Agro-biochemical Traits of Sugarcane Varieties Grown in the Brazilian Semi-arid Region

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Abstract

The objective of this research was to evaluate the productive response associate to biochemical indicators and oxidative enzymes activities involved in the water deficit resistance in eight sugarcane varieties (RB951541, RB931011, RB962962, RB867515, RB763710, RB72454, RB863129, and RB92579) grown in the semi-arid regions of Brazil. Compared to all other varieties, RB763710 was superior in the number of stems m^{-1}, mean height, and stem diameter, production of whole plant fresh biomass and stem. When biochemical responses were obtained, all sugarcane varieties had a statistically similar solubility and maturity, regardless of the specific maturity rate of each cultivar. The increase in soluble carbohydrate levels occurred in the most stress-sensitive varieties and the variety RB763710 had the highest proline content. A lower general correlation was observed between the production of fresh biomass of stalks and the enzymatic activity. Among the varieties, RB763710 had the highest enzymatic activities which possibly provided greater tolerance to water stress due to the ability to maintain the redox state in the leaves of plants under water deficit. The study of the adaptation mechanisms of sugarcane against water deficit has contributed to the development and diffusion of genotypes tolerant to rainfed conditions, thus contributing to increased productivity even under adverse conditions, allowing maintenance and optimization of the production chains of sugarcane in rainfed regions.

Keywords: *Saccharum* spp., water stress, enzymatic activity

1. Introduction

The economic, social and energy valorization of sugarcane (*Saccharum officinarum* L.) has promoted its cropping even in unfavorable and semi-arid regions due to its diverse uses such as energy matrix, sugar-alcohol, animal production and civil construction. Semi-arid regions have several conditions that can limit crop productivity such as water deficit, high temperatures, soil salinity and high luminosity (Choudhary et al., 2018; Pedroso et al., 2018).

Water stress is one of the main factors that reduces crop productivity (Ferreira et al., 2017; González-Chavira et al., 2018). Other types of stress are triggered as a consequence of water stress such as oxidative stress (due to reduced photosynthesis and increased respiration). Oxidative stress can increase the production of reactive oxygen species, such as singlet oxygen, hydrogen peroxide, superoxide and radical hydroxyl groups, which are capable of damaging vital cell structures and organelles, culminating in cell death, depending on the severity of the stress (Iqba et al., 2018).

As a defense mechanism against unfavorable conditions, plants produce antioxidant enzymes and other non-enzymatic compounds that are able to neutralize the damage caused due to stress (Jiang et al., 2016; Marcos et al., 2018). Antioxidative enzymes include catalase, superoxide dismutase, ascorbate peroxidase, polyphenoloxidase, peroxidase and glutathione reductase (Moura et al., 2018).

Several techniques can be used to identify crop varieties that are more drought resistant as well as others abiotic factors; simpler techniques such as through agronomic evaluations and sophisticated ones such as harness sing the biochemical characteristics of the plant and its enzymatic activity, which signal a possible tolerance of the crop to stress (Marcos et al., 2018). Evaluations of the antioxidative system have greatly aided in genetic improvement programs, as well as the identification of promising drought-tolerant genotypes (Hellal et al., 2018; Iqbal et al., 2018).

In this context, the study of the adaptation mechanisms of sugarcane to water stress has contributed to the development of tolerant genotypes capable of reducing productivity variation even under adverse conditions (Boaretto et al.; 2014; Marcos et al., 2018), making it possible to maintain and optimize sugarcane production chains in rainfed regions. We therefore aimed to evaluate the productive response associate to biochemical indicators and oxidative enzymes activities involved in the drought resistance in eight sugarcane varieties (RB951541, RB931011, RB962962, RB867515, RB763710, RB72454, RB863129, and RB92579) growing under natural semiarid conditions of Brazil, characterized by the water deficit cycles that severely affect the crop yield.

