AN EFFICIENT ANTIOXIDANT SYSTEM IS ASSOCIATED WITH LOWER PHOTOSYNTHESIS PHOTOINHIBITION AND GREATER TOLERANCE TO DROUGHT IN SUGARCANE CULTIVARS

UM EFICIENTE SISTEMA ANTIOXIDANTE ESTÁ ASSOCIADO A MENOR FOTOINIBIÇÃO DA FOTOSSÍNTESE E MAIOR TOLERÂNCIA À SECA EM CULTIVARES DE CANA-DE-AÇÚCAR

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ABSTRACT: The occurrence of seasonal droughts is one of the main factors that limit the sugarcane ratoon cycles, compromising sugarcane field longevity. The aim of this study was to evaluate the biochemical responses of sugarcane cultivars to drought stress in ratoon crop. Six cultivars were used: RB72910, RB99382, RB72454, RB92579, RB855536 and RB931011, and three water regimes based on soil available water content (SAWC) and defined as: control, 80 to 100% (SAWC); moderate water stress, 40 to 60% (SAWC), and severe water stress, 0 to 20% (SAWC). Cultivar RB72454 was most sensitive to water deficit. When under stress, this cultivar showed an increased production of hydrogen peroxide, but without concomitant increase in the activity of the antioxidant enzymes ascorbate peroxidase, catalase and superoxide dismutase. Oxidative stress led to lipid peroxidation and chlorophyll degradation, resulting in higher photochemical photoinhibition. On the other hand, cultivar RB92579 was the most tolerant to drought, with increased activity of antioxidant enzymes, which led to low lipid peroxidation, maintenance of photosynthetic pigments and photochemical activity. The antioxidant defense system triggered by ascorbate peroxidase, catalase and superoxide dismutase enzymes appears to be a key protection factor to photochemical complexes of chloroplast of sugarcane plants under water stress. The increase in the antioxidant system as well as the maintenance of photosynthetic pigments and cell membranes served as important criteria to indicate cultivars more tolerant to drought stress.

KEYWORDS: *Saccharum* spp. Photosynthesis. Chlorophyll. Photooxidation. Stress.

INTRODUCTION

In tropical countries, sugarcane is one of the most economically important crops due to its use in both the food industry and the production of renewable biofuels. The world sugarcane production is estimated 2.152 Mt in 2025, with planted area of 30.000.000 ha (FAO, 2016). However, in addition to the limited introduction of new areas for crop production, factors such as global warming and changes in precipitation patterns, even in traditionally cultivated areas, have increased the occurrence of drought events, which has significantly affected crop production (ST CLAIR; LYNCH, 2010; SILVA et al., 2013; VIEIRA et al., 2014). Therefore, the lack of water availability for irrigation has become one of the greatest challenges for the future of global agriculture. Meanwhile, one of the main strategies is the knowledge of species genotypes with greater capacity to tolerate water deficit (CIA et al., 2012; SILVA et al., 2012).

The occurrence of water deficit affects the absorption and utilization of light energy in photosystems through the degradation of the photosynthetic pigments (JALEEL et al., 2009). Under water deficit conditions, cultivars that manage to maintain stable pigment contents are reported as drought tolerant (SILVA et al., 2014; CHEN et al., 2016). Thus, reduction in leaf pigments affects the photosynthetic capacity and plant productivity (CARLIN et al., 2012; SILVA et al., 2012).

In addition, under conditions of water deficiency and high luminosity, plants suffer from the excess of electron flow through the photossystems, causing an over-excitation of the reaction centers of Photosystems I and II, leading to the production of reactive oxygen species (ROS) (CARVALHO, 2008). ROS affect the redox state in

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chloroplasts, triggering oxidative stress that causes negative effects on plant metabolism, such as oxidation of membrane lipids, proteins and nucleic acids (CARLIN et al., 2012; SALES et al., 2013). Under these conditions, plants may develop enzymatic and non-enzymatic mechanisms to increase tolerance or adaptation to stress conditions and minimize ROS that are produced as a result of stress (LI et al., 2011; WENG et al., 2015).

The oxidative damage caused by ROS significantly impairs growth and productivity of plants, which ultimately reduce the yield of agricultural crops such as sugarcane (SANTOS; SILVA, 2015). In this crop, more drought-tolerant cultivars generally have higher antioxidant capacity through the higher activity of enzyme such as superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT), and lower concentrations of hydrogen peroxide and lipid peroxidation (CIA et al., 2012; BOARETTO et al., 2014; CHEN et al., 2016). As a consequence of these physiological responses, plant growth is less affected by water stress, since the photosynthetic apparatus is protected by the antioxidant enzymes (BOARETTO et al., 2014; SALES et al., 2015).

Several studies with sugarcane have been conducted using biochemical approaches of enzymatic and non-enzymatic nature in the selection of cultivars more tolerant to different types of environmental stresses, among them, drought stress (BOARETTO et al., 2014; SANTOS; SILVA, 2015; SALES et al., 2015). However, most of them were made in first crop cycle, plant cane, making investigations in another crop cycle extremely important, since the culture is usually renewed only after five or more years of ratoon cropping and little is known about the mechanisms of drought tolerance in sugarcane ratoon crop. Thus, the aim of this study was to evaluate the implications of water stress on ratoon cane metabolism by quantifying photosynthetic pigments, antioxidant system enzymes and lipid peroxidation. Our hypothesis is that sugarcane cultivar with a better antioxidant system may be more tolerant and more productive under drought stress.

