DOI: 10.1007/s11099-015-0146-x PHOTOSYNTHETICA 53 (4): 547-554, 2015

Photosynthetic and antioxidant responses to drought during sugarcane ripening

C.R.G. SALES[*], P.E.R. MARCHIORI[*], R.S. MACHADO[*], A.V. FONTENELE[**], E.C. MACHADO[*], J.A.G. SILVEIRA[**], and R.V. RIBEIRO[***,+]

Laboratory of Plant Physiology 'Coaracy M. Franco', Center for Research and Development in Ecophysiology and Biophysics, Agronomic Institute (IAC), P.O. Box 28, 13012-970, Campinas SP, Brazil[*]
Laboratory of Plant Metabolism, Department of Biochemistry and Molecular Biology, Federal University of Ceará, 60455-970, Fortaleza CE, Brazil[**]
Department of Plant Biology, Institute of Biology, University of Campinas, P.O. Box 6109, 13083-970, Campinas SP, Brazil[***]

Abstract

Water deficit is an important exogenous factor that enhances the influx of sucrose into sugarcane (*Saccharum* spp.) stem internodes during ripening, when photosynthetic ability in supplying sinks is essential. The aim of this study was to test the hypothesis that drought tolerance in sugarcane is associated with an effective antioxidant protection during the ripening phase that might maintain a favorable redox balance in chloroplasts and protect photosynthesis under drought conditions. Two commercial sugarcane varieties, IACSP94-2094 (tolerant) and IACSP96-2042 (sensitive), with contrasting behavior under water deficit, were subjected to water withholding during the ripening stage. Our results revealed that the tolerant variety was less affected by water deficit, maintaining photosynthesis for a longer period and showing a faster recovery after rehydration as compared to the sensitive one. As consequence, the tolerant variety faced lesser excess of light energy at PSII. The maintenance of photosynthesis under water deficit and its fast recovery after rehydration resulted in the lower leaf H_2O_2 concentration and favorable redox status in the drought-tolerant genotype, which was associated with stimulation of superoxide dismutase during ripening. Our results also revealed that ferric superoxide dismutase isoforms were strongly enhanced under drought conditions, playing an important role in chloroplast redox homeostasis.

Additional key words: chlorophyll fluorescence; leaf gas exchange; oxidative stress; water stress.

Introduction

High sucrose accumulation occurs in stem internodes of sugarcane plants (*Saccharum* spp.) during ripening process, when mature and old leaves are physiologically senescent (McCormick *et al.* 2008). Such sucrose accumulation depends on source capacity; however, sugarcane photosynthesis decreases at this time due to feedback limitation by a high leaf sucrose content, nitrogen remobilization, and protein degradation due to senescence (Inman-Bamber *et al.* 2011, Wang *et al.* 2013). Besides these endogenous constrains, sugarcane plants also face water deficit during ripening under field conditions (Ribeiro *et al.* 2013, Sales *et al.* 2013). While water stress causes significant yield loss, when it occurs at the initial growth phase (Machado *et al.* 2009), mild water deficit during the ripening phase is desirable because it enhances the influx of sucrose into the stalk, maximizing sucrose production and its storage in internodes (Inman-Bamber 2004).

Regardless the plant cycle stage, a common response

Received 11 July 2014, *accepted* 25 March 2015.
[+]Corresponding author; tel: + 55–19–35216214, e-mail: rvr@unicamp.br
Abbreviations: AsA – ascorbic acid; AsA + DHA – total ascorbate concentration; APX – ascorbate peroxidase; C_i – intercellular CO_2 concentration; DAP – days after planting; DHA – dehydroascorbate or oxidized ascorbate; EXC – relative excess of light energy; FM – fresh mass; F_v/F_m – maximal quantum yield of PSII photochemistry; g_s – stomatal conductance; NPQ – nonphotochemical quenching; P_N – net photosynthetic rate; P_N/C_i – instantaneous carboxylation efficiency; REF – reference condition; ROS - reactive oxygen species; SOD – superoxide dismutase; TBARS – thiobarbituric acid-reactive substances; VPD_L – leaf-to-air vapour pressure deficit; WD – water-deficit condition; Φ_{PSII} – effective quantum yield of PSII photochemistry; Ψ_w – leaf water potential.
Acknowledgments: The authors acknowledge studentships (CRGS, PERM, AVF) and fellowships (ECM, JAGS, RVR) granted from the National Council for Scientific and Technological Development (CNPq, Brazil). This research was financed by the São Paulo Research Foundation (FAPESP, Brazil), through the Bioenergy Research Program (BIOEN, Grant #2008/57495–3).

to water deficit is the impairment of photosynthesis, which is limited by stomatal closure (Chaves *et al.* 2009) and biochemical reactions (Lawlor and Tezara 2009). As low photosynthetic rates increase excessive light energy, the redox status in chloroplasts can be changed and trigger oxidative stress. Under such condition, plants have evolved enzymatic and nonenzymatic mechanisms to scavenge the reactive oxygen species (ROS) that are formed (Asada 2006, Azevedo *et al.* 2011). In fact, water stress leads to large impairments in photosynthesis of sugarcane genotypes sensitive to drought (Ribeiro *et al.* 2013, Sales *et al.* 2013). On the other hand, drought-resistant cultivars exhibit high antioxidant capacity through high superoxide dismutase (SOD) and ascorbate peroxidase (APX) activities (Sales *et al.* 2013, Boaretto *et al.* 2014) which was not observed in drought-sensitive genotypes. As a consequence of these physiological responses, plant growth is less affected and sugarcane plants exhibit a better recovery of leaf gas exchange after rehydration (Sales *et al.* 2013).

