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Water Relation Studies in Water Stressed Sugarcane (*Saccharum officinarum* L.)

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ABSTRACT

This research was conducted to study the water relations and photosynthesis in mature leaf cells of drought sensitive (BL-4) and drought tolerant (CP 43/33) varieties of sugarcane (*Saccharum officinarum* L.). Moreover, it was aimed at selecting suitable indicators, which could be used to select drought resistant varieties. Turgor pressure of control and mannitol induced water stressed seedlings was measured by using a cell pressure probe. Almost similar turgor values were found in both varieties under control conditions. However, the decline in turgor was faster in BL-4 than in CP 43/33 with time after their exposure to 200 mol m^{-3} mannitol solution. Bulk leaf osmotic pressure and cell wall solutes were higher in BL-4 than in CP 43/33. CP 43/33 showed a better control in maintaining higher turgor and more balanced osmotic pressure under water stress. BL-4 exhibited higher net photosynthesis rate, stomatal conductance, sub-stomatal CO_2 and transpiration rate than CP 43/33. These results showed that CP 43/33 (at seedling stage) with high turgor, regulated osmotic adjustment and lower transpiration rate can be designated as drought tolerant.

Key words: Sugarcane; Drought Tolerance; Turgor Pressure; Osmotic Adjustment

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is a tropical crop and requires a warm and humid climate. It is cultivated in irrigated areas of Pakistan. In most of the sugarcane growing areas, irrigation water is insufficient to meet its requirements. Rainfall in Pakistan hardly supplements the water requirements of this crop. As a result, the crop is subjected to water stress during the peak growth stages and depresses tillering and cane formation resulting in low yields. In spite of all these factors sugarcane is the third most important economic crop of the country after wheat and cotton. It is grown on an area of 964.5 thousand hectares annually (Anonymous, 1997). Sugarcane varieties differ markedly in their growth behaviours and yield potentials under water stress conditions (Malik *et al.*, 1991). Water stress in soil causes reduction in the water potential gradient between soil and plant. This reduction results in decreased water and nutrient uptake (Schultz & Mathews, 1993), stomatal closure (Ray & Sinclair, 1997), reduction of photosynthesis and dry matter production (Zinselmeier *et al.*, 1995), which ultimately leads to reduced growth and low yield (Schonfeld *et al.*, 1988).

Drought often induces changes in plant water relation parameters such as cell turgor pressure (P), osmotic pressure (π) and water potential (Ψ) (Saliendra & Meinzer, 1991). Reduction in water potential induces stomatal closure resulting in a decline in the photosynthesis, leaf growth and ultimately yield. Saliendra and Meinzer (1991) reported a leaf water potential threshold of -0.8 to -0.9 MPa at which sugarcane shoot growth and stomatal opening began to decline and both were completely inhibited at -1.3 to -1.7 MPa. Most of the plants may avoid this situation by increasing cellular π (Arif & Tomos, 1995). An osmotic adjustment of -0.48 MPa in sugarcane has been reported when irrigation was withheld for five weeks (Saliendra & Meinzer, 1991). Such osmotic adjustment creates a water potential gradient that promotes entry of water into the cells leading to maintenance of turgor and ultimately growth, both in roots and shoots (Arif & Tomos, 1995).

The water relation parameters have been studied in sugarcane on whole plant or organ basis by using the pressure chamber (Saliendra & Meinzer, 1991). This type of research work is suited for relative studies. It can, however, be misleading for the absolute results. Use of a cell pressure probe (Hüsken *et al.*, 1978) makes it possible to measure P directly from individual cells. This technique along with osmometer measurements makes it possible to determine tissue osmotic pressure, cell wall solutes and water potential. The work on the development of drought tolerant sugarcane lines that are suitable for cultivation in areas of water deficits is a dire need of the countries like Pakistan. The present work aimed at exploring water relations and photosynthesis of a drought tolerant and a sensitive sugarcane cultivar under water stress conditions.