2. Methods

The experiment was conducted in field, under natural environment conditions of Brazilian semiarid in the period from february to october 2015, in the municipality of Triunfo (Latitude: 7°53′14.77″ S; longitude: 38°6′49.18″ W; altitude: 535.5 m), Pernambuco, Brazil. The climate, according to the classification of Köppen-Geiger (1928) is of the type BSh i.e., hot semi-arid. The average precipitation and temperature were 501.71 mm and 25.9 °C, respectively in 2015. During the study, plants were watered only by the rainfall with mean values relatively lower to required by culture, mainly in the months jun, july and august that presented precipitations by 25.03 mm, 24.34 mm and 25.13 mm, respectively. This volume of water provided by the rainfall is relatively lower than the demand required by the sugarcane, which should be around 1700 mm per year (Silva et al., 2012). Thus, it is worth noting that was not employed specific treatment for the water stress with different water levels. However, the plants were exposed to a water restriction during its growth cycle.
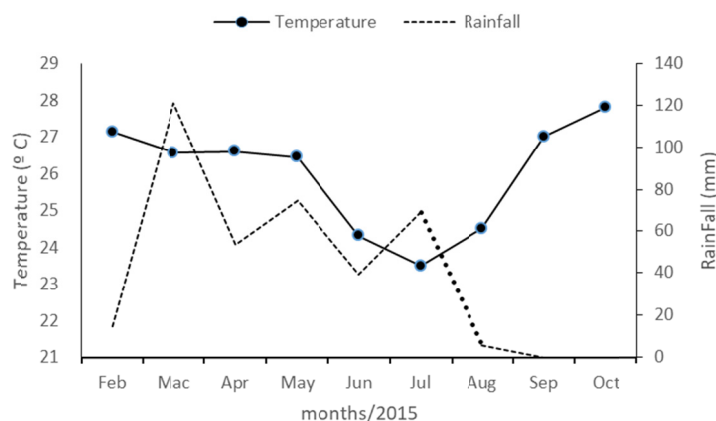

Figure 1. Average air temperature and rainfall in the municipality of Triunfo, Pernambuco, Brazil, from February to October 2015

The experiment was conducted as a randomized block design consisting of eight sugarcane varieties and three replicates. The experimental units (9 m long with 1 m spacing) were composed of four grooves with a total area of 36 m^2 consisted of two commonly known grasses *Andropogon gayanus* and *Urochloa mosambicensis* which presented an intermediate stage of degradation. Soil samples (0.2 m × 0.2-0.4 m depth) were characterized chemically according to the recommendation by EMBRAPA (2011) (Table 1).

Table 1. Chemical characterization of the soil of the experimental area located in the municipality of Triunfo, Pernambuco, Brazil

Depth	pH (H_2O)	P	K^+	Na^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H+Al	S.B
		mg dm^{-3}	--------------------------------- $cmol_c$ dm^{-3} ---------------------------------						
0.0-0.2 m	6.3	21.0	0.4	0.0	4.7	0.9	0.0	0.0	6.0
0.2-0.4 m	6.4	17.0	0.3	0.0	4.2	0.4	0.0	0.0	4.9
Depth	CTC	m	V	C	M.O.	Fe^{2+}	Cu^{2+}	Zn^{2+}	Mn^{2+}
	$cmol_c$ dm^{-3}	---------- % ---------		----- g kg^{-1} -----		------------------ mg dm^{-3} ------------------			
0.0-0.2 m	7.0	0.2	85.0	0.8	1.5	165.5	0.8	21.0	34.0
0.2-0.4 m	5.5	0.2	90.0	0.6	1.1	160.5	1.9	11.0	106.0

Note. S.B: Sum of bases; C.E.C: Cation exchange capacity; O.M.: Organic matter.

For plantation, plowing, and furrowing with a spacing of 1.0 m and approximately 0.3 m deep was performed. According to the results of the soil analysis, the experimental site was fertilized with 181, 142, and 134 kg ha^{-1} of urea, triple superphosphate, and potassium chloride, respectively based on the Manual of Recommendations for Fertilization for the State of Pernambuco (IPA, 1998). The planting of sugarcane was carried out by the "foot with tip method, using stems sectioned in smaller tops containing three to four buds, aiming at greater uniformity of the plants.

