



PROGRESSIVE CHANGES IN BIOCHEMICAL CHARACTERS OF SUGARCANE GENOTYPES UNDER SALINITY STRESS

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SUMMARY

Progressive stress responses were studied in contrasting sugarcane genotypes to elucidate the stress adaptative physiological and biochemical characters. Varieties showed differences with respect to parameters studied from the day four onwards. The tolerant variety Co 85019 maintained stability of plastid pigments (chlorophyll and carotenoids), higher proline concentrations and increased activity of oxidative enzymes. Sensitive genotype showed large reductions with regard to these characters. Lipid peroxidation, a measure of damage to the membrane system was higher in sensitive variety and difference between genotypes became significant from day four, indicating the progressive nature of adaptation in tolerant and its failure in sensitive varieties.

Key words: Carotenoids, chlorophylls, lipid peroxidation, oxidative enzymes.

INTRODUCTION

Soil salinity is regarded as one of the important abiotic stresses that limits yield in various crops. It has been reported that globally, one million hectares of sugarcane area is affected by salinity problem (Valdivia 1977, Rozeff 1995). Sugarcane is rated as moderately sensitive to salinity (Maas 1990). Under irrigated conditions productivity of sugarcane is considerably reduced due to salinity and sodicity. Salt tolerance mechanisms include array of characters, alterations and progressive adaptations resulting in stability of the genotype under stress situation. Progressive stress responses enlighten us about the metabolic changes during stress adaptation in tolerant types and any flaw that reflect on the metabolic failures resulting in sensitive behaviour. With an objective of studying progressive changes in sugarcane, an experiment was designed with a tolerant and a sensitive variety.

MATERIALS AND METHODS

Sugarcane genotypes Co 85019 (tolerant) and Co 95007 (sensitive) to salinity stress were used in the study. Single bud setts (12 numbers each/tray) were planted in plastic trays filled with field soil (electrical conductivity of 2.1 and pH of 7.9). Three replications were maintained for each variety and treatment. When the settlings reached 3-4 leaf stage, salinity stress of 250 mM NaCl was imposed. The plant samples were analysed at 0, 2, 4 and 7th days after imposition of salinity treatment. Leaf samples were collected when first leaf of sensitive genotype started exhibiting drying symptoms. The chlorophyll and carotenoids were estimated in a single extraction (Weybrow 1957). Leaf sample (0.5g) was extracted with 95% ethanol. Extraction was repeated till the residue was pigment free. All the extracts were filtered and combined. Peroxide free ether was added to it and the pigments were forced into the ether layer

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by flushing with distilled water. The ether layer was collected and volume was made 40 ml. Absorbance was recorded at 665, 649, 642.5 and 475 nm and pigment contents were computed using the following formula. Total chlorophyll: $5566.5 A_{(649)}$, Chl a: $1994.5 A_{(665)} - 173.4 A_{(642.5)}$, Chl b: $3528 A_{(642.5)} - 607 A_{(665)}$, Total carotenoids: $982.1 A_{(475)}$ - 0.0255 Chl a - 0.255 Chl b. Proline content of the leaves was estimated as per the method described by Bates *et al.* (1973).

Lipid peroxidation was measured by estimating thiobarbituric acid reactive substances (Heath and Packer 1968). Peroxidase (POX) activity was measured spectrophotometrically by monitoring the increase in absorbance (of the oxidized O-dianisidine), at 430 nm (Putter 1974). Total superoxide dismutase activity (SOD) was measured based on inhibition in the photochemical reduction of Nitro Blue Tetrazolium (NBT) (Beauchamp and Fridovich 1971). One unit of SOD was defined as the quantity of enzyme required to inhibit the reduction of NBT by 50% in a 1ml reaction volume. The reaction mixture contained 50mM sodium phosphate buffer, pH 7.8, 58 μ M NBT, 2.4 μ M riboflavin, 9.9 mM methionine and 0.025% tritonX-100.

RESULTS AND DISCUSSION

The visual symptoms, i.e. yellowing of younger leaves and salt injury in older leaves were noticed on day seven

in sensitive variety. Total chlorophyll, chlorophyll a and chlorophyll b contents decreased as the stress progressed from zero to seventh day (Table 1). The reduction was more in variety Co 95007 (60%) as compared to Co 85019 (45%). The rapid reduction in chlorophyll a and b is reflected on the chlorophyll a/b ratio and varietal as well as treatment effects were significant during the stress period. Reduction in chlorophyll concentration in response to salinity has been documented for sugarcane (Joshi and Naik 1980, Chandra *et al.*1993), barley, wheat, bajra (Kumar *et al.* 1981, Reddy and Vora 1985). Tolerant genotype maintained chloroplast stability and the injury due to stress was also marginal.

