ASSESSMENT OF CHLOROPHYLL AND LEAF RELATIVE WATER CONTENT AS INDICATORS OF DROUGHT TOLERANCE ON SUGARCANE INITIAL GROWTH

By

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**KEYWORDS: *Saccharum* spp., Water Stress,
SPAD Index, Photosynthetic Pigments, RWC.**

Abstract

WATER deficiency is the major environmental stress that affects sugarcane production worldwide. Physiological traits associated with drought tolerance can be suitable indicators for screening of drought tolerance of sugarcane clones to reduce the negative effects on crop yield. The aim of this study was to evaluate the ability of physiological parameters to identify drought tolerant and susceptible sugarcane clones. These parameters included estimated leaf chlorophyll content *via* SPAD index, photosynthetic pigments (chlorophyll *a*, *b*, *a* + *b* and the ratio of chlorophyll *a*/chlorophyll *b*) and leaf relative water content (RWC). The experiment was carried out under greenhouse conditions. Eight clones (CP92-675, HoCP85-845, HoCP01-523, L01-283, TCP87-3388, TCP89-3505, TCP02-4620 and TCP02-4624) variable in drought tolerance were used to assess the relation between these parameters and drought tolerance. Drought stress was imposed by withholding irrigation for 21 days and then rewatering for 6 days on plants grown at 90 days after planting. The results showed that during the drought stress the parameters SPAD index, chlorophyll *a*, *b*, *a* + *b* and RWC showed a progressive reduction independent of clone, but the decrease was much less in CP92-675, HoCP01-523, TCP87-3388 and TCP89-3505. After rehydratation, these clones recovered most rapidly from stress condition, although the initial values were not reached. HOCP85-845, TCP02-4624, TCP02-4620 and L01-283 showed a strong negative and residual effect of drought stress in terms of chlorophyll content and RWC, and L01-283's photosynthetic apparatus was most damaged because this clone did not show response after rewatering. Among chlorophyll parameters, chlorophyll a / *b* ratio was little affected by water deficit in the clones HoCP01-523, TCP89-3505, TCP87-3388 and CP92-675. Results suggest that chlorophyll and RWC could be considered to differentiate between drought tolerant and susceptible clones. We concluded that clones CP92-675, HoCP01-523, TCP89-3505 and TCP87-3388 performed as tolerant, HoCP85-845, TCP02-4620 and TCP02-4624 as intermediate, and L01-283 as drought susceptible during the initial growth.

Introduction

Sugarcane is a crop of global importance and it is cultivated between the 32º North and South parallels. In many areas over the world, rainfall is not sufficient for crop supply and water deficiency can limit crop production. Depending on the deficiency level, specifically for the sugarcane crop, growth and yield can decrease and result in great socioeconomic losses (Munns, 2002).

Drought is a multi-dimensional stress, which causes various morphological, physiological and biochemical effects on plants. The identification of markers linked to drought tolerance could be a useful tool to manage and adapt the sugarcane crop to adverse conditions (Domaingue, 1996; Blum, 1996; Jamaux *et al.*, 1997).

The degree of limitation of yield by environmental stresses varies even among genotypes within every species (Wolfe *et al.*, 1988; Aguilera *et al.*, 1999). Therefore, the ability to maintain key physiological processes, such as photosynthesis during moderate drought stress, is indicative of the potential to sustain productivity under water shortage.

Some parameters can be used as reliable indicators to evaluate efficiency of the photosynthetic system and yield performance of genotypes under water deficit.

According to Schlemmer *et al.* (2005), the plant's photosynthetic potential is directly proportional to the amount of chlorophyll present in the leaf tissue. Leaf relative water content (RWC) also seems to be positively correlated to the level of photosynthesis because the prevention of water stress needs a fine regulation of water loss, in order to maintain adequate CO_2 uptake (Colom and Vazzana, 2003). To our knowledge, little is known about changes in chlorophyll and relative water content under drought stress in sugarcane genotypes.

In this context, we studied the effects of water deficiency on chlorophyll and leaf relative water content of eight sugarcane clones differing in drought tolerance.

Materials and methods

Plant material and treatment

The experiment was carried out in a greenhouse belonging to Texas AgriLife Research/Texas A&M University, in Weslaco–TX–USA. We used isolated buds of eight sugarcane clones with varying drought tolerance–four tolerant varieties (HoCP85-845, HoCP01-523, TCP89-3505 and TCP02-4620) and four susceptible varieties (CP92-675, L01-283, TCP02-87-3388 and TCP02-4624).