Considering sugarcane responses to water shortage, most studies were carried out with young plants during the initial growth phase (Sales *et al.* 2013, Boaretto *et al.* 2014, Su *et al.* 2014). However, the evaluation of young plants may lead to a misleading concept about crop performance and yield under constraining conditions. This is partly due to the limited understanding of morpho-physiological mechanisms responsible for sugarcane acclimation to low water availability and their consequences to crop growth and development (Ribeiro *et al.* 2013). As seen before, sucrose accumulation occurs during the ripening stage and we do not know how sugarcane plants are able to overcome the deleterious effects of water deficit on metabolism at this important phenological stage, when sugarcane leaves are already senescent. The maintenance of photosynthetic activity under water deficit would be important for supplying the sink demand and maintaining sucrose accumulation in internodes during the ripening phase.

The aim of this study was to test the hypothesis that drought tolerance in sugarcane is associated with an effective antioxidant protection during the ripening phase that might maintain a favorable redox balance in chloroplasts. As the consequence, sugarcane photosynthesis is protected under drought conditions and shows a rapid recovery after rewatering.

Materials and methods

Plant material and growth conditions: Two sugarcane (*Saccharum* spp.) varieties were grown under greenhouse conditions, where the mean air temperature was 25.4°C and the maximum PPFD was approximately 1,800 μmol(photon) m^{-2} s^{-1}. The commercial sugarcane varieties, IACSP94-2094 and IACSP96-2042, were evaluated. While IACSP94-2094 is drought-tolerant (Ribeiro *et al.* 2013), IACSP96-2042 is drought-sensitive and exhibits a high yield (Machado *et al.* 2009). Plants were propagated by planting stalk segments with one bud in plastic pots (0.15 L) filled with soilless substrate composed by sphagnum peat and expanded vermiculite. After 35 days from planting (DAP) in plastic pots (2 March 2008), one plant of each variety was transplanted to eight tanks of 0.6 m^3 (2.0 × 0.5 × 0.6 m) inside a greenhouse (22°54'S; 47°05'W; altitude of 674 m), containing fertilized soil according to Van Raij *et al.* (1996). Plants were grown with the main stalk and three tillers until harvest.

At the beginning of the ripening stage (287 DAP, 9 November 2008), two treatments were imposed: the reference condition (REF), in which plants were maintained under well-watered conditions (30.7 ± 1.2% of soil moisture); and the water-deficit condition (WD), induced by withholding water. There were four tanks for each treatment. After the first plants exhibited null values of leaf CO_2 assimilation (at 300 DAP; soil moisture was 18.1 ± 0.8%), WD plants were rewatered (22 November 2008). The recovery capacity of WD plants was evaluated for three days after rehydration. The soil water content at 0.2 m depth was measured through the gravimetric method (v/v) at three critical times: before water withholding; at the maximum water deficit; and 48 h after reirrigation. The soil samples were collected around 06:00 h. After 48 h of rehydration, soil moisture increased from 18 ± 1% to 27 ± 1%.

The leaf water potential (ψ_w) was evaluated in four plants of each variety at predawn (06:00 h) with a pressure chamber *model 3005* (*Soilmoisture Equipment Corp*., Santa Barbara, CA, USA) and these data were previously published (Machado *et al.* 2009). No differences were found between sugarcane varieties, with ψ_w values varying from –0.23 ± 0.06 MPa in REF plants to –0.66 ± 0.19 MPa in WD plants at 300 DAP. Three days after rehydration, ψ_w values varied between –0.16 ± 0.04 MPa and –0.17 ± 0.03 MPa, considering both REF and WD plants, respectively.

Leaf gas exchange and chlorophyll (Chl) fluorescence: Gas exchange of the first fully expanded leaf with visible ligule was measured in four plants of each variety using an infrared gas analyzer *Li-6400* (*Licor*, Lincoln NE, USA), equipped with the Red/Blue light source *6400-02B* (*Licor*, Lincoln NE, USA). Evaluations were performed between 14:00 and 15:00 h, considering the natural variation of leaf-to-air vapour pressure deficit (VPD_L), in intervals of one to two days, from 288 DAP until 303 DAP. Measurements were taken under PPFD of 2,000 μmol (photon) m^{-2} s^{-1} and the air CO_2 partial pressure of 38.6 ± 1.0 Pa. Measurements of net photosynthetic rate (P_N), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were taken and the instantaneous carboxylation efficiency (P_N/C_i) was calculated (Machado

et al. 2009).

Chl *a* fluorescence emission was measured with a modulated fluorometer *PAM-2000* (*Heinz Walz GmbH*, Effeltrich, Germany) in three plants of each variety at the day of maximum water deficit (300 DAP). Using basic signals recorded before and after saturation pulses [λ < 710 nm, PPFD ~ 10,000 μmol(photon) m^{-2} s^{-1}, 0.8 s] and after exciting PSI with far-red light [λ = 735 nm, PPFD < 15 W m^{-2}, 3.0 s], some photochemical variables were estimated according to Baker (2008): the maximal quantum yield of PSII in dark-adapted (30 min) tissues (F_v/F_m); the effective quantum yield of PSII photochemistry (Φ_{PSII}), and the nonphotochemical quenching (NPQ) in light-adapted tissues. Relative excess of light energy (EXC) was calculated according to Bilger *et al.* (1995), as EXC = $[(F_v/F_m) - \Phi_{PSII}]/(F_v/F_m)$. All fluorescence parameters were evaluated between 14:00 and 15:00 h.

