on days 34 and 57, respectively) in Asc redox state were observed in stressed plants of BRS C 2501 relative to their control counterparts (Fig. 5b).
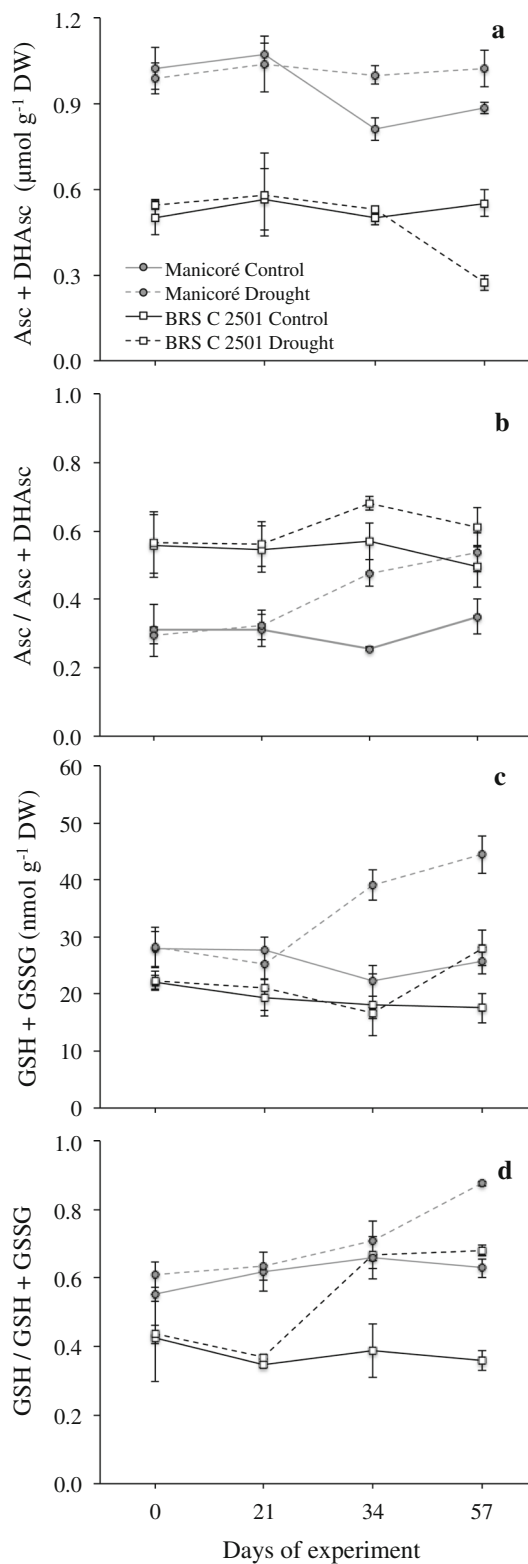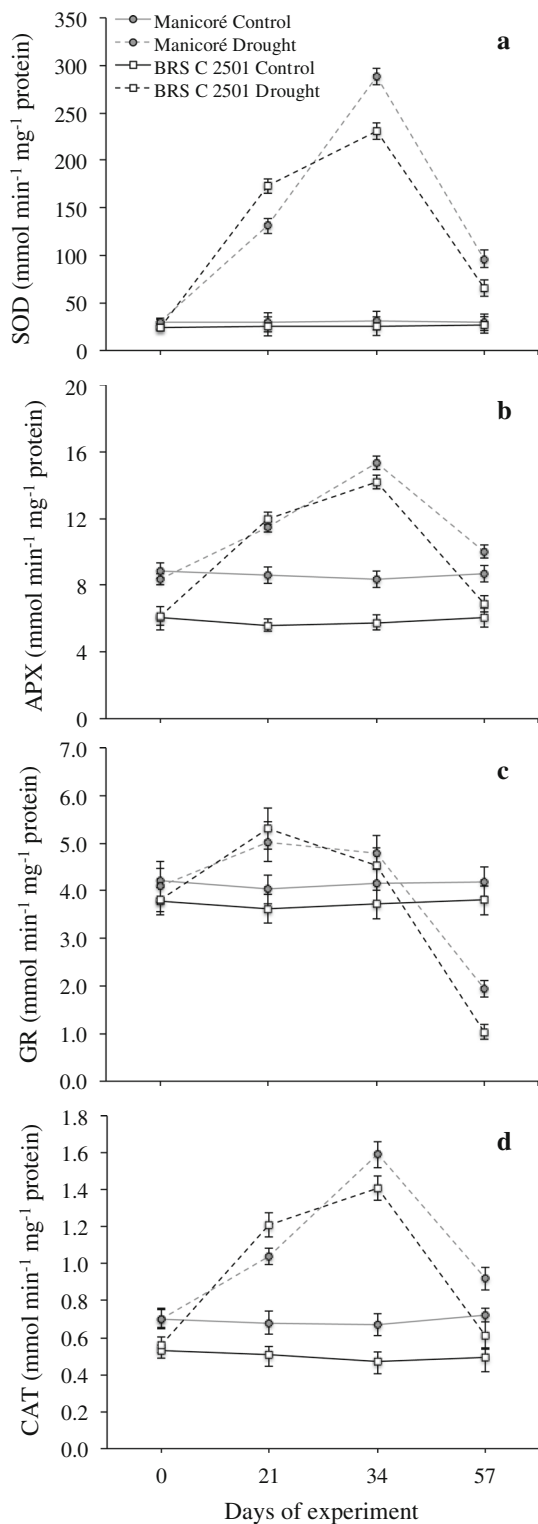
Fig. 4 Activities of superoxide dismutase (SOD; **a**), ascorbate peroxidase (APX; **b**), glutathione reductase (GR; **c**) and catalase (CAT; **d**) in two oil palm hybrids (BRS Manicoré and BRS C 2501) subjected to progressive drought. Data are the mean of six replicates ± SE

