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Salinity stress induced damage to lipids, proteins, primary photochemistry and possible protection by antioxidants in rice varieties from khazan fields of Goa

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ABSTRACT

Effect of salinity stress (50-150mM Nacl) was studied in three rice varieties (Jaya, Jyoti & Korgut) commonly grown in Goankhazan fields. Damage to primary photochemistry, lipids and proteins was studied through chlorophyll fluorescence (Fv/Fm, qP, NPQ & ETR), lipid peroxidation and protein oxidation. Possible role of antioxidant system (enzymatic & non-enzymatic) in protection against given salinity stress was studied. The study showed no significant damage to photosynthetic machinery. Rice variety Jyoti, showed more lipid peroxidation indicative of damage due to salinity stress, however showed better protection through accumulation of compatible solute such as proline. Antioxidant enzymes like ascorbate and APX showed no significant increase under salinity stress. The data obtained thus showed that influence of salinity stress may vary depending upon the varieties. All the three varieties are adapted to salinity stress, but Korgut and Jyoti appeared to be more tolerant to stress.

Key words: *Antioxidants, Fluorescence,Khazan, Peroxidation, Salinity*

Physiological processes from seed germination to plant growth are known to be affected severely due to salinity. Increase in salt concentration is known to cause water deficiency, ion toxicity, and nutrient deficiency and lead to plant death (Maggio *et al.*, 2010). Salinity also affects phenotypic and physiological processes by accumulation of Na^+ and Cl : This in turn results in hyper-ionic and hyper-osmotic stress inhibiting plant growth and development. It is causing damage to membranes and enhanced peroxidation of polyunsaturated fatty acids (PUFA) (Smirnoff, 1995).

Reactive oxygen species are often the results of salinity stress causing oxidative damage to plants (Smirnoff, 1995). The ROS have the ability to damage all the biomolecules such as proteins, lipids and nucleic acids (DNA, RNA)(Pawar and Pannerselvam, 2012). It also causes cell wall membrane damage & disruption of membrane lipids leading to metabolic impairment in the plant cell (Pawar and Pannerselvam, 2012). Harmful effects of (ROS) such as superoxide radicals (O^{-2}), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-) on plant proteins, photosynthetic pigments, lipids with membrane disruption is highly understood (Zhu 2009, Kundan Kumar *et al.* 2013). Moller and Kristensen (2004) have shown that oxidized proteins are the markers towards the oxidative stress. Oxidative modification of proteins under such stress impairs protein function, modifies the gene expression and affects the growth and development (Feechan *et al.*, 2005). In the chloroplast ROS damages photosystem II (Takahashi *et al.*2008) & hence it is necessary to regulate excess generation of ROS by the photosynthetic (CO_2) fixation. Salinity stress induced damage to photosynthesis is studied in terms of maximum quantum efficiency of PSII (Fv/Fm), photochemical quenching, and loss of excitation energy during electron transport as NPQ and overall electron transport rate (Amirjani 2010).

An efficient antioxidant networking system is operational in plants that respond to oxidative damage

(Foyer and Shigeoka, 2011). The antioxidant defenses in plants includes enzymatic antioxidants such as superoxide dismutase (SOD), peroxidase (POX), glutathione reductase (GR), and ascorbate peroxidase (APX) (Noctor and Foyer, 1998). The non enzymatic antioxidants includes carotenoids, total ascorbate, glutathione along with tocopherol, flavonoids and phenolics (Pallavi Sharma *et. al.* 2012). Amongst the non enzymaticantioxidative defense system, ascorbate–glutathione (ASC-GSH) cycle has been regarded as the most effective detoxifying mechanism (Anjum*et al.* 2011). These antioxidant systems play a major role in maintaining balance between H_2O_2 generation and scavenging of ROS in the chloroplast (Asada, 2006).

In Goa a large proportion of agricultural lands are khazan fields. Khazans are the low lying fields, subjected to fluctuations of salinity with frequent ingression of sea water from the estuaries. Rice is agronomically important staple foodcrop grown in these khazan fields. Jaya, Jyoti (hybrid) &Korgut (traditional non-hybrid) are the major rice varieties grown in these salinity affected lands. Not much work is done on these rice varieties and for the first time we report the impact of salinity stress on these rice varieties. The objective of this work hence, was to understand salinity induced damage to photosynthesis, proteins, membrane lipids and study protection by antioxidant enzymes if any.

