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Full Length Article

Genetic and Morpho-Physiological Differentiation of Sugarcane Genotypes under Drought Stress

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Abstract

Scarced water supply is the main limiting factor to the good growth and development of sugarcane, and implying the need to obtain germplasm tolerant to drought. The objective of this research was to screen out morpho-physiological traits to recognize tolerant and susceptible sugarcane genotypes. Ten sugarcane genotypes were grown under greenhouse conditions and subjected to water deficiency (-W), 50% of the moisture corresponding to the water holding capacity, at 75 days after planting (DAP) while well-watered (+W) conditions, around 22% soil moisture content (100% of water holding capacity), were maintained as control. Data were recorded at 75 days after the beginning of drought stress. Correlations were made to calculate the genetic distance between the genotypes and later to group them by Tocher's optimization method. The traits that contribute for genetic distance quantification were shoot dry matter (23.41%), chlorophyll content (15.12%), abaxial stomatal density (9.79%) and SPAD index (9.56%). The differentiation between the genotypes through their degree of tolerance to water deficit was evident, and the genotypes more tolerant to water restriction were: CTC2, RB92579, IAC91-5155 and SP89-1115. The genotypes CTC2 and RB92579 maintained higher values for most variables under drought stress along with the lowest reductions between +W and -W. These genotypes were superior due to their better resistance to drought than others. Seven groups were constituted by Tocher's method. In conclusion, the most promising crosses (that may generate descendants with high heterotic potential and better performance under drought stress) observed were SP89-1115 and CTC2, belonging to group II (emphasizing that both sugarcane breeding programs can be considered similar due to same germplas bank and the same cultivar selection methods and objetives), with RB92579 and IAC91-5155 belonging to group VI and IV, respectively.

Keywords: *Saccharum* spp.; Crossings; Drought tolerance; Screening; Tocher's method

Introduction

There are several abiotic stresses responsible for limiting the production of sugarcane (*Saccharum officinarum* L.), however water deficiency is considered the main one (Endres *et al*. 2019). The regions of expansion of sugarcane in Brazil are characterized by dry winter, with periods of up to six months of water deficit quite pronounced and more accentuated compared to regions traditionally occupied with the crop. An economical way to work around the problems caused by water deficit in crops is to use drought-tolerant genotypes (Meena *et al*. 2013).

The drought is a multidimensional stress, which causes several morphological, physiological and biochemical effects in sugarcane plants (Abbas *et al*. 2014; Santos *et al*. 2015; Ferreira *et al*. 2017). Thus, techniques that evaluate the morpho-physiological effects caused by water deficiency can be used to differentiate tolerant and susceptible genotypes (O'Neill *et al*. 2006; Silva *et al*. 2008, 2014a, 2018). Morphological variables such as height and number of tillers, volume and dry matter of roots, leaf area and stomatal density have already been evaluated in sugarcane to differentiate tolerant and drought susceptible genotypes (Jifon *et al*. 2005; Silva *et al*. 2008; Pincelli and Silva 2012).

Silva *et al*. (2007, 2018) have already studied non-destructive physiological variables such as estimated chlorophyll content (SPAD), maximum quantum efficiency of photosystem II, stomatal conductance and leaf area index. Moreover, the destructive variables as chlorophyll content (by spectrophotometry), relative leaf water content and leaf water potential were studied by Silva *et al*. (2007, 2014a, b). All of them presented a positive correlation with the drought stress.

Estimation of genetic divergence among sugarcane genotypes has been studied aiming the selection of progenitors for the formation of new hybrids or new segregating populations, from divergent genotypes with higher agronomic characteristics (Alam *et al*. 2017). The

purpose of grouping methods is to separate an original group of observations into subgroups, with the aim of obtaining homogeneity within and heterogeneity among the subgroups (Sneath and Sokal 1973). One of the most used optimization methods in the plant breeding area is that of Tocher.

In the context of changing climatic patterns in some regions of Brazil, there is a need of development tolerant sugarcane germplasm to drought. It will also give insight the applicability of physiological characterization of sugarcane cultivars under water deficiency within breeding program. Therefore, the objective of this research was to evaluate morpho-physiological traits in ten sugarcane genotypes grown under drought conditions. Nonetheless, genetic divergence will be evaluated between tolerant and susceptible genotypes through the use of multivariate analyzes, considering this analysis as a useful tool in the selection of drought-tolerant genotypes.

Materials and Methods

Site description and experimental design

This experiment was conducted in a greenhouse at the Department of Crop Production of the School of Agricultural Sciences, located in Botucatu city, São Paulo state, Brazil (22º51'01" S, 48º25'55" W, 800.1 m of altitude).

