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ORIGINAL ARTICLE

Differential responses of two rice varieties to salt stress

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Abstract Two rice varieties, viz. Nonabokra and Pokkali, have been evaluated for their responses to salinity in terms of some physiological and biochemical attributes. During the exposure to salinity (200 mM concentration of sodium chloride for 24, 48, and 72 h), a significant increase in sodium was recorded which was also concomitant with the changes of other metabolic profiles like proline, phenol, polyamine, etc. The protein oxidation was significantly increased and also varied between the two cultivars. The changes in activities of anti-oxidative enzymes under stress were significantly different to the control. The detrimental effects of salinity were also evident in terms of lipid peroxidation, chlorophyll content, protein profiles, and generation of free radicals; and these were more pronounced in Pokkali than in Nonabokra. The assessment and analysis of these physiological characters under salinity could unravel the mechanism of salt responses revealed in this present study and thus might be useful for selection of tolerant plant types under the above conditions of salinity.

Keywords Salinity · Antioxidative enzymes · Lipid peroxidation · Compatible solutes · Polyamine · Rice

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Abbreviations

DTT	Dithiothreitol
DW	Dry weight
EDTA	Ethylene diamine tetra acetic acid
FW	Fresh weight
GPX	Guaiacol peroxidase
M	Molar
MDA	Malondialdehyde
PMSF	Phenyl mercuric sulfonyl fluoride
Put	Putrescine
PVP	Polyvinylpyrrolidone
PVPP	Polyvinylpolypyrrolidone
ROS	Reactive oxygen species
SDS	Sodium dodecyl sulphate
Spd	Spermidine
Spm	Spermine
TCA	Trichloroacetic acid

Introduction

The adaptability of crop plants to environmental harshness depends upon the unfolding of genotypic plasticity and the resulting phenological and physiological expressions. These are also coordinated with environmental fluctuations and thereby accommodate such conditions partially or fully, whatever the case may be. The increase in such environmental stresses, like water deficit or excess, soil salinity, extreme temperature, chilling, etc., are the same constraints that essentially impair the plant's sustenance over a period of its life cycle. Salt deposition in different concentrations and duration is one of the major environmental extremes affecting the growth and development of plants, particularly those mesophytic crop species prone to salt injury by excess

salt deposition in the soil. In brief, salt deposition in the soil is sensed by plants in two ways: primarily through the changing of the water potential (ψ_w), and secondarily, the specific ion toxicity that disrupts the ion homeostasis of the tissues (Zhu 2001). The osmotic relationship and its different components are affected through inadequate water balance or more negative water potential in tissues and thus initiate a set of events both at the cellular and physiological levels. In general, a high level of Na^+ in soil is referred to as sodicity while alkalinity is considered as the composites of other salts in soil, Na^+ being the predominant one (Yokoi et al. 2002). The increased salinization poses a real threat to cultivatable land, since it may probably lead to the loss of 30% of land within the next 25 years and even of 50% by the year 2050. However, under this condition, plants can also show a wide range of responses to adjust to or combat the salinity. Accordingly, two common groups, namely halophytes and glycophytes, besides their degrees of sensitivity to salinity also show variability in tolerance at different levels to such environmental conditions. Withstanding osmotic stress and ion toxicity as primary effects, other detrimental effects are derived from perturbed cellular membrane integrity and the generation of toxic molecules, which initiate a gradual disruption of the cellular mechanism (Basu et al. 2009). It is well established that plants adapt multiple strategies for minimizing salt injury, and characteristically these are based on adjusting the water potential and, secondly, exclusion and/or sequestering the excess salt from sites of metabolism in cells, particularly organelles like chloroplast, mitochondria, etc. as those are very prone to oxidative damage. Moreover, acquisition of salt in excess becomes more detrimental to plants under high illumination, and it induces a high oxidative burst/exposure as it reduces the molecular oxygen by highly energized electrons (from the electron transport chain) and converts it into some over-oxidized moieties commonly referred as reactive oxygen species (ROS). An excessive generation of ROS, such as super oxide (O_2^-), peroxide (H_2O_2), and free radicals (OH^-) initiates rapid cell damage by triggering a chain of reactions, which make the plant susceptible to oxidative damage and more vulnerable to salt affecting tissues (Bascola et al. 2003). Thus, the chloroplast and mitochondria are the two most vulnerable to ROS. The cellular membranes and other macro-molecules, like proteins, nucleic acid, lipids, glycosides, etc., are more sensitive to cytosolic and organelle-specific ROS (Wang et al. 2009). Cellular impairment in terms of different metabolic events, which include chlorophyll attenuation, lipid peroxidation, protein carbonylation, oxidation of –SH groups of proteins and other organic moieties, reduction of enoic fatty acids, etc., are accelerated by these ROS and thus their cellular status or performance becomes retarded. Fortunately, plants are also attuned to such oxidative hazards partially or fully depending on the degrees of ROS accumulation in the tissues. This is accomplished by defolding the genetic plasticity through over-expression of both non-enzymatic and enzymatic pathways against these conditions. A variety of anti-oxidative enzymes, viz. super oxide dismutase (SOD), peroxidase, (POD), and catalase (CAT), operate in a sequential cascade reducing the ROS into inert or less reactive moieties, which can be either directly quenched or chelated by some proteins (Sairam and Tyagi 2004). Therefore, salinity or metal stress has appeared as another possible constraint in the form of oxidative stress to a plant's growth and development, and thus plants also accommodate some combined pathways for resistance against both salinity and oxidative stress (Roy Choudhury and Basu 2008).