MATERIALS AND METHODS

Plant material

Seeds of Jaya (salinity tolerant), Jyoti (salinity sensitive) & Korgut (salinity tolerant non-hybrid) rice varieties were obtained from ICAR old Goa.

Growth condition and salinity treatment

The seeds were surface sterilized with 0.1% Mercuric chloride ($MgCl_2$) solution for about 5 minutes, thoroughly washed with distilled water and were soaked overnight. The seeds were allowed to sprout for 2 days in muslin cloth prior to sowing in plastic pots containing soil: sand: peat (1:1:2 v/v). The seedlings were grown in the laboratory condition under illumination at 25^{0}C relative humidity (RH) of 70% and 200ìmol m^{-2} s^{-1} light intensity for 2 weeks. Two weeks old rice seedlings were then treated with salinity treatment of different NaCl concentration (50, 100, 150mM) and allowed to grow for next 12 days. Control plants were grown in distilled water alone. After 12 days of salinity treatment, the fully expanded leaf tissue from rice cultivars were then harvested and used to carry out different enzymatic assays.

Chlorophyll fluorescence measurement

Chlorophyll fluorescence measurement was done by using two weeks old rice seedlings (12 days) as described by Schrieber *et al.* 1986. Chlorophyll florescence in dark adapted leaves was excited, and measured using portable chlorophyll fluorometer Hansatek (UK). Photosynthetic efficiency (Fv/Fm) was determined after 10 min of dark acclimation of selected leaves using dark leaf clip. Actual quantum yield was measured on the fully expanded leaves that were illuminated with actinic light after dark adaptation. The florescence parameters like electron transport rate (ETR), Photochemical (qP) and Non-photochemical quenching (NPQ) were also measured.

Lipid peroxidation

Peroxidation of membrane lipids was studied as TBA-MDA adduct formation according to the method of (Sharma &Singhal, 1992). Tissue (0.1g) was homogenized using mortar and pestle in 1% TCA solution to a final volume of 5ml. The homogenate was centrifuged at 2000 rpm for 5 min.

Known concentration of the supernatant was then mixed with 2.5 ml of 0.5% TBA in 20% TCA and 2.5ml of incubation buffer composed of 50mM Tris-HCl and 175mM NaCl, pH 8.0. The contents of the tubes were then incubated at 95°C for 30 min, cooled and absorbance wasread at 600 and 532 nm. MDA was expressed as ìmol/g F.w.

Estimation of proline

Free proline was estimated at 520 nm according to the method of (Bates *et al.*1973) with L-proline as standard. Plant tissue (0.1 g) was homogenized in 5ml of 3% sulphosalicylic acid using mortar and pestle. The extract was centrifuged for 7 min at 2000rpm. 1ml of the supernatant was mixed with 1ml each of glacial acetic acid and acidic ninhydrin. The tube contents were vortexed for 5min and then incubated in a boiling water bath for an hour. The test tubes were then cooled to room temperature and placed on ice. 4ml of toluene was added to each test tube and mixed by vigorous shaking. The pink red chromophore developed in the

toluene layer was read at 520nm in a spectrophotometer. Amount of proline (ìmol/g. Fw) in the sample was calculated based on the standard curve.