MATERIALS AND METHODS

Plant material and experimental conditions. Two sugarcane cultivars, BL-4 (drought sensitive) and CP 43/33 (drought tolerant), were selected on the basis of their contrasting performance against drought (Malik *et al.,* 1991). The cultivars were sown in gravel culture in 9 dm^2 plastic pots in a wire house. The pots were watered daily and were fertilised once with 200 ml each of 2 mM Ca $(NO_3)_2$ and 1 mM K_2SO_4 (Michelena & Boyer, 1982). Six weeks old seedlings were transferred to hydroponic culture. The seedlings were grown on polystyrene sheets suspended over aerated full strength (100%) Hoagland solution (Hoagland & Arnon, 1950).

Stress induction and measurement of turgor pressure (P). After 60 days of germination, the seedlings were divided into two groups (i) water stress induced by 200 mM mannitol + full strength Hoagland solution and (ii) control in full strength Hoagland solution. To impose stress, the seedlings were transferred to mannitol + full strength Hoagland solution in a single step. Turgor was directly measured from individual lower epidermal cells from the mature zone of fully expanded upper most leaves by using the cell pressure probe (Hüsken *et al.,* 1978). Turgor of stress imposed plants was measured continuously from neighbouring cells up to 45 minutes of stress. Then the stress was removed by transferring the seedlings again to the full strength Hoagland solution. After the removal of stress, P was measured for further 15 minutes.

Measurement of osmotic pressure (π). To measure π, fully expanded upper most leaves from non stressed plants were rinsed with distilled water to eliminate salts and dust that may have accumulated on the leaves surface. The rinsed leaves were blotted by dry paper tissue and then were allowed to dry for 20 minutes. The leaves were then frozen. After thawing, individual leaves were crushed and sap was collected by centrifugation. The supernatants were collected in eppendorff tubes and their π was measured using a freezing point osmometer calibrated in mOsm kg^{-1} water. The values obtained in mOsm kg^{-1} water were converted to pressure unit (MPa) by dividing it by 407 mOsm kg^{-1} MPa^{-1} (Nobel, 1983). The π of tissue sap was assumed to be equivalent to individual cell π. Such observations were made from uppermost fully expanded leaves each from six non-stressed plants.

Osmotic pressure of cell wall solutes (π_w). The osmotic pressure of cell wall solutes (π_w) was determined by the difference of P, cell wall transpiration tension (P_w) and the tissue π (Arif & Tomos, 1995).

$$\pi_w = \pi - P + P_w$$

P_w was calculated by measuring P in a continuously transpiring leaf, and then after immersing the entire leaf in the hydroponics root medium for one hour. The P values increased and stabilised to a higher value. This increase in P is assumed to be a quantitative estimation of the cell wall transpiration tension (Arif & Tomos, 1993). P_w was measured for six individual uppermost fully expanded leaves of non-stressed plants of both varieties. The same leaves were then used for measurement of π.

Measurement of photosynthesis and transpiration parameters. The photosynthetic parameters like net photosynthetic rate, stomatal conductance and sub-stomatal CO_2 and transpiration rate of fully expanded leaves of non-stressed plants were measured by using a portable Infra-Red Gas Analyser system incorporating a Parkinson Leaf Chamber (Model LCA-3, Analytical Development Company, Hoddesdon, Herts, UK). The equipment measures the partial pressure of CO_2 entering and leaving the leaf chamber, the relative humidity of the air leaving the leaf chamber, the photon flux density incident on the chamber and the air temperature inside it. From these data, net photosynthesis rate, transpiration rate, stomatal conductance and the concentration of CO_2 in the sub-stomatal cavity were calculated. The data were recorded between 0900 to 1030 hours from non-stressed plants. The photosynthetic active radiation (PAR) was between 1300-1400 $\mu mol\ m^{-1}\ s^{-1}$ during the observation period.