Fig. 5 Total ascorbate (Asc + DHAsc; **a**), ascorbate redox state (Asc/Asc + DHAsc; **b**), total glutathione (GSH + GSSG; **c**), and glutathione redox state (GSH/GSH + GSSG; **d**) in two oil palm hybrids (BRS Manicoré and BRS C 2501) subjected to progressive drought. Data are the mean of six replicates ± SE

Under well-watered conditions, total glutathione (GSH + GSSG) concentration, averaged over the course of the experiment, was 26 % higher in BRS Manicoré than in BRS C 2501 (Fig. 5c). The total glutathione concentration in stressed BRS Manicoré plants was 75 % (day 34) and 72 % (day 57) higher than in control plants. By contrast, significant increase ($P < 0.05$) in total glutathione (60 %) in stressed BRS C 2501 plants was only at day 57 (Fig. 5c). From these results, the glutathione redox state (GSH/GSH + GSSG) in stressed plants of BRS Manicoré was 8 % (day 34) and 39 % (day 57) higher than in their respective control plants; and in stressed plants of BRS C 2501, it was higher by 72 % (day 34) and 90 % (day 57) than in their irrigated counterparts (Fig. 5d; Table 1).

Hydrogen peroxide and lipid peroxidation

The H_2O_2 concentrations in well-watered plants of BRS Manicoré were significantly lower (20 %) than in BRS C 2501 over the course of the experiment. In BRS Manicoré, significant ($P < 0.001$, Table 1) increases in H_2O_2 concentration triggered by drought were observed on days 34 (17 %) and 57 (64 %) (Fig. 6a). By contrast, these increases occurred earlier in BRS C 2501, as noted from day 21 (25 %) onwards, with a peak of concentration at day 34 (70 % higher than in control plants) (Fig. 6a).

The MDA concentrations in well-watered plants of both hybrids did not differ significantly over the experimental days (Fig. 6b; Table 1). This indicates that both materials have an intrinsic ability to control adequately ROS and prevent lipid membrane peroxidation under non-stressful conditions. There were no evident signs of lipid peroxidation in water-stressed plants of BRS Manicoré until day 34; however, a significant ($P < 0.001$, Table 1) drought-induced increase (15 %) in MDA concentration occurred on day 57 (Fig. 6b). In BRS C 2501, MDA accumulation due to drought stress was already noted on day 21. Overall, in this hybrid the increases in MDA in drought-stressed plants were more prominent compared to those found in BRS Manicoré, ranging from 31 % on day 34 to 50 % on day 57 relative to their control counterparts (Fig. 6b).