MATERIAL AND METHODS

Plant material and experimental conditions

The experiment was conducted under coordinates 9°28'S, 35°49'W, 127 m above sea level. The experimental design was a randomized complete block design in a 6 x 3 factorial scheme with six sugarcane cultivars and three water regimes, distributed in four blocks, totaling 72 experimental plots. The experimental plot consisted of a pot of 0.485 m of average diameter and 0.99 m of height, filled with approximately 180 kg of crushed, sieved and homogenized soil. Pots were distributed in a 1.0 x 1.0 m spatial arrangement between rows in the open air.

The Sugarcane cultivars used were RB72910, RB99382, RB72454, RB855536, RB92579 and RB931011. RB72910 is classified by the Brazilian Sugarcane Breeding Program / RIDESA as tolerant to drought stress, based on productivity data in experimental field, while RB99382 presents regular tillering and sprouting under stress. RB72454 has good stalk diameter, low fertility requirement, and sensitivity to water deficit (ENDRES et al., 2010; RIDESA, 2010). RB855536 presents high productivity and excellent sprouting when cultivated without water restriction (RIDESA, 2010). RB92579 presents mechanisms of drought stress tolerance and high productivity (ENDRES et al., 2010; RIDESA, 2010). RB931011 presents rapid growth, high stature, good development of stalks and tolerance to salinity (RIDESA, 2010; MEDEIROS et al., 2015).

Water regimes were based on the soil available water capacity and defined as: control, 80 to 100% (SAWC); moderate water stress, 40-60% (SAWC) and severe water stress, 0 to 20% (SAWC). The soil used was a cohesive Yellow Latosol. Physicochemical analyses were performed according to methodology of Embrapa (1997), and the water retention curve was estimated using the methodology of Richards (1965) which results are shown in Table 1.

The sugarcane billets used for planting (02/19/2014) were standardized taking into account age, health status and stem region of the cane. In order to guarantee better homogeneity of seedlings, billets were previously planted in plastic trays in greenhouse until seedlings had three leaves, suitable for transplanting. After 30 days after planting, three seedlings of uniform size were selected for each pot. At 240 days after planting, the plant-cane was harvested, beginning the second cycle. Subsequently, pots were irrigated near the field capacity until the moment of implantation of the water treatments that started 60 days after harvesting. Tillering thinning was performed, leaving six plants of uniform size in each pot.

Table 1. Chemical and physical properties of the soil used in the experiment

Analysis Soil	
Chemical	
Electrical conductivity	0.32 dS m^{-1}
pH	6.3
Ca^{2+}	7.61 cmolc kg^{-1}
Mg^{2+}	4.41 cmolc kg^{-1}
Na^{+}	0.26 cmolc kg^{-1}
K^{+}	0.18 cmolc kg^{-1}
P	49 mg kg^{-1}
Al^{3+}	0.00 cmolc kg^{-1}
Organic matter	35.60 g kg^{-1}
Physical	
Total porosity	56.2%
Real density	1.17 g cm^{-3}
Sand	637.6 g kg^{-1}
Silt	205.9 g kg^{-1}
Clay	156.5 g kg^{-1}
Textural classification	Franco clay sandy
Soil Tension (MPa)	Water content (%)
0.033	27.72
1.5	14.76
Available Water	12.96

During the water stress period, soil moisture was monitored in each pot by a soil moisture monitoring probe system (Moisture Meter PR2, Delta T Devices, England), which evaluates moisture every 10 cm to a depth of 40 cm. Water was replaced by a pressurized irrigation system, with one emitter per plot, leaving each water treatment in its moisture range (Figure 1A). Climatic data for the experimental period were obtained by an automatic agrometeorological station located approximately 200 m from the experiment. Data were recorded every ten minutes, obtaining information on rainfall (Figure 1B), mean air temperature (Figure 1C) and relative air humidity (Figure 1D).

At 22 days after stress, the maximum photochemical efficiency of PS II (Fv/Fm) in leaves +1 was performed. The numbering of leaves was based on Van Dillewijn's classification (1952). Subsequently, the median portion of same leaf was collected for biochemical analyses. Part of the collected leaf was conditioned in icebox for analysis of photosynthetic pigments, and another part was packed in aluminum foil, conditioned in liquid nitrogen, and later placed in a freezer at -70ºC until moment of enzymatic analyses.

Photochemical efficiency of PS II

The Fv/Fm ratio was determined using a portable light modulated fluorometer (PAM 2500, WALZ), according to procedures of Maxwell and Johnson (2000). These evaluations were performed between 12 a.m. and 1 p.m. in leaves pre-adapted to the dark (≈30 min) with the use of specific clips.

Content of photosynthetic pigments

The contents of chlorophyll a, b, and carotenoids were quantified using three 0.8 cm^2 discs of fresh leaves. The pigments were extracted in the dark at 4°C for 24 h in glass tubes protected with aluminum foil containing 5 mL of 80% acetone. Subsequently, the absorbance of extracts was quantified at wavelength of 480, 645, 663 nm with a spectrophotometer. The levels of chlorophyll *a*, *b*, and carotenoids were then calculated as described by Lichtenthaler (1987), and expressed in mg g^{-1} fresh weight. Subsequently, the total chlorophyll content (a + b), and the chlorophyll a/b ratio were calculated.