Ten sugarcane genotypes (SP91-1049, RB845210, RB855035, SP89-1115, SP80-1816, RB92579, IAC91-5155, IACSP94-4004, CTC2, IAC91-2195) were grown under well-watered (+W) and drought (-W) conditions. Around 22% moisture content (100% of water holding capacity) were maintained as well-watered conditions while under drought stress (-W) conditions the pots were maintained with 50% of water holding capacity. The cultivars IAC91-2195 and IAC91-5155 were used as a control of susceptibility and tolerance to water deficit, respectively. The experiment was laid out following completely randomized design under factorial arrangement and replicated four times. Sugarcane buds with same age were extracted from healthy plants. Three buds of each genotype were placed in pots of 22-L. Each pot containing 20-L of Plantmax® substrate (a sterile product made from expanded vermiculite and organic material, containing macro and micronutrients) and 55 g of the formulated fertilizer 8-28-16, which means 4.4, 15.4, and 8.8 g of N, P and K, respectively. After the emergence of the seedlings, there was paring, and only the primary tiller of one plant was kept per pot.

Sampling procedures, measurements and methods

From planting up to 74 days after planting (DAP) all pots received water in the same amount. At 75 DAP were started the treatments +W and -W. Pot moisture monitoring was performed three times daily by means of the ECH_2O meter (Decagon, DC, Washington, U.S.A.), coupled to Echo Check dielectric sensors (Decagon, Washington, DC, U.S.A.) inserted into the pots. Water was replaced in an adequate amount to maintain the water regime levels.

The measurements were taken at 75 days after the imposition of drought stress (DAT), when the plants were 150 days old. Initially the non-destructive evaluations were made and then the destructive ones. Grades from 0 to 2 were attributed for each variable, indicating the degree of tolerance to drought, being 0 (without tolerance), 1 (intermediate tolerance) and 2 (very tolerant).

The height of the stem was determined through a tape-measure, carrying out a measurement from the soil up to the height of the insertion +1 leaf. The number of green leaves was determined considering as green leaves those fully expanded, with at least 20% of green leaf area, starting at +1 leaf. For the calculation of the leaf area (LA), measurements of the diameter and length of the leaf blade in the middle part of the +3 leaf were carried out, using ruler and tape-measure, and the methodology of Hermann and Câmara (1999) was used (Equation 1):

$$LA = C \times L \times 0.75 \times (N+2) \quad (1)$$

Where C is the leaf length +3, L is the width, the crop correction factor is 0.75, and N is the number of open leaves with at least 20% green area.

For the counting of stomata, the methodology of Mazumdar *et al*. (1969) was used. The impression was withdrawal in four regions of the middle third of +1 leaf of each cultivar, two on each leaf face, with the impressions parallel to the leaf center rib. The impression with the shapes of the stomata was removed with colorless enamel and transparent tape. To realize the read, the adhesive tape was putted on a "Neubauer Chamber" and the counting of the stomata was performed in an area of 0.25 mm² in an optical microscope (Eclipse E200, Nikon, Shanghai, China), using the 10x magnification objective lens.

The SPAD (Soil Plant Analyzer Development) index was obtained using a portable chlorophyll meter (SPAD-502 Minolta Corp., Ramsey, New Jersey, U.S.A.). The readings were performed between 8 and 10 h, in the upper, middle and lower thirds of +1 leaf, after which the general mean of the different leaf parts was obtained.

The maximum photochemical efficiency of photosystem II (F_v/F_m) was measured by a portable fluorometer (Opti-Sciences, Inc., Hudson, NH, U.S.A.). Special clips for the +1 leaf darkening were used for 30 min and subsequently the value of the variable was obtained, according to the methodology of Maxwell and Johnson (2000), where F_m is the maximum intensity of the fluorescence in which all reactions of the photosystem II (FSII) close; F_0 is the minimum fluorescence intensity, when the FSII reaction centers are open; and F_v is the variable fluorescence, being calculated by the difference between the maximum and minimum fluorescence intensity of photosystem II ($F_v = F_m - F_0$). The readings were performed between 7 and 9 h.

The determination of the stomatal conductance (g_s, mol m^{-2} s^{-1}) and the CO_2 assimilation rate (A, μmolCO_2 m^{-2} s^{-1}) was performed using the Infra-Red Gas Analyzer (Li-6400XT, LI-COR, Lincoln, NE, U.S.A.). The readings were performed in the medium region of +1 leaf and determined between 8 and 10 h.