Discussion

Under full irrigation, both hybrids displayed similar photosynthetic performance as denoted by their similar values of A and g_s over the course of the experiment (Fig. 1). The magnitude of these values is consistent to that previously reported for irrigated plants of other *E. guineensis* hybrids (Cha-um et al. 2010; Suresh et al. 2010, 2012; Méndez et al. 2012, 2013). Under drought conditions, Ψ_{pd} decreased similarly over the course of the experiment

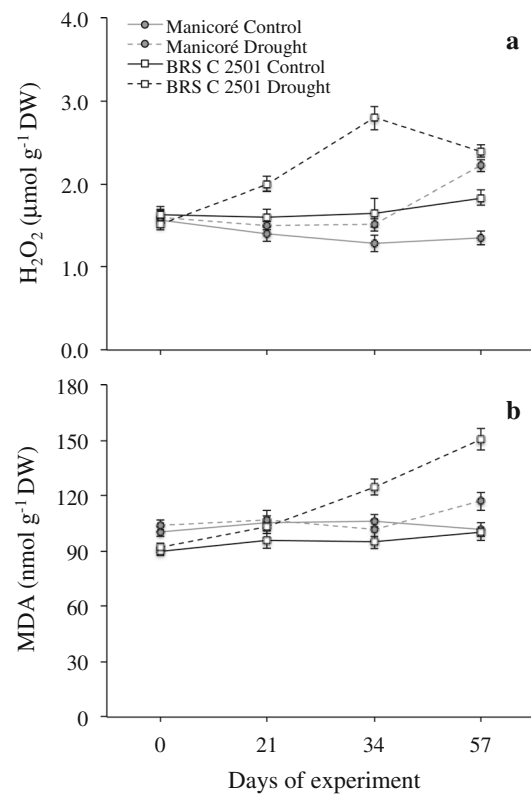
Fig. 6 Hydrogen peroxide (H_2O_2; a) and lipid peroxidation assessed as malondialdehyde equivalents (MDA; b) in two oil palm hybrids (BRS Manicoré and BRS C 2501) subjected to progressive drought. Data are the mean of six replicates ± SE

regardless of hybrids and, therefore, the observed differences in physiological and biochemical variables between hybrids should be primarily related to varying intrinsic abilities of each genotype to cope with progressive soil water shortage. Indeed negative values of A, and values of g_s approaching zero, were recorded earlier in BRS C 2501 than in BRS Manicoré (Fig. 1), suggesting a relatively improved physiological performance of the BRS Manicoré hybrid under long-term drought conditions.

The effects of drought reducing A have actually been explained in terms of increases in diffusive limitations (that can be further partitioned into stomatal and mesophyll restrictions) and biochemical limitations (Chaves et al. 2009; Flexas et al. 2012). Given that the decreases in A were accompanied by sharp reductions in g_s coupled with remarkable increases in C_i/C_a (Fig. 1), the anticipated lower influx of CO_2 into the leaves caused by stomatal closure cannot be considered as a primary factor associated with the reduction in A. In addition to stomatal constraints, mesophyll limitations might also have played a role in constraining CO_2 diffusion from the intercellular air spaces to the sites of carboxylation in the chloroplasts, as might be expected from the intrinsic co-regulation of stomatal and mesophyll conductance under drought stress (Flexas et al.

2008, 2012). While some diffusive limitations imposed by the mesophyll could not be excluded, we contend that such limitations, if at all, had only negligible importance in determining the photosynthetic capacity of drought-stressed plants under our experimental conditions. This suggestion is circumstantially supported by the remarkable increases in C_i/C_a (which is expected to largely overcome limitations to CO_2 diffusion throughout the mesophyll) and also because the carboxylation capacity of Rubisco was depressed, especially as the internal water status became less conducive to keep the metabolic activities of the plants. Therefore, the decreases in A in this current study may largely be linked to dysfunctions at the level of the biochemical reactions associated with CO_2 fixation, as suggested to occur under severe drought conditions due possibly to limitations in RuBP synthesis caused by ATP deficiency (Lawlor and Cornic 2002; Lawlor and Tezara 2009). Under these circumstances, decreases in A could not be prevented by an external CO_2 supply, thus reinforcing the role of non-diffusive factors as the prime cause of decreased photosynthetic capacity (Lawlor and Cornic 2002).

