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RESEARCH ARTICLE

Use of Physiological Parameters in Screening Drought Tolerance in Sugarcane Genotypes

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Abstract The physiological response of four commercial sugarcane genotypes to water stress was evaluated by measuring the photochemical efficiency of the photosystem II (chlorophyll *a* fluorescence ratio, F_v/F_m), estimated chlorophyll content (SPAD unit), leaf temperature (LT) and leaf relative water content (RWC). A field trial was established in the subtropical area with well-watered and water-stressed genotypes, in completely randomized blocks with four replicates in a $4 \times 2 \times 3$ factorial design (genotype × irrigation × evaluation date). Physiological measurements were done during a 90 day-period of formative stage of plants. The analysis of variance showed that the interaction of genotype × irrigation × evaluation date had a significant effect for three physiological markers tested, F_v/F_m, SPAD unit and RWC. Under non-stressed conditions, all genotypes showed similar responses for the four markers. Under water deficiency stress, two drought-tolerant genotypes, HOCP01-523 and TCP89-3505 displayed higher values for F_v/F_m, SPAD unit and RWC, and lower values for LT, and could be classified as tolerant. It is therefore possible to use these physiological water stress associated traits as scorable marker traits for selecting drought-tolerant sugarcane genotypes in future breeding programs.

Keywords *Saccharum* spp. · Screening · Chlorophyll *a* fluorescence · Physiological markers · Water stress

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Introduction

One of the main stress-causing factors in plants, and which significantly affects the development and productivity of sugarcane, is water deficit. Water deficit is common during the growth phases of the crop, leading to a temporary reduction in biomass accumulation, and also occurs mainly during the dry season, when the ground water is not available to the plant for a period of days, weeks, or even months, causing a decrease in physiological activities and in biomass accumulation of the plant (Levitt 1972).

Various criteria, based on soil moisture measurements and analysis of rainfall distribution, can be used to evaluate the momentary stress levels to which the plant is subjected. From the ecophysiological point of view, knowledge of the external factors alone is not enough for drawing accurate conclusions about the degree of drought (Larcher 2003). It is therefore important to understand the physiological processes that act on the plants, in conditions of stress caused by water deficit. Understanding how plants tolerate water deficit may significantly improve this situation in

future drought events and identifying mechanisms of water stress tolerance is crucial for the development of new tolerant commercial cultivars (Nepomuceno et al. 2001). The development of drought-tolerant cultivars has been one of the objectives of genetic improvement programs involving sugarcane (Inman-Bamber and Smith 2005), and requires the identification of important physiological mechanisms, for use as selection criteria (Smit and Singels 2006).

Varieties differ in their response to drought stress, and it is possible to identify more tolerant ones. This identification is essential, particularly in areas that has long period of water deficit. Rong-hua et al. (2006) showed that indirect and faster methods of measuring photosynthetic activity, such as the chlorophyll *a* fluorescence technique, and in particular, the maximum photochemical efficiency of photosystem II—PSII (which can be assessed via the variable-to-maximum chlorophyll *a* fluorescence ratio, F_v/F_m) and estimated chlorophyll content (SPAD unit), can be just as effective as the more time-consuming gas exchange techniques in revealing differences among drought-tolerant and susceptible genotypes of barley. The relationship between drought tolerance and chlorophyll fluorescence using portable fluorometer has been well established in sugarcane (Luo et al. 2004; Molinari et al. 2007; Silva et al. 2007). Other physiological parameters such as leaf temperature (LT) and relative water content (RWC) are also very responsive to water stress, and have been shown to correlate well with drought tolerance in sunflower, barley, wheat and wheeping lovegrass (Jamaux et al. 1997; Altinkut et al. 2001; Colom and Vazzana 2003).

Visual assessment of the agronomic performance and the overall varietal response to drought is the common method of selection for drought tolerance in sugarcane (Wagih et al. 2001). However, the outcome of this assessment varies significantly with changes in the environment. The use of physiological markers offers a relatively more accurate way of assessing drought tolerance, especially in field trials. Four physiological markers as fast tools to screen for drought tolerance in eight field-grown sugarcane genotypes were developed (Silva et al. 2007). In this study, four sugarcane genotypes previously classified based on their agronomic performance as drought-tolerant and drought-sensitive, for drought-associated physiological traits have been analyzed. The aim is to identify certain physiological marker traits that can be potentially employed for rapid selection of drought-tolerant genotypes.