The evaluated varieties were RB951541, RB931011, RB962962, RB867515, RB763710, RB72454, RB863129, and RB92579 chosen for having great representativity in the semi-arid region of Pernambuco (RIDESA, 2010) (Table 2). The sugarcane seeds were obtained from the Agronomic Institute of Pernambuco, Carpina Sugarcane Experimental Station in agreement with the Interuniversity Network for the Development of the Sugarcane Sector.

Table 2. General characteristics of the eight sugarcane varieties (*Saccharum officinarum* L.) to be evaluated agronomically and biochemically

Cultivar	AP	GR	GH	MA	RCE	SC	FC
RB951541	Averege	Fast	Erect	Precocious	Not	High	Averege
RB931011	Averege	Fast	Erect	Iate	Averege	High	Averege
RB962962	High	Fast	Erect	Iate	Not	High	Averege
RB867515	High	Fast	Erect	Iate	Averege	High	Averege
RB763710	High	Fast	Semi-decumbent	Iate	Not	Averege	Averege
RB72454	High	Regular	Erect	Iate	Not	High	Averege
RB863129	High	Regular	Semi-decumbent	Averege	Not	Averege	Averege
RB92579	High	Slow	Semi-decumbent	Iate	Averege	High	Averege

Note. AP: Agricultural productivity; GR: Growth rate; GH: Growth habit; MA: Maturation; RCE: Restriction to the culture environment; SC: Sucrose content; FC: Fiber content.

Source: RIDESA (2010).

Sugarcane planting, measurements, and collection of plant material for production, soluble solids content, and biochemical characteristics were carried out in October 2015. At the study site, the number of plants, stems m^{-1}, average stem height [(n = 10) measured at soil level to the sheath of the first leaf (+1)] and the stem diameter was measured as per Hermann and Câmara (1999). The production of fresh biomass of the stem and entire plant was obtained by weighing the plants present at the study site and expressed as mg ha^{-1}.

The content of soluble solids (Brix) was evaluated from the sugarcane juice extracted from the internodes of the second and third stem base, end of the stem, penultimate and antepenultimate internodes, and from the entire stem, enabling the monitoring of the maturity index of sugarcane. The broth was extracted with the aid of a mill. Following grinding, the broth was filtered (0.053 mm sieve) to remove bagasse and impurities. A bench refractometer (ABBE-ART-100) was used to determine the Brix using two drops on the refractometer prism.

The leaf samples were collected from the leaves without ribs, frozen in liquid nitrogen and kept at -80 °C for future biochemical analyses of soluble carbohydrates (CHO_{sol}), proline (PL) and enzymatic activity. The extraction and determination of CHO_{sol} was performed according to Dubois et al. (1956) with modifications: 0.05

g of the frozen material was macerated in 1.3 mL of distilled water and the extract centrifuged at $12,000 \times g$ for 21 min at 4 °C.

Subsequently, 25 μL of the supernatant was diluted in a solution containing 475.0 μL of distilled water, 500.0 μL of 5% phenol, and 2.5 mL of concentrated sulfuric acid. After standing for 10 min, the solution was stirred for a few seconds and then allowed to stand again for 20 min in beakers containing water at room temperature (25 °C). The readings were recorded using a spectrophotometer at 490 nm. The CHO_{sol} content was calculated from a standard curve prepared with 180 μg mL^{-1} glucose and the results were expressed as mg g^{-1} fresh leaf mass (FLM).

The extraction and determination of proline (PL) was performed according to a modified method described by Bates (1973). Fresh leaf tissues (0.5 g) were weighed from the central region of the leaf blade, without the ribs and packed in 10 mL of distilled water, followed by placing it in a bath for 1.0 h at 100 °C. The extracts were then filtered twice using Whatman® qualitative filter paper. A 1 mL aliquot of the filtrate, 1 mL of acidic ninhydrin (1.25 g L^{-1} ninhydrin, 30 ml L^{-1} of glacial acetic acid, and 20 ml L^{-1} of 6 M phosphoric acid) were added in a container with lids along with 1 mL of glacial acetic acid. The solution was homogenized and placed in the water bath for 1 h at 100 °C and then in an ice bath for 15 min to interrupt the reaction.

