

Some Growth, Photosynthetic and Anatomical Attributes of Sugarcane Genotypes under NaCl Salinity

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ABSTRACT

A tank experiment was conducted to study the effect of increased root zone salinity on some growth, photosynthetic and anatomical attributes of a tolerant (CPF-213) and a sensitive (L-116) genotype of sugarcane in comparison with a standard (CP-4333). Results revealed that although dry weight, water content, leaf area and photosynthetic parameters were adversely affected in all the genotypes, the effect of salinity was greater on the sensitive genotype. Determination of some leaf anatomical features revealed that applied salinity reduced the mesophyll area but the extent of reduction was greater in the sensitive genotype. A correlation of some photosynthetic attributes with anatomical features revealed that mesophyll area carried a significant ($r=0.998$; $P<0.01$) relationship with stomatal conductance. It was concluded that reduction in overall growth of the sugarcane leaves appeared due to effect of salinity, primarily on the gas exchange parameter and secondarily on the anatomical features.

Key Words: Anatomy; Growth; Photosynthesis; Salinity; Sugarcane

INTRODUCTION

Increased soil salinity has been taken as a noxious factor for most of the glycophytes. It induces specific changes at cell, tissue and organ levels. These changes are morphological, physiological and anatomical in nature (Cheesman, 1988; Lauchli & Epstein, 1990; Shannon, 1997; Isla *et al.*, 1998). Soil salinity reduces plant growth by perturbing different biochemical/ physiological processes (Zeng & Shannon, 2000). As much of the world's soils are salt-affected to a considerable extent (Cheesman, 1988), there has been keen interest in the development of crop plants displaying tolerance to the salinity (Rozeff, 1995; 1998).

It has been revealed from many studies that photosynthetic parameters are greatly affected by increased levels of salinity (Shabala *et al.*, 1998). The changes in the uptake of CO₂ and subsequently its assimilation by the leaves is greatly affected by salinity. This is mainly linked with the stomatal oscillation. At leaf level, the anatomical changes induced by salinity are smaller leaves, reduced stomatal frequency and changes in the mesophyll area (Javed *et al.*, 2000). All these factors indicate a close relationship with each other, and hence all of them play an important role in the reduction in final yield and productivity.

Sugarcane has been ranked as moderately sensitive to salinity (Shannon, 1997). However, genotypic differences are present in this species for salinity tolerance (Wahid *et al.*, 1997; Akhtar, 2000). It shows reduced growth under salinity, which can be assigned to reduced photosynthetic efficiency and occurrence of certain specific changes in the leaf (Meinzer *et al.*, 1994). This study reports changes in some growth photosynthetic and anatomical attributes of leaves of two differentially salt tolerant genotypes at grand

growth stage, in comparison with standard tolerant genotype, CP-4333 (Wahid *et al.*, 1997).

MATERIALS AND METHODS

Plant material. Genotypes of sugarcane (*Saccharum officinarum* L.) selected for this study were rigorously screened previously under NaCl salinity application. CPF-213 with salt tolerance limit (EC₅₀) of 18.5 dS m⁻¹ and L-116 with EC₅₀ of 11.2 dS m⁻¹ were declared salt tolerant and sensitive, respectively (Akhtar, 2000). CP-4333 designated as standard genotype, had previously been established as salt tolerant (Wahid *et al.*, 1997).

Experimental and growth details. Experiment was conducted in tanks (7×3.5×0.6 m (deep) filled with a loam soil (20 metric tonnes) and lined with double layer of polyethylene sheets, during 1994-95 and 1995-96. Experiment was laid out in completely randomized fashion with three replications. Physico-chemical characteristics of the soil determined with standard methods (Qureshi & Barrett-Lennard, 1998) were: Organic matter 1.2%, total N 0.72%, cation exchange capacity 16.7 meq 100 g⁻¹, pH 7.6, ECe 2.5 dS m⁻¹, sodium adsorption ratio 0.17, Na⁺ 2.48 mmol L⁻¹, Cl⁻ 8.4 mmol L⁻¹, SO₄ 3.6 mmol L⁻¹ and Ca+Mg 30 mmol L⁻¹, as determined from extracted solution of the soil paste. Twenty-four single noded sets of each genotype were planted in tank. After sprouting, 12 plants per tank were finally retained to maintain at least 30 cm row to row and plant to plant distance. Average temperature during the experimental year 1995-96 was between 36±5°C (summer March to September) and 17±6°C (winter October to February); relative humidity 62±3% (summer) and 78±8% (winter) and rainfall was 390 mm.