Protein Oxidation

Oxidation of Proteins was studied according to the method of Levine *et al*.1994. Plant tissue (200mg) was homogenized in 2ml of cold buffer containing (50mM 2- Morpholinoethanesulphonic acid (MES) in 1mM EDTA). The aliquot was then centrifuged at 15,000 g at 4<C for 15mins. The supernatant was used as an enzyme source and stored on ice. Absorbance of the supernatant was then checked at 260nm and 280nm, in order to determine the contamination by nucleic acids in the sample. Homogenization buffer was used as a blank. If the ratio of 260nm/280nm is <1, then removal of nucleic acids by adding 1% sterptomycinesulphate is required. Enzyme extract (1200µL) was pippeted out into two centrifuge tubes, one tube was labeled as sample tube (S#) and other one as control tube (C#). 800 µL of 10mM 2-4-dinitrophenyl hydrazine (DNPH) was then added in sample tube and 2.5mM HCL (4800 µL) in control tube. Both the tubes were incubated at room temperature for 1hr followed by vortexing during incubation after every 15 mins. This was followed by addition of 20% TCA (6ml) into the respective tubes and vortexed again for 5mins. The tubes were centrifuge at 10,000 rpm at 4<C for 15mins. Supernatant was then discarded and pellet wasresuspended in 10% TCA (6ml) and centrifuged again at 10,000rpm for 15mins. The pellet was retained after centrifugation followed by washing 3 times with freshly prepared ethanol: ethyl acetate (1:1). The pellet was thereafter centrifuged at 10,000rpm at 4<C for 15mins.This step was repeated two more times. After the final wash, the protein pellet was again suspended in 3ml of Guanidine hydrochloridefollowed by centrifugation at 10,000 rpm at 4<C for 10 minsand debries was removed. The absorbance was measured at 375 nm by using 6M guanidine hydrochloride as a blank.

Ascorbate and Dehydroxyascorbate Assay (ASC+DHA)

Ascorbate and Dehydroascorbate was measured using 2, 2-Bipyridal based calorimetric assay (Nakano & Asada, 1981). Leaf tissue (0.2g) was homogenized in liquid nitrogen (Liq. N_2) and extracted with 0.1 M HCL in 1mM EDTA (pH 7.0). After centrifugation for 2 mins at 5000 rpm, 20 µL of supernatant was taken for the assay with addition of 0.4 M phosphate buffer and 10mM DTT.

Samples were then incubated for 10 mins at room temperature to reduced oxidized ascorbate pool. Excess DTT was removed by addition of 0.5 % N-Ethylmaleimide (NEM). Buffer alone was used in place of DTT and NEM for the assay of reduced ascorbate content. Color reagent (80 µL) comprising of 4.6 % TCA, 15.3 % Ortho-phosphoric acid, 4 % 2, 2- Bipyridyl in 70 % ethanol and 0.6 % $FeCl_3$ was added to all the reaction tubes. Blanks and ascorbate standards were prepared using 6% TCA alone. All the assay tubes were again incubated at 42<"C for 45 mins and the absorbance was read at 520nm. Concentration of total and reduced ascorbate was calculated from the standard curve maintained with ascorbate. Concentration of dehydroascorbate (DHA) was calculated after subtracting the values of reduced ascorbate from those of total ascorbate.

Ascorbate Peroxidase (APX) enzyme activity

Ascorbate peroxidase (APX; EC 1.11.1.11) activity was determined according to the method of (Rama-devi& Prasad, 1998). Leaf sample (0.1 gm) was homogenized in 5mL of 50mM Tris HCL buffer (pH 7.8). The homogenate was centrifuged at 12000 rpm at 4 <C for 20 min and supernatant was used as enzyme source. The reaction mixture in a final volume of 3ml contained 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.5 mM sodium ascorbate, 0.1 mM H_2O_2, 2mMascorbate and 100µL enzyme. The decrease in absorbance at 290 nm for 1 min was recorded and the amount of ascorbate oxidized was calculated using extinction coefficient (2.8 mM^{-1}).

Tocopherol activity

Estimation of tocopherol in plant tissue was assayed by Emmerie-Engel reaction according to the method of (Rosenberg, 1992). Plant samples (0.25gm) were homogenized in 0.1N H_2SO_4 and allowed to stand overnight. The contents were shaken vigorously and filtered through whatman filter paper No. 1. The filtrate was used for the assay. 1.5 mL of plant extract, 1.5 mL standard (tocopherol) and 1.5 mL distilled water was pipetted out separately in three individual centrifuge tubes. Ethanol and xylene equivalent to 1.5 mL was added in the above tubes, mixed well and centrifuged.

After centrifugation 1 mL of the xylene layer was transferred into another centrifuge tube. To each of this tubes 1mL of 2,2-Bipyridyl reagent was added, mixed and 1.5mL of the mixture was spectrophotometrically assayed at 460 nm. The reaction mixture was then incubated for 15 mins after addition of 0.33 mL of $FeCl_3$ and mixed again. The development of red color was measured spectrophotometrically at 520nm using Thermo scientific UV-Visual spectro photometer.

