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COMPARATIVE LIPID PEROXIDATION, LEAF MEMBRANE THERMOSTABILITY, AND ANTIOXIDANT SYSTEM IN FOUR SUGARCANE GENOTYPES DIFFERING IN SALT TOLERANCE

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Role of plant antioxidant systems in salt stress tolerance was studied in four contrasting sugarcane genotypes. Salt stress imposed at various stages of crop growth resulted in an increase in lipid peroxidation and decrease in membrane stability, chlorophyll fluorescence ratio (fv/fm) and chlorophyll and carotenoid contents. The activity of antioxidant enzymes (ascorbate peroixdase, glutathione reductase, and superoxide dismutase) increased significantly under salt stress. The genotypes C 92038 and Co 85004, which had the highest ascorbate peroxidase, glutathione reductase, peroxidase, catalase and SOD activity, had the lowest lipid peroxidation (malondialdehyde content) and highest membrane stability, chlorophyll and carotenoid contents under salt stress, while the susceptible genotypes Si 94050 and Co 85036 exhibited lowest antioxidant enzyme activity, membrane stability, contents of chlorophyll and carotenoid with highest lipid peroxidation. The higher salt tolerance of varieties C 92038 and Co 85004 were related to higher membrane stability, significant higher chlorophyll and carotenoid contents, and maintenance of high fv/fm ratio under salt stress and lower lipid peroxidation of membranes. Hence, the relative tolerance of a genotype to salt stress as reflected by its lower lipid peroxidation, and higher membrane stability and pigment concentration, is related to the levels of activity of its antioxidant enzymes.

Key words: Ascorbate peroxidase, carotenoid, chlorophyll, lipid peroxidation, glutathione reductase, membrane stability index, chlorophyll fluorescence ratio, sugarcane, salt stress.

INTRODUCTION

Reactive oxygen species (ROS) such as the superoxide radical, hydrogen peroxide and hydroxyl radical can cause lipid peroxidation and consequently membrane injury which leads to leakage of cellular content, protein degrading, enzyme inactivation, pigment bleaching and disruption of DNA strands and thus cell death (Scandalios, 1993). Enhanced production of oxygen free radicals is responsible for peroxidation of membrane lipids and the degree of peroxides damage of cell was controlled by the potency of peroxidase enzyme system (Sairam and Tyagi, 2004). Plants have developed a series of both enzymatic and non-enzymatic detoxi-fication systems to counteract AOS, thereby protecting cells from oxidative damage (Sairam and Tyagi, 2004).

Tolerance to wide varieties of environmental stress conditions has been correlated with increased activity of antioxidant enzymes and levels of antioxidant metabolites (Davis, 1987). Plants protect the cellular and sub-cellular systems from the cytotoxic effects of these ROS in the form of enzymes such as superoxide dismutase, ascorbate peroxidase, peroxidase, glutothoine reductase and catalase and metabolites such as glutathione, ascorbic acid, α- tocopherol and carotenoids (Liebler et al., 1986). Modulation of the activities of these enzymes may be important in the resistance of plant to environ-mental stresses (Allen, 1995). Since sugarcane is an annual crop, identification of suitable adaptive mechanism at different growth stages of crop might help in early screening of genotypes for salt tolerance. Hence, the present study was conducted to evaluate the mechanism of adaptation to salt stress in the leaves of four sugarcane cultivars differing in salt tolerance.

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MATERIALS AND METHODS

Plant material and salt stress application

Sugarcane (*Saccharum officinarum*) cvs Co 85004 and C 92038 (salt stress tolerant), Si 94050 and Co 85036 (salt stress susceptible) were planted in earthen pots 60 × 45 cm size, filled with sand, loamy soil and farmyard manure in 2:1:1 ratio. Each pot was fertilized with 120, 60 and 60 kg ha^{-1} of N, P and K, respectively. Two seedlings were maintained in each pot. One set of pots maintained as control (T_1) and other set maintained as salt treatment (T_2) and each set of pots consist of four variety and 6 replications. Soil EC in the treated pots was monitored at fortnightly interval and salinization of pots was done whenever required to maintain the EC around 7 to 8 dSm^{-1}. The soil pH and EC were regularly determined in 1:2 soil water suspension 5 days after the salt treatment. Samples from control and treated plants were taken 5 days after imposition of stress at 3 different stages (formative, grand growth phase and maturity phases).