Cane was planted in 22 L pots, filled with vermiculite-based substrate and 55 g of the NPK formula 14-14-14. During the experiment, average air temperature was 18.1–22.6^{o}C, relative humidity was 60–70% and photosynthetic photon flux density (PPFD) was 600–800 µmol/m^2/s.

Plants were grown for 60 days after planting (DAP). After that period, water was not supplied for 21 days and then re-established for 6 days.

Evaluations

Physiological parameters were measured five times during the study: 0, 7, 14, 21 days after drought stress treatment (DAT) and again 7 days after relief of stress at 28 DAT.

Leaf chlorophyll content (SPAD index) 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 four measurements taken on the leaf + 1, i.e. first fully expanded leaf, from different plants in each plot was recorded.

For the evaluation of leaf chlorophyll and water content, four 1.3-diameter discs were taken from the leaf 1 of each plant with a cork borer and then stored in thermal boxes with ice.

Chlorophyll was extracted from two leaf discs submerged for 24 h in dimethyl formamide acid (DFA). The levels of *a*, *b* and *a* + *b* chlorophyll and carotenoids were determined according to Porra *et al.* (1989), with absorbance optical analysis in spectrophotometer on wave lengths of 480, 647 and 664 nm (Beckman DU-6400). Chlorophyll *a*/*b* ratio was calculated with the values previously determined.

Both remaining discs were used to evaluate leaf relative water content (RWC). Leaf disk fresh weight (Wf) was determined within 2 h of excision. The turgid weight (Wt) was obtained after hydration in deionised water for 24 h at room temperature.

Leaf discs were quickly blotted dry and oven-dried for 48 h at 80ºC before recording the dry weight (Wd). RWC was calculated from the following equation (Matin *et al.*, 1989):

$$RWC = [(W_f – W_d) \cdot (W_t – W_d)^{-1}] \times 100$$

Statistics

The experimental design was a completely randomised design, arranged in 40 combinations, within a two-factor factorial 8 x 5, where the first factor consisted of eight clones and the second consisted of five evaluation dates (0, 7, 14, 21 and 28 DAT), with three replicates. Variance analysis was applied for statistical procedure. Clones and evaluation dates were compared by the Tukey test.

Results and discussion

The ANOVA revealed that estimated chlorophyll content (SPAD), leaf chlorophyll *a* (Chl *a*), leaf chlorophyll *b* (Chl *b*), total leaf chlorophyll (Chl *a* + *b*), ratio chlorophyll *a*/*b* (Chl *a*/*b*) and leaf relative water content (RWC) were significantly affected by clone (C) and evaluation date (ED). C x ED interactions were also found to be significant for all six parameters. These results are in agreement with the ones reported by Colom and Vazzana (2003) for another C4 grass, *Eragrotis curvula*.

Drought stressed plants showed a progressive reduction of estimated chlorophyll content (SPAD index), during drought development (Figure 1). Positive effects of clone on drought response were evidenced by different results for three groups of clones after 21 days of stress. Clones HoCP01-523, TCP89-3505, CP92-675 and TCP87-3388 had their SPAD indexes less affected by water deficit (~35.50–41.73 SPAD units), whereas clones HoCP85-845, TCP02-4620 and TCP02-4624 showed intermediate values (~17.27–26.33 SPAD units).

The clone L01-283 was the most affected by drought stress (10.10 SPAD units). During rewatering, SPAD index increased for all clones, except for L01-283, but clones from the intermediate group reached lower SPAD (~ 28.70–33.27 SPAD units) than clones HoCP01-523, TCP89-3505, CP92-675 and TCP87-3388 (~ 40.93–46.27 SPAD units).

Chlorophyll degradation is one of the consequences of drought stress and may result from sustained photo-inhibition and photo-bleaching (Long *et al.*, 1994), and even though other plant processes, such as cell division and cell expansion, are the earliest to respond to water deficit stress (Dale, 1988), a decline in SPAD index is a sensitive and readily measurable trait that could be used to screen for stress tolerance (O’Neill *et al.,* 2006).

Silva *et al.* (2007) reported that the tolerance-susceptibility classification of a clone cannot be related to the average chlorophyll content, but rather, to the extent of the chlorophyll degradation under a stressful condition.