Statistical analysis

Analysis of variance (ONE WAY ANOVA) averages in case of data recorded on three varieties were analyzed.

RESULTS AND DISCUSSION

Damage to Photosynthesis (PSI& PSII)

Plant response to salt stress is very complex and extremely variable with wide range of changes at molecular, cellular and whole plant level (MadhavaRao *et al.* 2006).Chlorohyll florescence characteristics are an important tool used to determine the influence of various biotic and abiotic stresses on photosynthetic process (Stirbet and Govindjee, 2011). Maximum quantum efficiency of photosynthesis is determined by Fv/Fm ratio (SaeedSaeedipour 2009, Amirjani 2010). Salt induced effect on PSII is widely studied with contradictory results. Kalaji *et al.*(2011) have shown inhibitory effect of salinity stress on PSII activity while report by Belkhodja *et al.* (1994) and Jimenez *et al.* (1997) showed no significant effect on structure and function of PSII in response to NaCl treatment.Our results on efficiency of PSII (Fv/Fm), electron transport rate (ETR), photochemical quenching (qP) and non-photochemical quenching (NPQ) are shown in Fig. 1.The photosynthetic efficiency studied as Fv/Fm ratio showed no significant effect of salinity stress on the primary photochemistry of PSII. This indicates that the light reaction in these rice varieties was not affected due to salt stress. Similar observations are also reported by Dongsansuk *et al.* (2013) in KDML105, RD6 & Pokkali rice varieties with no significant differences between salt treated and non treated varieties.

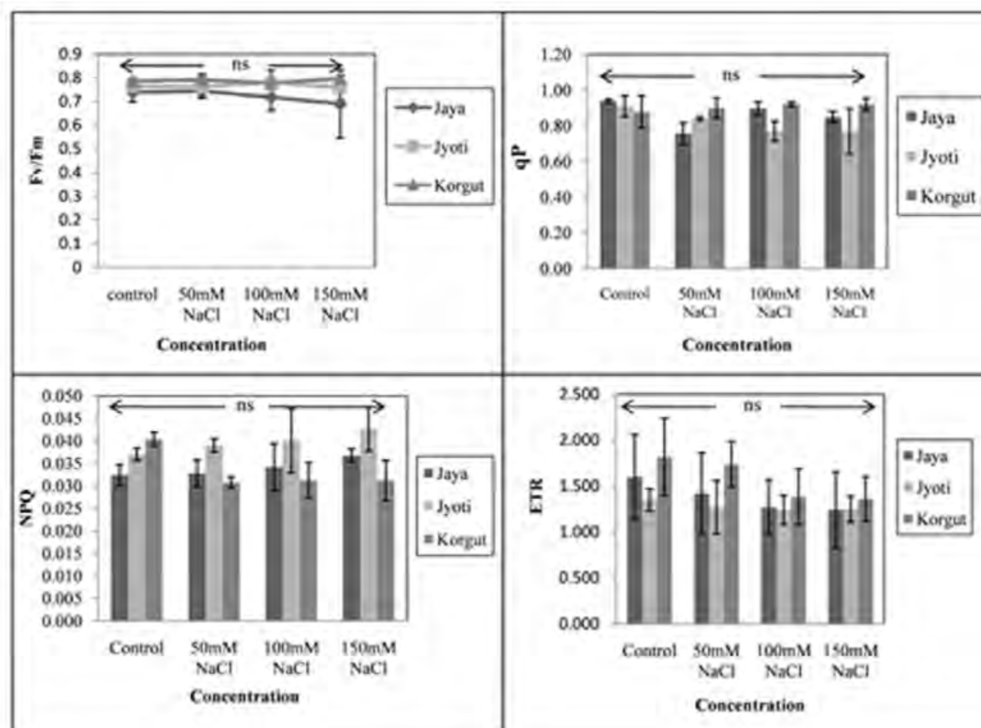

Fig. 1. Effect of salinity stress alone on photosynthetic efficiency (Fv/Fm), photochemical quenching (qP), Non- photochemical quenching (NPQ) and electron transport rate (ETR) in vivo. The values are mean of 3 experiments ± S.D (n=3). Statistical data shows non- significant difference at^{ns}P>0.05by one way ANOVA.