Rice is one of the major cereals. Like other crop species, it is also highly sensitive to salt but in a variable manner according to genotypic variability (Zhao and Yang 2008). The tall or semi-dwarf, lowland *indica* varieties display their variation in sensitivity according to the concentration of the salts, time of exposure, and also duration of the salt. The differential responses to salt by the two varieties are based on their potential for genotypic plasticity which is efficiently expressed into physiological attributes adopting or alleviating the shock for salt stress. However, in most the cases, not much is known about the local or traditional cultivars of *indica* rice which very often suffer from an unsatisfactory yield, reduced by more than 40% in different salt-affected fallows (Peng et al. 1999). Moreover, salt exposure provides early hazards for the rice seedling by creating poor establishment in the soil, reduced growth, impaired photosynthetic carbon fixation and very often reduced acquisition of other nutrients. Furthermore, inadequate dry matter accumulation at the seedling stage sets a limit on survivability. In addition, an oxidative burst or exposure is also very much more acute at the seedling stage in rice, particularly in traditional *indica* cultivars (Reddy et al. 2004). Therefore, superior plant types of those rice varieties are required to sustain against such salinity, as well as to realize a satisfactory yield. Although considerable efforts are made in the selection of salinity-tolerant crop species, progress has been meager primarily due to an inadequate understanding of the underlying mechanisms of salt tolerance. Moreover, inconsistency in the performance of rice varieties under saline conditions sets a limit on the ability of any variety to be used in the field for successive seasons and in practice only a few are found to be satisfactory for adoption. Therefore, a proper unraveling the pathways attributing tolerance to salt/salinity and the resulting selection of better plant types is worthy of study. This has led to the present investigation, which investigated the effects of the induction of salinity on some physiological and biochemical responses in two rice varieties (viz. Nonabokra and Pokkali) at the seedling stage, and

looked at their potential importance for producing reliable indices for screening salt tolerance, as well as their possible clarification.