Chlorophyll *a*, chlorophyll *b* and chlorophyll *a* + *b* showed similar results for the clones studied, either during drought stress or after water re-establishment. During drought, chlorophyll *a* decreased in all clones (Figure 2). After 21 days under withholding water, a significantly higher value of chlorophyll *a* was found in TCP 87-3388 (19.57 µg/cm^2), and the lowest value in L01-283 (8.29 µg/cm^2) and TCP02-4624 (8.54 µg/cm^2).

All the other clones showed intermediate performance. Chlorophyll *a* increased during rewatering in all clones, except L01-283 which did not show any recovery. Among the clones that increased chlorophyll *a*, only HoCP01-523, TCP89-3505, TCP87-3388 and CP92-675 reached values close to those observed in the beginning of the experiment.

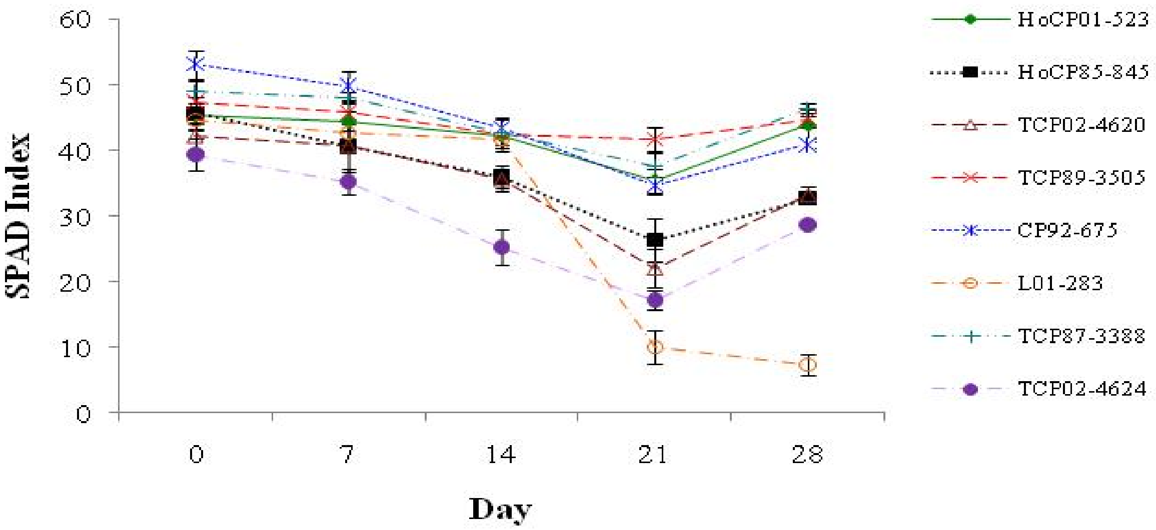

Fig. 1—Mean estimated chlorophyll content (SPAD index) (n = 3, ±S.E.) measured at 0, 7, 14, 21 and 28 days after onset of the drought experiment in eight sugarcane clones.