Photochemical quenching (qP)

Photochemical quenching (qP) is one of the important parameter that determines utilization of harvested energy by photochemical reactions (Sankhalkar and Sharma, 2005). Decline in qPwas observed in our study under salt stress. A non significant decline in qP in Jaya and Jyoti rice varieties was observed in comparison to their controls. However, an increase of 4.34 % at 150 mMNaCl stress was observed in Korgut indicating its better efficiency in synthesis of $NADPH_2$ & ATP. Decreased photochemical quenching under salinity stress may be involved in the protection against photo-damage during early dissipation with reduction in the relative quantum yield of PSII (Weis and Berry 1987; Krause and Weis, 1991). This maintains the adequate balance between photosynthetic electron transport and carbon fixation (Kafi, 2009). The results are thus in correlation with the findings of Netondo *et al.* (2004) that observed similar decline in sorghum under salinity stress. In another report a similar decline in qP was reported by Yasemin Ekmekc (2008) in cheakpea under draught stress. Cha-um and Kirdmanee (2009) showed reduced photochemical quenching in maize leaf tissue grown under salinity stress. Thus decline in qP thus indicate possible protective mechanism in plants against stresses.

Non Photochemical quenching (NPQ)

NPQ represents dissipation of excess excited energy away from the reaction centre thereby protection to PSII (Lima *et al*. 2002, Ashraf *et al.* 2013).Our results with non photochemical quenching (NPQ) studied as amount of excited energy lost as heat during photosynthetic electron transport showed non-significant increase of 13.51% and 15.62 % in Jaya, Jyoti respectively at 150 mMNaCl in comparison to its control. Korgutrice showed no significant change under salinity stress. The results thus indicates that Jyoti rice variety is comparatively better adapted to given salinity stress over other two varieties.This increase may represent the decreased demand for product of electron transport by dissipation of heat energy (Chaves *et al.,* 2009; Baker *et al.,* 2004). Similar increase in NPQ was reported in sorghum by Netondo *et al.* (2004)and in maize cultivars by Cha-um and Kirdmanee (2009) under salinity treatment.

Electron Transport Rate (ETR)

Electron transport rate (ETR) is the actual rate of electron flow, which is derived from quantum yield of PSII and is considered to be one of the very important photosynthetic parameter (Amirjani 2010). Our study on electron transport rate (ETR) did not showed any significant change with the increasing salinity treatment in Jaya, Jyoti and Korgut rice varieties. However, Korgut rice variety, showed maximum increase of 46% in ETR at 150 mMNaCl stress in comparison to its control. A linear decline in the ETR was observed in Jaya rice variety.Gao*et al.* (2008) has shown a linear relationship between O_2 production and carbon fixation and salinity effect on ETR through reduced oxygen production and carbon fixation. However, no changes in ETR were observed in our study.

Lipid peroxidation

Malondialdehyde (MDA) is studied as one of the parameters of damage to lipid molecule. When poly unsaturated fatty acids in the membrane undergo oxidation by the accumulation of free oxygen radicals large proportion of MDA are produced (Khan and Panda, 2008; Hernandez *et al*., 2000). Damage to the cellular membranes was studied by accumulation of the malondialdehyde (MDA) levels and results are shown in Figure 2. Our results with increasing salinity stress (50-150mM) showed significant increase in MDA content in all rice varieties studied. In comparison to

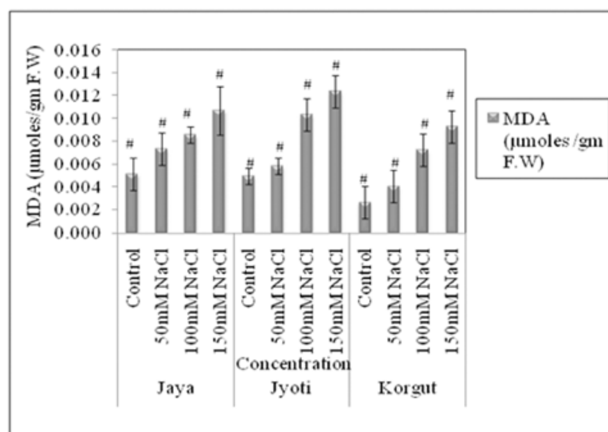

Fig.2. Effect of salinity stress alone on lipid peroxidation. The values are mean of 3 experiments, ± S.D (n=3). Statistical data shows highly significant difference at $^{\#}P<0.001$by one way ANOVA.