Leaf hydrogen peroxide, lipid peroxidation and ascorbate determinations: H_2O_2 concentration, lipid peroxidation, and ascorbate concentration were evaluated in the same leaf extract; evaluations were taken in four plants of each variety. After the gas exchange measurements at 300 and 303 DAP, leaves were collected, immediately frozen in liquid nitrogen and stored in ultrafreezer (–78°C) until biochemical determinations. Frozen leaves (0.1 g) were homogenized in 3 mL of trichloroacetic acid (TCA, 1% w/v). The mixture was centrifuged at 20,000 × *g* for 30 min at 4°C and the supernatant collected for analysis. The H_2O_2 concentration was measured by the titanium tetrachloride method (Brennan and Frenkel 1977). The leaf H_2O_2 concentration was calculated according to a standard curve, with absorbance being measured at 415 nm with a spectrophotometer model *Genesys 10S UV-Vis* (*Thermo Fisher Scientific*, Waltham MA, USA). The results were expressed as [μmol g^{-1}(FM)].

The thiobarbituric acid-reactive substances (TBARS) were determined in four plants of each variety according to Cakmak and Horst (1991), with minor modifications. One gram of fresh leaf tissue was homogenized in 3 mL of TCA (1.0%, w/v) at 4°C. The homogenate was centrifuged at 20,000 *g* for 15 min and 0.5 mL of the supernatant was added to 3 mL of 0.5% (v/v) thiobarbituric acid (TBA) in 20% TCA. The mixture was incubated at 95°C for 50 min and the reaction stopped by cooling tubes in ice water bath. Samples were then centrifuged at 9,000 × *g* for 10 min. The absorbance of supernatant was read at 532 nm and the value for nonspecific absorption at 660 nm was subtracted from readings at 532 nm, taken with the spectrophotometer cited previously. TBARS concentration was calculated using the absorption coefficient of 155 mM cm^{-1} and results were expressed as [nmol(MDA-TBA) g^{-1}(FM)].

The total ascorbate concentration (ascorbate + dehydroascorbate, AsA + DHA) was determined by adding the leaf extract to a mixture of potassium phosphate buffer (200 mM, pH 7.0) containing 2 mM dithiothreitol (DTT), 0.5% (w/v) N-ethylmaleimide, 10% (w/v) TCA, 45% (w/v) H_2PO_4, 4% (w/v) bipyridyl, and 5% (w/v) $FeCl_3$. The reaction was performed at 40°C for 30 min, and the absorbance was read at 525 nm with the spectrophotometer cited previously. The content of AsA+DHA and ascorbic acid (AsA) were estimated using L-ascorbate as standard and expressed as [μmol g^{-1}(FM)]. The oxidized ascorbate (DHA) concentration was obtained as the difference between the reduced AsA and the total ascorbate concentration (Kampfenkel *et al.* 1995).

SOD and APX activities in leaves: Fresh leaf samples (0.1 g) taken from four plants of each variety were macerated in presence of liquid nitrogen and extracted with 100 mM Tris-HCl buffer (pH 8.0) containing 30 mM DTT, 20% glycerol (v/v), 3% polyethylene glycol 6000 (w/v), and 1 mM ascorbate (Zimmermann *et al*. 2006). The extract was centrifuged at 14,000 × *g* for 30 min at 4°C and the supernatant was used as enzymatic extract. The activity of SOD (EC: 1.15.1.1) was determined in accordance to Giannopolitis and Ries (1977). One SOD activity unit (U.A.) was defined as the amount of enzyme required to inhibit 50% of p-nitroblue tetrazolium chloride (NBT) photoreduction, and the activity was expressed as [U.A. g^{-1}(FM) min^{-1}] (Beauchamp and Fridovich 1971). The activity of APX (EC: 1.11.1.1) was assayed by the method of Nakano and Asada (1981) as previously described (Sales *et al.* 2013) and it was estimated by considering the molar extinction coefficient of ascorbate (2.8 mM^{-1} cm^{-1}) and expressed as [μmol(AsA) g^{-1}(FM) min^{-1}].

Activities of SOD isoforms in gel of native electrophoresis: Fresh leaf samples (0.2 g) composed by subsamples taken from four plants of each variety were macerated in liquid nitrogen and extracted with 1 mL of 100 mM Tris-HCl buffer (pH 7.0) containing 1 mM EDTA. Native electrophoresis in polyacrylamide gel (10% for the main gel and 3.5% for the stacking gel) of samples containing 20 mg protein was performed according to Giannopolitis and Ries (1977). The identification of SOD isoforms was performed after 15 min of inhibition-specific assays in dark utilizing potassium phosphate buffer (pH 7.0) containing H_2O_2 or KCN inhibitors (Martinez *et al.* 2001).

Statistical design and data analysis: The experimental design was randomized in a 2 × 2 factorial scheme, with the causes of variation being sugarcane varieties (IACSP94-2094 and IACSP96-2042) and water conditions (REF and WD). Data were subjected to analyses of variance (*ANOVA*) and when statistical significance was detected, the mean values (n = 3 or 4) were compared by *Scott Knott*'s test (p<0.05), by using *Sisvar*® software *v.4.3* (Federal University of Lavras, Lavras MG, Brazil).