Extraction and Assay of Antioxidant Enzymes

Extracts for quantification of the activity of ascorbate peroxidase (APX) and catalase (CAT) enzymes and soluble proteins were obtained by cold maceration with N_2. About 0.1 g of leaf tissue was extracted in solution containing 300 mM potassium phosphate buffer (pH 7.5), 2 mM EDTA and 20 mM ascorbic acid with addition of 0.3 g PVPP, making a final volume of 2000 µL. The extract was centrifuged at 15,000*g* for 10 minutes at 4°C. While, extraction for quantification of superoxide

dismutase (SOD) was obtained from 0.1 g of frozen vegetable tissue, macerated in 2000 µL of the extraction medium containing 300 mM Potassium Phosphate Buffer (pH = 7.8). The extract was centrifuged at 15,000*g* for 10 min at 4°C.

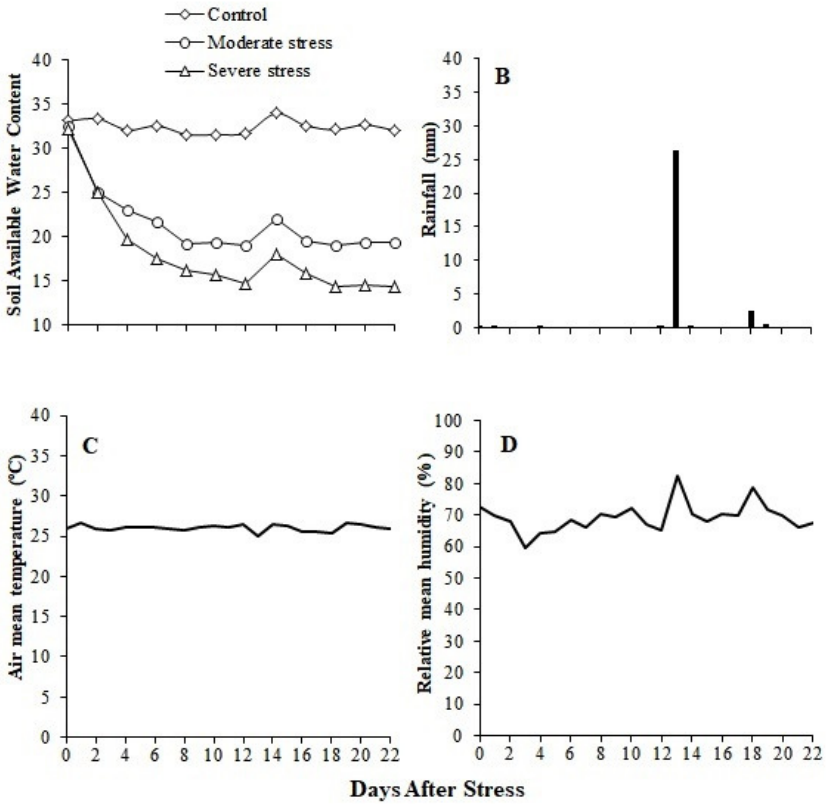

Figure 1. Soil moisture with sugarcane cultivars submitted to different water regimes in the second crops cycle (A), Rainfall (B) air mean temperature (C), and relative mean humidity (D) during the period experiment.

The APX activity was obtained according to methodology of Nakano and Asada (1981). Activity was measured using 50 µL of extract added of 334 µl of 300 mM potassium phosphate buffer (pH 7.5), 20 µl of 50 mM sodium ascorbate, 20 µl of 10 mM hydrogen peroxide and 1576 µL of deionized water. The change in absorbance after adding H_2O_2 was read at 290 nm for 1 min at every 10 s interval, using a spectrophotometer at 290 nm observing the decrease of ascorbate concentration, in triplicate. An ascorbate extinction coefficient of 2.8 mM^{-1} cm^{-1} at 290 nm was used. APX activity was expressed as units mg^{-1} of protein.

The CAT activity was quantified according to Havir and Mchale (1987), with modifications. 50 µL aliquots of the enzyme extract were added to 334 µL of 300 mM potassium phosphate (pH 7.5), 250 µL of 12.5 mM hydrogen peroxide and 1366 µL of distilled water incubated at 30°C. Readings of absorbance were taken at 240 nm for 1 min and the decrease of H_2O_2 concentration was observed, in triplicate. An H_2O_2 extinction coefficient of 36 mM^{-1} cm^{-1} at 240 nm was adopted. One unit of CAT activity was defined as the degradation of 1 µM H_2O_2 in one minute and expressed as units mg^{-1} of protein.

The SOD activity was quantified by the ability of the enzyme to inhibit nitrotetrazolium blue photoreduction (NBT) according to the method of Giannopolitis and Ries (1977), with modifications. 50 µL of the enzyme extract was added to 2950 µL of the reaction medium composed of 300 mM potassium phosphate buffer (pH 7.8), 14 mM methionine, 100 mM EDTA, 75 µM NBT, and 2 µM riboflavin. The sample plus the reaction medium were illuminated with a 15 W fluorescent lamp for 4 minutes, and then the absorbance at 560 nm was quantified using a spectrophotometer, in triplicate. One unit of SOD was defined as the amount of enzyme required to inhibit 50% of NBT

photoreduction. SOD activity was expressed as units mg^{-1} protein. The protein concentration was determined using bovine serum albumin as the standard (BRADFORD, 1976).

Hydrogen peroxide content and Lipid Peroxidation

The hydrogen peroxide (H_2O_2) content was determined according to Velikova et al. (2000). About 0.1 g of fresh frozen material was macerated in an acid solution composed of 2000 µL of 0.1% (trichloroacetic acid) TCA and centrifuged at 10,000g for 10 min at 4°C. In test tubes containing 700 µl of 10 mM potassium phosphate buffer (pH 7.0) and 1000 µl of 1 M potassium iodide, 300 µl of the supernatant was added and the mixture incubated for 10 min at 30°C. Absorbance was then quantified at 390 nm in triplicate. The content of H_2O_2 was quantified based on the standard curve and the results expressed in µmol g^{-1} FW.