The total chlorophyll content (CC) was evaluated by removing two leaf discs of 0.69 cm², with a cork borer, from the +1 leaf, that were placed in vials containing 2 mL of N, N-dimethylformamide (DMF). The solution was protected from light for 24 h, being subsequently withdrawn 1 mL of the chlorophyll extract diluted in 1 mL of deionized water, for the spectrophotometer reading at wavelengths 480, 647 and 664 nm, according to the methodology of Wellburn (1994).

The relative water content (RWC) was determined by weighing two 0.69 cm² leaf discs extracted from the same +1 leaf, and the fresh tissue mass (Wf) was determined by means of an analytical balance. The mass of the turgid tissue (Wt) was obtained after hydration of the discs for 24 h in deionized water, followed by removal of excess water with tissue paper from the turgid discs. The mass of the dry tissue (Wd) was obtained after drying the leaf discs in an air forced circulation stove oven 60ºC, for 48 h. The methodology of Jamaux *et al*. (1997) was used to calculate the RWC (Equation 2):

$$RWC = [(Wf - Wd) \times (Wt - Wd)^{-1}] \times 100 \qquad (2)$$

The leaf water potential (Ψw) was performed at the tops of +1 leaf, using a Scholander pressure chamber (Soil Moisture Equipment, Santa Barbara CA, USA), between 10 and 14 h, the hottest period of the day, in which the lowest values of leaf water potential are observed.

The shoot and roots dry matter masses (SDM and RDM) were obtained at 150 DAP. The plants were separated into aerial part and roots, both parts were conditioned in forced air circulation oven at 70ºC until constant mass. The mass of the dry matter was determined by means of a precision scale.

Data analysis

Data were analyzed using two-way ANOVA and, in cases of significance, the Tukey's test was followed to separate treatments means at $P \leq 0.05$). The genetic divergence among the cultivars was calculated using the generalized distance of Mahalanobis as a measure of dissimilarity, and the Tocher's optimization method was used as a grouping technique. The genetic-statistical analyses were processed through the GENES software.

Results

Morphological traits

Sugarcane genotypes, water regime and interaction among them had significant effect on stem height, number of green leaves, leaf area, abaxial stomatal density, and root and shoot dry weight of sugarcane, excepting the non-significant effect of water regime on adaxial stomatal density and interactive effect on number of green leaves (Table 1).

Except for stomatal density in the abaxial face that showed an increase in -W treatment (Table 2); the other traits showed a significant reduction under drought conditions (Tables 2 and 4). The highest stem height in -W was noticed for the cultivar SP91-1049 (146.7 cm); whereas the lowest (80.2 cm) was noticed for IAC91-2195 (Table 2). The minimum percentage reduction in stem height growth, compared to +W, was noticed for CTC2 (19.04%), while the maximum was noticed for IAC91-2195 (52.4%).

The maximum number of green leaves at -W was observed for IAC91-2195 (6.8) and IACSP94-4004 (6.2), while the minimum (3.8) was observed for SP89-1115 and SP80-1816 (Table 2). The lowest reduction, compared to +W, was noticed for RB855035 (26.0%), while the highest was noticed for SP91-1049 (50.9%). In case of LA, the highest values in -W treatment were noticed for SP91-1049 (0.62 m²) and SP89-1115 (0.57 m²), while the lowest were noticed for RB855035 (0.32 m²) and IAC91-5155 (0.39 m²) (Table 2). The lowest percentage reduction was presented in SP89-1115 (27.8%), whereas the highest was observed for IAC91-5155 (55.6%). The stomatal density in the adaxial face had a marked effect of cultivars, because only RB855035 and IACSP94-4004 presented a statistical difference between +W and -W (Table 2).

The maximum stomatal densities in the abaxial face, in -W, were observed for IAC91-2195 (198) and SP89-1115 (197); whereas the minimum stomatal densities in the abaxial face were observed for RB855035 (168) and SP91-1049 (169) (Table 2). The highest percentage increase of this trait under -W was noticed for IAC91-2195 (33.7%), while the lowest was noticed for RB855035 (12.7%). The highest SDM in -W was produced by RB855035 (182 g), while the lowest was produced by IAC91-2195 (66.4 g) (Table 2). The lowest percentage reduction in SDM was observed for IAC91-5155 (47.3%), whereas the highest was observed for IAC91-2195 (75.8%). In case of RDM under -W, cultivar IAC91-2195 was on top with maximum value of 167.1 g, while the minimum values of RDM were observed in RB845210 (98.9 g) and IAC91-5155 (99.7 g) (Table 2). The lowest percentage reduction in this trait, compared to +W, was noticed for IAC91-5155 (20.3%), and the highest was observed for RB845210 (48%).

