INFLUENCE OF SALINITY STRESS ON PHOTOSYNTHESIS AND ANTIOXIDATIVE SYSTEMS IN TWO COTTON VARIETIES

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Summary. The effect of the progressive increase in salinity on photosynthesis, antioxidant enzymes and the contents of proline and glycine betaine in two cotton varieties, Arya-Anubam and LRA-5166 was investigated. Plants were treated with four different salt concentrations (mixed salts: NaCl, $MgSO_4$, and $CaCl_2$): 0 mM (control), 50 mM, 100 mM, and 150 mM. Photosynthetic rate and activities of RuBP carboxylase and sucrose phosphate synthase (SPS) decreased with increasing salinity. In addition, the activities of key antioxidative enzymes, superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR) also significantly increased. Quantitative differences in the contents of proline and glycine betaine were also noticed in the two varieties subjected to salinity. The results indicated that plants of variety Arya-Anubam exhibited higher adaptive potential under salinity stress as judged by increased photosynthetic rate and activities of photosynthetic and antioxidative enzymes as well as higher accumulation of proline and glycine betaine when compared to variety LRA-5166.

***Key words*:** antioxidant enzymes, cotton, photosynthesis,

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photosynthetic enzymes, salinity stress.

***Abbreviations*:** APX - ascorbate peroxidase, EDTA – ethylene diamine tetraaceticacid, GR - glutathione reductase, GSH – reduced glutathione, GSSG – oxidized glutathione, NBT – nitroblue tetrazolium, ROS – reactive oxygen species, RuBP – ribulose-1,5-bisphosphate carboxylase/oxygenase, SOD - superoxide dismutase, SPS - sucrose phosphate synthase.

INTRODUCTION

Soil salinity is a major constraint limiting agricultural productivity on nearly 20 % of the cultivated area and half of the irrigated area worldwide (Zhu, 2001). Salt stress has been reported to cause an inhibition of growth and development, reduction in photosynthesis, respiration and protein synthesis in sensitive species (Boyer, 1982; Meloni et al., 2003; Pal et al., 2004). An important consequence of salinity stress in plants is the excessive generation of reactive oxygen species (ROS) such as superoxide anion (O^{-}_{2}), hydrogen peroxide (H_2O_2) and the hydroxyl radicals ($OH^{\bullet}$) particularly in chloroplasts and mitochondria (Mittler, 2002; Masood et al., 2006). Plants possess a number of antioxidant enzymes like superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR) for protection against the damaging effects of ROS (Asada, 1992; Prochazkova and Wilhelmova, 2007). Membrane disorganization, metabolic toxicity due to ROS and attenuated nutrients are the factors, which initiate more catastrophic events in plants subjected to salinity stress (Frommer et al., 1999; Zhu, 2000; Cost et al., 2005).

Accumulation of metabolites that act as compatible solutes is one of the probable universal responses of plants to changes in the external osmotic potential. Metabolites with osmolyte function like sugar alcohols, complex sugars and charged metabolites are frequently observed in plants under unfavorable conditions (Hasegawa et al., 2000; Satiropoulos, 2007). Proline and glycine betaine are known to serve as compatible osmolytes, protectants of macromolecules and also as scavengers of ROS under stressful conditions (Hellman et al., 2000; Ashraf and Foolad, 2007). Cotton

varieties are commercially important crops worldwide and their practical utilization ranges from fibres of textile industry and cotton seed oil. Arya-Anubam and LRA-5166 are two important cotton varieties grown in India. Elucidation of the biochemical and molecular mechanisms by which cotton plants tolerate environmental stresses is necessary for breeding and genetic engineering approaches to improve cotton performance under stress. Although agricultural aspects of cotton are well known, little information exists on physiological and biochemical characteristics of cotton with particular reference to photosynthesis and stress metabolism, which control productivity and survival. The main objectives of the present study were to evaluate the photosynthetic potential and antioxidative systems in two varieties of cotton as influenced by salinity stress. Further, we attempted to understand the relationship between salinity stress and osmolyte protection in the leaves of the tested plants.