Lipid peroxidation was determined through measurement of the malondialdehyde (MDA) content, as described by Cakmak and Horst (1991) with minor modifications. About 0.1 g of fresh leaf tissue was macerated with 2000 µL of 0.1% TCA. The homogenate was then centrifuged at 10,000g for 10 min at 4°C. Aliquots of 300 µL of the supernatant were added to 1700 µL of the reaction medium composed of 0.5% (w/v) TBA and 20% TCA (w/v), and incubated at 90°C for 60 min with reaction interruption by rapid cooling in ice bath. The absorbance was read in triplicate at 532 nm and non-specific absorbance at 600 nm was subtracted from it. The MDA content was calculated by using an extinction coefficient of 155 mM^{-1} cm^{-1} and expressed in nmol g^{-1} FW.

Statistical analysis

Data were submitted to analysis of variance and when significant effect was detected, the means were compared by the Tukey test at $p < 0.05$, both between water regimes and between cultivars. Pearson correlations were calculated to determine the relationship among variables analyzed in the studied period.

RESULTS

The pigment content varied among sugarcane cultivars even without water stress (Figure 2). In general, there is a reduction in chlorophyll content when plants were submitted to water stress (Figures 2A, B, C, D). However, the reduction was different among cultivars. Moderate stress was sufficient to reduce chlorophyll *a* content in all cultivars except for RB855536 and RB92579 (Figure 2A). The cultivar RB72454 was the one that most reduced chlorophyll *a* under moderate stress, with reduction of 40%. Under severe stress, all cultivars reduced the chlorophyll *a* content, with the highest reduction of 43.2% in RB72454.

The chlorophyll *b* content was not affected by water regime in cultivar RB855536 (Figure 2B). Under moderate stress, cultivar RB72454 had the largest reduction, 35.1%, in relation to control, while cultivar RB92579 reduced only 8.9% in the same conditions. Under severe stress, the reduction was 50.7% in cultivar RB72454 and only 22.1% in RB92579.

Cultivar RB72454 had reduction of 38.8% of total chlorophyll content under moderate stress, while the reduction was only 8.9% in RB92579 in relation to control (Figure 2C). Under severe stress, the reduction was 45% in cultivar RB72454, and only 14.5% in RB92579.

The Chl *a/b* ratio was not affected by water regimes in cultivars RB855536, RB92579 and RB931011 (Figure 2D). On the other hand, under severe stress, the Chl *a/b* ratio increased 21.5% in cultivar RB99382, 17.2% in RB72910 and 12.7% in RB72454 compared to control.

The carotenoid content remained unchanged between control treatment and moderate stress in cultivars RB99382 and RB92579. Meanwhile, cultivar RB72454 was the one that most reduced the carotenoid content, 32.4% under moderate stress and 49.1% under severe stress in relation to control (Figure 2E). The carotenoid content of cultivar RB92579 reduced only 13.5% under severe stress. Chlorophyll *a* and *b* had high correlation with total Chl in all water conditions (Table 2). On the other hand, the carotenoid content only correlated with the chlorophylls when plants were under stress.

The maximum photochemical efficiency (Fv/Fm) was affected only under severe stress, where cultivars RB72910 and RB72454 had reductions of 12.2 and 31.3%, respectively, in relation to control treatment (Figure 2F).

The activity of APX, CAT and SOD enzymes in leaves did not vary among cultivars when under control conditions, but increased under drought stress (Fig. 3A, B, C). The activity of the APX enzyme had increase of 29.3% in cultivar RB92579 under moderate stress and 59.8% under severe stress, compared to plants under control conditions (Figure 3A). A similar increase of APX activity was observed in RB72910. On the other hand, cultivars RB99382 and RB72454 had no alteration in APX activity when under stress.