Results

Leaf gas exchange and photochemical activity: P_N, g_s, and P_N/C_i were reduced in both sugarcane varieties due to WD (Fig. 1); however, drought sensitivity was different when comparing genotypes. The WD treatment did not reduce those parameters until thirteen days of water withholding in IACSP94-2094 (Fig. 1*A,C,E*), while IACSP96-2042 presented significant reductions in P_N (~ 57%), g_s (~ 57%), and P_N/C_i (~ 61%) after ten days of treatment, at 297 DAP (Fig. 1*B,D,F*). Both varieties presented a large reduction in leaf gas exchange at the maximum stress (300 DAP); however, the recovery pattern was distinct in both varieties. After rehydration, IACSP94-2094 recovered the initial values of leaf gas exchange after two days, while IACSP96-2042 recovered photosynthesis after three days of rehydration (Fig. 1). When considering the total carbon gain during the experimental period, IACSP94-2094 was less affected by drought as compared to IACSP96-2042 (Fig. 1*A,B*).

The water deficit reduced F_v/F_m in IACSP96-2042; it was not noticed in IACSP94-2094 (Fig. 2*A*). In fact, IACSP94-2094 showed higher F_v/F_m than that of IACSP96-2042 regardless of water condition. Both varieties exhibited significant reduction in Φ_{PSII} (Fig. 2*B*) and increase in NPQ (Fig. 2*C*) due to WD treatment, but no differences between genotypes were found. EXC increased 1.5 times in IACSP94-2094 due to water deficit, while in IACSP96-2042, the increase was 2.4 times (Fig. 2*D*).

Antioxidant metabolism: Leaf H_2O_2 concentration increased due to water deficit in both genotypes, with IACSP96-2042 showing higher values than that of IACSP94-2094 (Table 1). Water deficit also caused increases in lipid peroxidation in both genotypes and TBARS was slightly higher in IACSP94–2094 as compared to IACSP96-2042, regardless of water regime (Table 1). The ascorbate content increased only in water-stressed IACSP96-2042 and slightly decreased in IACSP94-2094 (Fig. 3*A*). As the DHA content increased in both genotypes due to water deficit, there is evidence that water stress likely induced ascorbate synthesis. Under water deficit, the proportion of AsA in relation to DHA in IACSP94-2094 decreased more when compared to IACSP96-2042 (Fig. 3*C*).

SOD activity increased by 28% in IACSP94-2094 and only by 6% in IACSP96-2042 under water deficit (Fig. 4*A*). In general, IACSP94-2094 showed lower SOD activity

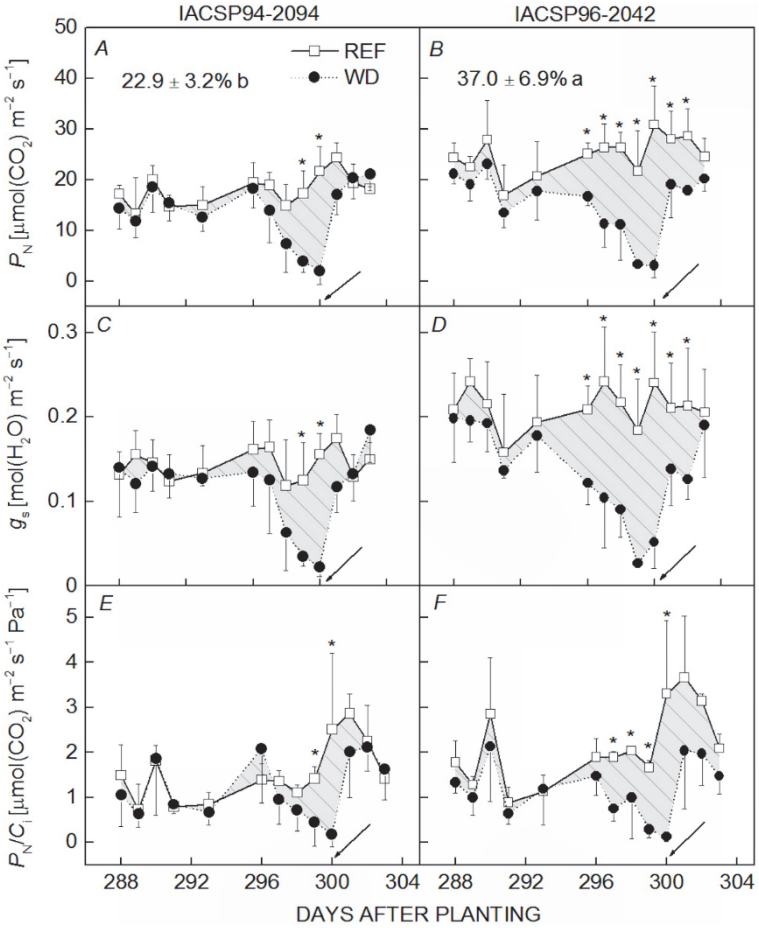

Fig. 1. Net photosynthetic rate (P_N) (*A*,*B*), stomatal conductance (g_s) (*C*,*D*), and instantaneous carboxylation efficiency (P_N/C_i) (*E*,*F*) in IACSP94-2094 (*A*,*C*,*E*) and IACSP96-2042 (*B*,*D*,*F*) sugarcane varieties growing under well-watered (REF) and water-deficit (WD) conditions. Measurements were taken between 14:00 and 15:00 h in intervals of one to two days, from 288 days after planting (DAP) until 303 DAP. Mean values of four replicates (± SD) were compared by he *Scott-Knott* test (p<0.05) and * indicates statistical differences between watering regimes. *Arrows* indicate the maximum stress (300 DAP). The relative reductions in integrated P_N throughout the experimental period are shown in *A* and *B*.