Materials and methods

To satisfy the above objectives as proposed, an experiment was conducted in the laboratory of Plant Physiology and Plant Molecular Biology, Department of Botany, University of Kalyani, Kalyani, West Bengal, India. The seeds of two rice varieties, namely Pokkali and Nonabokra (reported as tolerant and moderately sensitive, respectively; 110–120 days duration; photo insensitive; semi-dwarf and nitrogen-responsive; Roychowdhury et al. 2007), were collected from the farm of Bose Institute, Kolkata, India. The viability of the seeds was tested by a germination test as per Hong and Ellis (2004). Briefly, 100 g of seeds from each variety were soaked for a prolonged period in distilled water and allowed to germinate under laboratory conditions of optimum temperature (27 ± 1°C) and relative humidity around 80% in a seed germinator. Seven-day-old seedlings (seedling age was recorded from the day of radical emergence) were transplanted into Hoagland's nutrient medium (Hoagland and Arnon 1950) on a metal net in a polythene tray (2–2.5 l capacity). In such conditions, the seedlings were allowed to grow for 10 days in the open air to attain their full growth under natural conditions of day and night periods during the month of June (average temperature = 30°C; relative humidity = 70–80%, photoperiod = 14/10 h light/dark). Next, the entire set was transferred into a fresh media as before, but containing salt (sodium chloride) at 200 mM/l concentration, i.e. equivalent to electrical conductivity of 18 ds m^{-1} as suggested by Roy et al. (2005). For each variety, 30 plants were given salt treatments in 3 replications following a randomized block design. A controlled set was maintained in which salt was withdrawn. Plants were allowed to grow up to 72 h, in both cases under the conditions described above. Intermittent destructive sampling (i.e. excision of all leaves from the stems of seedlings) were carried out at intervals of 24, 48 and 72 h. Plant samples were immediately frozen in liquid nitrogen and stored at −80°C in a deepfreeze (Thermo, USA) for biochemical analysis. For each analysis, equal amounts of fresh tissues were taken from both control and treated plants and the following parameters were measured under identical laboratory conditions.

Estimation of Na^+/K^+

The concentrations of sodium (Na^+) and of potassium (K^+) were estimated from the leaf samples after the treatments. The samples were dried at 80°C and digested with tri-acid mixture ($HCl:HNO_3:HClO_4$ = 1:1:1) and analyzed for Na^+/K^+ concentration withan atomic absorption spectrophotometer (Schimadzu, Model No. A6800) as described by Liu et al. (2008).

Estimation of chlorophyll

The chlorophyll content was estimated from the harvested leaf samples according to Arnon (1949). Fresh leaf samples was crushed thoroughly with 80% acetone and centrifuged at 3,000*g* for 10 min at 4°C (Hermle, Model No. Z323K). Supernatant was taken as the source of chlorophyll and was estimated by reading the absorbance at 645 and 663 nm with a UV–V Spectrophotometer (Cecil, Model No. CE7200).

Estimation of proline

Proline was extracted from 1 g of fresh leaf tissue in 3% aqueous sulphosalicylic acid and reacted with acid-ninhydrin solution (0.8 g stannous chloride in 500 ml 0.2 M citrate buffer, pH 5.4 and 20 g ninhydrin in 500 ml 2-methoxyethanol). Estimation of proline from the reaction mixture was done according to Bates et al. (1973) and expressed as micromole/gram of fresh tissue.

Total phenolics

For this analysis, 500 mg of tissue was crushed in 80% hot ethanol and centrifuged at 10,000*g* for 15 min at 4°C. The supernatant was evaporated to dryness in a water bath and finally dissolved in a minimum volume of water. An aliquot of 0.5 ml was treated with 5 times diluted Folin–Ciocalteu reagent followed by addition of 20% sodium carbonate. The mixture was incubated in a boiling water bath for 1 min. The colur was read at 650 nm and phenol content was derived with gallic acid as standard according to Mohsen and Ammar (2009).

Lipid peroxidation

For the lipid peroxidation, malondialdehyde (MDA) content was determined from leaf homogenate, extracted with 20% trichloroacetic acid (TCA) containing 0.5% thiobarbituric acid (TBA). MDA content was determined by reading the absorbance at 532 nm and corrected for non-specific absorbance at 600 nm (Heath and Packer 1968).