Treatment application. Two salinity levels i.e., 8 and 12 dS m⁻¹ were developed at maturity stage (170 days after

planting) using sodium chloride (99% pure). The salinity levels were gradually developed (by dissolving NaCl in tap water) in six days. The plants were irrigated with subsoil water ($EC=0.8 \text{ dS m}^{-1}$; $SAR=4.9$) whenever needed. Agronomically recommended doses of N, P and K (150, 100 and 100 kg ha^{-1} , respectively) were splitted to add at three intervals i.e., 50 and 150 days after planting. The ECE of the soil was checked at the termination of experiment, which was slightly higher than the original ECE of the soil.

Growth measurements. Plants were harvested 80 days after the application of salinity. Harvested plants were separated in to leaves, stem and roots. The roots were carefully cleaned by keeping overnight in running tap water. Dry weights of young leaf, old leaf and roots were recorded after drying continuously at 70°C . Area of young and old leaf was measured of intact plants as maximum length \times maximum width \times 0.68 (correction factor calibrated for all leaves).

Anatomical measurements. Third fully expanded leaf samples of all the genotypes were used for preparing hand cut thin sections. These tissues were preserved in formalin: acetic acid: alcohol: water (10:5:35:50) for 24 h and then stored in 70% alcohol until use (Wahid *et al.*, 1998). Free hand cut sections were dehydrated in alcohol series and stained with safranin and light green stains. Measurements of various cells and tissues were taken with an ocular micrometer and exact values were calculated with a factor derived by comparing ocular with stage micrometers.

Photosynthetic measurements. Photosynthetic attributes of third fully expanded leaves were determined for photosynthetic rate (A), substomatal CO_2 concentration (C_i), transpiration rate (E) and stomatal conductance (gs), with an infrared gas analyzer (IRGA, Model LCA-4, UK).

Statistical analysis. Data of different parameters were analyzed using MSTAT-C programme to determine

statistically significant difference among the genotypes, treatments and their interactions.

RESULTS AND DISCUSSION

Dry weight. All the genotypes indicated significant ($P<0.01$) differences for dry weight of young leaf, old leaf, stem and root under increased salinity. The interaction of genotype \times salinity was also significant ($P<0.01$) for the parts, except for the old leaf (Table I). Dry weight of young leaves and roots in CP-4333 and CPF-213 was enhanced at 8 dS m^{-1} as compared to respective control, but not in L-116 and for old leaf and stem. However, at 12 dS m^{-1} , all the genotypes indicated marked reduction in dry mass of all the parts, but the extent of reduction was greater in L-116 when compared with CP-4333 and CPF-213.

Water content. There was a significant ($P<0.01$) reduction in water content of young and old leaf, stem and root under increased salinity. No interaction ($P>0.05$) between genotypes \times salinity was noted in old leaf, stem and roots for this parameter, except the young leaf where significant ($P<0.01$) interaction was seen (Table I). CP-4333 and CPF-213 were able to maintain higher water content in all parts even at 12 dS m^{-1} but L-116 failed to do so and indicated much decreased water content at 12 dS m^{-1} (Table I).