their respective controls (150 mMNaCl stress) this increase was 107%, 150% and 246% in Jaya, Jyoti and Korgut respectively. When these three varieties were compared amongst themselves, Jyoti showed highest MDA content while Korgut had the least appearing to be more salinity tolerant.Work by Ben Amor *et al.*(2005) and Chaparzade *et al.(*2004) have shown that increase in lipid peroxidation may be due to the poor or inability of antioxidants to scavenge reactive oxygen species. These results are also in correlation to our result which showed significant ($^{\#}P< 0.001$) increase in lipid peroxidation with a decline in enzymatic antioxidant like APX and non enzymaticantioxidant like ascorbate.

Proline content

Stress responsive amino acid such as proline is shown to be up-regulated under drought (Hare P.D*et al.* 1998) or salinity stress (Munns R. 2005, Rhodes D *et al.* 2002). Effect of salt stress on proline accumulation are shown in Figure 3. Significant ($^{\#}P<0.001$) increase in proline content was observed in all three rice varieties with Jyoti showing highest level of proline 349% followed by Jaya 294% and Korgut 159%. Our results indicate that Jyoti is significantly tolerant to given salinity stress and are in correlation with number of reports that showproline accumulation suggesting its possible adaptation to salinity (Shafi *et al.*2011, Misra and Saxena, 2009).

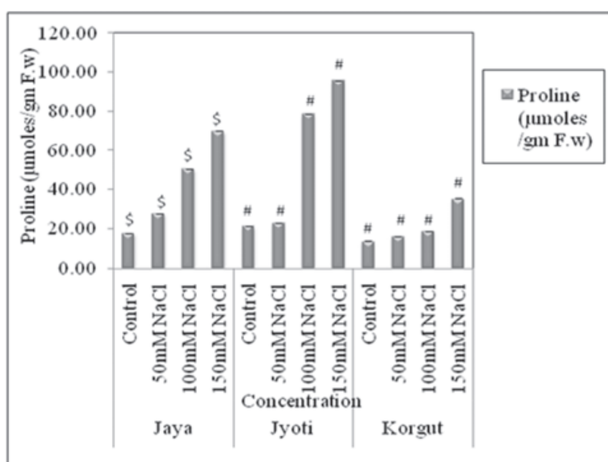

Fig. 3. Effect of salinity stress alone on proline content. The values are mean of 3 experiments, ± S.D (n=3). Statistical data shows highly significant and significant difference at $^{\#}P<0.001$&$^{\$}P>0.01$ by one way ANOVA.

Protein oxidation

Reactive oxygen species (ROS) generated under salinity stress, attacks proteins and other biomolecules directly or indirectly through carbonylation and nitrosylation (Pallavi Sharma *et al.,* 2012).Under stressful condition injured or damaged tissues are known to affects the proteins by oxidizing them (Moller and Kristensen 2004). Our results of salinity induced (50- 150mM NaCl) protein oxidation are shown in Table 1.An increase of 263.5%, 275.56% & 113.34% in protein oxidation was observed in Jaya, Jyoti and Korgut rice varieties respectively. However, the increase was significant (*P<0.05) with 47.12nmol/ml in Jaya at 150mM NaCl stress in comparison to its control. The results showed linear increase in oxidation of protein and isthus correlatedtodecline inenzyme activity in all the three rice varieties studied. From the above results Jyoti rice variety appeared to be better adapted to the given salinity stress.