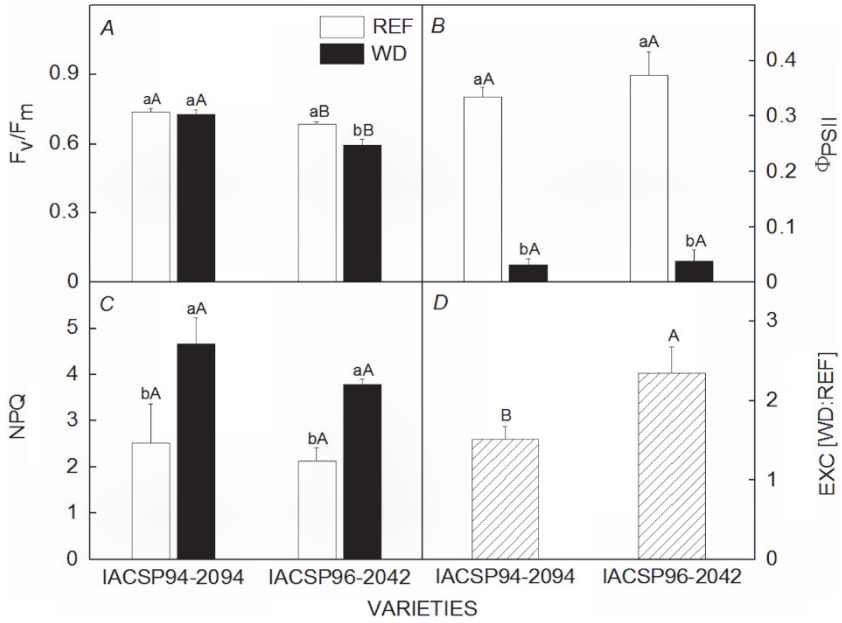

Fig. 2. Maximal (F_v/F_m) (*A*) and effective (Φ_{PSII}) (*B*) quantum yield of PSII photochemistry, nonphotochemical quenching (NPQ) (*C*), and relative excess of light energy (EXC) (*D*) in IACSP94-2094 and IACSP96-2042 sugarcane varieties growing under well-watered (REF) and water-deficit (WD) conditions. Measurements were taken at 14:00 h, after 14 days of water withholding (maximum stress at 300 DAP). In *D*, EXC was estimated in each treatment and then the ratio WD/REF was calculated. Mean values of three replicates (+ SD) were compared by the *Scott-Knott* test ($p<0.05$). Different *lowercase letters* indicate statistical differences between watering regimes in the same genotype, whereas *capital letters* indicate differences between genotypes in the same water regime.

Table 1. Leaf H_2O_2 concentration and lipid peroxidation (TBARS) in IACSP94-2094 and IACSP96-2042 sugarcane varieties growing under well-watered (REF) and water-deficit (WD) conditions. Measurements were taken after 14 days of water withholding (maximum stress). Mean values of four replicates (± SD) were compared using the *Scott-Knott* test ($p<0.05$). As interaction between genotypes and water regimes was not significant, *different letters* indicate statistical differences between genotypes or water regime.

Variable	Variety	Water regime		Mean
		REF	WD	
H_2O_2 [μmol g^{-1}(FM)]	IACSP94-2094	13.9 ± 0.5	14.6 ± 0.6	14.3 ± 0.7[b]
	IACSP96-2042	15.0 ± 0.7	16.7 ± 0.2	15.7 ± 1.0[a]
	Mean	14.3 ± 0.8[b]	15.9 ± 1.2[a]	
TBARS [nmol(MDA-TBA) g^{-1}(FM)]	IACSP94-2094	51.9 ± 3.1	62.1 ± 2.3	56.9 ± 6.1[a]
	IACSP96-2042	42.7 ± 1.7	53.1 ± 3.2	47.9 ± 6.1[b]
	Mean	47.2 ± 5.3[b]	57.6 ± 5.5[a]	

than that of IACSP96-2042 under both water regimes (Fig. 4*A*). The APX activity strongly increased by water deficit in both cultivars, with IACSP94-2094 presenting higher constitutive activity than that in IACSP96-2042 (Fig. 4*B*). Relative increase in APX due to water deficit was higher in IACSP96-2042 than that in IACSP94-2094. The SOD zymogram revealed details about the activities and identification of some SOD isoforms and we numbered arbitrarily each isoform based only in the order of appearance on gel (Fig. 5). IACSP94-2094 exhibited six SOD isoforms, corresponding to one Mn-SOD, two Fe-SOD, and three Cu/Zn-SOD. IACSP96-2042 exhibited eight SOD isoforms under well-watered conditions: one Mn-SOD, two Fe-SOD, and five Cu/Zn-SOD (Fig. 5). There was an increase in Fe-SOD (II) in both cultivars under WD treatment. While IACSP96-2042 showed increases in three Cu/Zn-SOD isoforms (III, IV, and V), IACSP94-2094 exhibited a slight stimulation of two Cu/Zn-SODs (VI and VIII) under water stress. It is important to notice that most of SOD activity was exhibited by two Fe-SOD isoforms, which are probably located in chloroplasts.

Discussion

Studies with sugarcane plants under water deficit are widely reported, but most of them were carried out at the initial or maximum growth phases. In general, plants are grown in pots, where root growth may be limited (*e.g.*, Sales *et al.* 2013, Boaretto *et al.* 2014, Su *et al.* 2014). In this study, sugarcane plants were grown in large containers and their physiological response to water deficit was evaluated during the ripening phase. This condition is closer to that one faced by plants under field conditions; differential responses related to antioxidant metabolism and photosynthesis were revealed when using two sugarcane genotypes with contrasting response to drought (tolerant *vs*. sensitive).