Physiological traits

The sugarcane genotypes, water regime and interaction among then had significant effect on all the studied physiological traits as well (Table 3). The higher level of Ψw_L in -W was maintained by cultivar SP91-1049 (-1.21 MPa); whereas the minimum value of Ψw_L was noticed for IACSP94-4004 (-1.99 MPa) (Table 4). The lowest Ψw_L reduction, compared to +W, was observed for CTC2

Table 1: Statistical summary of morphological variables of ten sugarcane genotypes grown under different water regimes

Source of variation		SH (cm)	NGL (nº)	LA (m²)	SDAD (mm^{-2})	SDAB (mm^{-2})	DM (g)	
		F values						
	DF						Shoot	Root
Replications	3	3.59ns	2.14ns	0.71ns	0.06ns	0.24ns	0.88ns	6.97ns
Genotypes	9	22.97**	9.33**	8.57**	3.42**	25.61**	41.31**	26.41**
Water Regime (W)	1	383.74**	169.33*	206.24**	3.25ns	905.86**	1,489.06**	243.36**
C × W	9	3.30**	1.89ns	2.47*	2.63*	9.32**	7.43**	3.33**
CV (%)		8.49	15.90	17.60	10.59	3.26	9.69	12.05

SH: stem height, NGL: number of green leaves, LA: leaf area, SDAD: adaxial stomatal density, SDAB: abaxial stomatal density, DM: shoot and root dry matter mass, ns: not significant; *: significant at $P \leq 0.05$; **: significant at $P \leq 0.01$

Table 2: Morphological variables of ten sugarcane cultivars submitted to adequate water regime (+W) and to water deficit (-W) conditions

Variables		Cultivars									
		SP91-1049	RB845210	RB855035	SP89-1115	SP80-1816	RB92579	IAC91-5155	IACSP94-4004	CTC2	IAC91-2195
SH (cm)	+W	200.1a	138.7a	169.7a	178.7a	209.7a	172.3a	209.5a	151.5a	173.8a	168.6a
	-W	146.8b	99.3b	122.3b	126.6b	137.8b	122.8b	140.1b	109.6b	140.7b	80.2b
NGL (nº)	+W	10.4a	8.8a	7.5a	5.8a	7.2a	9.2a	9.5a	9.2a	9.5a	9.2a
	-W	5.1b	5.0b	5.5b	3.8b	3.8b	5.7b	5.7b	6.2b	6.1b	6.8b
LA (m²)	+W	1.23a	0.99a	0.57a	0.79a	0.89a	0.99a	0.88a	0.98a	0.78a	0.82a
	-W	0.62b	0.51b	0.32b	0.57b	0.56b	0.45b	0.39b	0.50b	0.56b	0.48b
SDAD (mm^{-2})	+W	98a	89a	77b	95a	85a	88a	79a	79b	77a	81a
	-W	84a	79a	90a	97a	78a	96a	81a	97a	79a	92a
SDAB (mm^{-2})	+W	142b	145b	149b	173b	139b	150b	146b	148b	163b	148b
	-W	169a	191a	168a	197a	171a	191a	195a	180a	190a	198a
SDM (g)	+W	410.1a	240.3a	362.6a	368.3a	331.5a	354.9a	225.1a	251.2a	335.1a	275.2a
	-W	145.5b	76.1b	182.0b	174.5b	168.4b	149.7b	118.6b	80.5b	138.3b	66.4b
RDM (g)	+W	148.2a	190.2a	165.5a	180.1a	140.2a	151.4a	125.2a	180.1a	245.2a	250.9a
	-W	101.9b	98.9b	112.3b	105.9b	100.9b	104.2b	99.7b	114.7b	154.7b	167.1b

Different letters between water regime and within the same variable indicate significant differences at $P \leq 0.05$
SH: stem height, NGL: number of green leaves, LA: leaf area, SDAD: adaxial stomatal density, SDAB: abaxial stomatal density, SDM: shoot dry matter mass, RDM: root dry matter mass

Table 3: Statistical summary of physiological variables of ten sugarcane genotypes grown under different water regimes

Causes of Variation		Ψw_L (MPa)	RWC (%)	CC (μg cm^{-2})	SPAD	Fv/Fm	g_s (mol m^{-2} s^{-1})	A (μmol CO_2 m^{-2} s^{-1})
	DF	F values						
Replications	3	0.23ns	2.42ns	1.91ns	1.04ns	2.65ns	2.03ns	0.83ns
Cultivars	9	13.49**	6.00**	16.14**	14.24**	10.94**	22.17**	8.23**
Water Regime (W)	1	673.03**	528.23**	649.21**	719.50**	280.25**	1,038.22**	996.57**
C × W	9	7.27**	5.91**	6.63**	3.57**	5.25**	19.72**	6.03**
CV (%)		11.38	2.61	11.65	6.29	3.49	20.73	20.20