Chlorophyll fluorescence ratio (fv/fm) and membrane stability

The **c**hlorophyll fluorescence ratio (fv/fm) was recorded in top, middle, and bottom of selected leaves of each treatment and variety by using plant efficiency analyzer (model - Haustach) and expressed in fv/fm ratio. The sugarcane leaf membrane stability index (MSI) was determined according to the method of Premachandra et al. (1990). Leaf discs (0.1 g) were thoroughly washed in running tap water and double distilled water and thereafter, placed in 10 ml of double distilled water at 40°C for 30 min. After that, the EC was recorded by Conductivity Bridge (make - Systronics; model - 306) (C_1). Subsequently, the same samples were placed in the boiling water bath (100°C) for 10 min and their EC was recorded as above (C_2). The membrane stability index (MSI) was calculated as, MSI (%) = (1-(C_1 /C_2) × 100 (Sairam et al., 1997).

Estimation of chlorophyll and carotenoid

Chlorophyll (Chl) and carotenoid (Car) were estimated by extracting the leaf material in 80% acetone. Absorbances were recorded at 665, 645 and 470 nm. Chl a, Chl b and total Chl were calculated as described by Arnon (1949), and Car content was estimated according to the method of Lichtenthaler and Wellborn (1983). The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) content, a product of lipid peroxidation, following the method of Heath and Packer (1968). A leaf sample (0.5 g) was homogenized in 10 ml of 0.1% trichloro acetic acid (TCA). The homogenate was centrifuged at 15000 g for 5 min. To a 1.00 ml aliquot of the supernatant, 4.00ml of 0.5% thiobarbituric acid (TBA) in 20% TCA was added. The mixture was heated at 95°C for 30 min and then quickly cooled in ice bath. After centrifugation at 10000 g for 10 min, the absorbance of the supernatant was recorded at 532 nm. The value for non-specific absorbance at 600 nm was subtracted. The MDA content was calculated by its extinction coefficient of 155 mM^{-1} $cm^{-1}$and expressed as nmol MDA per gram fresh weight.

Enzyme extraction and assays

Enzyme extracts for ascorbate peroxidase (APX), glutathione reductase (GR) and peroxidase (POX) were prepared by grinding0.25 g of leaf material with 10 ml of chilled 0.1 M potassium phosphate buffer (pH 7.5) containing 0.5 mM EDTA and 0.5 mM ascorbic acid in a pre chilled mortar and pestle. The extract was filtered through cheese cloth and the filtrate was centrifuged for 15 min at 20000 ppm. The supernatant is referred to henceforth as the enzyme extract. All operation was carried out at 4°C. The ascorbate peroxidase activity (APX) was assayed according to the method of Nakano and Asada (1998) by recording the decrease in absorbance at 290 nm, as ascorbate was oxidized. The reaction mixer contains 50 mM potassium phosphate buffers (pH 7), 0.5mM ascorbic acid, 0.1 mM EDTA, 0.1 mM H_2O_2 and 0.1 ml of diluted enzymes in a total volume of 3.00 ml. The reaction was started with the addition of H_2O_2 and absorbance was recorded at 290 nm spectrophotometrically, for 5 min.

GR was assayed by the method of Smith et al. (1988). The reaction mixture contained 1.0 ml of 0.2 M potassium phosphate buffer (pH 7.5) containing 1.0 mM EDTA, 0.5 ml of 3.00 mM DTNB (5, 5-dithiobis 2-nitrobenzoic acid) in 10 mM potassium phosphate buffer (pH 7.5), 0.1 ml 2.0mM NADPH, 0.1 ml of enzyme extract and distilled water to make up a final volume of 3.0 ml. The reaction was initiated by adding 0.1 ml of 2.00 GSSG (oxidized glutathione) and the increase in absorbance at 412 nm was recorded at 25°C over a period of 10 min on a UV -1601, UV –VIS spectrophotometer.

POX activity was estimated at 25°C in a 3.0- ml cuvette containing 60 mM potassium phosphate buffer (pH 6.1), 16 mM guaiacol, 2.0 mM H_2O_2 and 0.1 ml diluted (10 times) enzyme extract (Castillo et al., 1984). The increase in absorbance was recorded at 470 nm spectrophotometerically, over a period of 10 min.

Catalase activity (CAT) of leaves was determined following the method of Luck (1974). One gram of the sample was extracted in 0.067 M phosphate buffer (pH 7.0). A known volume of the extract was added to the experimental cuvette containing 3 ml H_2O_2–PO_4 buffer. The time taken for percent change in absorbance (Δt), at 240 nm was recorded for calculating the enzyme activity and expressed as enzyme units g^{-1} min^{-1} tissue. All the operations were carried out at 0 to 5°C.

Superoxide dismutase (SOD) activity was assayed spectrophotometerically, as the inhibition of photochemical reduction of nitro-blue tetrazolium (NBT) at 560 nm (Beauchamp and Fridovich, 1971). The reaction mixer contained 33 µm NBT, 10 mM L-methionine, 0.66mM EDTA.Na_2, and 0.003mM riboflavin in 0.05 M Na-phosphate buffer (pH 7.8). The riboflavin was added last. The test tubes containing reaction mixture were shaken and waited for 10 min under, 300 µmol m^{-2} s^{-1} irradiance at room temperature. The reaction mixture with no enzyme developed maximum color due to maximum reduction of NBT. A non-radiated reaction mixture did not develop color and served as the control. The reduction of NBT was inversely proportional to the SOD activity. One unit of SOD was defined as the amount of enzyme that inhibits 50% NBT photoreduction. The specific enzyme activity for all the enzymes was expressed as units mg^{-1} $protein^{-1}$ FW.