Materials and Methods

The study was conducted in the subtropical region of south Texas, near Weslaco (26° 12′ N, 97° 57′ W, elevation 18.90 m), Texas, USA, during the 2005–2006 growing season in a commercial field with a sandy clay loam soil type. The experiment was arranged in a complete block design within a three-factor factorial, where the first factor consisted of four genotypes; the second consisted of two irrigation levels (wet and dry), and the third consisted of three evaluation dates (0, 45, and 90 days after water deficit imposition), with four replicates.

The four contrasting sugarcane genotypes assessed in this study were selected based on their performance in relation to drought. Thus, HOCP01-523 and TCP89-3505 were chosen as drought-tolerant genotypes, and TCP87-3388 and HOCP93-776 as susceptible genotypes, according to previous assessment by the authors of their agronomic parameters under water stress (Silva et al. 2008). Each genotype was planted in three rows, 3 m long, and 1.5 m apart, on November 14th, 2005. Data were only collected from the central row plants.

Two irrigation treatments (well-watered and drought) were initiated at 180 days after planting. The well-watered plots were irrigated at 50% depletion of available soil moisture (DASM), whereas the water-stressed plots were irrigated at 80% DASM. Soil moisture depletion was monitored periodically with a neutron probe.

Physiological parameters were measured three times during the study: at 0, 45, and 90 days after the start of irrigation treatments (dat) on cloudless days and between approximately 0900 and 1500 h. Chlorophyll *a* fluorescence characteristics were measured on a section of the first upper leaves using a pulse amplitude modulation fluorometer (Model OS5-FL, Opti-Sciences, Tyngsboro, Massachusetts, USA). On each evaluation date, at least four leaves per plot were dark-adapted for 30 min using leaf clips (FL-DC, Opti-Science) before fluorescence measurements. The F_v/F_m ratio was determined following the procedures of Maxwell and Johnson (2000), in order to quantify the level of drought-induced photoinhibition.

Leaf chlorophyll content (SPAD unit) was estimated nondestructively, using a SPAD-502 chlorophyll meter (Minolta Corp., Ramsey, New Jersey, USA). This index was selected preferentially, due to the close relationship between the readings of the portable chlorophyll meter and leaf chlorophyll content (Markwell et al. 1995), and because it has been used as a reliable nondestructive tool for rapid screening for drought tolerance in sugarcane (Silva et al. 2007). The average of five measurements taken on the first upper leaves from different plants in each plot was recorded.

LT readings were collected using a hand-held infrared thermometer (Model OS530HR, Omega Engineering Inc., Stamford Connecticut, USA) with leaf emissivity set at 0.95. During each LT measurement, the natural leaf orientation with respect to the sun was maintained to avoid shade effects.

For determination of leaf RWC, leaf disks (1.3 cm diameter each) were collected with a cork borer from the same leaf samples used for F_v/F_m, SPAD unit and LT measurements. Five disks per plant were collected, and quickly transported to the laboratory on ice in glass vials. Leaf disk fresh weight (W_f) was determined within 2 h of excision. The turgid weight (W_t) was obtained after hydration in deionized water for 24 h in the dark at room temperature. Leaf discs were quickly blotted dry and oven-dried for 48 h at 80°C before recording the dry weight (W_d). RWC was calculated from the following equation (Matin et al. 1989):

$$\text{RWC} = \left[(W_f - W_d)\,(W_t - W_d)^{-1}\right] \times 100$$

The data were subjected to an analysis of variance (ANOVA) and where appropriate, mean separation was performed using the least significance difference (LSD; $P < 0.05$) procedures of the SPSS statistical package (SPSS Student version 15.0). Genotypes and irrigation treatments were considered as fixed effects and replication as a random effect. Evaluation dates were repeated observations in the analysis.

Results and Discussion

Air temperatures during the study period (November 2005 to August 2006) ranged from ~10 to ~34°C (Table 1) and cumulative rainfall during this period was 158.2 mm. The wet and dry treatments received an additional 301.0 and 185.0 mm, respectively in irrigation. Thus, the total water inputs were 459.2 and 343.2 mm for the wet and dry treatments, respectively, applied in 8 (wet) and 5 (dry) irrigation events.