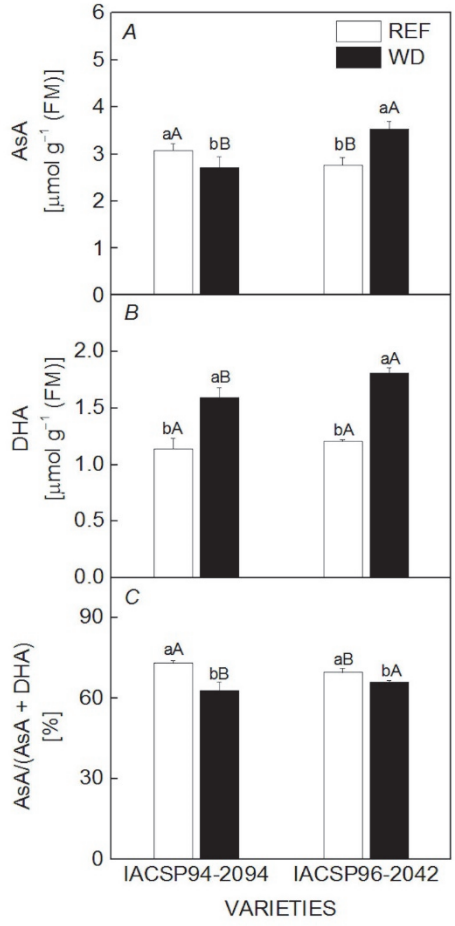

Fig. 3. Reduced (AsA) (*A*) and oxidized (DHA) (*B*) ascorbate and ratio of AsA to total ascorbate content [AsA/(AsA+DHA)] (*C*) in IACSP94-2094 and IACSP96-2042 sugarcane varieties growing under well-watered (REF) and water-deficit (WD) conditions. Measurements were taken after 14 days of water withholding (maximum stress at 300 DAP). Mean values of four replicates (+ SD) were compared by the *Scott-Knott* test (p<0.05). Different *lowercase letters* indicate statistical differences between watering regimes in the same genotype, whereas *capital letters* indicate differences between genotypes under the same water regime.

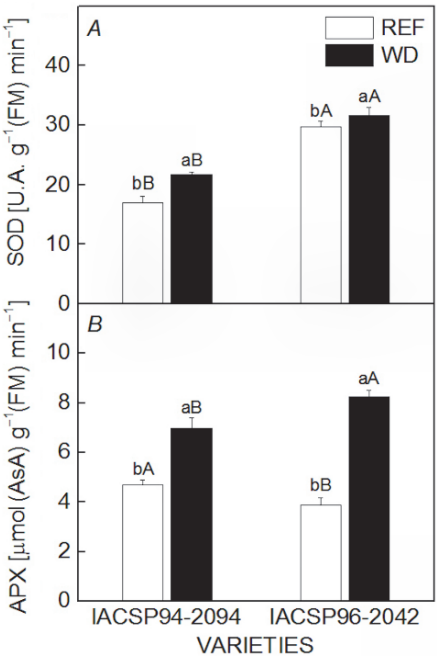

Fig. 4. Activity of superoxide dismutase (SOD) (*A*) and ascorbate peroxidase (APX) (*B*) in IACSP94-2094 and IACSP96-2042 sugarcane varieties growing under well-watered (REF) and water-deficit (WD) conditions. Measurements were taken after 14 days of water withholding (maximum stress at 300 DAP). Mean values of four replicates (+ SD) were compared by the *Scott-Knott* test test (p<0.05). Different *lowercase letters* indicate statistical differences between watering regimes in the same genotype, whereas *capital letters* indicate differences between genotypes under the same water regime.

The water deficit caused strong decrease in g_s (Fig. 1*C,D*), reducing the CO_2 diffusion into the leaf and limiting photosynthesis. Besides this initial limitation, we may argue that photosynthesis was also reduced due to biochemical impairment as revealed by significant reduction in instantaneous carboxylation in both genotypes (Fig. 1*E,F*). Both stomatal and biochemical limitations were more intense in IACSP96-2042, with stomatal closure and reduction in P_N/C_i occurring earlier in IACSP96-2042 than in IACSP94-2094. In fact, photosynthesis of IACSP94-2094 was significantly reduced only after thirteen days of water withholding (Fig. 1*A*). After rehydration, the leaf gas exchange in IACSP94-2094 recovered to the initial values one day earlier than that in IACSP96-2042. Such a response in IACSP96-2042 is the evidence of high photosynthetic sensitivity to drought (Fig. 1*A,B*).

As light energy is absorbed by photosynthetic pigments and CO_2 assimilation is strongly impaired under water deficit, an imbalance between photochemistry and CO_2 carboxylation reactions occurs. In these conditions, plants should cope with an excess of energy in thylakoid