RESULTS

Lipid peroxidation (LPO) as MDA content, estimated at formative and grand growth phase of the crop, showed increasing trend over the stage as well as NaCl stress condition (Figure 1a). Under control, in both stages, C92038 showed minimum value of 350.25 and 452.25 nmol malondialdehyde g^{-1}f.wt of LPO while, Co 85036 showed maximum values (490.25 and 676.56 nmol malondialdehyde g^{-1}f.wt.) in FP and GGP respectively. Under NaCl stress condition, an increase in LPO activity was found to be higher in Si 94050 (60.25%) over control followed by Co 85036 (50.69%) while, C 92038 recorded minimum increase in LPO activity (29.42%) at GGP (Figure 1b).

Chlorophyll (Chl) and carotenoid content showed a

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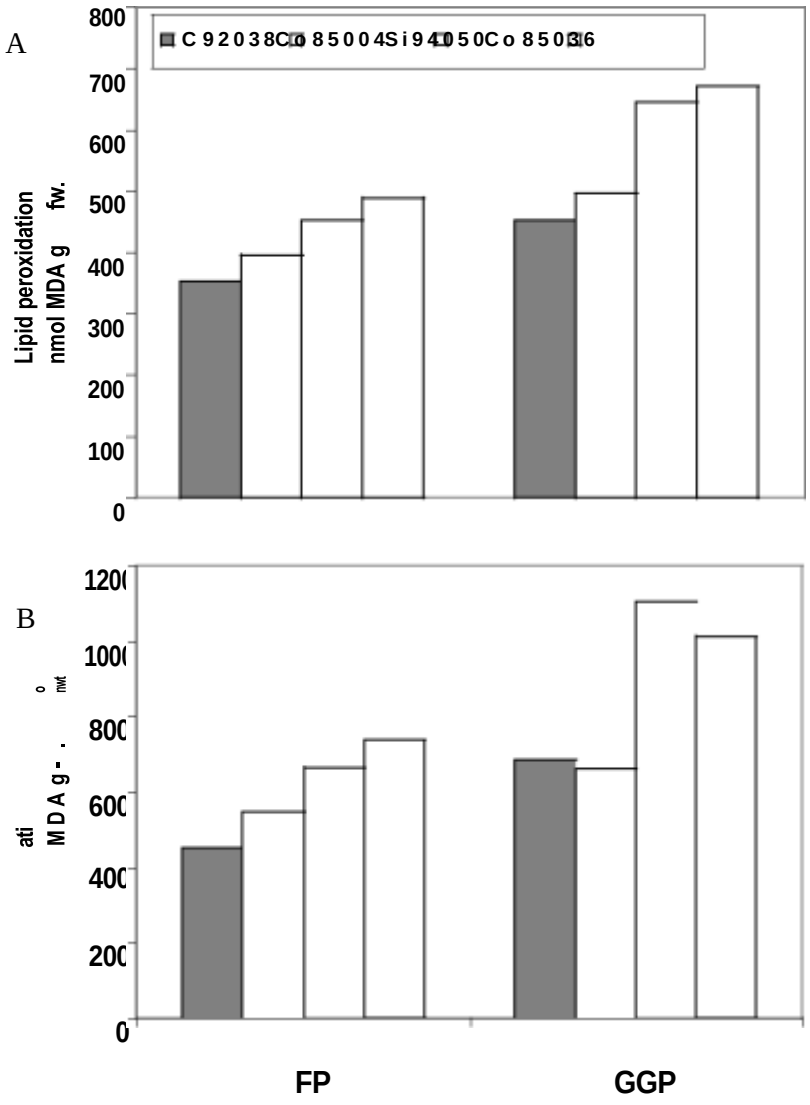

Figure 1. Effect of salt stress on Lipid peroxidation; a (control); b (NaCl stress) in sugarcane genotypes. Data are significant at P = 0.05. (FP- formative phase, GGP- grand growth phase).

decreasing trend with age, under both control and salt stress condition (Figure 2a, b). Under controlled condition, Co 85036 and Co 85004 were higher in chlorophyll at second and third stages, respectively, while carotenoid content was also highest in Co 85036 in all the stages, followed by Si 94050. A marked reduction in Chl and carotenoid content was noticed under salt stress condition, in all the stages. However, the reduction was found to be greater in Co 85036 and Si 94050, particularly at GGP and maturity phase of the crop. The genotype Co 85004 and C 92038 were able to maintain higher Chl and carotenoid content even at GGP and maturity phases of the crop.