Estimation of protein

Extraction of protein from the frozen leaf samples was done by grinding them in liquid nitrogen into a fine powder which was homogenized with protein extraction buffer

(50 mM Tris–Cl, pH 7.5; 250 mM sucrose; 25 mM KCl; 5 mM $MgCl_2$; 3 mM EDTA; 1 mM PMSF as protease inhibitor; 1 mM DTT; 0.5% β-mercaptoethanol (in 1:2 ratio). The homogenate was centrifuged at 15,000*g* for 20 min at 4°C. The supernatant was saved and bovine serum albumin used as standard.

Separation of total protein by sodium dodecyl sulfate polyacrylamide gel electrophoresis

The total protein was isolated from 1 g of tissue crushing in 10 ml buffer containing 1.5 mM Tris–HCl, pH 6.8; 1 mM sucrose; 3 mm $MgCl_2$; 1.0 mM bovine serum albumin; 0.1% PVPP; 0.1 mM DTT; 1.0 mM PMSF under cold condition. The homogenate was centrifuged at 10,000*g* at 4°C for 15 min. The supernatant was concentrated by lyophilization at −40°C. The protein was quantified with Bradford reagent (Bradford 1976). A protein sample of 50 µg was loaded into a denaturing polyacrylamide gel containing 10% sodium dodecyl sulfate (SDS). The gel was casted as 10% and 5% as resolving and stacking gel, respectively (Sambrook and Russell 2001). The protein was run at constant voltage (10 V/lane) under cold condition. The protein was visualized by staining the gel with Coomassie brilliant blue (G-250; Bio-Rad) and viewed in a gel Doc system (Bio-Rad Gel Doc^{TM} XR).

Enzyme assays

Steady state measurements for the activity of guaiacol peroxidase or GPX (EC 1.11.1.7) were assayed spectrophotometrically using *O*-dianisidine as electron donor and hydrogen peroxide (H_2O_2) as substrate. The enzyme extract was prepared by thoroughly homogenizing the tissues in liquid nitrogen followed by 0.1 M potassium phosphate buffer (pH 7.0) under cold condition. The homogenate was centrifuged at 15,000*g*, at 4°C for 15 min. The protein content of the supernatant was concentrated using 80% ammonium sulphate $(NH_4)_2SO_4$ precipitation followed by dialysis and lyophilization (Sambrook and Russell 2001). The protein was measured as per Bradford (1976). The concentrated protein samples were used as the source of enzyme and incubated in an assay mixture containing (0.1 M phosphate buffer, pH 6.5; 1.5 mM *O*-dianisidine; 0.2 M H_2O_2; 50 µg of protein) at 37°C. The absorbance was recorded at 430 nm using an extinction coefficient of *O*-dianisidine (26.2/mM/cm). One unit of enzyme activity was determined as the amount of enzyme required to change the absorbance by 0.1 per unit time (Hu et al. 2009). For detection of isoenzymic profiles of GPX, the enzymatic protein was extracted from 1 g of tissue ground in liquid nitrogen and extracted with 5.0 ml buffer containing 1.0 mM Tris–HCl (pH 7.7), 10 mM $MgCl_2$, 1.0 mM DTT, 0.1 mM PMSF, 0.1 mM EDTA, 0.1 mM leupeptin, 0.1 mM BSA and 2% PVP under cold condition. The extract was centrifuged at 20,000*g*, for 15 min at 4°C and the supernatant was dialyzed in a cellulose acetate bag against a dialysis buffer containing 10 mM KCl, 0.1 mM EDTA, 1 mM PMSF, 1 mM β-ME under 4°C for 6–8 h. The protein was concentrated under vacuum and dissolved in 0.5 ml of the dialysis buffer. The protein was run in a non-denaturing 10% polyacryl amide gel at 5 V/lane under cold condition (Ammar et al. 2008). For detection of polypeptides, the gel was incubated in an assay mixture containing 50 mM Potassium phosphate buffer, 0.5 mM *O*-dianisidine and 0.5% H_2O_2 until orange-brown bands were visualized.