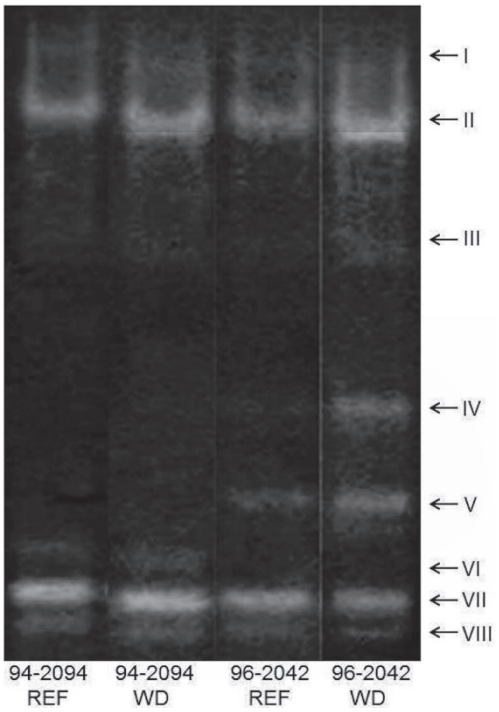

Fig. 5. Superoxide dismutase isoforms by nondenaturing 10% PAGE in leaves of IACSP94-2094 and IACSP96-2042 sugarcane varieties growing under well-watered (REF) and water-deficit (WD) conditions. Measurements were taken after 14 days of water withholding (maximum stress at 300 DAP). The bands corresponding to the SOD isoforms were identified after gel treatment with KCN and H_2O_2 inhibitors. (I) Mn-SOD, (II) Fe-genotype SOD, (III-VI) Cu/Zn-SOD, (VII) Fe-SOD, and (VIII) Cu/Zn-SOD. The gel is representative of three runs.

membranes otherwise photochemical damage might occur due to reduced quinone accumulation (Foyer and Noctor 2000). While IACSP96-2042 reduced its F_v/F_m, IACSP94-2094 maintained high F_v/F_m under water deficit (Fig. 2*A*). This pattern suggests that IACSP94-2094 preserved the PSII integrity under drought, which was not true in IACSP96-2042. The maintenance of F_v/F_m found in the drought-tolerant genotype was related to the increase in NPQ under water stress (Fig. 2*C*) and lower EXC when compared to the sensitive genotype (Fig. 2*D*). Increase in NPQ is considered as a strategy of photoprotection through dissipation of excessive energy as heat under conditions of low electron transport rate (Horton *et al.* 1996, Niyogi *et al.* 1998). Apparently, the similar increase in NPQ (~ 45%) in both genotypes subjected to drought (Fig. 2*C*) was enough to preserve PSII integrity in IACSP94-2094, but not in IACSP96-2042. This fact was probably associated with the larger reduction of photosynthesis (integrated P_N) in IACSP96-2042, which led to higher EXC (Fig. 1*A*).

Efficient use of absorbed light energy that reaches the photosystems is essential to drain the most of electrons and minimize the production of ROS in chloroplasts. In oxygenic metabolism, ROS are commonly produced during the photosynthetic electron transport chain and their production may be enhanced due to low activity of electron sinks (Foyer and Shigeoka 2011). EXC increased due to water deficit and was higher in IACSP96-2042 as compared to IACSP94-2094 (Fig. 2*D*), suggesting that this latter genotype had better controlled excessive energy under drought. Such ability is associated with the maintenance of photosynthesis, which reduces the excess of light energy at the PSII level. On the other hand, IACSP96-2042 exhibited higher sensitivity of photosynthesis and higher EXC than that of IACSP94-2094, suggesting that the NPQ increase in IACSP96-2042 was not sufficient to deal with the large increase of EXC (Figs. 1*A,B*; 2*C,D*).

As compared to IACSP94-2094, higher excess of energy at PSII found in IACSP96-2042 was associated with higher leaf H_2O_2 concentration (Fig. 2*D*, Table 1). Regarding the response to water deficit, both genotypes presented increase in SOD activity (Fig. 4*A*); that is considered the first line of defense for protection against the formation of superoxide radicals, acting as an electron sink in plants under stress conditions (Mittler 2002). However, SOD activity was more stimulated by water deficit in IACSP94-2094 as compared to IACSP96-2042 (Fig. 4*A*). Regardless of water regime, both genotypes showed the high Fe-SOD activity (Fig. 5), indicating that the chloroplast SOD isoforms were crucial to drought tolerance in sugarcane leaves at the ripening stage when the photosynthetic efficiency was reduced and leaf senescence proceeded. The total APX activity increased under water deficit in both varieties (Fig. 4*B*), whereas AsA concentration increased only in IACSP96-2042 (Fig. 3*A*). This was the evidence of newly synthesized ascorbate, which maintain the high AsA concentration even with the increasing APX activity (Silva *et al.* 2010). Under depletion of AsA inside chloroplast, APX losses the efficiency to reduce H_2O_2; this was prevented in both genotypes.

In conclusion, the tolerant variety, IACSP94-2094, was less affected by water deficit during ripening, as revealed by the maintenance of photosynthesis for a longer period and a fast recovery after rehydration. Then, IACSP94-2094 faced less excessive energy at PSII level under water deficit. Even under lesser energetic pressure, IACSP94-2094 showed the same increase of NPQ and a higher stimulation of SOD activity as compared to IACSP96-2042 under water deficit. Such physiological changes protected IACSP94-2094 from oxidative damage caused by drought.

References

Asada K.: Production and scavenging of reactive oxygen species in chloroplasts and their functions. – Plant Physiol. **141**: 391-396, 2006.

Azevedo R.A., Carvalho R.F., Cia M.C. *et al*.: Sugarcane under pressure: an overview of biochemical and physiological studies of abiotic stress. – Trop. Plant Biol. **4**: 42-51, 2011.

Baker N.R.: Chlorophyll fluorescence: a probe of photosynthesis *in vivo*. – Annu. Rev. Plant Biol. **59**: 89-113, 2008.

Beauchamp C., Fridovich I.: Superoxide dismutase: improved assay applicable to acrylamide gels. – Anal. Biochem. **44**: 276-287, 1971.