MATERIALS AND METHODS

Cotton seeds from varieties Arya-Anubam and LRA-5166 were procured from PASIC, Pondicherry. Seeds with uniform size were selected and the plants were grown in pots containing red and clay soil. After 20 days, seedlings were thinned and three plants of uniform vigor were maintained in each pot. The maximum irradiance (PAR, 400-700 nm) available during growth was 1800-2000 μmol $m^{-2}s^{-1}$ on a clear day. Daily maximum and minimum temperatures were 29-33 ^{o}C and 20-22 ^{o}C, respectively. Plants were watered for the first 20 days after germination.

The mixed salts used to obtain the required salinity were NaCl, $MgSO_4$ and $CaCl_2$ and they were applied to the plant as a basal dose. The experiment was arranged in a complete randomized design and replicated thrice. The following concentrations of the salts were applied: 0 mM (control), 50 mM (low salinity), 100 mM (medium salinity) and 150 mM (high salinity). Sampling was done after 30 days of salinity treatment.

Photosynthetic rates in the leaves were determined by following the incorporation of $^{14}CO_2$ as described by Sunder and Reddy (2000) and the rates of CO_2 fixation were expressed as nmolg^{-1}fresh weight s^{-1}.

All enzyme extractions were performed at 4 ^{o}C. The leaves (10 g) were

homogenized with three volumes of grinding medium which consisted of 100 mM Tris-HCl (pH 7.8), 5 mM DTT, 10 mM $MgCl_2$, 1 mM EDTA, 5 mM magnesium acetate and 1.5 % PVP-40. The homogenate was squeezed through four layers of cheesecloth and then centrifuged at 18,000 *g* for 30 min. The supernatant was used as the crude enzymes preparation and used for the assays of protein content by the method of Bradford (1976).

Ribulose-1,5-bisphosphate carboxylase (EC 4.1.1.39) activity was assayed at 30 °C by the incorporation of $^{14}CO_2$ into acid stable products (Lorimer et al., 1977) and the radioactivity was measured using a liquid scintillation counter. Sucrose phosphate synthase (SPS, EC 2.4.1.14) was assayed at 30 °C by measuring the production of UDP (Huber, 1981). The activity of SOD (EC 1.15.1.1) was assayed by measuring its ability to inhibit the photochemical reduction of nitro-blue tetrazolium (NBT) according to the method of Beauchamp and Fridovich (1971). The reaction mixture (3 ml) contained: 50 mM K-phosphate buffer (pH 7.8), 10 mM methionine, 1.17 mM rifoflavin, 56 mM NBT and suitable enzyme extract. The reaction started after switching on the light. The reaction was allowed to take place for 30 min and stopped by switching off the light. The absorbance was measured at 560 nm. A_{560} was plotted as a function of fresh matter equivalent of enzyme extract used in the reaction mixture. From the resultant graph fresh matter equivalents of enzyme extract corresponding to 50 % inhibition of the reaction was read and considered as one enzyme unit. APX (EC 1.11.1.11) activity was estimated by monitoring the decline in absorbance at 240 nm according to Nakano and Asada (1981). Each 3 ml reaction medium contained: 50 mM K-phosphate buffer, (pH 7.0), 0.1 mM H_2O_2 and 20 µl enzyme extract. The amount of ascorbate oxidized was calculated using the extinction coefficient of 2.8 $mM^{-1}cm^{-1}$ and the activity was expressed as mmol ascorbate oxidized mg^{-1}protein min^{-1}. GR (EC 1.6.4.2) activity was measured by oxidized GSH – dependent oxidation of NADPH using the method of Foyer and Halliwell (1976). The reaction mixture contained: 25 mM Tris-$MgCl_2$ (pH 7.6), 5 mM NADPH, 50 mM GSSG and 1ml enzyme extract. The change in absorption at 340 nm (E=6.2 $mM^{-1}cm^{-1}$) was recorded over 2.5 min. The enzyme activity was expressed as µmol NADPH oxidized mg^{-1}protein min^{-1}.

The content of proline was estimated according to Bates et al. (1973).