After reaching room temperature, 2 mL of toluene was added and the solution was homogenized for 15 seconds. After 30 min of homogenization, the spectrophotometer readings were recorded at 520 nm. For quantification of the free proline, a standard curve was prepared using proline. The results were expressed in μmol g^{-1} FLM.

For the enzymatic activities the foliar extract was obtained according to methodology proposed by Silva (1981) and Simões et al. (2015). Estimates of the enzymatic activity of peroxidase were performed using the method described by Urbanek, Kuzniak-Gebarowska, and Herka (1991), using guaiacol and H_2O_2 as substrates. The activity of the polyphenoloxidase was verified by the oxidation of pyrogallol according to Kar and Mishra (1976) and the catalase activity measured according to the recommendations of Havir and Mchale (1987). Superoxide dismutase activity was measured by using the method proposed by Giannopolitis and Ries (1977), and Beauchamp and Fridovich (1971). The enzymatic activities were expressed in U.A. min^{-1} g^{-1} FLM.

The data obtained from productivity, biochemistry, and enzymatic activity were subjected to tests of normality, homoscedasticity, and analysis of variance. The averages were compared by the Scott-Knott test ($p \leq 0.05$), using SISVAR software 5.6. In addition, Pearson's correlation between general production data, and proline and enzymatic activities was performed. Log-transformed data was used to construct the scatter plot by main coordinate analysis obtained on the basis of the similarity matrix of the Jaacard Coefficient using PAST 1.9 software.

3. Results

The sugarcane variety RB763710 was significantly superior in all agronomic variables such as the number of stems m^{-1}, mean height, and main stem diameter, production of whole plant fresh biomass and stem, followed by RB962962, RB867515, and RB72454 (Table 3).

Table 3. Production characteristics of different sugarcane varieties (*Saccharum officinarum*) grown in the rainfed conditionsbetween February and October 2015, Triunfo, Pernambuco, Brazil

Sugarcane varieties	NSM	MHS	MSD	PFB	FBS
	m linear	m	cm	-------------- Mg ha^{-1} --------------	
RB951541	22.000 b	0.904 b	2.695 b	138.093 c	106.036 c
RB931011	18.000 c	0.868 b	2.665 b	89.750 d	72.446 d
RB962962	23.666 b	1.259 a	3.046 a	208.753 b	166.291 b
RB867515	22.000 b	1.270 a	2.792 b	187.060 b	134.266 b
RB763710	27.333 a	1.139 a	3.045 a	267.130 a	208.160 a
RB72454	23.666 b	0.876 b	3.201 b	186.900 b	138.055 b
RB863129	17.000 c	1.332 a	2.717 a	156.406 c	117.068 c
RB92579	23.000 b	1.002 b	2.526 b	157.696 c	118.155 c
CV (%)	12.35	9.25	7.93	18.11	18.96

Note. NSM: Number of stems per linear meter; MHS: Mean height of stem; MSD: Main stem diameter; PFB: Production of whole plant fresh biomass; FBS: Production of fresh biomass of stem. The averages followed by the same letter in the column do not differ by Scott-Knott's test at 5% probability.

The varieties with the highest number of shoots m^{-1} and the diameter of the main stem showed the highest yields of fresh whole plant biomass and height (Table 3), a fact proven when evaluating the correlation between the variables (Table 4).

All evaluated varieties of sugarcane presented Brix and similar maturity index (Figure 2), independent of the specific maturation rate of each cultivar (Table 2). The increase in soluble carbohydrate levels occurred in the most stress-sensitive varieties and the variety RB763710 was highlighted in proline production (Table 5).