Bilger W., Schreiber U., Bock M.: Determination of the quantum efficiency of photosystem II and non-photochemical quenching of chlorophyll fluorescence in the field. – Oecologia **102**: 425-432, 1995.

Boaretto L.F., Carvalho G., Borgo L. *et al.*: Water stress reveals differential antioxidant responses of tolerant and non-tolerant sugarcane genotypes. – Plant Physiol. Bioch. **74**: 165-175, 2014.

Brennan T., Frenkel C.: Involvement of hydrogen peroxide in the regulation of senescence in pear. – Plant Physiol. **59**: 411-416, 1977.

Cakmak I., Horst W.J.: Effect of aluminum on lipid-peroxidation, superoxide-dismutase, catalase and peroxidase- activities in toor-tips of soybean (*Glycine max)*. – Physiol. Plantarum **83**: 463-468, 1991.

Chaves M.M., Flexas J., Pinheiro C.: Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. – Ann. Bot.-London **103**: 551-560, 2009.

Foyer C.H., Noctor G.: Oxygen processing in photosynthesis: regulation and signaling. – New Phytol. **146**: 359-388, 2000.

Foyer C.H., Shigeoka S.: Understanding oxidative stress and antioxidant functions to enhance photosynthesis. – Plant Physiol. **155**: 93-100, 2011.

Giannopolitis C.N., Ries S.K.: Superoxide dismutases: I. Occurrence in higher plants. – Plant Physiol. **59**: 309-314, 1977.

Horton P., Ruban A.V., Walters R.G.: Regulation of light harvesting in green plants. – Annu. Rev. Plant Phys. **47**: 655-684, 1996.

Inman-Bamber N.G.: Sugarcane water stress criteria for irrigation and drying off. – Field Crop. Res. **89**: 107-122, 2004.

Inman-Bamber N.G., Jackson P.A., Hewitt M.: Sucrose accumulation in sugarcane stalks does not limit photosynthesis and biomass production. – Crop Pasture Sci. **62**: 848-858, 2011.

Kampfenkel K., Van Montagu M., Inzé D.: Extraction and determination of ascorbate and dehydroascorbate from plant tissue. – Anal. Biochem. **225**:165-167, 1995.

Lawlor D.W., Tezara W.: Causes of decreased photosynthetic rate and metabolic capacity in water-deficient leaf cells: a critical evaluation of mechanisms and integration of processes. – Ann. Bot.-London **103**: 561-579, 2009.

Machado R.S., Ribeiro R.V., Marchiori P.E.R. *et al.*: [Biometric and physiological responses to water deficit in sugarcane at different phenological stages.] – Pesqui. Agropecu. Bras. **44**:1575-1582, 2009. [In Portuguese]

Martinez C.A., Loureiro M.E., Oliva M.A. *et al.*: Differential responses of superoxide dismutase in freezing resistant *Solanum curtilubum* and freezing sensitive *Solanum tuberosum* subjected to oxidative and water stress. – Plant Sci. **160**: 505-515, 2001.

McCormick A.J., Cramer M.D., Watt D.A.: Culm sucrose accumulation promotes physiological decline of mature leaves in ripening sugarcane. – Field Crop. Res. **108**: 250-258, 2008.

Mittler R.: Oxidative stress, antioxidants and stress tolerance. – Trends Plant Sci. **7**: 405-410, 2002.

Nakano Y., Asada K.: Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. – Plant Cell Physiol. **22**: 867-880, 1981.

Niyogi K.K., Grossman A.R., Björkman O.: *Arabidopsis* mutants define a central role for the xanthophyll cycle in the regulation of photosynthetic energy conversion. – Plant Cell **10**: 1121-1134, 1998.

Ribeiro R.V., Machado R.S., Machado E.C. *et al.*: Revealing drought-resistance and productive patterns in sugarcane genotypes by evaluating both physiological responses and stalk yield. – Exp. Agr. **49**: 212-224, 2013.

Sales C.R.G., Ribeiro R.V., Silveira J.A.G. *et al.*: Superoxide dismutase and ascorbate peroxidase improve the recovery of photosynthesis in sugarcane plants subjected to water deficit and low substrate temperature. – Plant Physiol. Bioch. **73**: 326-336, 2013.

Silva E.N., Ferreira-Silva S.L., Fontanele A.V. *et al.*: Photosynthetic changes and protective mechanisms against oxidative damage subjected to isolated and combined drought and heat stresses in *Jatropha curcas* plants. – J. Plant Physiol. **167**: 1157-1164, 2010.

Su Y., Guo J., Ling H. *et al.*: Isolation of a novel peroxisomal catalase gene from sugarcane, which is responsive to biotic and abiotic stresses. – PLoS One **9**: 1-11, 2014.

Van Raij B., Cantarella H., Spironello A. *et al.*: [Sugarcane]. – In: Van Raij B., Cantarella H., Quagglio J.A. *et al.* (ed.): [Recommendation of Fertilization and Liming to the State of São Paulo]. Pp. 237-239. IAC, Campinas 1996. [In Portuguese]

Wang J., Nayak S., Koch K. *et al.*: Carbon partitioning in sugarcane (*Saccharum* species). – Front. Plant Sci.: doi: 10.3389/fpls.2013.00201, 2013.

Zimmermann P., Heinlein C., Orendi G. *et al.*: Senescence-specific regulation of catalases in *Arabidopsis thaliana* (L.) Heynh. – Plant Cell Environ. **29**: 1049-1060, 2006.