Leaf tissue (5 g) was homogenized in 3 % sulfosalysilic acid and filtered. To 2 ml of filtrate, 2 ml of acid ninhydrin and 2 ml of glacial acetic acid were added and incubated for 1h in a boiling water bath followed by an ice bath. To this 4 ml of toluene was added and mixed vigorously and the chromophore containing toluene was aspirated from the aqueous phase and the absorbance was measured at 520 nm. A standard curve was obtained using a known concentration of authentic proline. The content of glycine betaine was estimated in leaf tissues according to Storey and Jones (1977). Leaf tissue (5 g) was homogenized in 25 ml of isopropyl alcohol and centrifuged at 3000 *g* for 10 min. The supernatant was dried in vacuum at 4 ^{o}C. The residue was washed twice in chloroform (20 ml) and distilled water (5 ml). The two phases of six bulked washes were partitioned by centrifugation and the upper aqueous layer was removed. The remaining lipid layer was washed thrice with 20 ml of MeOH:H_2O (1:1). The combined aqueous layers were evaporated to dryness in a hot water bath and re-dissolved in distilled water (5 ml). Potassium triiodide solution (0.2 ml) was added to 1ml of the above extract and the mixture was incubated for 90 min on an ice bath with intermittent shaking. Ice cold distilled water (2 ml) was added to the mixture followed by 2 ml of 1,2-dichloroethane and the two layers were mixed by constant stream of air bubbles for 5min, while the temperature was maintained at 4 ^{o}C. The absorbance of the lower organic layer was measured at 365 nm.

Five independent determinations were used for statistical analysis. Student's t-test and analysis of variance (ANOVA) were used for analyzing significant differences between control and treated plants ($p<0.05$).

RESULTS AND DISCUSSION

Our results showed that the photosynthetic rate of the two cotton varieties measured at different salt concentrations (50, 100 and 150 mM) varied significantly after 30 days of treatment (Fig. 1). The highest photosynthetic rate and the highest activity of RuBP carboxylase in controls as well as in plants treated with different salt concentrations were observed in plants of var. Arya-Anubam (Fig. 1 and 2). Although photosynthetic rates and RuBP carboxylase activity decreased with increasing salinity in both cotton

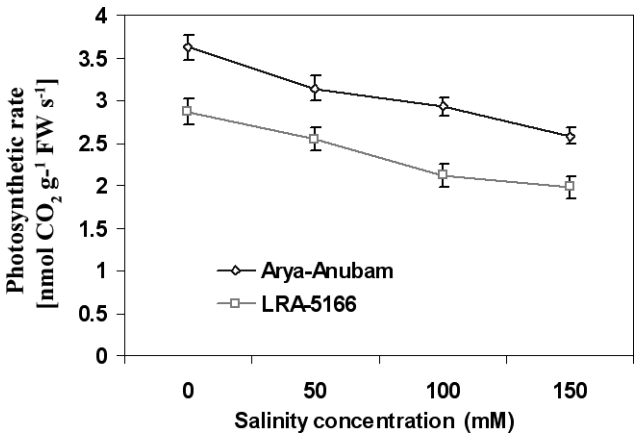

Fig. 1. Effect of salinity stress on photosynthetic rate in two different cotton varieties. Each value represents the mean ± S.E. obtained from five independent measurements, $p<0.05$.

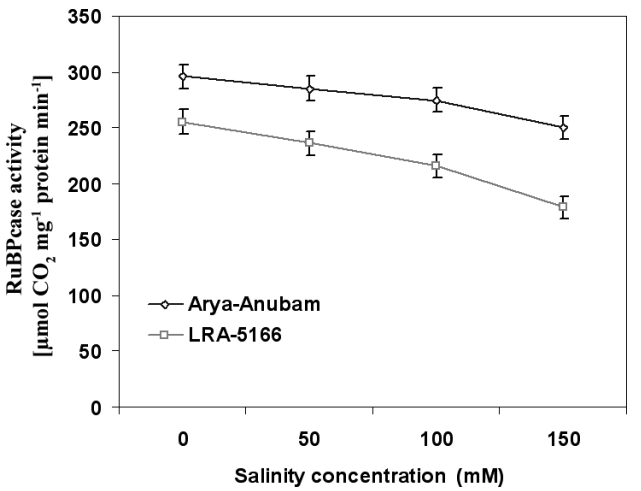

Fig. 2. Changes in RuBPcase activity in two different cotton varieties subjected to salinity stress. Each value represents the mean ± S.E. obtained from five independent measurements, $p<0.05$.