RESULTS AND DISCUSSION

BL-4 showed almost similar P (0.43 MPa) to CP43/33 (0.42 MPa) under control conditions (Fig. 1). There was a rapid and continuous decline in P in BL-4 when water stress was imposed to roots being 0.04 MPa after 45 minutes of stress. However, within 15 minutes of stress removal, the original P was restored. In CP43/33, P decreased to 0.08 MPa after 10 minutes of stress. Thereafter, the cells started recovering their P, still under stress. P restored up to 0.22 and 0.25 MPa after 20 and 30 minutes of stress, respectively, and then stabilised for the remaining period of measurement. Original P was, however, regained within 15 minutes of the removal of stress as in the case of BL-4 (Fig. 1). BL-4 exhibited higher (1.16 MPa; 0.63 MPa) bulk leaf and cell wall solutes osmotic pressure than CP 43/33 being 0.93 MPa and 0.44 MPa, respectively (Table I).

Almost similar photosynthesis rates were observed (Table II) in both BL-4 (13.65 μmol CO_2 m^{-2} s^{-1}) and CP 43/33 (13.51 μmol CO_2 m^{-2} s^{-1}). However, the transpiration rate (0.5 mmol m^{-2} s^{-1}) was higher in BL-4 than in CP 43/33 (0.4 mmol m^{-2} s^{-1}). BL-4 exhibited a higher stomatal conductance (0.21 mol m^{-2} s^{-1}) than CP 43/33 (0.16 mol m^{-2} s^{-1}) (Table. II). A higher concentration of sub-stomatal CO_2 (114.23 μL L^{-1}) in BL-4 was measured than in CP 43/33 (105 μL L^{-1}) (Table II).

It is evident from the results that both the cultivars (BL-4 & CP 43/33) had almost similar P which declined sharply (Fig. 1) after the stress was imposed. The first symptom of water stress is a decline in cell P (Saliendra & Meinzer, 1991) and it happens so when plants loose water faster than it is transported into the roots (Sharkey *et al.*, 1990). The P decreased linearly in BL-4 (drought sensitive) and never recovered during the observation period. However, when stress was removed the original P was restored within 15 minutes. In CP 43/33 (drought tolerant), P decreased more rapidly during the first 10 minutes of stress and then started recovering while still under stress. Schultz and Mathews (1993) observed that plants could adjust to water stress and continue their growth by maintaining the necessary P for growth and favourable water potential gradient between roots and the growing medium for water uptake. The recovery in P is possible when cells start synthesising organic (e.g. proline, glycinebataine, sucrose) and accumulating inorganic (e.g. K^+, Cl^-) osmotica (Delauney & Verma, 1993). This results in increased osmotic pressure within the symplast than the apoplast. As a result, a favourable water potential gradient is created for water uptake into the cells and helps maintaining the turgor necessary for stomatal opening (Saliendra & Meinzer, 1991) and growth (Arif & Tomos, 1993). However large genotypic variations in osmotic adjustments have been reported in wheat (Morgan, 1977; Moustafa *et al.*, 1996) and sugarcane (Saliendra & Meinzer, 1991). Abscicic acid (ABA) also accumulates in response to the physical phenomena of loss of water (Bray, 1993). More tolerant cultivar either enhances the release of ABA by a faster reduction of π or the stomata are more sensitive to an increased concentration of free ABA. The dynamics of ABA at very early stages of water stress might be a useful indicator for selecting drought tolerant lines as well. The results of the present study show that CP 43/33 (drought tolerant) has a capability of restoring its P under stress condition, which may be due to better osmotic adjustment mechanism. These results are in agreement with previous studies on sugarcane genotypes

Fig.1. Effect of mannitol induced water stress on turgor pressure in epidermal cells of uppermost fully expanded sugarcane leaves