Table 1 Monthly maximum and minimum mean air temperature, total rainfall and wet and dry irrigation treatments from November 2005 to August 2006

Growth period	Temperature		Rain fall	Irrigation	
	Max. (°C)	Min. (°C)	(mm)	Wet treatment (mm)	Dry treatment (mm)
Nov. 05	25.0	11.8	12.4	0	0
Dec. 05	18.8	10.4	9.4	0	0
Jan. 06	23.9	11.4	1.0	39.0	39.0
Feb. 06	23.2	10.4	1.5	35.0	35.0
Mar. 06	27.1	17.6	7.4	40.0	40.0
Apr. 06	30.5	20.5	2.5	44.0	44.0
May 06	31.6	21.8	41.4	30.0	0
Jun. 06	33.2	22.6	3.3	40.0	27.0
Jul. 06	33.5	24.0	70.2	47.0	0
Aug. 06	34.3	24.6	9.1	26.0	0
Total	–	–	158.2	301.0	185.0

The ANOVA revealed that PSII photochemical efficiency (F_v/F_m), SPAD unit, LT and leaf RWC were affected by genotype (G), irrigation level (I) and evaluation date (D) (Table 2). G × I and I × D interactions were found to be significant for all four parameters, while G × D interaction was significant only for F_v/F_m and RWC. A significant G × I × D interaction was found for the three parameters, F_v/F_m, SPAD unit and RWC. These results are in agreement with those reported by Silva et al. (2007) in sugarcane.

Table 2 Analysis of variance, means, least significance differences (LSD) and coefficient of variation (CV) for PSII photochemical efficiency (F_v/F_m), estimated chlorophyll content (SPAD unit), leaf temperature (LT) and leaf relative water content (RWC) for four sugarcane genotypes grown under well-watered and drought conditions, with measurements taken on three dates during 2006

Treatment	Physiological attributes			
	F_v/F_m	SPAD	LT	RWC
Genotype	Unit	Unit	°C	%
HOCP01-523	0.824 a	40.45 b	30.09 c	89.30 a
TCP89-3505	0.822 ab	43.01 a	30.53 bc	87.99 b
TCP87-3388	0.811 b	41.62 ab	32.47 ab	86.66 c
HOCP93-776	0.795 c	41.36 ab	32.89 a	85.19 d
Irrigation level				
Well-watered	0.822 a	44.12 a	30.60 b	89.35 a
Drought-stressed	0.804 b	39.10 b	32.39 a	85.22 b
Evaluation date				
0	0.821 a	43.14 a	29.04 c	88.77 a
45	0.815 a	41.74 a	31.80 b	87.66 b
90	0.803 b	39.94 b	33.65 a	85.42 c
F				
Genotype (G)	19.32**	3.32*	7.14**	35.92**
Irrigation level (I)	32.38**	74.74**	11.89**	196.87**
Evaluation date (D)	11.95**	10.19**	26.53**	44.83**
G × I	10.49**	9.96**	3.83*	19.25**
G × D	5.59**	1.11 ns	0.76 ns	16.56**
I × D	8.32**	21.45**	3.26*	63.05**
G × I × D	2.69*	2.85*	1.08ns	5.50**
LSD				
Genotype	0.011	2.16	1.94	1.10
Irrigation level	0.006	1.15	1.04	0.49
Evaluation date	0.009	1.70	1.53	0.86
CV (%)	1.85	6.84	8.09	1.65

Means with the same letter, in the same column and within the same attribute, are not significantly different at a probability level of 0.05 (Tukey LSD)

ns not significant

* $P < 0.05$

** $P < 0.01$

On average, the genotypes showed different responses to F_v/F_m (Table 2), HOCP01-523 presented the highest value, and there was no significant difference in relation to TCP89-3505, which in turn, did not differ from genotype TCP87-3388. The lowest value was obtained for HOCP93-776. According to Hall and Rao (1994), values for photosystem II (PSII) photochemical efficiency (F_v/F_m) of around 0.83 signify healthy plants. However, this level is very close to the values found for genotypes HOCP01-523 and TCP89-3505, indicating that they were not affected by the water stress applied for a period of 90 days, while HOCP93-776, water stress led to a decrease in F_v/F_m. The F_v/F_m values, under well-watered conditions, were higher than those of the sub-drought conditions, showing that the plants under this condition did not show damage in their photosynthetic apparatus. In terms of evaluation dates, the F_v/F_m values decreased as the drought period increased, with the evaluation at 90 dat presenting the lowest value.