Table 4. Correlation of production variables of sugarcane varieties (*Saccharum officinarum*) RB951541, RB931011, RB962962, RB867515, RB763710, RB72454, RB863129 and RB92579 cultivated under rainfed conditions between February and October 2015, Triunfo, Pernambuco Brazil

Variables	MHS	MSD	PFB	FBS
NSM	-0.049	-0.264	0.815*	0.820*
MSD		0.071	0.462	0.443
PBF			0.671*	0.676*
FSB				0.992*

Note. NSM: Number of stems per linear meter; MHS: Mean height of stem; MSD: Main stem diameter; PFB: Production of whole plant fresh biomass; FBS: Production of fresh biomass of stem. * Significant correlation at the 5% level.
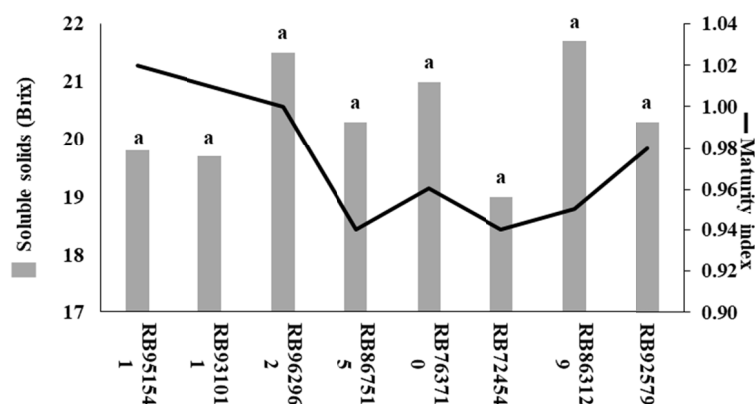

Figure 2. Soluble solids (° Brix) and maturation index of different sugarcane varieties (*Saccharum officinarum*) cultivated in the dry season between February and October 2015, Triunfo, Pernambuco, Brazil

Table 4. Biochemical characteristics and enzymatic activity of different sugarcane varieties (*Saccharum officinarum*) cultivated in the dry season between February and October 2015, Triunfo, Pernambuco, Brazil

Sugarcane varieties	CHO$_{sol}$	PL	POD	PPO	CAT	SOD
	mg g^{-1}MFF	µmol g^{-1} MFF	--------------------- U.A. min^{-1} g^{-1} MFF --------------------			
RB951541	2.380 a	0.122 c	1.005 d	1.406 b	0.017 c	31.937 a
RB931011	2.309 a	0.374 a	0.945 d	1.630 b	0.002 e	32.253 a
RB962962	2.388 a	0.296 b	0.952 d	1.382 b	0.014 c	25.897 c
RB867515	2.028 a	0.097 c	1.290 c	1.754 b	0.011 c	28.976 b
RB763710	2.106 a	0.345 a	1.724 a	2.714 a	0.040 a	31.977 a
RB72454	2.340 a	0.079 c	1.128 c	1.165 b	0.034 b	32.881 a
RB863129	2.336 a	0.342 a	1.160 c	1.597 b	0.017 c	31.147 a
RB92579	2.248 a	0.263 b	1.446 b	1.647 b	0.012 c	29.726 b
CV (%)	3.56	9.43	7.41	7.47	12.45	14.89

Note. CHO$_{sol}$: Soluble carbohydrates; PL: Proline; POD: Peroxidase; PPO: Polyphenoloxidase; CAT: Catalase; SOD: Superoxide dismutase. Means followed by the same letter in the column do not differ by Scott-Knott's test at 5% probability.

Low overall correlation was observed between fresh shoot biomass production and enzymatic activity (Table 5). Among all the sugarcane varieties, RB763710 obtained the highest biomass yields and showed the highest enzymatic activities (Table 5), differing in similarity of the other cultivars when evaluating proline production and enzymatic activity (Figure 3).