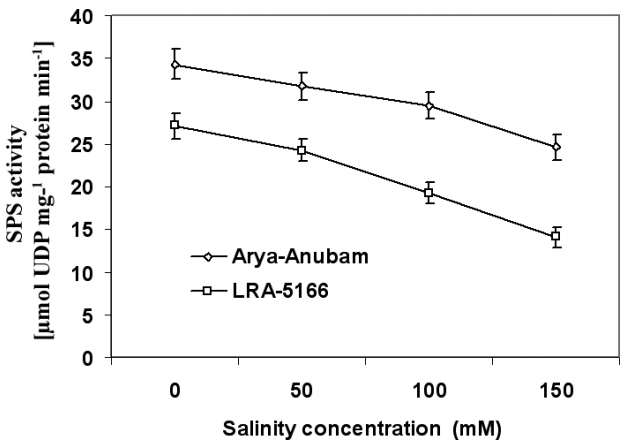

Fig. 3. Changes in sucrose phosphate synthase activity in two different cotton varieties subjected to salinity stress. Each value represents the mean ± S.E. obtained from five independent measurements, $p<0.05$.

varieties, var. Arya-Anubam showed a significant decrease in photosynthetic rates and RuBP carboxylase activity only at a concentration of 150 mM compared to the respective controls. Distinct differences were found in the activities of RuBPcase and SPS in var. LRA-5166 at all salt concentrations compared to var. Arya-Anubam. The effect of salinity stress on the activities of photosynthetic enzymes might be a secondary effect mediated by the reduced CO_2 partial pressure in the leaves caused by stomatal closure (Lawler and Cornic, 2002; Meloni et al., 2003; DeRidder et al., 2007). We also presume that salinity stress might affect the biochemistry of photosynthesis by causing disorientation of the lamellar system of chloroplasts and loss of chloroplast integrity leading to a decrease in the activities of photosystems. Variations in the activities of SPS were found between the two cotton varieties (Fig. 3). SPS of var. Arya-Anubam decreased at 150 mM (24.59 µmol/mg pro/min) when compared to the control (34.31 µmol/mg pro/min). Thus, var. Arya-Anubam manifested better photosynthetic potential than var. LRA-5166 even under high salinity stress conditions.

Resistance to salinity occurs when a plant withstands the imposed stress and this may arise from either tolerance or a mechanism that permits avoidance of the stress. The loss of the ability to scavenge free radicals during stress is generally attributed to a decrease in the activity of antioxidant enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR) (Pastori and Trippi, 1993; Mittova et al., 2002). Even under optimal conditions many metabolic processes produce ROS. The production of toxic derivatives is increased as a result of all types of abiotic or biotic stresses. Plants possess efficient systems for scavenging active oxygen species, thus protecting them from destructive oxidative reactions (Foyer et al., 1994). As part of this system, antioxidant enzymes are key elements in the defense mechanisms. Many changes have been observed in the activities of antioxidant enzymes in plants under salt stress. The activity of antioxidant enzymes has been reported to increase under saline conditions in the case of salt tolerant cotton (Meloni et al., 2003), shoot cultures of rice (Fadzilla et al., 1997), cucumber (Lechno et al., 1997), wheat shoot (Menogeuzzo and Navari-Izzo, 1999) and pea (Hernandez et al., 1999). In this study, the activities of antioxidant enzymes showed a progressive increase in both cotton varieties at all salt concentrations

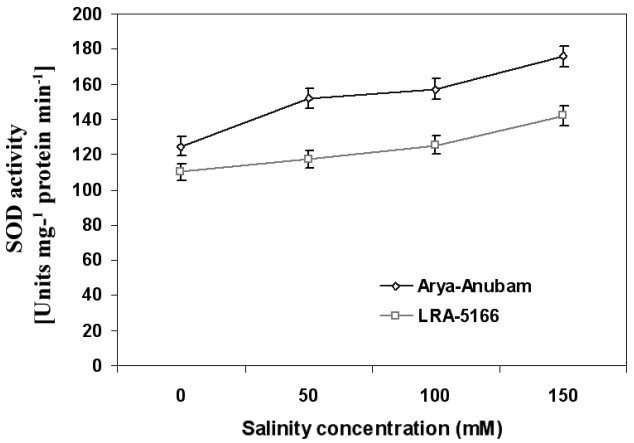

Fig. 4. Changes in superoxide dismutase activity in two different cotton varieties subjected to salinity stress. Each value represents the mean ± S.E. obtained from five independent measurements, $p<0.05$.