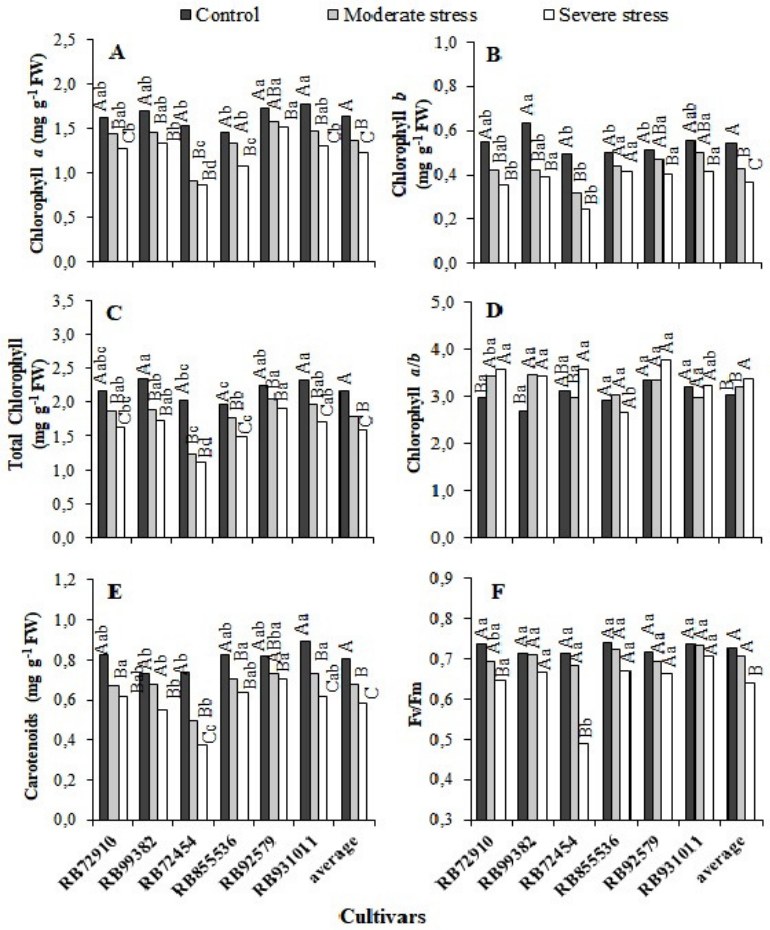

Figure 2. Concentrations of Chlorophyll *a* (A), Chlorophyll *b* (B), total Chlorophyll (C), ratio Chlorophyll *a*/*b* (D), carotenoids (E) and Photochemical efficiency PSII, Fv/Fm (F) in sugarcane cultivars under different water regimes: control, moderate stress, and severe stress. Different capital letters denote significant differences between water treatments in each cultivars; different lowercase letters denote significant differences between cultivars in each water treatments by Tukey test ($p<0.05$).

The CAT activity increased slightly under stress conditions, where cultivar RB92579 had the highest increase under severe stress, of 33.1% in relation to control (Figure 3B). The CAT activity had a positive correlation only with Chl *a* in plants under control conditions. In plants under stress, CAT activity also has a correlation with total Chl, carotenoids and APX (Table 2).

Similar to APX and CAT activity, SOD increased its activity with the imposition of stress (Fig. 3C). Cultivar RB92579 had the highest increase in SOD activity, 19.7% under moderate stress, and 57.8% under severe stress, in relation to control. On the other hand, the SOD activity in cultivar RB72454 had the lowest increase of 18% even under severe stress. The SOD activity also had no correlation with pigments and other antioxidant enzymes in plants under control treatment, but had a correlation under moderate stress, and this correlation increased under severe stress (Table 2).

The H_2O_2 content and lipid peroxidation not changed among cultivars in non-stressed plants, but increased with the intensity drought stress (Figure 3). The H_2O_2 content had the higher increase in cultivar RB72454, 38.4% under moderate stress, and 62.5% under severe stress, in relation the control plants (Figure 3D). The same occurred with the lipid peroxidation of cultivar RB72454 that had of increase 34% under moderate stress, and 47.2% under severe stress (Figure 3E). In contrast, lipid peroxidation in the other cultivars increased only under severe stress, with the lower increase in cultivar RB92579, of 22.8%. It is interesting observe that H_2O_2 content and lipid peroxidation started have negative correlation with chlorophyll and carotenoids when plants were under moderate stress and remained under severe stress (Table 2). Lipid peroxidation also had a negative correlation with the activity of antioxidant enzymes under severe stress, while lipid peroxidation and H_2O_2

content had a positive correlation in plants under stress.