Table 5. Correlation of the biochemical variables and enzymatic activity with the production of sugarcane varieties (*Saccharum officinarum*) RB951541, RB931011, RB962962, RB867515, RB763710, RB72454, RB863129 and RB92579 cultivated in the dry season between the months of February to October 2015, Triunfo, Pernambuco, Brazil

Variables	PL	POD	PPO	CAT	SOD
FSB	0.899*	0.090	0.128	0.030	0.524
PL		0.756*	0.255	0.696*	0.853*
POD			0.012	0.139	0.722*
PPO				0.316	0.738*
CAT					0.374

Note. FSB: Fresh stalk biomass; PL: Proline; POD: Peroxidase; PPO: Polyphenoloxidase; CAT: Catalase; SOD: Superoxide dismutase. * Significant correlation at the 5% level.
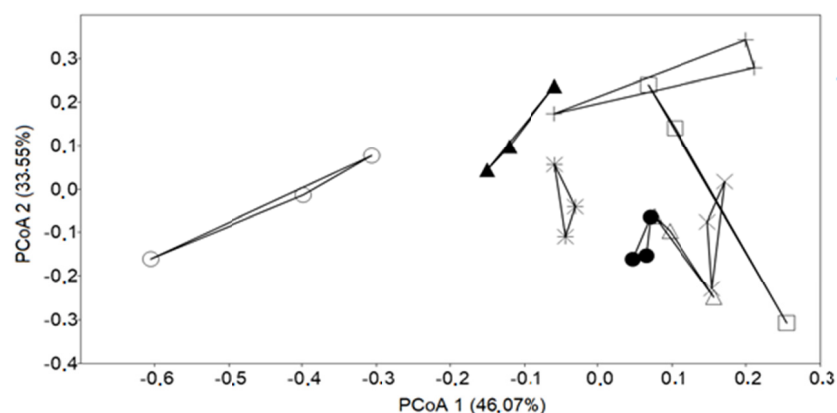

Figure 3. Principal coordinate analysis (CPoA) obtained from the similarity matrix based on the Jaccard Coefficient, estimated on the basis of proline production and peroxidase, polyphenoloxidase, catalase and superoxide dismutase activity of sugarcane varieties (*Saccharum officinarum*) ×: RB951541; Δ: RB931011; □: RB962962; ▲: RB867515; ○: RB763710, +: RB72454, ●: RB863129 and *RB92579 cultivated under dry season between February and October 2015, Triunfo, Pernambuco, Brazil

4. Discussion

Water deficit represent major environmental factor restrictive for crop yield, adverse effect that occur mainly in semiarid areas. When exposed to natural precipitation under field conditions the plants experience periods of water stress due to natural rain cycles. During these water deficit cycles may occur several metabolic disturbances related to key physiological processes necessary to yield, such as water relations, nutrient unbalance and photosynthesis (Li et al., 2018). This disturbances can affect plant growth and development as well as induced secondary damages like as oxidative stress, compromising index relative to crop quality and yield (Kumar et al., 2019). Thus, the understanding of how the water deficit should affect production components associated with key metabolic processes, may be essential to adopt corrective strategies of these damages for crop under field conditions.

Genetic resistance to drought stress in plants is a multigenic character, considered relatively complex by involve a net metabolic process necessary to maintain growth under conditions of water deficit. Thus, the identification and exploitation of varieties that are more resistant to drought represent a viable strategy for obtaining crops that are more resistant to drought. In the presente study, varieties different sugarcane were cultivate under field conditions and evaluated relative its growth capacity associate to oxidative protection efficiency during relative water deficit. The sugarcane varieties RB763710, RB962962, RB867515, and RB72454 are characterized by

high productivity and rapid growth however the variety RB763710 showed the highest average plant height even under water stress conditions, which has a semi-decumbent growth habit. The results observed in this study corroborate with those found by Silva et al. (2011), and Oliveira et al. (2015) that showed stem diameters of 2.450 and 2.486 cm for the varieties RB 92579 and RB72454, respectively. In both studies, the varieties were subjected to water stress, similar to this current study.

It should be emphasized that the maturity indices found in this current study represent sugarcane maturity according to the classification of Cesnik and Miocque (2004). Oliveira et al. (2011), and Cardozo and Sentelhas (2013) showed the negative influence of increased water availability on these varieties; hence, sugarcane plants submitted to rainfed conditions tend to accelerate their maturation.