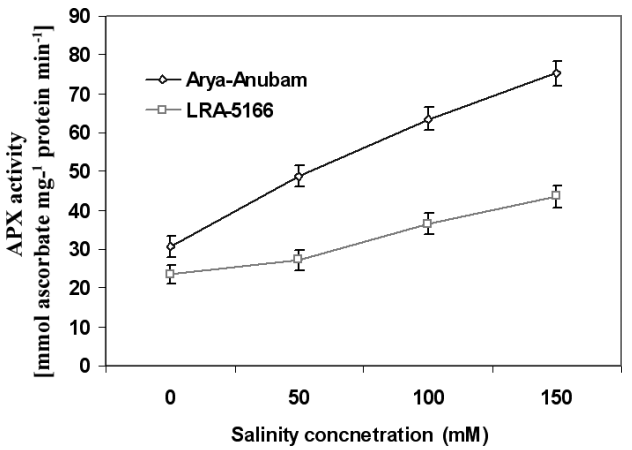

Fig. 5. Changes in ascorbate peroxidase activity in two different cotton varieties subjected to salinity stress. Each value represents the mean ± S.E. obtained from five independent measurements, $p<0.05$.

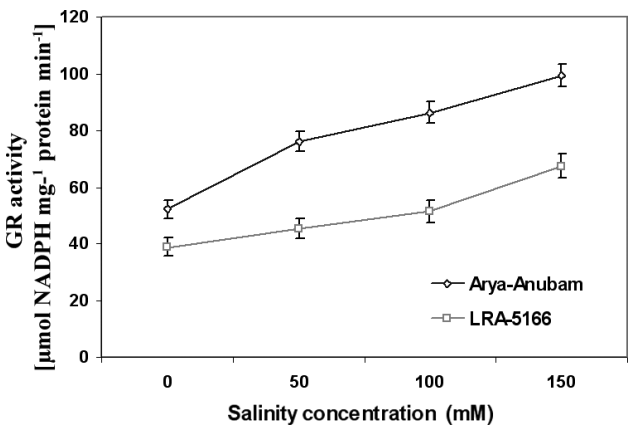

Fig. 6. Changes in glutathione reductase activity in two different cotton varieties subjected to salinity stress. Each value represents the mean ± S.E. obtained from five independent measurements, $p<0.05$.

applied (Figs. 4-6). However, the activities of all enzymes were markedly higher in var. Arya-Anubam than in var. LRA-5166 at all salinity levels. For example, var. Arya-Anubam showed a significantly increased activity of SOD (175.93 units/mg pro/min), APX (75.32 mmol/mg pro/min) and GR (99.47 µmol/mg pro/min) at 150 mM salt concentration when compared to control plants (124.63 units/mg pro/min, 30.65 mmol/mg pro/min, 52.19 µmol/mg pro/min, respectively). SOD is considered as a key enzyme in the antioxidant defense system as it regulates the concentration of O_2^- and H_2O_2. This enzyme is present in all aerobic organisms and in all sub-cellular compartments susceptible to oxidative stress (Bowler et al., 1992). In several cases transgenic plants overexpressing SOD showed increased tolerance to oxidative treatments and became more resistant to photoinhibition when exposed to different abiotic stresses (Smirnoff, 1993). Different levels of SOD activity might occur depending on stress intensity, species or genotype, growth conditions, stress period, plant age (Sgherri et al., 2000). Anyhow, a higher degree of protection against oxidative damage should require a fast removal of H_2O_2 by other scavenging systems, thus minimizing H_2O_2 toxicity and the formation of the highly toxic hydroxyl radicals (Perl et al., 1993). The intercellular level of H_2O_2 produced under stress conditions is regulated by catalases and peroxidases. Ascorbate peroxidases (APX) can scavenge H_2O_2 that is inaccessible for catalase because of their high affinity for H_2O_2 and their presence in different subcellular locations (Noctor et al., 2002). The analysis of the activity of APX in the two cotton varieties showed a significantly high activity in var. Arya-Anubam when compared to var. LRA-5166 (Fig. 5). Regeneration of the oxidized ascorbate is a critical component of the antioxidant scavenging system in plants. The activity of GR was also relatively high in var. Arya-Anubam (Fig. 6). The elevated levels of GR might be able to increase the ratio of $NADP^+$ to accept electrons from the photosynthetic electron transport chain (Peltzer et al., 2002; Reddy et al., 2004). Gosset et al., 1994 reported that in the salt tolerant cotton genotype, GR activity significantly increased under salinity stress.