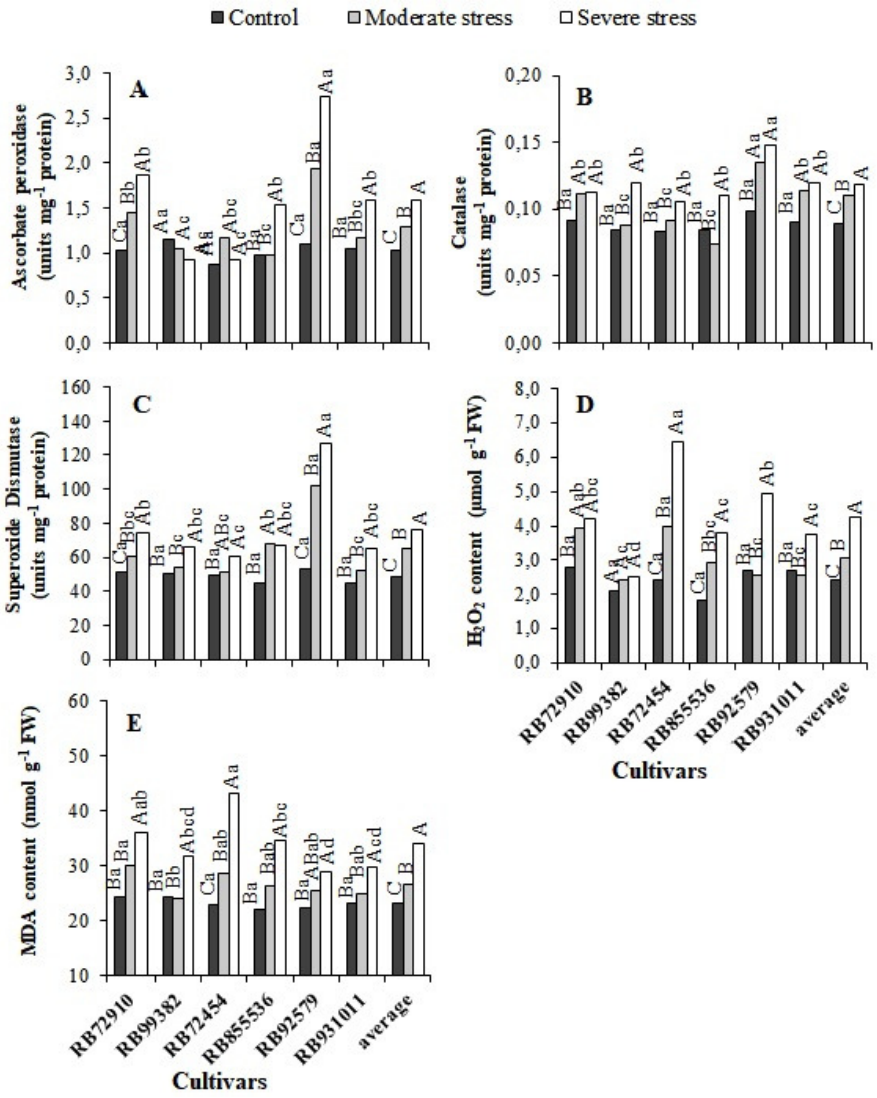

Figure 3. Antioxidant enzymes: ascorbate peroxidase (A), catalase (B) and superoxide dismutase (C); content of Hydrogen peroxide, H_2O_2 (D) and Lipid peroxidation, MDA (E), in sugarcane cultivars under different water regimes: control, moderate stress, and severe stress. Different capital letters denote significant differences between water treatments in each cultivars; different lowercase letters denote significant differences between cultivars in each water treatments by Tukey test ($p<0.05$).

Table 2. Pearson correlation coefficients between chlorophyll *a* (Chl *a*), chlorophyll b (Chl b), total chlorophylls (Chl a+b), ratio of chlorophyll a to chlorophyll *b* (Chl*a*/*b*), Carotenoids (Carot), maximum photochemical efficiency of photosystem II (Fv/Fm), ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD), content of hydrogen peroxide (H_2O_2) or lipid peroxidation (MDA) in ratoon sugarcane crop submitted to appropriate water regime (Control), Moderate stress and Severe stress.

Variables	Chl b	Chl t	Chl a/b	Car	Fv/Fm	APX	CAT	SOD	H_2O_2	MDA
Control										
Chl a	0.52**	0.94**	0.12	0.03	-0.26	0.50*	0.41*	-0.02	0.38	0.31
Chl b		0.78**	-0.76**	-0.29	-0.29	0.52**	0.03	-0.14	-0.00	0.38
Chl t			-0.21	-0.09	-0.30	0.57**	0.31	-0.07	0.28	0.38
Chl a/b				0.33	0.13	-0.24	0.24	0.17	0.30	-0.21
Carot					0.56**	-0.09	0.05	-0.11	0.16	-0.34

Fv/Fm						-0.08	0.02	-0.24	0.03	-0.09
APX							0.32	-0.13	-0.00	0.02
CAT								-0.12	0.30	0.12
SOD									0.38	0.13
H_2O_2										0.11
Moderate stress										
Chl a	0.77**	0.98**	0.31	0.72**	0.14	0.35	0.49*	0.45*	-0.60**	-0.37
Chl b		0.86**	-0.35	0.55**	0.36	0.13	0.38	0.29	-0.55**	-0.46*
Chl t			0.15	0.71**	0.20	0.31	0.48*	0.43*	-0.62**	-0.41*
Chl a/b				0.21	-0.30	0.29	0.14	0.18	-0.04	0.17
Carot					0.14	0.33	0.44*	0.49*	-0.48*	-0.40*
Fv/Fm						-0.38	-0.15	-0.32	-0.30	-0.43*
APX							0.74**	0.73**	-0.03	0.11
CAT								0.54**	-0.12	-0.04
SOD									-0.31	-0.14
H_2O_2										0.76**
Severe stress										
Chl a	0.52**	0.97**	0.37	0.64**	0.21	0.62**	0.69**	0.63**	-0.44*	-0.80**
Chl b		0.71**	-0.58**	0.65**	0.39	0.38	0.38	0.24	-0.55**	-0.61**
Chl t			0.14	0.71**	0.28	0.61**	0.68**	0.59**	-0.51**	-0.83**
Chl a/b				-0.12	-0.18	0.17	0.24	0.33	0.19	-0.07
Carot					0.40	0.75**	0.53**	0.58**	-0.40*	-0.67**
Fv/Fm						-0.00	-0.13	-0.14	-0.45*	-0.22
APX							0.72**	0.87**	0.08	-0.48*
CAT								0.82**	-0.10	-0.66**
SOD									0.15	-0.44*
H_2O_2										0.48*

** significant at P<0.01; *Significant at P<0.05

DISCUSSION

The reduction in chlorophyll content as drought stress intensifies suggests that pigment degradation is linked to the degeneration of chloroplasts as a response to stress and may be associated with drought tolerance index (JALEEL et al., 2009; CHEN et al., 2016). In sugarcane, the maintenance of chlorophyll content in cultivar RB92579 and degradation in RB72454 under water stress conditions indicate that cultivars respond distinctly to stress. The reduction in the chlorophyll content under drought stress is considered a typical symptom of oxidative stress, resulting from photooxidation and degradation of pigments (FAROOQ et al., 2009; JUNG, 2004), expressively in more sensitive cultivars (CHEN et al., 2016). Such a response probably occurred in cultivar RB72454, which markedly reduced the chlorophyll content.

On the other hand, the maintenance of the chlorophyll content in cultivar RB92579 was an important response in tolerance to water stress. In sugarcane under water deficit, more tolerant cultivars have similar responses or little degradation of chlorophylls in relation to well-hydrated plants (SILVA et al., 2012; SILVA et al., 2014). In addition to sugarcane, it has been reported in other grasses that water stress also affects the chlorophyll content, as in maize (KHOLOVÁ et al., 2011; CHEN et al., 2016) and wheat (NIKOLAEVA et al., 2010). Among the several changes of metabolic functions caused by drought stress, the reduction of pigments is one that reflects very much in plant yield, since these are responsible for the absorption and transfer of light energy to photosystems (FAROOQ et al., 2009; JALEEL et al., 2009).