Sales et al. (2012) showed that the CHO_{sol} contents remained constant for the tested varieties and their performance in the deterioration of the starch reserves of the plant when water stressed. Patade et al. (2011) found results different from those observed in this study (Table 4), where there was an increase in the levels of CHO_{sol} in the varieties more sensitive to stress (water and salinity).

Proline has been shown to have a strong correlation with increased drought tolerance (Balestro et al., 2017; Castañeda et al., 2018). However, Mansour and Ali (2017), and Marcos et al. (2018) argued that since proline is produced under stressful conditions, its absence or low production by the plant may indicate less stress.

The accumulation of proline in plant tissues is associated with a reduction in the concentration of toxic ions and the increase of water volume in the cytosol, besides protecting the cell membranes from oxidative stress (Merwad et al., 2018). Proline has been shown to decrease the osmotic potential and, consequently, maintain water and cell turgescence potential near adequate levels, being extremely important for the growth and development of plant tissues (Jungklang et al., 2017; De la Torre-González et al., 2018).

The low correlation between fresh stem biomass production and enzymatic activity in the present study may have been due to the greater tolerance to water stress as a consequence of the ability to maintain the redox state in the leaves of plants under water deficit.

Peroxidase integrates the oxidoreductase group, which catalyzes a large number of oxidative reactions, using peroxide as a substrate, or in some cases oxygen as a hydrogen acceptor; a process of high importance for the adaptation of plants to water stress (Freitas et al., 2008) as also observed in this current study with sugarcane.

Polyphenoloxidase is an enzyme that catalyzes the oxidation reaction of phenols in quinones, acting in an aerobic environment, located in the plastids (Kuwabara & Katoh, 1999). The action of polyphenoloxidase reduces the amount of superoxide, causing oxidative damage in the plants, thus being an indicative of varieties tolerant to water restriction. However, the responses may vary between cultivars, species, tissues analyzed, duration and the magnitude of stress (Campos et al., 2004).

Superoxide dismutase plays an important role in the adaptation and survival of stressed plants, and the first line of defense against reactive oxygen species forming superoxide for hydrogen peroxide, generating lower levels of lipid peroxidation (Scandalios, 2005; Amaro, 2018). Superoxide produced in excess as a result of the stress caused by the water restriction, is extremely reactive and can be transformed into a hydroxyl radical, which is the most harmful of all the radicals, with the capacity to lead to deleterious changes in primary and secondary metabolism and mutations, leading to cell death and in high severity, plant death (Vranova et al., 2002).

The catalase enzyme has amain auxiliary function in the ascorbato-glutathione cycle, acting toward the detoxification caused by the hydrogen peroxide, in the cells of the plants (Sheikh-Mohamadi et al., 2018). Catalase activity increases according to the amount of hydrogen peroxide within the cells (Kumar et al., 2018). The values observed in this current study were low because sugarcane is a C4 plant possessing minimum photorespiratory ability; contrastingly C3 plants are more involved in the removal of hydrogen peroxide (Tolbert et al., 1969). Benesová et al. (2012) observed that the variety more tolerant to water stress had greater catalase activity and vice versa thus corroborating with the results observed in this current study.

Water deficit is one of the main factors against crop production (Ferreira et al., 2017), but not the only one, since other types of stress are capable of causing damage and organicity, cellular (Maia et al., 2012). Aiming at favorable conditions, plants may produce several antioxidant enzymes and other non-enzymatic compounds that seek to neutralize stress-induced damage (Cia et al., 2012; Marcos et al., 2018).

The results showed interspecific differences in the agronomic performance and the physiological responses of the studied cultivars. The variables that obtained the best tolerance to drought were those selected by slow maturation and fast growth rate. This pattern indicates the need for adjustments in the period of planting,

management and cultivation time in a semi-arid environment, aiming at the supply of water during periods of high water demand, considering the expected product, fiber, biomass or sugar.

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