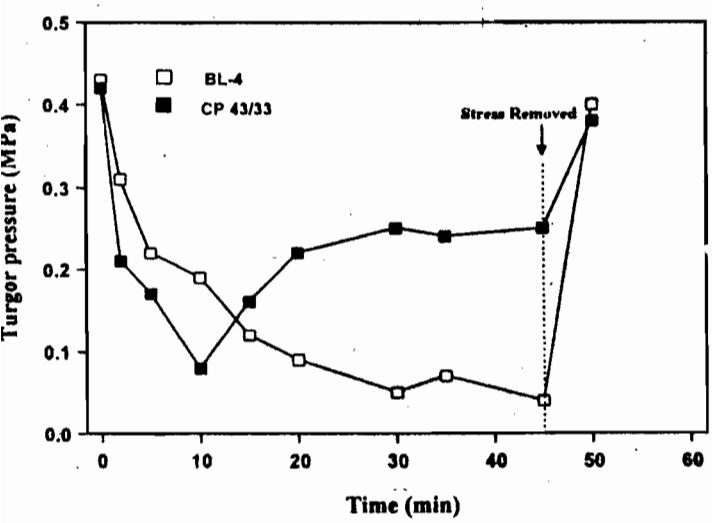

Table I. Osmotic pressure of bulk leaf and cell solutes of seedlings of two sugarcane cultivars in full strength Hoagland solution

Cultivar	Bulk leaf O.P.(MPa) (Means ± S.D.)	Cell wall Solute O.P.(MPa) (Means ± S.D.)
BL-4	1.16 ± 0.16	0.63 ± 0.06
CP 43/33	0.93 ± 0.06	0.44 ± 0.02

S.D.= Standard Deviation; O.P.= Osmotic Pressure

Table II. Photosynthetic measurements in sugarcane leaf

Parameter	BL-4 (n=6; Means ± S.D.)	CP 43/33 (n=6; Means ± S.D.)
Stomatal Conductance (mol m^{-2} s^{-1})	0.21 ± 0.08	0.16 ± 0.04
Sub-Stomatal CO_2 (μL L^{-1})	114.23 ± 16.36	105 ± 11.24
Net Photosynthesis Rate(μmol CO_2 m^{-2} s^{-1})	13.65 ± 01.92	13.51 ± 2.29
Transpiration Rate (mmol m^{-2} s^{-1})	0.51 ± 0.10	0.40 ± 0.06

S.D.= Standard Deviation; O.P.= Osmotic Pressure

subjected to water stress by Saliendra and Meinzer (1991). They proposed that differences among sugarcane cultivars in the rates of water use or in efficiency of extracting soil water might be largely due to osmotic and elastic adjustments in response to drought. Munns (1988) challenged the belief that the significance of osmotic adjustment lies in its effects on turgor. However, many other scientists (Morgan, 1977; Arif & Tomos, 1995; Schultz & Mathews, 1993; Moustafa *et al.,* 1996) have stressed importance of turgor in osmotic adjustment. In sugarcane, genotypic variation seems to exist in osmotic adjustment to water stress at seedling stage. This could be used as a possible selection criterion to breed sugarcane varieties with an improved drought stress tolerance.

The drought sensitive cultivar (BL-4) exhibited higher transpiration rate and stomatal conductance than drought tolerant cultivar (CP43/33). While almost similar photosynthesis rates in both the cultivars were measured (Table II). Higher transpiration rate and stomatal conductance are associated with more rapid depletion of soil water, when the net photosynthetic rate is similar (Saliendra & Meinzer, 1991). It means that for converting the same amount of CO_2 into organic compounds, BL-4 requires more water than CP 43/33. This shows that CP 43/33 has more control on its stomatal conductance. Working on maize hybrids, Nissanka *et al.* (1997) reported that photosynthesis tended to be slightly higher, and transpiration and stem water potential tended to be slightly lower in a drought tolerant than in a drought sensitive hybrid. So, higher transpiration rates and stomatal conductance as well as sub-stomatal CO_2 of a genotype at same photosynthesis rate could be indicators for drought sensitivity.

CONCLUSIONS

It is concluded from the results that sugarcane cultivar CP 43/33 has more ability in maintaining higher turgor pressure and osmotic adjustment under drought than BL-4. CP43/33 also had less stomatal conductance and transpiration rate. Although these results are based on the comparison of only two cultivars of sugarcane, such studies should be expanded to a wider range of cultivars and populations.

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