Effect of salinity stress on total ascorbate (ASC+DASC)

Plants ability to synthesize complex enzymatic antioxidant systems as possible defense mechanisms against abiotic stresses is well known (Pallavi Sharma *et al.* 2012). Low molecular weight compounds like ascorbate, glutathione and tocoplerol are non-enzymatic antioxidants playing important role in cellular compartmentationand disposal of H_2O_2that is in turn harmful (Pallavi Sharma *et al.* 2012, GaberAbogadallah 2010). Antioxidants also influence plant growth and development (Pinto &Gara 2004). Increase in the level of non enzymatic antioxidants under stress is directly correlated to reduced ROS (Wook Kim *et al.* 2011).The total ascorbate (ASC+DASC) was calculated as µmoles gm^{-1}F.w and is shown in Figure 4. With increasing salt stress (50-150mM NaCl) non-significant ($^{ns}P>0.05$) decline of 27.90 %, 13.88 % and 21.73 % in total ascorbatecontent was observed in Jaya, Jyoti&Korgutresp. When the rice varieties were compared to each other Korgut rice showed higher total ascorbate (0.54 µmoles gm^{-1}F.w) followed by Jaya (0.31µmoles gm^{-1}F.w) and Jyoti(0.31µmoles gm^{-1}F.w) rice varieties. The study undertaken by us showed a decline in total ascorbate content in all the three rice varieties. Work by Ashrafuzzaman(2013) has also

Table 1. Effect of salinity stress alone on protein oxidation. The values are mean of 3 experiments ± S.D (n=3). Statistical data shows significant difference at (*P<0.01) and non-significant difference at (nsP>0.05) by one way ANOVA.

Concentration	Jaya	Jyoti	Korgut
	Protein oxidation (nmol/ml)		
Control	12.96+7.53*	3.56+0.87ns	9.7+1.08 ns
50Mm NaCl	35.61+9.08*	4.51+0.512 ns	24.02+8.02 ns
100mM NaCl	48.07+11.70*	6.63+1.08 ns	16.44+5.77 ns
150mM NaCl	47.12+12.02*	13.37+5.34 ns	20.69+2.49 ns

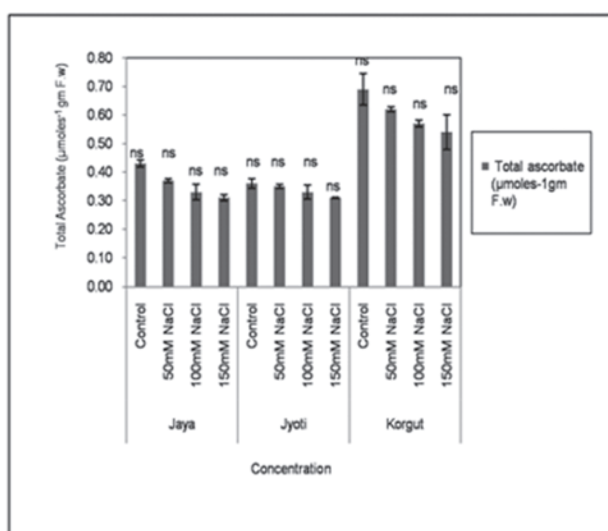

Fig. 4. Effect of salinity stress alone on total ascorbate (ASC+ DASC).The values are mean of 3 experiments, ± S.D (n=3).Statistical data shows non-significant difference at nsP>0.05 by one way ANOVA.

reported a similar decline in ascorbate content in BRRI dhan 29 salt sensitive rice variety. Tolerant variety Korgut accumulated comparatively higher ascorbate content compared to that of Jaya and Jyoti. We have also observed higher GSH content in the above rice varieties (data not shown). Presence of higher level of total and reduced ascorbate (ASA+DHA) was observed in CSR10 rice cultivar (Satpal and Tripathy *et al.* 2012) explaining its potential to destroy the harmful effects of ROS. Sufficient evidencesare available which shows increase in glutathione overlaps with antioxidant functions of ascorbate and glutathione (Niyogi *et al.,* 2004).