In addition to likely compromising the biochemical ability for CO_2 fixation, the drought stress could also have provoked a range of dysfunctions at the photochemical level, as denoted from the remarkable decreases in Chl a and Chl b pools under drought stress regardless of plant hybrids. Decreases in both Chl a and Chl b (up to 50 %) were previously reported in potted $E.$ $guineensis$ plants subjected to progressive water deficit imposed by withholding irrigation (Cha-um et al. 2013) and in seedlings subjected to water deficit induced by mannitol or polyethylene glycol in a culture medium (Cha-um et al. 2010, 2012); these decreases were linearly associated with reductions in both maximum and actual quantum yield of PSII which, in turn, were directly related to decreases in A (Cha-um et al. 2010, 2012, 2013). In any case, given that A was almost suppressed in this current study due to the imposed stress, it is unlikely that photochemical disorders associated with pigment degradation have contributed significantly for impairing the CO_2 fixation at the biochemical level.

Notably, the decreases in A were not accompanied by commensurate decreases in Rubisco total activity irrespective of plant hybrids. This response, altogether with the marked increases in GLO_X (Fig. 3b) and CAT (Fig. 4d) activities under drought stress, suggests that photorespiration occurred at high rates under drought conditions, particularly in BRS Manicoré. Increased photorespiration rates, impaired CO_2 fixation and the likely maintenance (or even increase as in BRS Manicoré) of mitochondrial respiration (indirectly evidenced by NAD^+-MDH activity in stressed plants, Fig. 3c) are expected to increase ROS

production that must be detoxified to avoid oxidative stress (Mittler 2002; Miller et al. 2008). Here, it is immediately evident that BRS C 2501 plants triggered more markedly its enzymatic antioxidant system (higher relative increases and higher absolute activities of SOD, APX, GR, CAT and GLO_x) earlier (day 21) than did BRS Manicoré. Nonetheless, such a triggering failed to BRS C 2510 could successfully cope with drought stress as compared to BRS Manicoré given that ROS (e.g., H_2O_2 pools) and MDA (a marker for oxidative stress) pools increased earlier (with greater decreases in total Chl) in BRS C 2510 than in BRS Manicoré (Fig. 6). Given these facts, the earlier up-regulation of the enzymatic antioxidant system in BRS C 2510 should precisely represent its increased susceptibility to drought events compared with BRS Manicoré. Furthermore, with the progress of drought stress (particularly on day 34), BRS Manicoré was better able to cope with oxidative stress through a more robust antioxidant system, as denoted by its higher absolute activities of SOD, APX, GR and CAT relative to BRS C 2501. Furthermore, regardless of drought severity, this hybrid displayed increased pools of total carotenoids (that may play a key role in photoprotection by xanthophylls engaged in sustained thermal energy dissipation; Logan et al. 2007) coupled with lower Chl $a + b$/Car ratio, which suggests a more adequate balance between light capture and dissipation, a fact which might be of utmost importance to avoid the creation of an oxidizing environment within chloroplasts (Pompelli et al. 2010). In addition, BRS Manicoré also displayed higher total Asc and GSH pools (coupled with similar Asc and improved GSH redox states) than did BRS C 2501 (Fig. 5). As a final consequence, BRS Manicoré was better able to limit oxidative stress in its leaf tissues than did its BRS C 2501 counterpart, even on day 57 when Ψ_{pd} reached values below -4.0 MPa. Such a low Ψ_{pd} is indicative of a severe drought stress; under these circumstances the unequivocal down-regulation of the enzymatic antioxidant system in parallel to the up-regulation of non-enzymatic antioxidants might represent different strategies of oil palm plants to cope with drought-induced oxidative stress depending on the severity of drought events.

In summary, both hybrids displayed similar water statuses, and thus differences in their abilities to cope with drought stress are unlikely to have been associated with varying plant water relations, but were rather associated with biochemical traits especially those related to an improved antioxidant system under drought stress. In this context, it is tempting to conclude that BRS Manicoré is better able to tolerate drought than BRS C 2501 by triggering multiple antioxidant strategies involved both in ROS scavenging (SOD, APX, GR and CAT) and dissipation of excess energy and/or reducing equivalents

(photorespiration, NAD$^+$-MDH, Car, ascorbate and glutathione pools) particularly under severe drought stress. We believe that our data provide valuable resources for traits of physiological importance that can be used in oil palm-breeding programs to select hybrids with improved performance in drought-prone regions.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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