The membrane stability index (Figure 2c) in leaves of sugarcane cultivars under control and salt stress condition showed a decreasing trend with age in all

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the genotypes. MSI was higher in Co 85004 and it was closely followed by C 92038. The Si 94050 and Co 85036 recorded lowest values in all the stages. While in control, Si 94050 recorded higher values and Co 85004 recorded lowest values in all the stages.

Under control, irrespective of the genotypes, ascorbate peroxidase (APO) activity showed increasing trend with age. In all the stages, Si 94050 recorded higher and it was closely followed by C 92038 and lowest recorded in Co 85036. Under salt stress condition, significant geno- typic change in APO activity was noticed in all the stages. The genotypes C 92038 and Co 85004 were showed increasing trend in APO activity, while, Si 94050 and Co 85036 were showed declining trend in all the stages of the crop growth (Figure 3a).

There was more than 50% increase in glutathione

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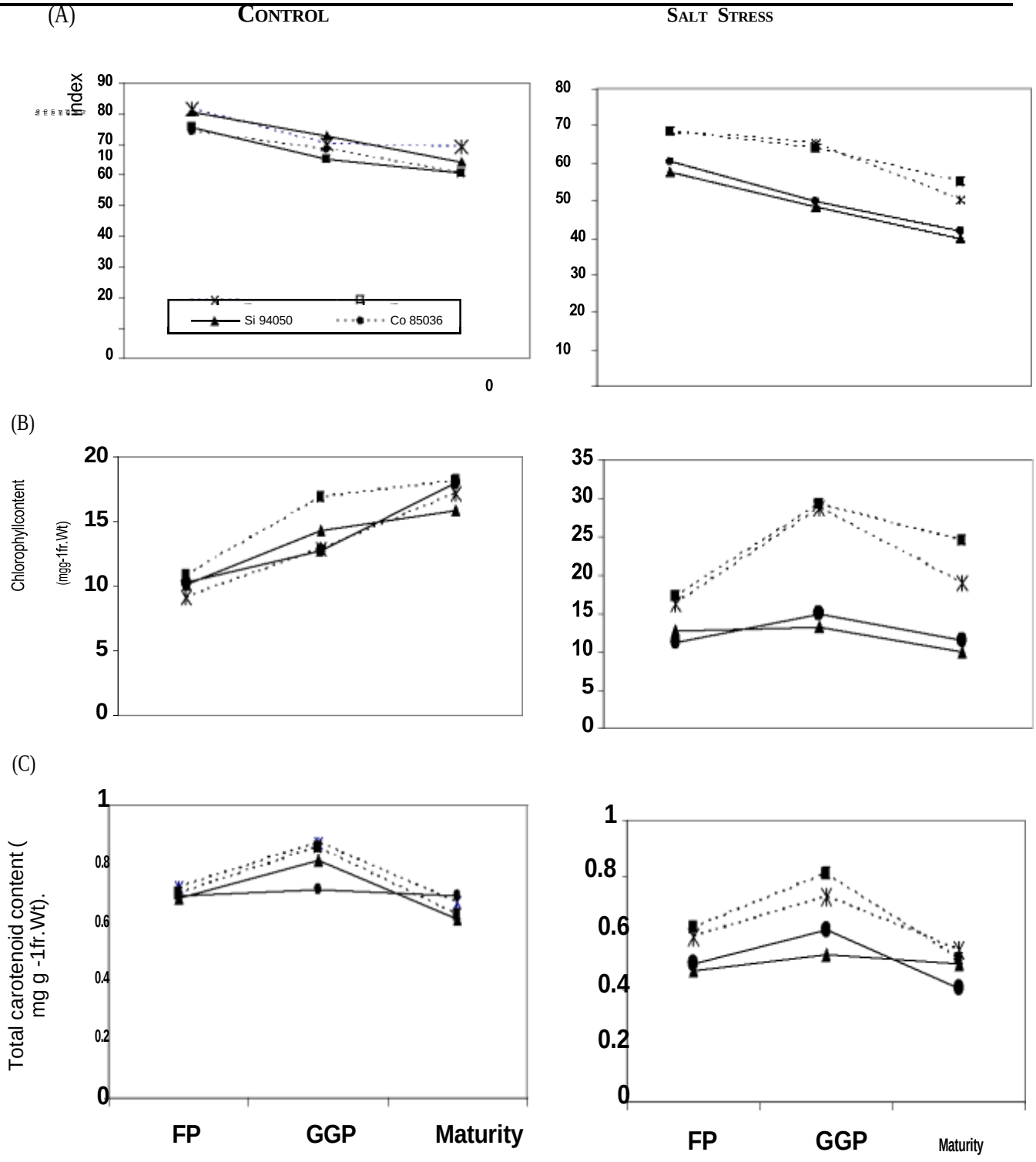