Effect of salinity stress alone on ascorbate peroxidase (APX) activity

Enzymatic antioxidant like ascorbate peroxidase was studied and the results of APX activity are shown in Table 2.It was observed that with increasing salinity stress (50-150mM NaCl) APX activity in Korgut rice showed non-significant (nsP>0.05) increase of 44% in comparison to its control. Jaya and Jyoti rice varieties however showed a decline of 3.84 % and67.74 % in APX activity. APX enzyme is primarily involved in detoxification of H_2O_2 into H_2O and O_2 (Maruta *et al* 2010).Research findings by YousefSohrabi (2012) showed a significant increase in APX activity in leaf tissues of *Glycine max* L. Salinity induced increase in rate of APX activity in salt tolerant Pokkali rice variety was compared to salt sensitive BRRI dhan 29 rice variety by Hossain*et al.* (2013) and their results indicated detoxification of H_2O_2 with increasing APX activity in presence of ascorbate serving as electron donor. APX thus play an important role in lowering of H_2O_2 and lipid peroxidation level thereby providing protection.

Effect of salinity stress on tocopherol content

Results with non-enzymatic low molecular weight antioxidant tocopherol are shown in Figure 5.Within the chloroplast, tocopherol role in protection against oxidative stress to membrane, lipids and other

Table 2. Effect of salinity stress alone on ascorbate peroxidase (APX). The values are mean of 3 experiments, ± S.D (n = 3). Statistical data shows non- significant difference at $^{ns}P>0.05$ by one way ANOVA.

Concentration	Jaya	Jyoti	Korgut
	APX (U/Litre)		
Control	0.52±0.02 ^{ns}	0.93±0.12 ^{ns}	0.25±0.12 ^{ns}
50Mm NaCl	0.73±0.08 ^{ns}	0.72±0.13 ^{ns}	0.27±0.13 ^{ns}
100mM NaCl	0.63±0.02 ^{ns}	0.41±0.08 ^{ns}	0.35±0.15 ^{ns}
150mM NaCl	0.54±0.10 ^{ns}	0.3±0.14 ^{ns}	0.36±0.14 ^{ns}

biomolecules is wellunderstood (Ivanov *et al*. 2003). We observed highly significant increase in tocopherol content in all three rice varieties. At higher salinity stress, an increase of 0.86 µg gm^{-1}F.w, 0.67µg gm^{-1}F.w and 0.60µg gm^{-1} F.w in Jaya, Jyoti and Korgut was observed. A recent report by Orabi *et al.,* (2014) showed a similar pattern of increase in tocopherolin two cultivars of *Viciafaba* under salinity stress. We hypothesize that the increase in tocopherolcontent under salt stressmay possibly be due to the networking of ascorbate-glutathione cycle along with other antioxidant enzymes providing protection against oxidative stress.

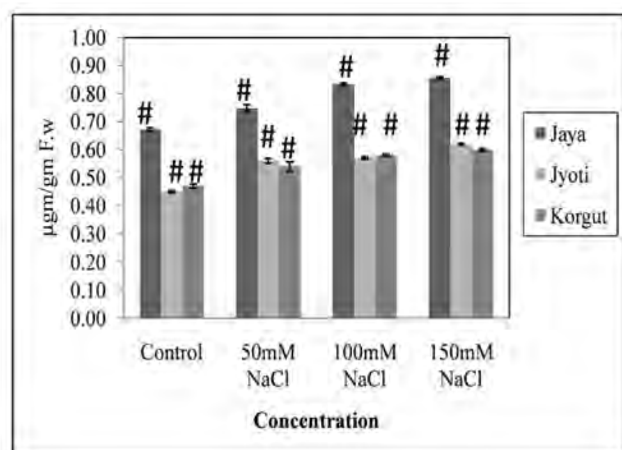

Fig. 5. Effect of salinity stress alone on tocopherol. The values are mean of 5 experiments ± S.D (n=5). Statistical data shows highly significant difference at ($^{\#}P<0.01$) by one way ANOVA.

From the present study it can be concluded that level of salinity stress and damage associated with it differs with rice varieties. Significant damage to lipids and proteins was observed, however no significant damage to photosynthetic system was seen under salinity stress. The studied rice varieties showed protection at cellular level through accumulation of compatible solute like proline. Antioxidants like tocopherol, ascorbate and APX were also seen to be providing protection under given stress condition. Amongst the three varieties studied Jyoti and Korgut appeared to be comparatively better tolerant to salinity stress.

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