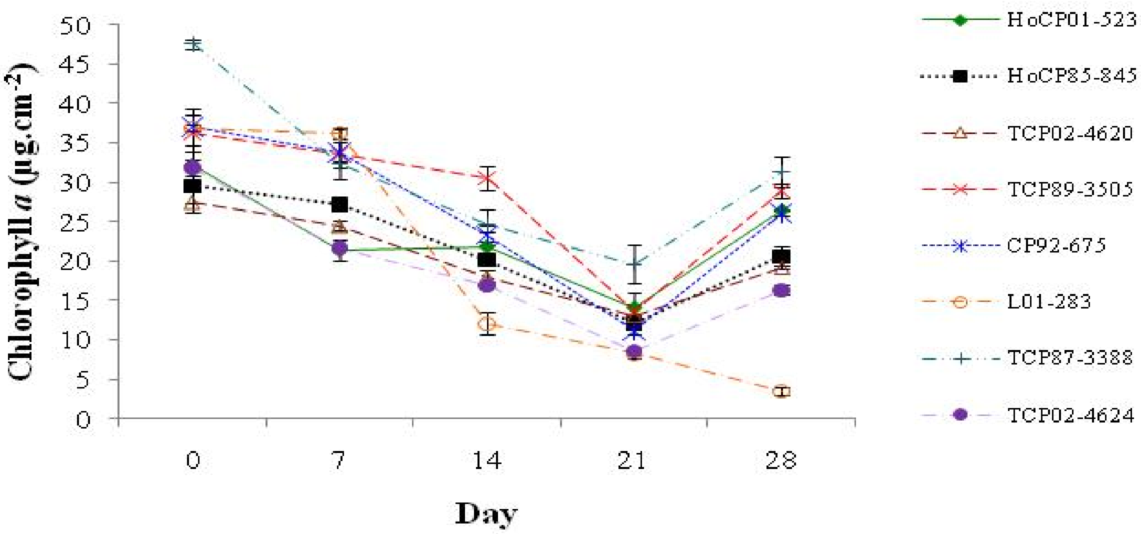

Fig. 2—Mean chlorophyll *a* (n = 3, ±S.E.) measured at 0, 7, 14, 21 and 28 days after onset of the drought experiment in eight sugarcane clones.

Similarly to the results for chlorophyll *a*, chlorophyll *b* and chlorophyll *a* + *b* decreased after drought for all clones. However, clones varied in degree of stress impact on chlorophyll *a* and *a*+*b* up to 21 days after water interruption and also at 6 days after water re-establishment (Figures 3 and 4). Similar results were observed by Pardo and Delgado (1989), who also reported different contents of chlorophyll *a* and *b* of two sugarcane clones under drought. These authors stated that the clone susceptible to drought immediately decreased chlorophyll rates. Therefore, according to the results of this work, the clone TCP87-3388 showed the lowest decrease of chlorophyll *b* after 21 DAT (6.76 µg/cm^2). A lower value of chlorophyll *b* was observed for the clone TCP89-3505 (3.49 µg/cm^2), but this material was not significantly different from HoCP85-845, HoCP01-523, L01-

283, TCP02-4620 and TCP02-4624. Nevertheless, after water re-establishment, values of chlorophyll *b* (Figure 3) and *a* + *b* (Figure 4) were almost completely restored for the clone TCP89-3388. Additionally, chlorophyll *b* and *a* + *b* significantly increased for the clones HOCP01-523, CP92-675, TCP87-3505 and TCP02-4620. The clones HOCP85-845 and TCP02-4624 had intermediate restoration of chlorophyll values. Irreversible damage can be caused by drought in the integrity of photosynthetic pigments, and this was evidenced in the clone L01-283 that did not show any recovery after water re-establishment. Colom and Vazzana (2003) reported drought affecting chlorophyll content in leaves of *Eragrotis curvula* and the susceptible clone showed the largest reduction.

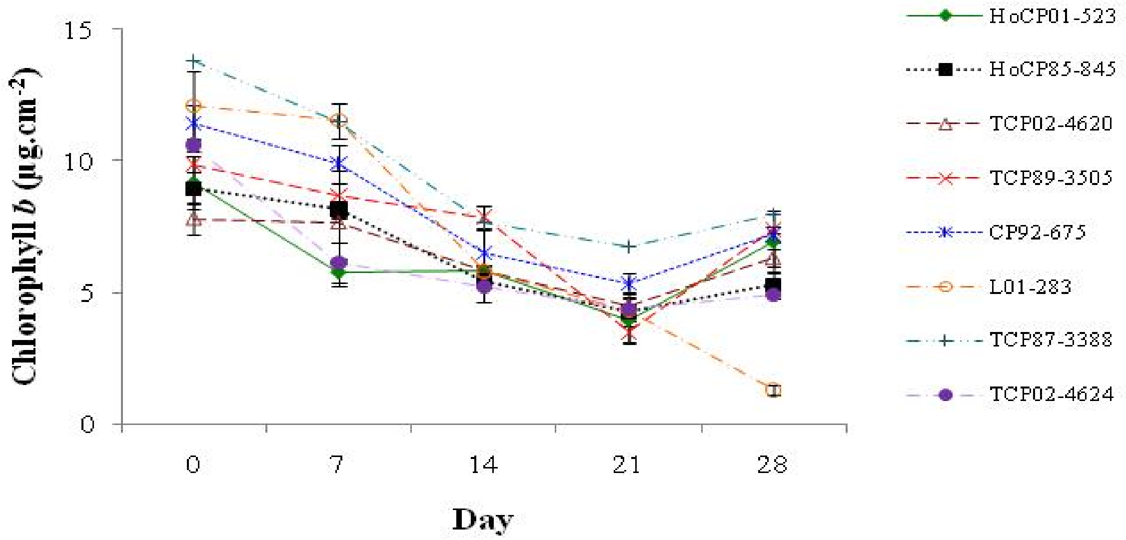

Fig. 3—Mean chlorophyll *b* (n = 3, ±S.E.) measured at 0, 7, 14, 21 and 28 days after onset of the drought experiment in eight sugarcane clones.