Figure 2. Effect of salt stress on contents of; (a) chlorophyll; (b) carotenoid; (c) membrane stability index of sugarcane genotypes. Data significant at P= 0.05.

reductase activity (GR) as noticed under salt stress condition in comparison to control (Figure 3b). However, on overall, the increase was found to be maximum in C 92038

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(62.10%) followed by Co 85004(58.20%) while Si 94050 and Co 85036 recorded lesser increase of 10.50 and 12.35% respectively. Peroxidase activity (POX) showed similar trend as that of GR activity in all the stages. In control, except maturity phase, C 92038 was

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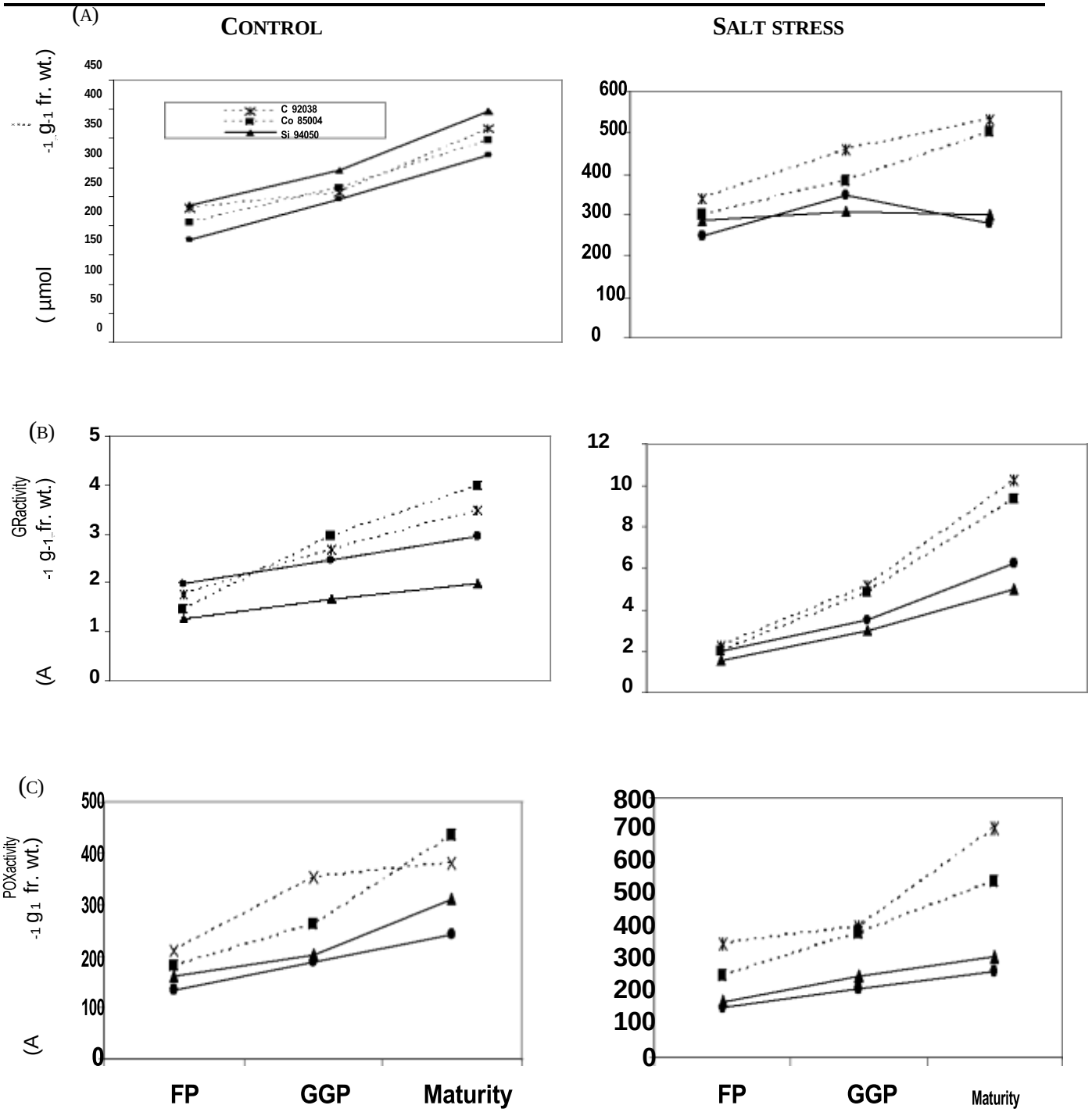

Figure 3. Effect of salt stress on the activity of; (a) ascorbate peroxidase; (b) glutathione reductase; (c) peroxidase of sugarcane genotypes. Data are significant at P= 0.05.

recorded higher POX activity and it was closely followed by Co 85004. However, under salt stress a 35.20% increase in enzyme activity was noticed. Among the varieties, increase in enzyme activity was found to be maximum in C 92038 (42.50%) followed by Co 85004 (38.50%).