Ψw_L: leaf water potential, RWC: relative water content, CC: total chlorophyll content, SPAD: estimation of chlorophyll content via SPAD unit, Fv/Fm: maximum photochemical efficiency of photosystem II, g_s: stomatal conductance, A: CO_2 assimilation rate: ns: not significant; *: significant at $P \leq 0.05$; **: significant at $P \leq 0.01$

(51.6%), while the highest was observed for SP89-1115 (168%). The maximum values of RWC in the leaf under -W were observed for RB92579 (86.6) and SP80-1816 (85.4), while the minimum value (75.7) was observed for IAC91-2195 (Table 4). The lowest RWC reduction, compared to +W, was noticed for RB92579 (7.08%), whereas the highest reduction was noticed for SP91-1049 (18.08%).

In case of CC in -W, cultivar RB92579 was on top with maximum value of 44.4 μg cm^{-2}, while minimum number of CC of 11.1 μg cm^{-2} was observed in SP80-1816 (Table 4). The lowest percentage reduction of CC was noticed for RB92579 (27.6%), and the highest was observed for SP80-1816 (78.7%). The highest value of SPAD index under water restriction was noticed for RB855035 (36.81), while the lowest was noticed for IACSP94-4004 (22.0) (Table 4). The minimum percentage reduction of SPAD index was observed in RB855035 (18.2%), whereas the maximum was observed in IACSP94-4004 (44.3%). The maximum values of F_v/F_m under -W were observed in IACSP94-4004 (0.78) and RB855035 (0.72); whereas the minimum values of F_v/F_m were observed in CTC2 (0.67), SP91-1049 and SP89-1115 (0.68) (Table 4). The lowest percentage reduction, under water deficit, occurred in IACSP94-4004 (3.8%), while the highest occurred in SP91-1049 (17.07%).

The highest g_s in -W (0.03 mol m^{-2} s^{-1}) was noticed for RB855035, SP91-1049 and RB92579; while the lowest g_s (0.01 mol m^{-2} s^{-1}) was noticed for IAC91-5155 (Table 4). The smaller percentage reduction of g_s was observed in CTC2 (71.4%) and the highest percentage reduction occurred in RB845210 (90.0%). In case of A, cultivar CTC2 was on top with maximum value of 4.47 μmol cm^{-2} s^{-1}, while the minimum number of A of 1.52 μmol cm^{-2} s^{-1}

Table 4: Physiological variables of ten sugarcane cultivars submitted to adequate water regime (+W) and to water deficit (-W) conditions

Variables		Cultivars									
		SP91-1049	RB845210	RB855035	SP89-1115	SP80-1816	RB92579	IAC91-5155	IACSP94-4004	CTC2	IAC91-2195
$\Psi w_{L\ (MPa)}$	+W	-0.59a	-0.71a	-0.81a	-0.67a	-0.70a	-0.95a	-0.78a	-0.98a	-0.91a	-0.82a
	-W	-1.21b	-1.31b	-1.84b	-1.80b	-1.38b	-1.65b	-1.37b	-1.99b	-1.38b	-1.69b
RWC (%)	+W	95.1a	94.7a	93.9a	94.1a	95.1a	93.2a	92.2a	91.1a	90.1a	90.8a
	-W	77.9b	80.6b	82.5b	83.0b	85.4b	86.6b	81.0b	80.0b	80.3b	75.7b
CC ($\mu g\ cm^{-2}$)	+W	55.0a	52.8a	65.4a	51.0a	52.2a	61.4a	44.2a	53.1a	56.1a	56.2a
	-W	22.3b	21.3b	33.3b	18.4b	11.1b	44.4b	29.5b	26.9b	36.9b	30.5b
SPAD	+W	42.1a	44.8a	45.0a	42.2a	39.5a	44.2a	40.3a	39.5a	42.1a	43.1a
	-W	32.08b	29.1b	36.81b	29.3b	26.4b	30.2b	24.2b	22.0b	31.06b	30.2b
F*v*/F*m*	+W	0.82a	0.81a	0.81a	0.79a	0.76a	0.79a	0.77a	0.81a	0.78a	0.80a
	-W	0.68b	0.70b	0.72b	0.68b	0.69b	0.69b	0.69b	0.78a	0.67b	0.71b
g_s (mol $m^{-2}\ s^{-1}$)	+W	0.13a	0.20a	0.14a	0.08a	0.13a	0.11a	0.06a	0.10a	0.07a	0.18a
	-W	0.03b	0.02b	0.03b	0.02b	0.02b	0.03b	0.01a	0.02b	0.02b	0.02b
A (μmol $CO_2\ m^{-2}\ s^{-1}$)	+W	14.61a	19.92a	14.91a	17.73a	19.85a	22.11a	18.17a	14.22a	22.51a	20.11a
	-W	3.79b	3.14b	2.01b	2.19b	1.52b	3.78b	3.84b	2.28b	4.47b	3.78b