Total carotenoids reduced under salinity in both the varieties and the reduction was significant as the duration of stress increased from zero to fourth day (Table 1). Variation in carotenoids content became significant with progression of the stress (on seventh day). Carotenoids, apart from being an accessory pigment protect the chlorophyll pigments from photooxidation. Chlorophyll a/b ratio was unaltered on second day in the variety Co 85019 and increased on fourth day. The trend in chlorophyll a/b was not similar between the varieties as the stress intensified. In variety Co 85019, the ratio was more or less similar during zero to seventh day suggesting a more stable pigment system while initial increase up to 4th day followed by decrease on 7th day in Co 95007 implied a drastic reduction in pigment resulting in ineffective stability of the pigment system.

Table 1. Leaf pigment content (mg g⁻¹ fw) in response to NaCl treatment

| Days after NaCl treatment | Total chlorophyll | | Chl a | | Chl b | | Total carotenoids | | Chl a/b | | Chl/Car | |
|---------------------------|-------------------|-----------|------------|-----------|------------|-----------|-------------------|-----------|---------|------|---------|------|
| | V1 | V2 | V1 | V2 | V1 | V2 | V1 | V2 | V1 | V2 | V1 | V2 |
| 0 day | 2.139 | 1.992 | 1.705 | 1.468 | 0.401 | 0.425 | 0.441 | 0.328 | 4.25 | 3.29 | 4.85 | 6.07 |
| 2 nd day | 1.983 | 1.315 | 1.615 | 1.071 | 0.382 | 0.251 | 0.411 | 0.302 | 4.22 | 4.26 | 4.82 | 4.35 |
| 4 th day | 1.236 | 0.847 | 0.933 | 0.784 | 0.175 | 0.140 | 0.235 | 0.177 | 5.33 | 5.60 | 4.40 | 4.78 |
| 7 th day | 1.147 | 0.688 | 0.774 | 0.474 | 0.133 | 0.122 | 0.202 | 0.165 | 5.81 | 3.88 | 4.68 | 4.77 |
| | SEd | CD | SEd | CD | SEd | CD | SEd | CD | | | | |
| V | 0.005 | 0.01 | 0.009 | 0.02 | 0.004 | 0.009 | 0.001 | 0.002 | | | | |
| Day | 0.003 | 0.007 | 0.006 | 0.014 | 0.003 | 0.006 | 0.001 | 0.002 | | | | |
| Vx Day | 0.007 | 0.015 | 0.013 | 0.028 | 0.006 | 0.013 | 0.001 | 0.004 | | | | |

V1= Co 85019, V2=Co 95007

Proline is a compatible osmolyte that accumulates in greater proportion under abiotic stress. Proline content increased from zero day up to seventh day under salinity in both varieties (Fig. 1). The increase was only marginal on second and fourth day in both the varieties. With the progression of stress (on seventh day), the proline level was high and varietal difference was significant. Salt tolerant cultivars of barley accumulated over 80% proline than control and the level increased only by 40% in a sensitive cultivar (Kumar *et al.* 1981). However, Lutts *et al.* (1999) suggested that the accumulation of proline is a symptom of salt injury in salt sensitive cultivar. In the present study increasing trend of proline content with the progress of stress, suggests its protective and stabilizing role under stress.

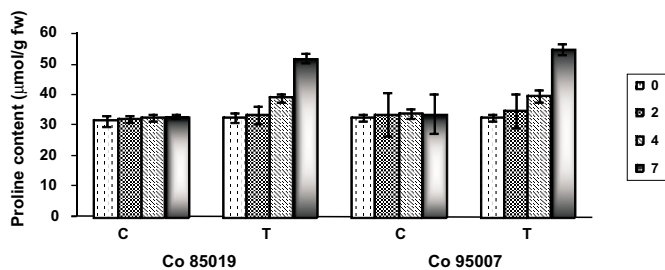


Fig. 1. Proline content in sugarcane as influenced by salinity. C and T indicate control and salinity treatment, 0, 2, 4 and 7 represent sampling on respective days

The oxidative enzymes, viz. peroxidase, superoxide dismutase, and ascorbate peroxidase etc. are reported to increase under the influence of high salinity. General and nonspecific peroxidases expressed as oxidized O-dianisidine, increased from zero day to seventh day and the increase was more or less uniform for both the varieties (Fig 2). A significant increase in peroxidase activity was recorded as an influence of salinity. The observation on peroxidase activity was similar to that reported for wheat and rice (Sgherri *et al.* 2000, Srivalli *et al.* 2003). The superoxide dismutase activity estimated as photo-chemically inhibited nitroblue tetrazolium compound (NBT), increased under influence of salinity. The varietal behavior differed under salinity. In var. Co 85019 the increase in activity was gradual from zero through seventh day (Fig. 3), while in variety Co 95007 an initial increase on second day followed by steep increase in seventh day. On seventh day the activity