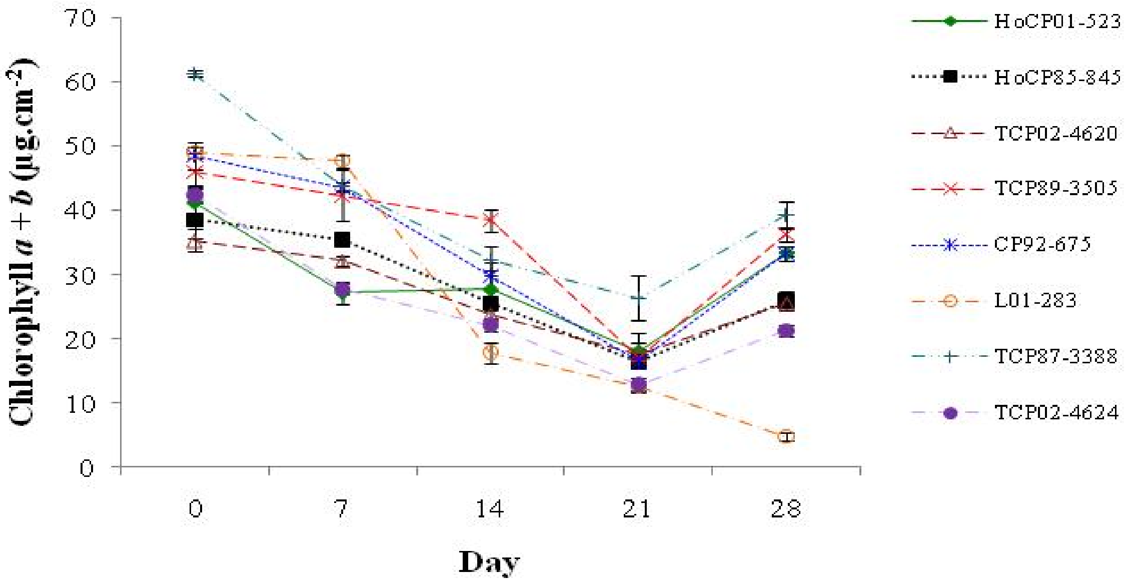

Fig. 4—Mean chlorophyll *a* + *b* (n = 3, ±S.E.) measured at 0, 7, 14, 21 and 28 days after onset of the drought experiment in eight sugarcane clones.

Chlorophyll *a* / *b* ratios responded distinctively depending on the clones under water deficiency (Figure 5). The clones HoCP01-523, TCP89-3505 and CP92-675 showed constant results, around 3.0–3.5 during all the period. For the clones HoCP85-845 and TCP87-3388, this ratio was constant up to 14 DAT, and slightly decreased, up to 21 DAT, to about 2.8–3.0, increasing after water re-establishment, until reaching the initial values. As for the clones TCP02-4620 and TCP02-4624, the ratio was also constant up to 14 DAT, but decreased to 2.0 between 14 and 21 DAT.