Plants accumulate compatible osmolytes such as proline and glycine betaine when they are subjected to salinity stress and they appear to protect plants from such stresses (Zhu, 2001). The osmolytes are known to

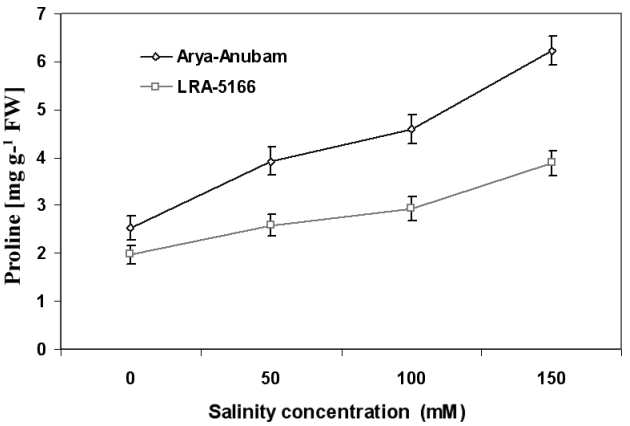

Fig. 7. Effect of salinity stress on the content of proline in two different cotton varieties. Each value represents the mean ± S.E. obtained from five independent measurements, $p<0.05$.

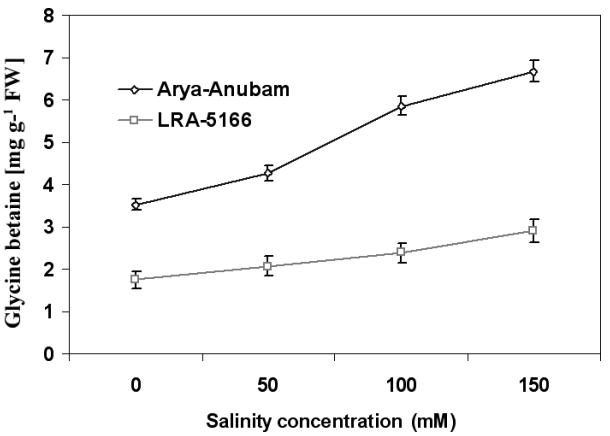

Fig. 8. Effect of salinity stress on the content of glycine betaine in two different cotton varieties. Each value represents the mean ± S.E. obtained from five independent measurements, $p<0.05$.

function in protecting macromolecules by stabilizing protein structure and/or scavenging ROS produced under stress conditions (Mitysik et al., 2002). Proline is a dominant organic molecule that acts as a mediator of osmotic adjustment under salinity stress, a stabilizer of sub-cellular structures, a sink for energy and even a stress-related signal. It is also involved in cell osmoregulation, protection of proteins during dehydration and can act as an enzymatic regulator during stress conditions (Rontein et al., 2002). Glycine betaine has strong osmoprotective properties and is known to confer tolerance to salinity stress (McNell et al., 2001). The quantitative estimation of proline and glycine betaine in the two cotton varieties is shown in Figs. 7 and 8. In var. Arya-Anubam proline and glycine betaine

contents were significantly increased by 6.22 mg/g FW, 6.68 mg/g FW, respectively at 150 mM salt concentration when compared to control plants (2.53 mg/g FW, 3.53 mg/g FW, respectively). On the other hand, in var. LRA- 5166, the enhancement of proline and glycine betaine contents was 3.88 mg/g FW and 2.92 mg/g FW when compared to control plants (1.97 mg/g FW, 1.75 mg/g FW, respectively). Although the two cotton varieties accumulated more proline and glycine betaine with increasing salinity stress, var. Arya-Anubam possessed comparatively higher amounts of proline and glycine betaine, providing evidence for an efficient role of these metabolites as osmoprotectants under salinity stress. The data in the present study demonstrated that plants of var. Arya-Anubam exhibited higher adaptive potential under salinity stress as judged by photosynthetic carbon assimilation, antioxidant defense metabolism and accumulation of osmoprotectants when compared to var. LRA-5166.