Leaf area. All the genotypes indicated significant ($P<0.01$) difference for the area of young leaf, indicating significant ($P<0.01$) impact of salinity (Table I). Moreover, the interaction of both these factors was also significant ($P<0.01$). For old leaf, there was no significant ($P>0.05$) difference among genotypes, salt treatment and their interaction. Under natural conditions, CP-4333 had low area of young leaf, which was slightly enhanced under 8 dS m^{-1} ,

Table I. Changes in some growth parameter of sugarcane genotypes under levels of salinity

| Genotypes | Salt levels (dS m^{-1}) | Dry weight (g) | | | | Water content (%) | | | | Area (cm^2) | |
|--|---------------------------------------|----------------|--------------------|---------|--------------------|-------------------|--------------------|--------------------|--------------------|------------------------|--------------------|
| | | Young leaf | Old leaf | Stem | Root | Young leaf | Old leaf | Stem | Root | Young leaf | Old leaf |
| CPF-213 | Control | 2.47 | 4.63 | 188.4 | 7.03 | 68.4 | 56.4 | 70.6 | 72.3 | 137.3 | 198.3 |
| | 8 | 3.07 | 4.27 | 185.3 | 8.08 | 65.3 | 54.3 | 66.3 | 68.4 | 146.5 | 196.5 |
| | 12 | 1.80 | 3.77 | 161.9 | 5.37 | 58.4 | 53.6 | 58.6 | 62.6 | 89.5 | 191.9 |
| CP-4333 | Control | 3.00 | 4.83 | 201.3 | 8.50 | 70.4 | 57.3 | 71.4 | 75.4 | 154.9 | 200.1 |
| | 8 | 3.03 | 4.23 | 188.9 | 9.73 | 66.3 | 55.3 | 65.3 | 67.4 | 152.1 | 196.1 |
| | 12 | 2.73 | 3.51 | 172.6 | 6.07 | 57.6 | 52.6 | 56.6 | 62.1 | 108.3 | 191.1 |
| L-116 | Control | 3.08 | 4.70 | 202.6 | 5.83 | 69.6 | 54.8 | 72.4 | 74.6 | 149.0 | 198.2 |
| | 8 | 2.10 | 3.10 | 168.4 | 4.90 | 59.3 | 52.3 | 60.4 | 67.5 | 95.8 | 191.3 |
| | 12 | 1.70 | 2.70 | 143.3 | 2.70 | 47.9 | 52.6 | 50.9 | 54.0 | 78.8 | 189.8 |
| Significance of variance sources (F-values) | | | | | | | | | | | |
| SOV | df | | | | | | | | | | |
| Genotypes (G) | 2 | 51.61** | 43.95** | 7.65** | 27.44** | 4.62** | 0.74 ^{NS} | 2.06** | 3.44** | 86.3** | 0.23 ^{NS} |
| Salinity (S) | 2 | 24.55** | 33.30** | 38.99** | 0.63 ^{NS} | 28.91** | 1.85 ^{NS} | 31.85** | 27.41** | 23.5** | 1.63 ^{NS} |
| GxS | 4 | 5.31** | 0.56 ^{NS} | 4.40** | 4.12** | 2.44** | 0.10 ^{NS} | 1.08 ^{NS} | 1.41 ^{NS} | 12.0** | 0.07 ^{NS} |

** Significant at $p<0.01$ and NS, non-significant

while rest of the genotypes showed reduced leaf area over control at this level. However, at 12 dS m⁻¹ all the genotypes displayed a reduced leaf area, but a greater reduction was observed in L-116.

Photosynthetic parameters. The genotypes and salinity levels indicated significant (P<0.01) differential responses for all the photosynthetic parameters i.e. photosynthetic rate (A), sub-stomatal concentration (Ci), rate of transpiration (E) and stomatal conductance (gs) (Table II). The interaction of genotypes and salinity levels were also significant (P<0.01) for these parameters except for stomatal conductance where no interaction was noted. For all these parameters, CPF-213 and CP-4333 were the best to have greater A, Ci, E and gs, while L-116 indicated a poor

Anatomical studies. All the genotypes differed significantly (P<0.01) for interveinal distance, vascular bundle area, mesophyll cell area and epidermal cell area under increased levels of salinity (Table III). The exception was mid vein size, where no difference (P>0.05) was found among genotypes but, here again, applied salinity affected it significantly (P<0.01). Significant (P<0.01) interaction of genotypes and salinity was found only for mesophyll cell area while no interaction (P>0.05) was noted for other parameters. The cane genotypes showed a reduction in all these parameters but the impact was greatly prevalent on L-116 (Table III). On the contrary, CP-4333 indicated a minimum reduction in these parameters.