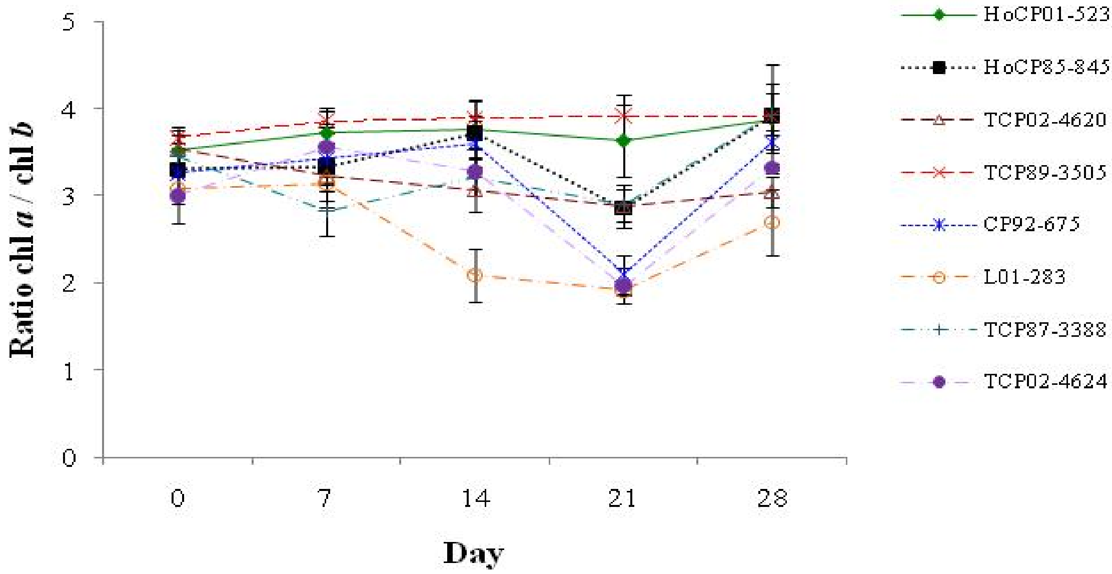

Fig. 5—Mean ratio chlorophyll *a*/chlorophyll *b* (n = 3, ±S.E.) measured at 0, 7, 14, 21 and 28 days after onset of the drought experiment in eight sugarcane clones.

The clone L01-283 showed chlorophyll values similar to the initial ones up to 7 DAT, decreasing to 2.0 between 7 and 21 DAT, and values increased after water re-establishment up to the initial value. According to the results, it is possible to infer that tolerant clones show a mechanism of defence against drought, keeping chl *a* / chl *b* ratio about 3.0–3.5. A second group of clones show this ratio up to 14 DAT, and they could be considered intermediately tolerant. The sensitive clones would be the ones that do not show the established values during drought.

Contradictory results can be found in literature, in relation to effects of water deficiency on the chlorophyll *a* / *b* ratio. Pardo and Delgado (1989) concluded that the chl *a* / chl *b* ratio decreased for sugarcane clones that were tolerant to drought due to an increase in the chlorophyll *b* content, as a possible mechanism of defence. Other authors showed that this ratio was not affected neither for tolerant nor susceptible clones of *Eragrotis curvula* under water deficiency (Colom and Vazzana, 2003).

RWC reduced as drought evolved, but the response differed among clones (Figure 6). After 21 DAT, the clone HoCP01-523 showed lower decrease in RWC values (81.2%), followed by the TCP89-3505 (78.5%) and HoCP85-845 (76.5%).

Lower values were observed for the clones L01-283 (60.3%) and TCP02-4624 (63.5%). During water re-establishment, RWC values increased for all clones. The clones HoCP01-523, HoCP85-845, TCP02-4620, TCP89-3505 and TCP87-3388 reached the values reported before drought. The clones CP92-675 and TCP02-4624 intermediately restored the original values, whereas, L01-283 showed the lowest results (70.6%).

The characteristic of maintaining a higher amount of water in the leaves during drought results in higher tolerance for some clones, because the higher capacity of saving water does not limit the CO_2 absorption so much and, consequently, the efficiency of the photosynthetic process, due to better preservation of the integrity of the PSII system (Maxwell and Johnson, 2000). Then, it can be inferred that the clones with lower decrease in the RWC and higher capacity to restore the initial conditions after water re-establishment can be considered tolerant. Besides, Silva *et al.* (2007) reported that drought tolerance was found to be positively correlated to RWC in sugarcane under drought conditions. Therefore, this parameter might be used as a selection criterion to differentiate sugarcane clones under drought stress.