Different letters between water regime and within the same variable indicate significant differences at $P \leq 0.05$

Ψw_L: leaf water potential, RWC: relative water content, CC: total chlorophyll content, SPAD: estimation of chlorophyll content via SPAD unit, F*v*/F*m*: maximum photochemical efficiency of photosystem II, g_s: stomatal conductance, *A*: CO_2 assimilation rate

Table 5: General analysis of the cultivars with sum of the grades of all analyzed variables and classification in levels of tolerance under water deficit conditions

Cultivars	Variables													
	SH	NGL	LA	SDAB	SDM	RDM	Ψw_L	RWC	CC	SPAD	F*v*/F*m*	g_s	*A*	Total
SP91-1049	1	0	1	0	1	1	1	0	0	2	0	0	2	9*
RB845210	1	0	0	2	0	0	2	0	0	0	1	0	0	6*
RB855035	1	1	1	0	2	2	0	1	1	2	1	1	0	13**
SP89-1115	1	1	2	1	2	0	0	2	0	1	2	1	1	14***
SP80-1816	1	0	2	0	2	1	1	1	0	0	1	0	0	9*
RB92579	1	1	0	2	2	1	0	2	2	2	2	2	1	18****
IAC91-5155	1	1	0	2	1	1	2	2	2	0	1	1	1	15***
IACSP94-4004	2	2	0	0	0	1	0	0	1	0	2	0	1	9*
CTC2	2	2	2	2	2	1	2	2	2	2	0	1	1	21****
IAC91-2195	0	2	0	2	0	0	0	0	0	0	1	0	1	6*

*: up to 9; **: 10-13; ***: 14-17; ****: 18-21. SH: height of stem, NGL: number of green leaves, LA: leaf area, SDAB: abaxial stomatal density, SDM: shoot dry matter mass, RDM: root dry matter mass, Ψw_L: leaf water potential, RWC: relative water content, CC: total chlorophyll content, SPAD: estimation of chlorophyll content *via* SPAD unit, F*v*/F*m*: maximum photochemical efficiency of photosystem II, g_s: stomatal conductance, *A*: CO_2 assimilation rate.

was observed in SP80-1816 (Table 4). The lowest percentage reduction, compared to +W, were noticed for SP91-1049 (74.05%), while the highest were noticed for SP80-1816 (92.3%).

Genetic dissimilarity and grouping by Tocher optimization

After the study of the response of ten genotypes to the 14 morphological and physiological traits, the sum of the grades obtained in each studied trait and the classification for susceptibility or tolerance was made according to this: sum of grades from 0 to 9, susceptible; from 10 to 13, slightly tolerant; from 14 to 17, medium tolerance, and from 18 to 21, very tolerant (Table 5). For the adaxial stomatal density there was no evident response, since only two genotypes had statistical differences, therefore, this trait was not considered in the Table with the indicative tolerance grades. The differentiation between the genotypes through their degree of tolerance indicated that four genotypes *i.e*., CTC2, RB92579, IAC91-5155 and SP89-1115 were more drought tolerant in descending order of tolerance.

First of all, we calculated the matrices of variances and residual covariance of the fourteen morpho-physiological characters of the ten sugarcane cultivars to enable the calculation of Mahalanobis Distance (D^2) and schematization of the Mahalanobis matrix, of dimension 10, according to the methodology described by Rao (1952). Thus it was observed that the greatest genetic distances were between 6 (RB92579) and 5 (SP80-1816) cultivars, 8 (IACSP94-4004) and 3 (RB855035), and 5 (SP80-1816) and 3 (RB855035); and the lowest between 2 (RB845210) and 7 (IAC91-5155), 2 (RB845210) and 8 (IACSP94-4004), 6 (RB92579) and 9 (CTC2), and 7 (IAC91-5155) and 9 (CTC2) (Table 6).