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In control, both CAT and SOD activity showed increasing trend with the age in all the genotypes (Figure 4a, b). In control, the genotype response was almost identical in all the stages. While under salinity condition, (Figure 4a, b) a clear cut genotypic variation in CAT and SOD activity was noticed ant it showed increasing trend

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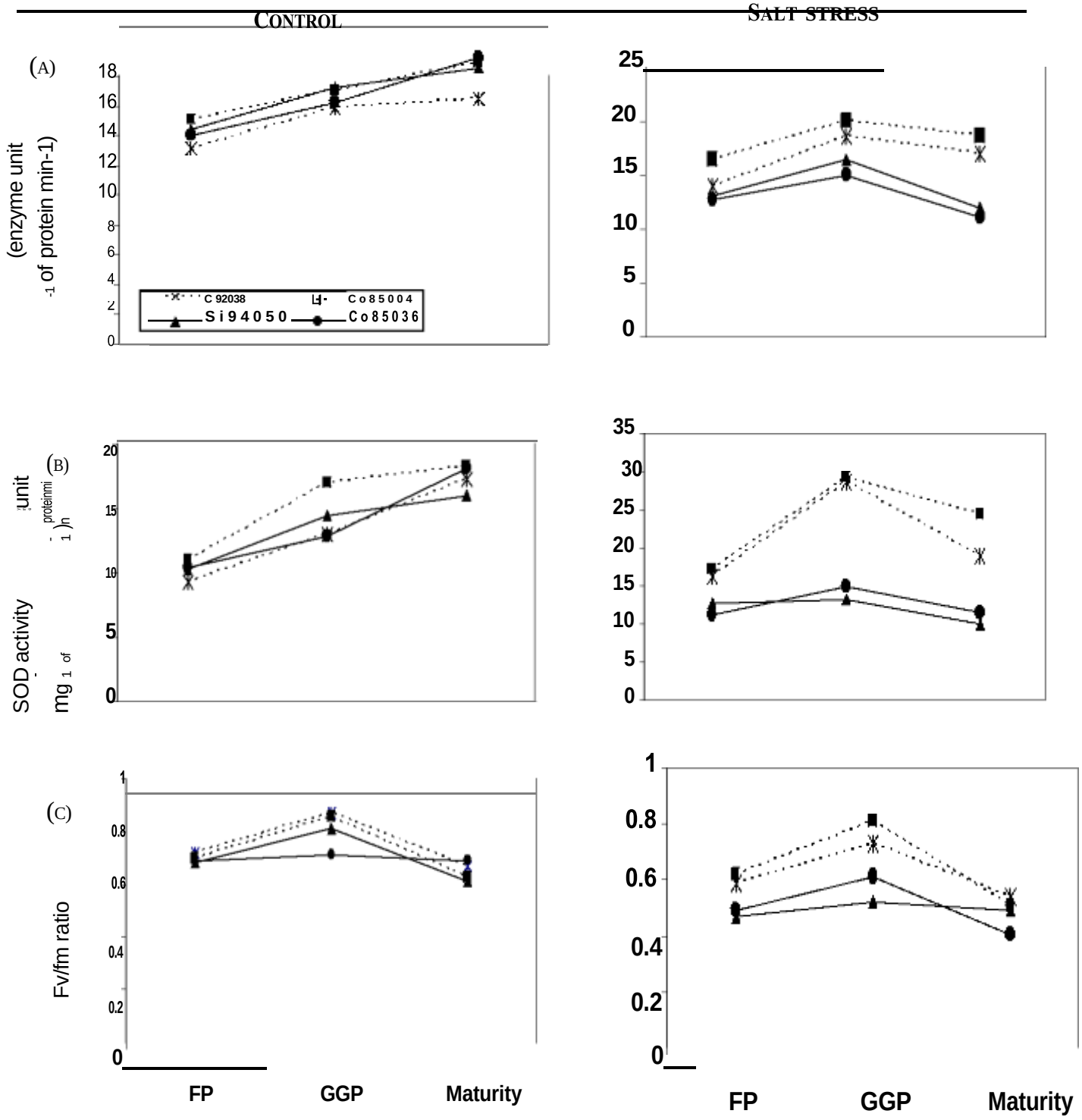

Figure 4. Effect of salt stress on; (a) CAT; (b) SOD activity; (c) fv/ fm ratio of sugarcane genotypes at three important growth stages. Data are significant at P= 0.05.

up to GPP and it declined towards maturity phase. Here again, the Co 85004 and C 92038 were recorded higher in the enzyme activity in all the stages compared to Si 94050 and Co 85036. Irrespective of the treatments, an increase in chlorophyll florescence ratio (fv/fm) was

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noticed up to GGP and declined towards maturity (Figure 4c). In control, the genotypes response was almost identical, while under salinity stress, Co 85004 was recorded highest fv/fm ratio in all the stages and it was closely followed by C 92038. Lowest value was recorded

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in Co 85036 followed by Si 94050.