The four genotypes varied in their SPAD unit response to drought (Table 2). The genotype TCP89-3505 showed the highest SPAD unit value, and the HOCP01-523, the lowest SPAD unit value. Schlemmer et al. (2005) observed that the use of the SPAD-502 meter on plant tissue under water stress resulted in an underestimate of chlorophyll content compared with the chlorophyll extraction procedure, suggesting that water stress does not affect chlorophyll content. However, we observed in this study and in a previous report (Silva et al. 2007) that the tolerance-susceptibility classification of a genotype is not related to the average chlorophyll content, but rather, to the extent of the chlorophyll degradation under the stressful condition (Fig. 2). Under water deficit conditions, the average SPAD unit value was significantly lower than for the well-watered conditions. There was a gradual reduction in SPAD unit as stress increased with values lower at 90 dat than those at 0 and 45 dat.

LT showed, on average, a certain distinction among the genotypes, with the formation of two groups, i.e., the genotypes HOCP01-523 and TCP89-3505 displayed average temperature values of around 30–30.5°C during the evaluation period, while TCP87-3388 and HOCP97-776 showed average temperatures of around 32.4–32.9°C (Table 2). O'Neill et al. (2006) found average temperatures that are around 2.5°C warmer for the deficit versus adequate water level in corn. Silva et al. (2007) demonstrated that water stress generally resulted in a higher increase in LT in susceptible genotypes. This is based on the principle that increasing plant water deficits led to stomatal closure, decreased leaf transpiration cooling, and consequently, increased LT relative to well-watered plants (O'Neill et al. 2006). In terms of interaction of irrigation level by evaluation date, the opposite situation for LT is observed as compared to that for F_v/F_m and SPAD unit. Under water deficit, the LT values were higher than under well-watered conditions and reached the highest at 90 dat evaluation date.

The effect of environment temperature on the evaluation date for LT measurements was found to be non significant in this study. As shown in Table 1, the variation in environment temperature during the evaluation period (May–August 2006) was only 2.7°C. Also, environment temperature was run as a covariate in our ANOVA, and results were not found to be significantly different from those obtained when ANOVA was performed without considering environment temperature as a covariate.

Jamaux et al. (1997) suggested that RWC is positively correlated with water stress tolerance in sunflower. As higher is the RWC value under water-limited conditions, the higher is the genotype tolerance. As shown in Table 2, the highest RWC value is displayed by the drought-tolerant genotype HOCP01-523, while the lowest is observed for the drought-sensitive genotype HOCP97-776. RWC was the physiological attribute that best differentiated both the tolerant and the susceptible genotypes. Differences in RWC were also found on the interaction of irrigation level by evaluation date. Genotypes with no water deficit treatment in the first evaluation date showed high RWC values, but that were decreased significantly with a concurrent increase in days under water deficit treatment. According to Altinkut et al. (2001) and Schlemmer et al. (2005), RWC in barley, wheat and corn with adequate water was approximately 90%. On the other hand, under water stress conditions, RWC ranged from 45 to 58% for barley, 56 to 72% for wheat and around 65% for corn. The smaller reduction found for sugarcane may be an indication that this species is more tolerant to drought.

The values for F_v/F_m, SPAD unit, LT and RWC by genotype, irrigation level and evaluation date after treatment are shown in Figs. 1, 2, 3 and 4.