The calculation of the contribution of each evaluated trait, as well as its relative contribution to the calculation of genetic dissimilarity, revealed that the four traits with the greatest contribution in the calculated value of genetic dissimilarity between the accesses were shoot dry matter (23.41%), chlorophyll content (CC) (15.12%), abaxial stomatal density (9.79%) and SPAD index (9.56%) (Table 7). The information obtained through the genetic dissimilarity matrix enabled the grouping of the 10 cultivars studied in seven distinct groups (Table 8).

Table 6: Dissimilarity matrix based on Mahalanobis distance (D^2) among 10 sugarcane genotypes under water deficit conditions

Genotypes	1	2	3	4	5	6	7	8	9
2	110.52								
3	160.40	282.00							
4	86.99	83.36	147.93						
5	75.72	148.86	302.09	154.10					
6	216.18	145.27	183.73	137.63	309.94				
7	131.77	52.11	213.82	102.29	199.81	82.58			
8	207.36	58.11	303.64	135.87	251.38	170.32	92.20		
9	92.99	112.31	117.83	86.23	204.33	64.10	64.79	174.76	
10	250.09	96.59	252.85	150.57	404.43	140.73	105.56	76.88	135.46

(1): SP91-1049; (2): RB845210; (3): RB855035; (4): SP89-1115; (5): SP80-1816; (6): RB92579; (7): IAC91-5155; (8): IACSP94-4004; (9): CTC2; (10): IAC91-2195.

Table 7: Relative contribution (RC) of 14 morphological and physiological variables for the calculation of the genetic dissimilarity of 10 sugarcane genotypes under water deficit conditions

Variables	S.j	RC (%)
Shoot dry matter mass	1,656.60	23.41
Chlorophyll content	1,070.40	15.12
Abaxial stomatal density	692.63	9.79
Estimation of chlorophyll (SPAD)	683.05	9.56
Water potential	576.59	8.15
Stomatal conductance	530.25	7.49
Green leaves	491.26	6.94
Foliar area	424.12	5.99
Root dry matter mass	225.56	3.19
Adaxial stomatal density	189.42	2.68
F*v*/F*m*	154.73	2.19
Relative water content	153.99	2.18
Stem height	131.54	1.86
CO_2 assimilation rate	96.38	1.36

S.j: Contribution of the variable X to the value of Mahalanobis distance between cultivars *i*e., *i'*, RC: Relative Contribution, F*v*/F*m*: maximum photochemical efficiency of photosystem II

Table 8: Grouping by Tocher optimization of ten sugarcane cultivars under water deficit conditions

Groups	Cultivars
I	SP91-1049, SP80-1816
II	S989-1115, CTC2
III	IACSP94-4004, IAC91-2195
IV	IAC91-5155
V	RB855035
VI	RB92579
VII	RB845210

Groups and cultivars in bold indicate drought tolerant and genetically distant cultivars

Discussion

Drought stress impaired sugarcane growth and development, and divergent genotypes behaved differently due to their different genetic makeup (Guan *et al*. 2015; Chen *et al*. 2016). In sugarcane, the water deficit promotes restrictions on cell division, number of green leaves, leaf area, elongation rate of leaves and stems, emission of new tillers, and on the accumulation of dry matter; reflecting penalty in the final yield (Inman-Bamber and Smith 2005; Vieira *et al*. 2014). Likewise, significant reductions in the growth of all studied genotypes under water stress conditions were observed in this study (Table 2).

According to Inman-Bamber (2004), the number of green leaves can be used as an indicator of the effect of this stress on sugarcane. In this sense, leaf area also can be an indicative of tolerance to water deficit, since sugarcane cultivars with greater number of green leaves have larger leaf area. Cultivars considered susceptible in this work had greater reductions of leaf area in water deficit conditions, which lead to decrease in interception of solar radiation, transpiration, stomatal conductance and photosynthesis. In addition to early leaf senescence, all this in turn decreases the CO_2 assimilation and thus the accumulation of biomass (Santos and Carlesso 1998; Ferreira *et al.* 2017; Silva *et al*. 2018).