Chlorophylls degradation may result from photochemical disturbances caused by excess light in the PSII reaction centers (KHOLOVÁ et al., 2011; SALES et al., 2015), as observed in our

results by the decreased Fv/Fm and chlorophylls in cultivar RB72454. This may also be related to the low activity of antioxidant enzymes observed in this cultivar. The reduction of total chlorophyll content caused by drought stress in sugarcane plants is one of the main factors limiting photosynthetic activity because it affects the photochemical efficiency (SILVA et al., 2014; SANTOS; SILVA, 2015). In sugarcane cultivars susceptible to drought stress, the decrease of Fv/Fm was related to disturbances in PSII reaction centers, compromising crop productivity (CARLIN et al., 2012; SALES et al., 2015).

The maintenance of carotenoids in cultivar RB92579 under water stress suggests that this cultivar has greater protection against photochemical injury during oxidative stress, minimizing chlorophyll degradation. The correlations between carotenoids and chlorophyll under stress indicate that the maintenance of carotenoids was essential in helping against damage caused by stress, since carotenoids play additional roles in photoprotection, helping plants to tolerate stress adversities (JALEEL et al., 2009; KHOLOVÁ et al., 2011). Thus, it is expected that cultivars that maintain higher amounts of carotenoids during stress have greater protection against photooxidation (SILVA et al., 2014) as observed in cultivar RB92579 in our experiment, and also reported in more drought-tolerant maize cultivars (KHOLOVÁ et al., 2011; CHEN et al., 2016). On the other hand, increased carotenoid degradation associated with low Fv/Fm, low enzymatic activity and increased lipid peroxidation may indicate damage to the photosynthetic apparatus, which probably occurred in cultivar RB72454 in our study. Greater degradation of carotenoids was observed in sensitive sugarcane cultivars under drought stress (SILVA et al., 2014).

The responses of APX, CAT and SOD enzymes to drought stress vary depending on the cultivar analyzed, since several adaptive mechanisms are presented by different cultivars (CIA et al., 2012), as observed in our study. In sugarcane under drought stress, the activity of antioxidant enzymes allows photoprotection of the photosynthetic apparatus (SALES et al., 2013; BOARETTO et al., 2014), probably because it helps maintaining pigments, the Fv/Fm, and inhibiting the increase of lipid peroxidation (CARVALHO, 2008; ANJUM et al., 2016).

Increased antioxidant enzyme activity has been reported in cultivars that are more tolerant to water deficit when compared to drought sensitive cultivars, suggesting that the antioxidant system plays an important role in plant tolerance to water deficit as observed in rice (LUM et al., 2014) and maize (CHEN et al., 2016). The high APX activity in cultivar RB92579 indicates good performance of this cultivar against oxidative stress caused by drought stress, resulting in the maintenances of pigments, photoinhibition, and low lipid peroxidation. Increased APX activity in cultivar RB92579 was also observed in plants under saline stress (MEDEIROS et al., 2014). APX activity appears to be rapidly activated in stressed plants with the purpose of alleviating photochemical damage (SALES et al., 2013). In wheat cultivars after three days under water deficit increased APX activity in the leaves of stressed plants was observed, which helped to decrease lipid peroxidation (NIKOLAEVA et al., 2010). The increase of APX activity has been observed in sugarcane cultivars more tolerant to water deficit (NGAMHUI et al., 2015). These authors observed that APX activity in the most tolerant cultivar was 15% higher than in the most sensitive cultivar.

On the other hand, higher H_2O_2 content and low APX activity in the cultivar RB72454 under stress may have contributed to the increase of photochemical damage, as observed by the increased photoinhibition, greater degradation of chlorophylls and lipid peroxidation in this cultivar. This fact can be confirmed by the positive correlations of APX with Chl *a*, total Chl and Car in our work. These correlations suggest that the APX activity contributed to maintenance of pigment levels under stress conditions in cultivar RB92579 but not in RB72454. In other studies with sugarcane under water deficiency, the increase of APX activity in drought-tolerant cultivars, suggest that this enzyme is efficient in the relief of oxidative stress (SANTOS; SILVA, 2015; NGAMHUI et al., 2015).

CAT was less affected by the presence of stress, which may have occurred because this enzyme is presented essentially in peroxisomes. In this case, C4 plants, such as sugarcane, photorespiration is minimal, and consequently exhibit lower CAT activity than C3 plants (CARMO-SILVA et al., 2008). However, even at low concentration, it is suggested that this enzyme has an essential participation in ROS removal, together with APX and SOD, as observed in cultivar RB92579. CAT is the second enzyme of antioxidant defense, acting mainly on the decomposition of H_2O_2 produced by SOD (MITTLER, 2002). In maize cultivars, higher CAT activity was found in the most drought-tolerant cultivar (ANJUM et al., 2016), indicating that the increased activity of this

enzyme protects against oxidative damage caused by stress (CIA et al., 2012).