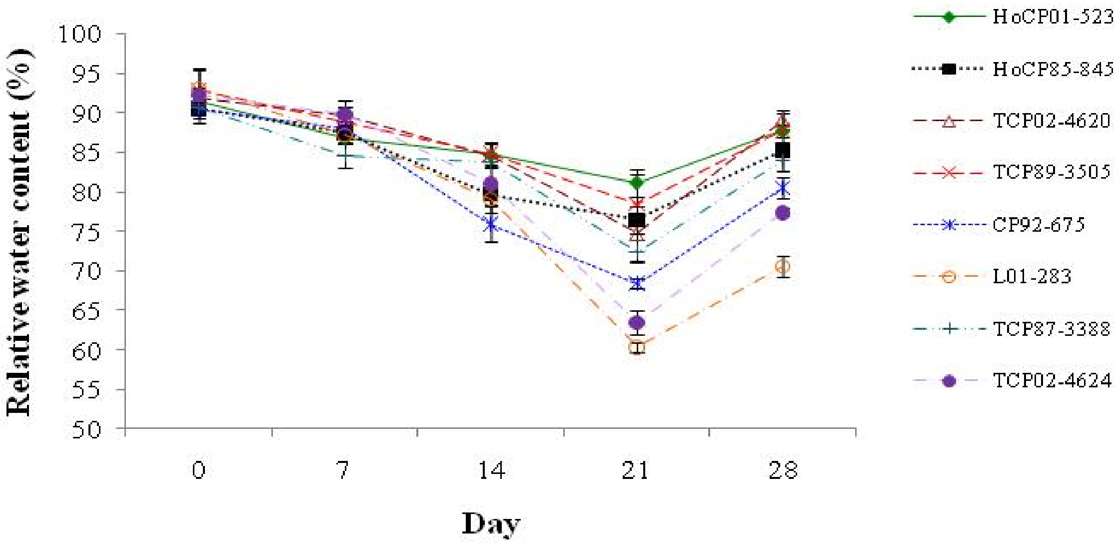

Fig. 6—Mean leaf relative water content (RWC) (n = 3, ±S.E.) measured at 0, 7, 14, 21 and 28 days after onset of the drought experiment in eight sugarcane clones.

The present study showed that it is possible to distinguish sugarcane clones under water deficiency conditions during initial growth measuring chlorophyll and leaf relative water content. Therefore, these traits could be considered as useful tools during the crop breeding procedure in order to make this process faster and cheaper. Besides, this information should be used in hybridisation programs to find parents with greater ability to transfer drought tolerance genes as expressed in different agronomic traits.

In conclusion, according to the results of this experiment, it was possible to separate the eight clones studied into three groups. The clones CP92-675, HoCP01-523, TCP89-3505 and TCP87-3388 showed lower chlorophyll degradation and higher capacity to preserve water in the leaves during the initial growth; so, they can be considered as tolerant to drought. The clones HoCP85-845, TCP02-4620 and TCP02-4624 were intermediately tolerant, whereas, the L01-283 was susceptible to drought due to irreversible chlorophyll degradation.

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ÉVALUATION DE LA CHLOROPHYLLE ET DE LA TENEUR EN EAU RELATIVE DES FEUILLES COMME INDICATEURS DE LA TOLÉRANCE A LA SÉCHERESSE SUR LA CROISSANCE PRECOCE DE LA CANNE À SUCRE

Par

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MOTS-CLÉS: *Saccharum* spp., Stress Hydrique,
Indice SPAD, Pigments Photosynthétiques, RWC.

Résumé

LE MANQUE d'eau est le stress environnemental principal qui affecte la production de canne à sucre dans le monde entier. Les caractéristiques physiologiques directement liées au stress hydrique sont des indicateurs appropriés pour le criblage de variétés de canne à sucre tolérantes à la sécheresse. Le but de cette étude était d'évaluer la capacité de ces paramètres physiologiques à identifier des variétés de canne tolérantes ou sensibles à la sécheresse Ces paramètres incluaient la teneur estimée en chlorophylle des feuilles à l'aide de l'indice SPAD, les pigments photosynthétiques (chlorophylles *a*, *b*, *a* + *b* et le rapport a/*b*) et la teneur en eau relatives des feuilles (RWC). L'expérience a été effectuée sous serre. Huit variétés (CP92-675, HoCP85-845, HoCP01-523, L01-283, TCP87-3388, TCP89-3505, TCP02-4620 et TCP02-4624) de tolérance variable à la sécheresse ont été employées pour évaluer la relation entre ces paramètres et tolérance de sécheresse. Sur des cannes plantées âgées de 90 jours , un stress hydrique fut appliqué en arrêtant l'irrigation pendant 21 jours, puis en puis en irrigant de nouveau pendant 6 jours. Pendant le stress hydrique, une diminution progressive de l'indice de SPAD, des chlorophylles *a*, *b*, *a* + b et du RWC a été observée quelque soit la variété. Cependant la diminution fut moindre pour les variétés CP92-675, HoCP01-523, TCP87-3388 et TCP89-3505. Après réhydratation, ces 4 variétés récupérèrent plus rapidement, bien que les valeurs initiales des paramètres ne furent pas atteintes. HOCP85-845, TCP02-4624, TCP02-4620 et L01-283 ont montré un effet négatif et résiduel élevé du stress hydrique en termes de teneur en chlorophylle et RWC. Le système photosynthétique de L01-283 fut le plus endommagé, cette variété n'ayant pas montré de réponse lors de la remise en eau. Parmi des paramètres liés à la chlorophylle, le rapport des chlorophylles a/*b* a été peu affecté par le stress hydrique chez les variétés HoCP01-523, TCP89-3505, TCP87-3388 et CP92-675. Ainsi la chlorophylle et RWC pourraient être considérés comme des paramètres capables de différencier des variétés sensibles et tolérantes à la sécheresse. Nous en avons conclu que les variétés CP92-675, HoCP01-523, TCP89-3505 et TCP87-3388 étaient tolérantes, HoCP85-845, TCP02-4620 et TCP02-4624 intermédiaires, et L01-283 sensible à la sécheresse pendant la croissance initiale.