In case of stomatal density, Bertolino *et al*. (2019) affirm that tolerant plants may respond to water deficiency by emitting new leaves with greater stomatal density, but with smaller diameter of stomata. This allows the air around it to become more humid, increasing the resistance to air movement of the layer adjacent to the leaf epidermis, thus avoiding further damage to gas exchange. However, for SDAD the water regime had little interference, so it allowed inferring that this trait does not receive a pronounced interference of the water deficit. From this, it can be inferred that the number of green leaves, the leaf area and dry matter mass were traits indicative of water deficiency, since cultivars considered tolerant, such as CTC2, SP89-1115 and RB92579, performed well in these variables. However, the stem height, even varying among cultivars, did not follow a standard that could be related to a level of stress tolerance, and the results showed that the use of adaxial and abaxial stomatal density is not recommended as an indicative of tolerance to water deficit. The chlorophyll is the main pigment responsible for the capture of the light energy used in the photosynthesis process; and chlorophyll contents in sugarcane cultivars, though cultivars had divergent response, were decreased under drought stress (Table 4). The decrease in chlorophyll content under water deficit is considered a characteristic symptom of oxidative stress caused by photo oxidation and pigments degradation (Farooq *et al*. 2009), more expressed in susceptible cultivars (Chen *et al*. 2016). Silva *et al*. (2014a) and Kumar *et al*. (2019) also verified values lower than 40 for SPAD index in sugarcane under water

deficiency as were observed in this study. Silva *et al*. (2007; 2011; 2018) affirmed that SPAD index readings lower than 40 evidenced the beginning of chlorophyll degradation due to water restriction, thus affecting the photosynthetic apparatus of sugarcane.

According to Silva *et al*. (2007) and Silva *et al*. (2018), ability of sugarcane plants to maintain high F_v/F_m value under water deficiency indicates the maintenance of high radiation use efficiency and carbon assimilation. The cultivars considered tolerant which had lower reductions of F_v/F_m; this suggests a greater capacity of these cultivars to resist to photoinhibitory conditions under water deficiency. Thus this trait was reliable for differentiating between drought-tolerant sugarcane cultivars, with the benefit of being non-destructive. Stomatal closure, strategy used by plants to reduce water loss through transpiration, compromises photosynthetic carbon assimilation, due to the reduction in CO_2 influx. In this study, all cultivars showed reduction in g_s when submitted to stress, although combined with a strong varietal effect and great genotypic variability, as verified by Santos *et al*. (2015). This suggests that the response is intrinsic to each cultivar. Despite, CO_2 assimilation rate was efficient in differentiating cultivars between tolerant and susceptible, and can be used in studies as a tool indicative of tolerance.

Significant reductions in Ψw_L were also found by Medeiros *et al*. (2013) in sugarcane under water stress. According to results of this study, cultivars which maintained higher levels of Ψw_L, obtained higher stem height and dry matter mass, as CTC2 and IAC91-5155. The reduction of RWC of the leaves is considered as a good indicator of plants water conditions under water stress, once elementary changes in water balance induce cell damage (Hussain *et al*. 2018). These changes can paralyze the growth and even lead to death of plants (Zhang *et al*. 2014). In this context, plants that can maintain higher values of Ψw_L and RWC under water deficiency are considered tolerant (Santos *et al*. 2015; Silva *et al*. 2018). Thus, Ψw_L and RWC could be used as indicators to select drought tolerant sugarcane cultivars.

In the plant breeding area there are methods of grouping or projections of distances in two-dimensional graphs, which are used by breeders, based on the coordinates obtained from the chosen genetic dissimilarity measure (Cruz and Carneiro 2006). Among the optimization methods most used in plant breeding, stands out the one of Tocher, that is used as an optimization grouping technique and has as basic principle to maintain homogeneity within and heterogeneity between the formed groups (Rao 1952). The studied cultivars grouping showed the first two groups, I and II, (closest to each other) with the three "SP" cultivars and with the only "CTC" cultivar present in the experiment. It is emphasized that the sugarcane breeding program "SP" (COPERSUCAR) started to adopt the CTC acronym (Sugarcane Technology Center) since 2004, so it can be considered the same program due to the same germplasm bank and the same cultivar selection methods and objectives.

While in groups III and IV, furthest from the first two and closest to each other, there are the three "IAC" cultivars, and the groups V, VI and VII include the three "RB" cultivars; evidencing the genetic distance between the groups and, consequently, between the cultivars. Based on the grouping obtained, group II contains two cultivars evaluated as tolerant, SP89-1115 and CTC2, and the other two tolerant are IAC91-5155 and RB92579 from groups IV and VI, respectively.

Conclusion

The morpho-physiological traits of sugarcane were efficient to differentiate tolerant and susceptible genotypes to water deficiency. Under water deficiency, the genotypes that stood out for most of the morpho-physiological variables were RB855035, SP89-1115, SP80-1816, RB92579 and CTC2. The multivariate analysis and genetic grouping showed that the most promising crosses were: SP89-1115 and CTC2, both belonging to group II, crossed with RB92579 or IAC91-5155 of groups VI and IV, respectively. The descendants of these crosses might be able to obtain better results under drought conditions.

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