References

Asada, K., 1992. Ascorbate peroxidase-a hydrogen peroxide scavenging enzyme in plants. Physiol. Plant., 85, 235-241.

Ashraf, M., M.R. Foolad, 2007. Role of glycine betaine and proline in improving plant abiotic stress resistance. Environ. Exp. Bot., 59, 206-216.

Bates, L.S., R.P. Waldem, I.D. Teare, 1973. Rapid determination of free proline for water stress studies. Plant and Soil., 39, 205-208.

Beauchamp, C.O., I. Fridovich, 1971. Superoxide dismutase; Improved assays and an assay applicable to acrylamide gels. Anal. Biochem., 44, 276-287.

Bowler, C., M. Van Montagu, D. Inze, 1992. Superoxide dismutase and stress tolerance. Ann. Rev. Plant Physiol. Plant Mol. Biol., 43, 83-116.

Boyer, J.S., 1982. Plant productivity and environment. Science., 218, 443-448.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye

binding. Anal. Biochem., 72, 248-253.

Cost, P., A. Neto, M. Bezerra, T. Prisco, E. Filho, 2005. Antioxidant–enzymatic system of two *Sorghum* genotypes differing in salt tolerance. Braz. J. Plant Physiol., 17, 139-147.

DeRidder, B.P., M. Salvucci, 2007. Modulation pf Rubisco activase gene expression during heat stress in cotton (*Gossyoium hirsutum* L.) involves post-transcriptional mechanisms. Plant Sci., 172, 246-252.

Fadzilla, N.M., R.H. Finch, R.H. Burdon, 1997. Salinity, oxidative stress and antioxidant responses in shoot cultures of rice. J. Exp. Bot., 48, 325-331.

Foyer, C.H., P. Descourvieres, K.J. Kunert, 1994. Protection against oxygen radicals: an important defence mechanism studied in transgenic plants. Plant and Cell Environ., 17, 507-523.

Foyer, C., B. Halliwell, 1976. The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. Planta., 133, 21-25.

Frommer, W.B., U. Ludewig, D. Rentsch, 1999. Taking transgenic plants with a pinch of salt. Science., 285, 1222-1223.

Gosset, D.G., E.P. Millhollon, M.C. Lucas, 1994. Antioxidant response to NaCl stress in salt-tolerant and salt-sensitive cultivars of cotton. Crop Sci., 34, 706-714.

Hasegawa, P.M., R.A. Bressan, J.K. Zhu, H.J. Bohnert, 2000. Plant cellular and molecular responses to high salinity. Annu. Rev. Plant Physiol. Plant Mol. Biol., 51, 463-499.

Hellman, H., D. Funk, D. Rentsch, W.B. Frommer, 2000. Hypersensitivity of an *Arabidopsis* sugar signaling mutant toward exogenous proline application. Plant Physiol., 123, 779-790.

Hermandez, J.A., A. Campilio, A. Jimenez, J.J. Alarcon, F. Sevilla, 1999. Response of antioxidant systems and leaf water relations to NaCl stress in pea plants. New Phytol., 141, 214-251.

Huber, S.C., 1981. Interspecific variation in the activity and regulation of leaf sucrose phosphate synthase. Z Pflanzenphysiol., 102, 443-450.

Lawlor, D.W., G. Cornic, 2002. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. Plant Cell Environ., 25, 275-294.

Lechno, S., E. Zamki, E. Tel-or, 1997. Salt stress-induced responses in cucumber plants. J. Plant Physiol., 150, 206-211.

Lorimer, G.H., M.R. Badger, T.J. Andrews, 1977. D-ribulose 1,5-bisphosphate carboxylase-oxygenase. Improved methods for activation and assay of catalytic activities. Anal. Biochem., 78, 66-75.

Masood, A., N.A. Shah, M. Zeeshan, G. Abraham, 2006. Differential response of antioxidant enzymes to salinity stress in two varieties of *Azolla* (*Azolla pinnata* and *Azolla tilieuloides*). Env. Exp. Bot., 58, 216-222.