The higher SOD activity in response to drought stress in cultivar RB92579 also indicated that this cultivar has good tolerance to drought stress. In others sugarcane cultivars under water stress, the SOD activity increased 28% in the tolerant cultivar, and only 6% in the drought-sensitive cultivar (SALES et al., 2015). The increase in SOD activity was also recorded in plants of different species under water stress, such as maize (ANJUM et al., 2016), rice (LUM et al., 2014) and faba bean (SIDDIQUI et al., 2015). The increase in SOD activity in cultivar RB92579 was also found under saline stress (MEDEIROS et al., 2014). The combined action of APX, CAT and SOD enzymes can be visualized by the positive correlation of their activity, only when plants were under moderate and severe drought stress. In addition, APX, CAT and SOD correlations with Chl *a*, total Chl and Car under severe stress indicate that enzymes are efficient in protecting the photosynthetic apparatus of stressed plants, helping maintaining pigments and membranes health.

H_2O_2 is formed by the dismutation of $O_2^{\bullet}$ by SOD, and can be eliminated by CAT and various peroxidases such as APX (MITTLER, 2002; BOARETTO et al., 2014). Thus, H_2O_2 concentration is also related to SOD activity (WENG et al., 2015), as found in our study, in which cultivar RB92579 had high SOD activity but low H_2O_2 production, specifically under severe stress. Thus, it can be suggested that the H_2O_2 produced by SOD was efficiently removed by the action of APX and CAT. Cia et al. (2012) observed that there was an increase in H_2O_2 concentration in all sugarcane cultivars at the beginning of stress. The increase of H_2O_2 concentration with the imposition of stress can be related to the lower tolerance of cultivars to water deficit, as seen in cultivar RB72454, which had high H_2O_2 production and simultaneously exhibited lower CAT, APX and SOD activity. In other studies, it was also observed that H_2O_2 contents were higher in drought sensitive cultivar of sugarcane (BOARETTO et al., 2014; SALES et al., 2015) and faba bean (SIDDIQUI et al., 2015).

ROS production can induced lipid peroxidation, protein degradation, DNA fragmentation, and cell death (ANJUM et al., 2016; SIDDIQUI et al., 2015), which probably occurred with cultivar RB72454 that showed increase MDA under stress. Similarly, increased lipid peroxidation under drought stress was found in maize, most notable in the sensitive cultivar (ANJUM et al., 2016), and in woody species, where lipid peroxidation was proportional to the stress intensity (LIU et al., 2011). High MDA concentrations suggest less protection of plants against photochemical oxidation during stress, associated with the increase of H_2O_2 and low activity of APX, SOD and CAT enzymes as observed in different studies (LIU et al., 2011; BOARETTO et al., 2014; MEDEIROS et al., 2014; SIDDIQUI et al., 2015). Similar results we can see in drought sensitive sugarcane, in wherein an inverse correlation between the activities of antioxidant enzymes and MDA in plants under severe stress, and a positive correlation between H_2O_2 and MDA in plants under moderate stress were observed. On the other hand, cultivar RB92579 that showed lower lipid peroxidation among cultivars when under severe stress indicates that this cultivar was benefited by the increase of the activity of antioxidant enzymes. Similar results were found in the same cultivar under saline stress, in which the low lipid peroxidation was attributed to the increased activity of antioxidant enzymes (MEDEIROS et al., 2014).

CONCLUSIONS

Sugarcane cultivars have different levels of tolerance to drought. Among the cultivars used RB92579 was the most tolerant to drought stress. This tolerance to water deficit is associated with an increase in the activity of antioxidant enzymes to maintain low ROS levels. Less ROS leads to lower lipid peroxidation and maintenance of photosynthetic pigments, causing less photoinhibition, which did not occur in cultivars more sensitive to drought, such as RB72454.

The antioxidant defense system triggered by ascorbate peroxidase, catalase and superoxide dismutase enzymes, as well as the maintenance of membrane integrity and photosynthetic pigment content can be consider as important criteria for selection of drought tolerance among sugarcane cultivars.

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RESUMO: A ocorrência de secas sazonais é um dos principais fatores que limitam a longevidade do canavial. Esse trabalho teve como objetivo avaliar as respostas bioquímicas de cultivares de cana-de-açúcar em seu segundo ciclo de cultivo a condições de estresse hídrico. Para tanto, foram utilizadas seis cultivares: RB72910, RB99382, RB72454, RB92579, RB855536 e RB931011, e três regimes hídricos, baseado na capacidade de água disponível (CAD) e definidos como: controle, 80 a 100% (CAD); estresse hídrico moderado, 40 a 60% (CAD), e estresse hídrico severo, 0 a 20% (CAD). A cultivar RB72454 se mostrou a mais sensível ao déficit hídrico. Essa cultivar mostrou aumento na produção de peróxido de hidrogênio, mas sem aumento concomitante das enzimas antioxidantes ascorbato peroxidase, catalase e superóxido dismutase. Esse estresse oxidativo levou a peroxidação de lipídeos das membranas e degradação das clorofilas, resultando na diminuição da eficiência fotoquímica do PSII. Por outro lado, a cultivar RB92579 foi a mais tolerante à seca, com aumento de atividade das enzimas antioxidantes, que levou a baixa peroxidação de lipídeos, manutenção dos pigmentos fotossintéticos e da eficiência fotoquímica do PSII. O sistema de defesa antioxidante desencadeado pelas enzimas ascorbato peroxidase, catalase e superóxido dismutase parece ser o fator chave de proteção à fotoinibição de plantas de cana-de-açúcar sob estresse hídrico. O aumento do sistema antioxidante, bem como a manutenção dos pigmentos fotossintéticos e das membranas celulares serviram como critérios importantes para indicar cultivares mais tolerantes ao estresse hídrico.

PALAVRAS-CHAVE: *Saccharum* spp. Fotossíntese. Clorofila. Fotoxidação. Estresse.

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