Under well-watered conditions, all the genotypes maintained F_v/F_m values without significant variation throughout the 90 dat. However, under water deficit conditions, F_v/F_m declined in the drought-susceptible genotypes TCP87-3388 (0.83 at 0 dat to 0.78 at 90 dat) and HOCP93-776 (0.82 at 0 dat to 0.73 at 90 dat). There was little variation in the F_v/F_m values for the drought-tolerant genotypes HOCP01-523 and TCP89-3505; values for both genotypes varied from 0.82 to 0.81, at 0 and 90 dat, respectively (Fig. 1). Genty et al. (1987) reported similar observations in cotton, where PSII photochemical efficiency was not affected under mild water stress, but was hindered under severe water stress. Maxwell and Johnson (2000) further associated photoinhibition with an over-reduction of PSII. Also, it is well known that a sustained decrease in F_v/F_m reflects a photoinhibitory damage in response to environmental stress. Silva et al. (2007) were

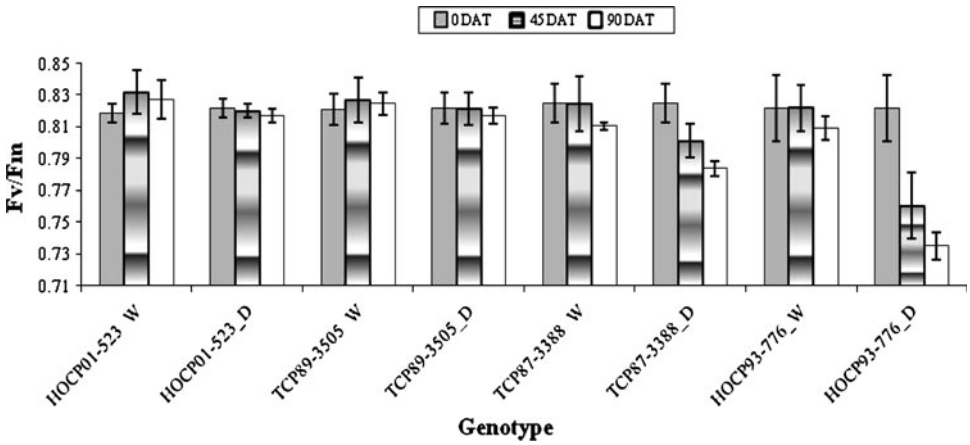

Fig. 1 Mean PSII photochemical efficiency (F_v/F_m) for four sugarcane genotypes under two water treatments (*W* well-watered and *D* drought) during three evaluation dates in Weslaco, TX, USA. Data represent means (±standard error) of four observations

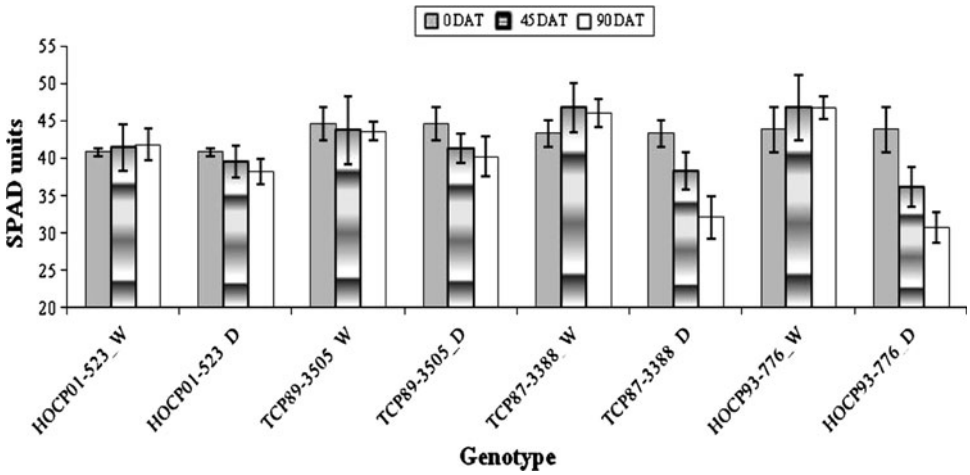

Fig. 2 Mean chlorophyll content (SPAD unit) for four sugarcane genotypes under two water treatments (*W* well-watered and *D* drought) during three evaluation dates in Weslaco, TX, USA. Data represent means (±standard error) of four observations