DISCUSSION

Results of the present study showed that the overall 20% reduction in chlorophyll fluorescence ratio (fv/fm) was observed under salinity conditions. However, the reduction in chlorophyll fluorescence efficiency was found to be more in susceptible genotypes (Co 85036 and Si 94050). It might be due to the reduction of photochemical quenching in PSII due to salt stress. Several reports have proposed these measurements for screening salt tolerance in different crop species (Smillie and Nott, 1982; Belkhodja et al., 1994; Taishi et al., 2000).

The stress induced increase in leaf membrane damage, reduced uptake of CO_2 as a result of closer stomatal, decreased hydrolytic enzyme activity and increased lipid peroxidation level; it may stimulate for-mation of AOS such as superoxide, hydrogen peroxide, and hydroxyl radicals. Among AOS, superoxide is converted by SOD enzyme into H_2O_2, which is further scavenged by CAT and various peroxidases. APOX and GR also play a key role by reducing H_2O_2 to water through the Halliwell-Asada pathway (Noctor and Foyer, 1998). Allen (1995) also reported that much of injury to plants caused by various stresses is associated with oxidative damage at cellular level such as cell membrane damage.

In present study, higher membrane stability and chlorophyll fluorescence ratio (fv/fm) and lower level of lipid peroxidation in resistant genotypes (Co 85004 and C 92038) might be due to the increased activities of antioxidant enzymes (APO, GR, POX, CAT and SOD) which act as a damage control system and thus provide protection from oxidative stress, otherwise, which would cause damage of cell membrane and protein, DNA structure and inhibit the photosynthesis as reported under water stress condition (Sairam and Saxena, 2000; Sairam and Tyagi, 2004). Lower lipid peroxidation and higher membrane stability (lower ion leaching) have also been reported in tolerant genotypes of wheat (Kraus et al., 1995) and Rice (Tijen and Ismail, 2005).

Carotenoids are accessory pigments in photosynthetic systems and their levels are altered during physiological and pathological conditions. Besides their function as light harvesting pigments that contribute to photosynthe-sis, the carotenoids have another function in thylakoids lamellae which is to protect chlorophylls against oxidative destruction by O_2 when irradiance level is high (Aono et al., 1993). Kanhaiya (1997) showed that higher levels of carotenoid and total chlorophyll (resistant genotypes) might prove an index for superior juice quality under saline conditions. It was evident from the present results that the genotypes (Co85004 and C 92038) which possess highest total chlorophyll and carotenoids content under salinity conditions signifying lower pigment bleaching and favors better adaptation under saline condition.

GR, APX, CAT, POX and SOD are involved in the scavenging of the products of oxidative stress, such as hydrogen peroxide generated in the chloroplast (Kraus et al., 1995; Jagtap and Bhargava, 1995) and thus help in ameliorating the adverse effects of oxidative stress. Higher APO and POX activity have been correlated with the relative tolerance of many crop plants (Allen, 1995; Wang et al., 2000; Sairam and Saxena, 2000). In the present investigation, the ROS scavenging activity was much higher in resistant cultivars Co 85004 and C 92038 under NaCl salt stress than that of susceptible cultivars (Co 85036 and Si 94050). The above results are in correlation to the findings of Olmos et al. (1994) who reported that tolerance of wide varieties to various environmental stress conditions is associated with activity of antioxidant enzymes.

A perusal of the results shows that the higher free radical scavenging capacity in resistant genotypes, which are, C 92038 and Co 85004 associated with lowest lipid peroxidation and highest membrane thermostablity, PSII efficiency and chlorophyll and carotenoid contents. Thus, it can be summarized that the relative tolerance of a genotype to salt stress as reflected by its lower lipid peroxidation, and higher membrane stability and pigment concentration, is related to the levels of activity of its antioxidant enzymes (Kraus et al., 1995; Jagtap and Bhargava, 1995; Sairam and Tyagi, 2004).

REFERENCES

Allen RD (1995). Dissection of oxidative stress tolerance using transgenic plants. Plant Physiol., 107: 1049-1054.

Aono M, Saji H, Fujiymma K, Sugaita M, Kondo N, Tanaka K (1993). Decrease in activity enhances paraquat sensitivity in transgenic *Nicotiana tabacum*. Plant Physiol., 107: 645-648.