McNell, S.D., M.L. Nuccio, M.J. Zeimark, A.D. Hanson, 2001. Enhanced synthesis of choline and glycine betaine in transgenic tobacco plants that overexpress phosphoethanolamine N-Methyltransferase, Proc. Nat..Acad. Sci. USA., 98, 10001-10005.

Meloni, D.A., M.A. Oliva, C.A. Martinez, J. Cambraia, 2003. Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. Environ. .Exp. Bot., 49, 69-76.

Menogeuzzo, S., F. Navari-Izzo, 1999. Antioxidative responses of shoot and roots of wheat to increasing NaCl concentrations. J. Plant Physiol., 155, 274-280.

Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci., 7, 405-410.

Mittova, V., M. Tal, M. Volokita, M. Guy, 2002. Salt stress induces up – regulation of an efficient chloroplast anti oxidant system in the salt-tolerant wild tomato species *Lycopersicon pennelli* but not in the cultivated species. Physiol. Plant., 115, 393-400.

Mitysik, J., B. Alia, P. Mohanty, 2002. Molecular mechanism of quenching of reactive oxygen species by proline under stress in plants. Current Sci., 82, 525-532.

Nakano, Y., K. Asada, 1981. Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. Plant Cell Physiol., 22, 867-880.

Noctor, G., S. Veljovic-Jovanovic, C.H. Foyer, 2002. Peroxide processing in photosynthesis: antioxidant coupling and redox signaling. Philos. Trans. R. Soc. London., 355, 1465-1475.

Pal, M., D.K. Singh, L.S. Rao, K.P. Singh, 2004. Photosynthetic characteristics and activity of antioxidant enzymes in salinity tolerant and sensitive rice cultivars. Indian J. Plant Physiol., 9, 407-412.

Pastori, G.M., V.S. Trippi, 1993. Antioxidative protection in a drought-resistant maize strain during leaf senescence. Physiol. Plant., 87, 227-231.

Peltzer, D., E. Dreyer, A. Polle, 2002. Differential temperature dependencies of antioxidative enzymes in two contrasting species: *Fogus sylvatica* and *Coleus blumei*. Plant Physiol. Biochem., 40, 141-150.

Perl, A., R. Perl–Treves, S. Galili, D. Aviv, E. Shalgi, 1993. Defense in transgenic potato expressing tomato Cu, Zn superoxide dismutase. Theor. Appl. Genet., 85, 568-576.

Prochazkova, D., N. Wilhelmova, 2007. Leaf senescence and activities of the antioxidant enzymes. Biol. Plantarum., 51, 401-406.

Reddy, A.R., K.V. Chaitanya, M. Vivekanandan, 2004. Drought-induced response of photosynthesis and antioxidant metabolism in higher plants. J. Plant Physiol., 161, 1189-1202.

Rontain, D., G. Basset, A.D. Hanson, 2002. Metabolic engineering of osmoprotectant accumulation in plants. Met. Eng., 4, 49-56.

Sgherri, C.L.M., M. Maffei, P. Navari-Izzo, 2000. Antioxidative enzymes in wheat subjected to increasing water deficit and rewatering. J. Plant Physiol., 157, 273-279.

Smirnoff, N., 1993. The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol., 125, 27-58.

Sotiropoulos, T.F., 2007. Effect of NaCl and $CaCl_2$ on growth and contents of minerals, chlorophyll, proline and sugars in the apple rootstock M4 cultured *in vitro*. Biol. Plantarum., 51, 177-180.

Storey, R., W.R.G. Jones, 1977. Quaternary ammonium compounds in plants in relation to salt tolerance. Phytochemistry., 16, 447-453.

Sundar, D., A.R. Reddy, 2000. Low night temperature induced changes in photosynthesis and rubber accumulation in *Guayule* (*Parthenium argentatum* Gray). Photosynthetica, 38, 421-427.

Zhu, J.K., 2000. Genetic analysis of plant salt tolerance using *Arabidopsis*. Plant Physiol., 124, 941-948.

Zhu, J.K., 2001. Plant salt tolerance. Trends Plant Sci., 6, 66-71.