Sugarcane is prone to salinity at various growth stages

Table II. Changes in some gas exchange parameters of third fully expanded leaves of sugarcane genotypes under increased levels of salinity

| Genotypes | Salt levels (dS m ⁻¹) | Photosynthetic rate (A) (μmol m ⁻² s ⁻¹) | Sub-stomatal CO ₂ conc. (Ci) (μmol mol ⁻¹) | Transpiration rate (E) (mol m ⁻² s ⁻¹) | Stomatal conductance (gs) (mol m ⁻² s ⁻¹) |
|--|-----------------------------------|---|---|---|--|
| CPF-213 | Control | 9.29 | 81.54 | 1.18 | 0.17 |
| | 8 | 6.03 | 60.53 | 1.09 | 0.12 |
| | 12 | 5.26 | 54.90 | 1.07 | 0.10 |
| CP-4333 | Control | 8.91 | 79.35 | 1.26 | 0.18 |
| | 8 | 7.59 | 62.30 | 0.97 | 0.15 |
| | 12 | 5.45 | 54.90 | 0.90 | 0.11 |
| L-116 | Control | 8.92 | 77.13 | 1.22 | 0.15 |
| | 8 | 4.26 | 44.11 | 0.88 | 0.08 |
| | 12 | 2.57 | 32.14 | 0.66 | 0.04 |
| Significance of variance sources (F-values) | | | | | |
| SOV | df | | | | |
| Genotypes (G) | 2 | 29.87** | 348.64** | 35.92** | 5.29** |
| Salinity (S) | 2 | 119.13** | 1533.60** | 153.95** | 16.86** |
| GxS | 4 | 7.75** | 56.17** | 16.84** | 1.54 ^{NS} |

** Significant at p<0.01 and NS, non-significant

Table III. Anatomical characteristics of third fully expanded leaf of differentially salinity tolerant sugarcane genotypes under increased NaCl salinity

| Genotypes | Salt levels (dS m ⁻¹) | Epidermal cell area (μ ²) | Mesophyll cell area (μ ²) | Interveinal distance (μ) | Mid vein size (μ) | Vascular bundle area (μ ²) |
|--|-----------------------------------|---------------------------------------|---------------------------------------|--------------------------|--------------------|--|
| CPF-213 | Control | 28.58 | 272.27 | 116.67 | 591.67 | 280.00 |
| | 8 | 26.83 | 272.27 | 96.67 | 566.66 | 249.92 |
| | 12 | 23.04 | 225.13 | 75.00 | 441.67 | 215.83 |
| CP-4333 | Control | 30.04 | 298.47 | 110.00 | 600.00 | 249.92 |
| | 8 | 26.83 | 267.00 | 83.33 | 561.66 | 224.58 |
| | 12 | 24.21 | 246.07 | 76.67 | 500.00 | 180.83 |
| L-116 | Control | 26.83 | 272.23 | 117.22 | 608.33 | 180.83 |
| | 8 | 22.85 | 251.30 | 71.66 | 470.00 | 140.00 |
| | 12 | 18.66 | 193.73 | 38.33 | 460.00 | 110.83 |
| Significance of variance sources (F-values) | | | | | | |
| SOV | df | | | | | |
| Genotypes (G) | 2 | 8.46** | 14.94** | 37.13** | 0.56 ^{NS} | 59.58** |
| Salinity (S) | 2 | 8.46** | 14.94** | 37.13** | 17.84** | 59.58** |
| GxS | 4 | 0.93 ^{NS} | 8.64** | 0.56 ^{NS} | 0.63 ^{NS} | 1.03 ^{NS} |

** Significant at p<0.01 and NS, non-significant

response for these parameters under increased root zone salinity.