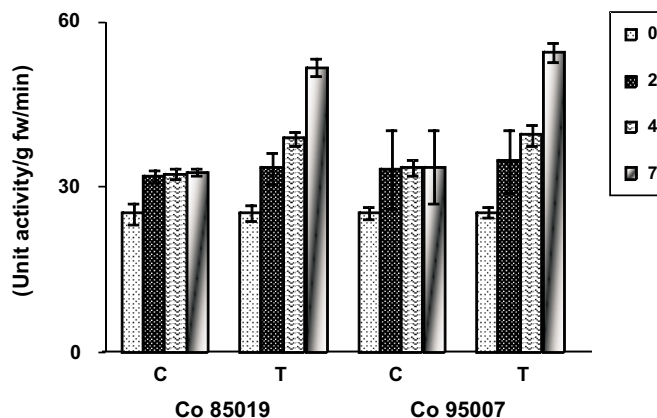


Fig. 2. Progressive change in peroxidase activity under salinity. C and T indicate control and salinity treatment, 0, 2, 4 and 7 represent sampling on respective days.

reached peak. The logarithmic curve explains the hike in the activity of SOD under salinity. The control plants exhibited least activity suggesting normal physiological functioning in control condition. The increase in activity of SOD was several fold in both the varieties with Co 95007 showing higher activity indicating the severity of the stress experienced by reactive oxygen species scavenging system. The role of SOD in the protection of membranes from salt damage through scavenging toxic oxygen radicals is well established (Shaaltiel and Gressel 1986). The results are in conformity with earlier reports (Gupta *et al.* 1993).

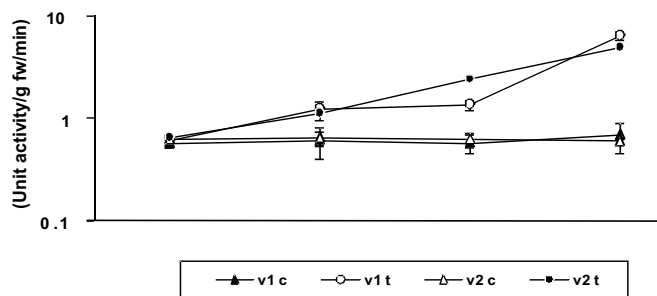


Fig. 3. Super oxide dismutase activity as influenced by salinity. v1c and v1t represent Co 85019 control and salt treatment and v2c, v2t represent Co 95007 control and salinity, 0, 2, 4 and 7 indicate sampling days.

Lipid peroxidation estimated as malon dialdehyde (MDA) content increased from zero day through seventh day. The initial peroxidation values of lipid was uniform

up to fourth day in both the varieties (Fig. 4). However, on day seventh the MDA content was highest in Co 95007 indicating maximum injury to membrane lipids which is lethal to tissue survival. The MDA content was 2.2 mg/g in Co 95007 on seventh day, an increase of 3.5 fold over control. Although increase in MDA content was observed in Co 85019 the increase was moderate on day seventh (2.3 fold) indicating lesser damage to the membrane system. In sugarcane, rice and rye increase in lipid peroxidation level, under stress, with varietal variation has been reported (Venkataramana *et al.* 1997, Shalata and Tal 1998). Biochemical studies indicated the altered trend in metabolic behavior under salinity treatment. Although remarkable variation may not be expected in short term experiments (Munns 2002), the visual salt injury observed in sensitive variety Co 95007, is supported by drastic reduction in pigment content, proline level, SOD activity and higher lipid peroxidation. In the present study, the parameters included are numerically too few to explain the tolerance mechanism; however their relevance in describing the varietal variability is significant.

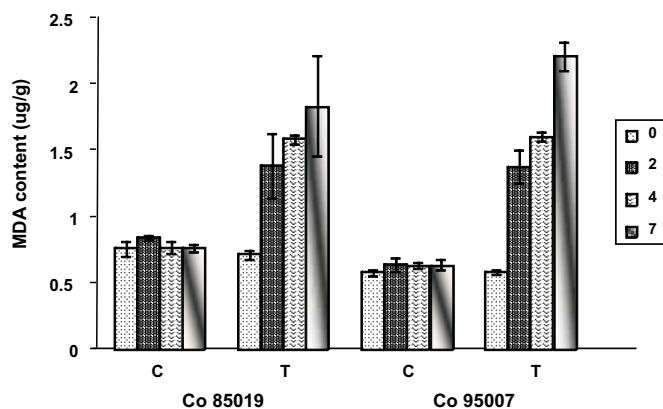


Fig. 4. MDA (malondialdehyde) content in sugarcane genotypes. C and T indicate control and salinity treatment, 0, 2, 4 and 7 represent sampling on respective days.

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