Arnon DI (1949).Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris* . Plant Physiol., 24: 1

Belkhodja R, Morales FAA, Medrano H, Abadia J (1994). Effects of salinity on chlorophyll fluorescence and photosynthesis of barley (*Hordeum vulgare* L.) grown under a triple-line- source sprinkler system in the field. Photosynthetica, 36(3): 375-387.

Beauchamp C, Fridovich I (1971). Superoxide dismutases: Improved assays and assay applicable to acrylamide gels. Analyt. Biochem., 44: 276-287.

Castillo FJ, Panel C, Greppin H (1984). Peroxidase release induced by ozone in *Sedum album* leaves. Plant Physiol., 74: 846-851.

Davis KJA (1987). Protein damage and degradations by oxygen radicals. J. Biol. Chem., 262: 9895-9901.

Heath RL, Packer L (1968). Photo oxidation in isolated chloroplast .I. Kinetics and Stoichiometry of fatty acid peroxidation. Arch. Biochem. Bio. Physic., 125: 189-198.

Jagtap VB, Bhargava D (1995). Variation in the antioxidant metabolism of drought tolerant and drought susceptible varieties of *Sorgum bicolar* (L) exposed to high light, low water and high temperaturestress. J. Plant Physiol., 145: 195-197.

Kanhaiya L (1997). Biochemical studies in relation to adoptability of

International Journal of
DATA SCIENCE AND IOT MANAGEMENT SYSTEM
Peer Reviewed, Referred & Indexed Journal
ISSN: 3068-272X www.ijdim.com Original Research Paper

sugarcane under salinity stress. Joint convention of STAI & DSTA. pp.15-18.

Kraus TE, Mckersie BD, Fletcher RA (1995). Paclobutrazol induced tolerance of wheat leaves to paraquat may involve increased antioxidant enzyme activity. J. Plant Physiol., 145: 570-576.

Liebler DC, Kling DS, Reel DJ (1986). Anti oxidant protection of phospholipids bilayers by ά – tocopherol control of tocopherol status

and lipid peroxidation by ascorbic acid and glutathione. J. Bio. Chem., 261: 1214-12118.

Lichtenthaler HK, Wellborn JA (1983). Determination of total Carotenoide and Chlorophyll a and b of leaf extracts in different solvents,. Biochem. Soc. Trans., 11.591-592.

Luck H (1974). Estimation of catalase. In: Methods in Enzymatic Analysis**.** (Eds). Luck. H. Bergmeyer Academic Press, New York. p. 885.

Nandigama, N. C. (2022). Machine Learning–Enhanced Threat Intelligence for Understanding the Underground Cybercrime Market. International Journal of Intelligent Systems and Applications in Engineering. Internet Archive. https://doi.org/10.17762/ijisae.v10i2s.7972.

Noctor G, Foyer CH (1998). Ascorbate and glutathione: keeping active oxygen under control. Annu. Rev. Plant Physiol. Plant Mol. Bio., 49: 249-279.

Olmos E, Hernandez TA, Sevilla F, Hellin E (1994). Induction of several anti xidant enzymes in the selection of salt tolerant cell line of *Pisum sativum*. J. Plant Physiol., 144: 594-595.

Premachandra GS, Saneoka H, Ogata (1990). Cell membrane stability an indicator of drought tolerance as affected by applied N in soybean. J. Agric. Soc. Camp., 115: 63-66.

Sairam RK, Deshmukh PS, Shukla DS (1997). Increased antioxidant enzyme activity in response to drought and temperature stress related with stress tolerance in wheat genotypes, Abstract: National Seminar (ISSP), IARI, New Delhi. p. 69

Sairam RK, Saxena DC (2000). Oxidative stress and Antioxidants in wheat genotypes: Possible mechanism of water stress tolerance. J. Agron. Crop Sci., 184: 55-61.

Nandigama, N. C. (2023). Enhanced Fingerprint Recognition system using hybrid Feature Fusion with Deep learning and Machine learning Optimization. Research Journal of Nanoscience and Engineering, 6(1), 9–15. https://doi.org/10.22259/2637-5591.0601003.

Scandalios JG (1993).Oxygen stress and superoxide dismutases. Plant Physiol., 101: 7-12.

Smith IK, Vierheller TL, Thorne CA (1988). Assay of glutathione reductase in crude tissue homogenate using 5, 5- dithiobis . Anal. Biochem., 175: 408-413.

Smillie RM, Nott R (1982). Salt tolerance in crops plants monitored by chlorophyll fluorescence *In vivo*. Plant Physiol. 70: 1049-1054.

Taishi U, Katsuyoshi S, Morio K, Takao V (2000). Enhancement of salt tolerance in soybean with NaCl pretreatment. Physiol. Plant., 110: 59-63.

Tijen D, Ismail T (2005). Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. Environ. Exp. Bot., 53: 247-257.