like many other crops (Wahid *et al.* 1997; Rozeff, 1998; Akhtar, 2000). A more clearly evident impact of salinity is a

reduction in the overall growth of plant. This becomes more critical, when there is greater effect on leaves as well as root. Various studies have reported a greater impact of salinity on the shoot than root growth (Zeng & Shannon, 2000). This study indicated a greater effect of salinity on the shoot growth, particularly on the area and dry weight of leaf while root growth was comparatively less affected in the standard and tolerant genotypes, while the sensitive genotype indicated a severely depressed growth of these parameters (Table I). This corroborates with the finding of Rozeff (1998) and those of Plaut *et al.* (2000). These findings provoked the determination of some photosynthetic and anatomical characteristics, the changes in which are crucial under saline conditions. The decline in growth under salinity is considered mainly by the reduced photosynthetic efficiency of this crop (Meinzer *et al.*, 1994; Plaut *et al.*, 2000).

Salinity exposed plants often show a considerable reduction in water uptake. It results in the lowering of water content of various tissues including the leaves (Curtis & Lauchli, 1987; Colmer *et al.*, 1995). In these studies, the water content of leaves was significantly reduced in all the genotypes (Table I). Anatomical studies revealed that there was a considerable reduction in the size of mid vein and other vascular tissues (Table III). Moreover, the distance between the veins was also reduced considerably. These findings led the authors to conclude that reduction in the water and ion-conducting tissues caused a reduction in the transport of water. Moreover, reduced area of xylem and phloem cells offered more resistance to the flow of water, which required more energy to transport any quantity of water from root to the leaves (Joly, 1989). This ultimately resulted in greatly hampered growth performance by the sensitive genotype as compared to the tolerant and standard genotype. One of the important consequences of salinity is its impact on the mesophyll area, which tends to spill over to the reduced photosynthetic rate (Longstreth & Nobel, 1979). In this study, the standard and tolerant genotypes indicated the ability to curtail the reduction in surface area of leaves under salinity, which probably brought about a reduction in the size of epidermal cells (Table III). A reduction in the mesophyll area of the genotypes when correlated with stomatal conductance revealed a significant positive ($r=0.998$; $p<0.05$) relationship. This indicated that the initial impact of salinity was on the reduction in the size of mesophyll cells and reduced photosynthetic efficiency of the plant.

One among the many adverse effects of salinity is the reduction in the size of various tissues and cells (Solomons *et al.*, 1986; Reinhardt & Rost., 1995). In many plants, it has been reported that the most salt sensitive tissues include reduction in the mesophyll tissue, vascular bundles and cortical tissues (Curtis & Lauchli, 1987; Gucci *et al.*, 1998). Longstreth and Nobel (1979) recorded a significant increase

in the mesophyll area to cell area in the sensitive species. In this study, it was noted that irrespective of salinity tolerance potential of genotypes, applied salinity substantially affected almost all the tissues under investigation. There was significant reduction in the epidermal cell area (dermal tissue), a considerable reduction was noted in the mesophyll area (ground tissue) and interveinal distance, mid-vein size and vascular bundles were all substantially reduced (vascular tissue). However, there were significant genotypic differences (Table III). CPF-213 and CP-4333 indicated almost comparable responses to applied salinity; whereas, L-116 was very highly affected, showing maximum decrease in the parameters studied here. A reduction in mesophyll area under salinity carries significance in that, it reduces the photosynthetic capacity of leaves either by reducing the activities of enzymes or causing toxicity to photosynthetic membrane. This is plausible because enzymes either from halophytes or glycophytes are equally affected by salinity (Ohta *et al.*, 1988).

CONCLUSIONS

Growth reduction in sugarcane genotypes under salinity was primarily assignable to the inhibitory effect on the gas exchange parameters and the expansion of mesophyll area. All these changes reduced the photosynthetic efficiency. However, a reduced effect of salinity on the standard and tolerant genotypes suggests that both can be successfully grown under low to moderately saline areas.

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