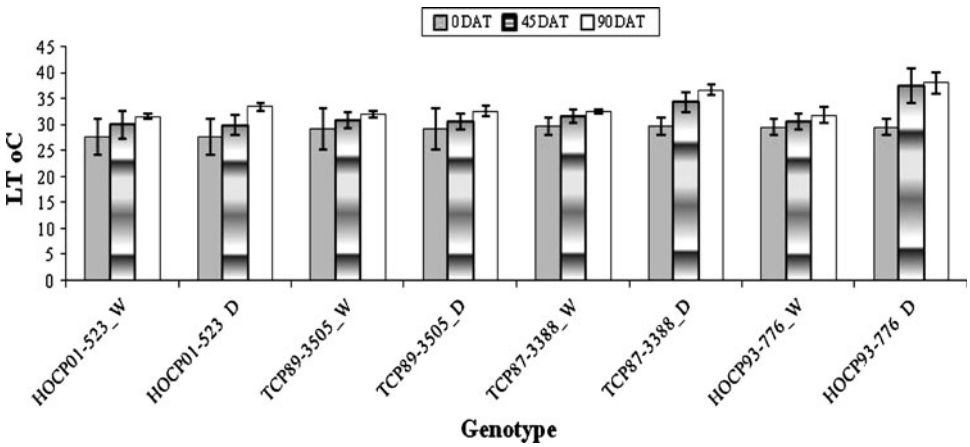

Fig. 3 Mean leaf temperature (LT) for four sugarcane genotypes under two water treatments (*W* well-watered and *D* drought) during three evaluation dates in Weslaco, TX, USA. Data represent means (±standard error) of four observations

able to classify eight sugarcane genotypes into two groups, according to their response to water stress. They reported that the most tolerant genotypes maintained higher F_v/F_m values under water stress, concluding that this parameter would be a good tool for screening genotypes for this characteristic. Our results indicate that F_v/F_m is associated with drought tolerance, since it could make a clear distinction between the drought-tolerant genotypes HOCP01-523 and TCP89-3505, and the drought-sensitive genotypes TCP87-3388 and HOCP93-776.

SPAD unit was affected by drought in both tolerant and susceptible genotypes during drought development (Fig. 2). However, susceptible genotypes showed significantly lower SPAD unit values than the tolerant ones at both 45 and 90 dat. Under well-watered conditions, SPAD unit values were similar for both evaluation dates in

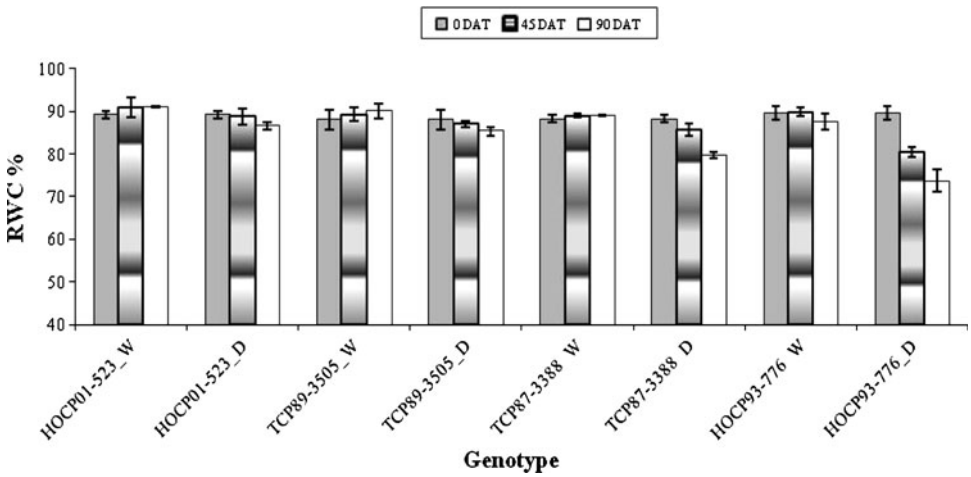

Fig. 4 Mean leaf relative water content (RWC) for four sugarcane genotypes under two water treatments (*W* well-watered and *D* drought) during three evaluation dates in Weslaco, TX, USA. Data represent means (±standard error) of four observations

drought-tolerant and drought-susceptible genotypes. Therefore, water stress affected leaf chlorophyll content (SPAD unit). In this study, we observe that the initial estimated leaf chlorophyll content does not necessarily mean that the plant has better drought tolerance, since the genotypes TCP89-3505, TCP87-3388 and HOCP93-776 displayed a SPAD unit of around 43-44, while the genotype HOCP01-523 showed little variation around 40 for this parameter (Fig. 2). Schlemmer et al. (2005) found no effect of drought on SPAD unit in corn; however, O'Neill et al. (2006) found that SPAD unit was the only one of the four attributes to be affected by drought conditions in corn between 90 and 98 dat after planting. Our data indicate that SPAD unit is a good physiological marker that correlates positively with drought tolerance. This supports the results obtained by Silva et al. (2007) who found a consistent relationship between SPAD unit values and tolerant-susceptible classification of sugarcane genotypes, and proposed that this technique could be promising for selecting genotypes for drought tolerance in a rapid, non-destructive way.