EVALUACIONES DE CLOROFILA Y CONTENIDO RELATIVO DE AGUA EN LA HOJA COMO INDICADORES DE TOLERANCIA A LA SEQUÍA DURANTE EL CRECIMIENTO INICIAL DE LA CAÑA

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PALABRAS CLAVE: *Saccharum* spp., Stress Hídrico, Índice SPAD, Pigmentos Fotosintéticos, RWC.

Resúmen

LA DEFICIENCIA de agua es el estrés ambiental más importante que afecta a la producción mundial de caña de azúcar. Las características fisiológicas asociadas con la tolerancia a la sequía pueden ser usadas como indicadores de la tolerancia a la sequía de los clones de caña de azúcar y de esta manera reducir los efectos negativos sobre el rendimiento del cultivo. El objetivo de este estudio fue evaluar la utilidad de usar parámetros fisiológicos para identificar clones de caña de azúcar tolerantes y susceptibles a la sequía. Estos parámetros incluyeron contenido estimado de clorofila en las hojas calculado por índice SPAD, pigmentos fotosintéticos (clorofila A, B, A + B y la relación de la clorofila a / clorofila b) y el contenido relativo de agua en la hoja (RWC). El experimento se llevó a cabo bajo condiciones de invernadero. Ocho clones (CP92-675, HoCP85-845, HoCP01-523, L01-283, TCP87-3388, TCP89-3505, TCP02-4620 y TCP02-4624), variables en cuanto a tolerancia a la sequía fueron utilizados para evaluar la relación entre estos parámetros y tolerancia a la sequía. El estrés hídrico fue impuesto por deprivación de riego por 21 días y posterior irrigación durante 6 días a plantas de 90 días de edad. Los resultados mostraron que durante el periodo de stress por sequía los parámetros índice SPAD, clorofila A, B, A + B y RWC mostraron reducción progresiva independientemente del clon, pero la reducción fue mucho menor en CP92-675, HoCP01-523, TCP87-3388 y TCP89-3505. Después de la rehidratación, estos clones se recuperaron más rápidamente de la condición de estrés, aunque los valores iniciales no fueron alcanzados. HOCP85-845, TCP02-4624, TCP02-4620 y L01-283 mostraron un efecto residual negativo causado por la fuerte sequía en términos de contenido de clorofila y RWC, y el aparato fotosintético de L01-283 fue el más dañado ya que este clon no mostró respuesta después del riego. Entre los parámetros de la clorofila, la relación clorofila a / b fue poco afectada por el déficit de agua en los clones HoCP01-523, TCP89-3505, TCP87-3388 y CP92-675. Los resultados sugieren que la clorofila y la RWC podrían ser considerados para diferenciar entre clones tolerantes y susceptibles a la sequía. Concluimos que los clones CP92-675, HoCP01-523, TCP89-3505 y TCP87-3388 se comportaron como tolerantes, HoCP85-845, TCP02-4620 y TCP02-4624 como intermedios, y L01-283 como susceptible a la sequía durante su crecimiento inicial.