LT is another physiological marker found to correlate well with drought. At 45 dat, two groups of genotypes, tolerant and susceptible, could be identified under stress conditions using LT as a physiological marker. LT was significantly affected in the drought-sensitive genotypes TCP87-3388 and HOCP93-776 (Fig 3). Despite the increase in LT in all the genotypes, this increase was higher in the susceptible plants and the difference intensified with time. After 90 days of water stress, the variation in LT in HOCP93-776 was 29.6 to 38.1°C. These results show that the LT marker correlates strongly with drought susceptibility, and support those obtained by Silva et al. (2007), although measurements need to be carefully recorded since that LT is more sensitive to variations in wind and sunlight reflection. O'Neill et al. (2006) provided evidence that LT is a proven indicator of plant water stress; stomatal closure is one of the first adaptive responses to increasing water stress, as the plant attempts to reduce transpiration losses through the leaves, and prevent the development of lethal water deficits in its tissues. The gradual LT increase in both genotype groups can indicate a reduction in the stomatal aperture of between 45 and 90 dat, and the highest LT in the susceptible genotypes could reflect a stomatal closure.

The genotypes TCP87-3388 and HOCP93-776 showed a strong reduction in RWC during the water stress period, and therefore were classified as drought-susceptible (Fig. 4). The genotypes HOCP01-523 and TCP89-3505 also suffered a reduction with time, but this was less marked, and therefore, were classified as drought-tolerant. Both groups, under well-watered conditions, maintained approximately 90% RWC. For the susceptible genotypes, RWC decreased to about 80–85% after 45 days of drought stress, reaching about 73–79% at 90 dat. However, the tolerant genotypes showed less decrease in their RWC over time of water stress; their RWC was reduced on average to 87% at 45 dat and to 85% at 90 dat. According to Colom and Vazzana (2003), the plants that are more tolerant to drought stress have a higher capacity to save water during periods of drought and therefore display higher RWC values. This occurs through a decrease in their CO_2 uptake and photosynthetic biochemical processes, and a consequent reduction in photoinhibition and damage to the PSII system. Our data indicate that the RWC is a drought-associated marker that can be used for the classification of drought-tolerant and drought-susceptible sugarcane genotypes. They corroborate the results reported by Silva et al. (2007) on the correlation of RWC with drought tolerance as well as with productivity; the selection of genotypes with higher RWC values under water stress corresponded to the selection of the more productive plants.

In summary, our results suggest that the four physiological markers SPAD unit, F_v/F_m, RWC and LT, are drought-associated and can be used to differentiate among

sugarcane genotypes based on their tolerance or susceptibility to water stress. SPAD unit, F_v/F_m and RWC correlated strongly with drought tolerance while LT associated with drought susceptibility. O'Neill et al. (2006) obtained similar differential responses to drought in corn hybrids using LT, chlorophyll content and chlorophyll fluorescence, and concluded that these measurements could be used to distinguish tolerant hybrids from susceptible ones. The authors also reported similar patterns with the agronomic and photosynthetic responses of the hybrids to deficit and adequate water conditions, indicating that tolerant hybrids have a higher yield than susceptible ones, under stress conditions. It is therefore important to select for physiological markers that could either confer adaptation and higher yield under water stress conditions or be associated with drought tolerance, and could potentially be used to routinely screen genotypes and parental plants for selection of new drought-tolerant genotypes in breeding programs.

Conclusions

Four physiological markers, PSII photochemical efficiency (F_v/F_m), SPAD unit, LT and relative leaf water content (RWC) were found to be drought-associated in sugarcane under our conditions. They are of value for rapid selection of drought-tolerant genotypes in plant variety selection programs, and for modeling crop growth to sustain high yield production. Selection of plants for drought tolerance is otherwise difficult due to the genetic complexity of the drought trait.

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