Polyamine determination

Polyamine determination was done according to Zhao and Yang (2008). A leaf sample was homogenized with 5% perchloric acid ($HClO_4$) under cold condition followed by centrifugation at 26,000*g* for 15 min at 4°C. The supernatant and reference polyamines (Spd, Put, Spm) were dansylated and extracted with benzene. The polyamines were separated from the extract on a HPTRC silica gel 60 plate (E-Merck) with chloroform:triethylamine (100:20) as solvent. The spots of dansylated polyamines were identified from the R_f values with respect to those of the standards. The spots were identified under UV and scraped off and eluted with ethyl acetate (Botella et al. 2000). The polyamines were quantified from the fluorescence measured at 337 nm excitation and 495 nm emission by a fluorescence spectrophotometer (Perkin-Elmer, MmPF 44B) and expressed as µM/g of FW.

Determination of endogenous H_2O_2 concentrations

Hydrogen peroxide (H_2O_2) concentration was determined according to the method by Loreto and Velikova (2001). Leaf samples of 0.5 g were homogenized in 3 ml of 1% (W/V) TCA. The homogenate was centrifuged at 10,000*g* for 10 min at 4°C. Subsequently, 0.75 ml of supernatant was added to 0.75 ml of 10 mM K-phosphate buffer (pH 7.0) and 1.5 ml of 1 M potassium iodide (KI) solution freshly prepared. H_2O_2 concentration of the supernatant was evaluated by comparing its absorbance at 390 nm to a standard calibration curve. The concentration of H_2O_2 was calculated from a standard curve plotted in the range from 100 to 1,000 µM/ml H_2O_2 and expressed as µM/g FW.

Statistical analysis

All the observations were recorded with three replications ($n = 3$) and data were expressed as mean ± SE.

The statistical analysis was performed by one-way ANOVA analysis, taking $P \leq 0.05$ as significant.

Results

From the performances of the plants under the salinities used in the present experiment, some important findings regarding the physiological and biochemical changes were found which might be useful to justify and analyze the varietal difference in tolerance to salt stress. Irrespective of varieties, the plants recorded significant changes in their physiological attributes as a function of the duration of salt exposure, i.e. 24, 48 and 72 h. Since salinity in this experiment was experienced by the plants in the form of sodium chloride (concentration of 200 mM), so sodium (Na^+) uptake in the tissues would be the preliminary concern for the plants. Thus, the results with respect to Na^+ uptake are significantly ($P \leq 0.05$) variable between the two rice cultivars, Nonabokra and Pokkali, for different durations (Fig. 1). Notably, Pokkali had nearly 1.7 times more accumulation of Na^+ than the control, compared with that of Nonabokra which was 1.2 times over the control (Fig. 1). Furthermore, the tissue-specific accumulation of sodium ions (Na^+) was also statistically correlated with the duration of stress exposure ($r = 0.872$). Accumulation of Na^+, secondarily induces the membrane injury which manifests as the loss of electrolytes and potassium ion (K^+) which is the most important cationic osmolyte. Thus, the loss of K^+ was also recorded in the present experiment. The loss under salinity of K^+, the most important ion for osmo-regulation in leaf tissues, in this experiment was also significantly ($P \leq 0.05$) variable between the two varieties as compared to control (Fig. 2). The loss of K^+ was noticed in both cases throughout the period of stress: Pokkali was the highest being 2.0 times that of the control at 72 h, while in Nonabokra it was 1.8 times. Thus, Nonabokra showed a higher tolerance to salinity with a smaller loss of K^+. Regardless of the varieties, the maximum accumulation of Na^+ and loss of K^+ had both taken place at 72 h of the stress period (Figs. 1 and 2). Salinity otherwise induces the perturbation of osmotic status of the tissues and, to combat this, plants tend to accumulate some osmolytes, of which proline is the most important. Likewise, estimation of proline recorded significant ($P \leq 0.05$) variations irrespective of the rice varieties when exposed to salt compared with the control (Fig. 3). Although varietal

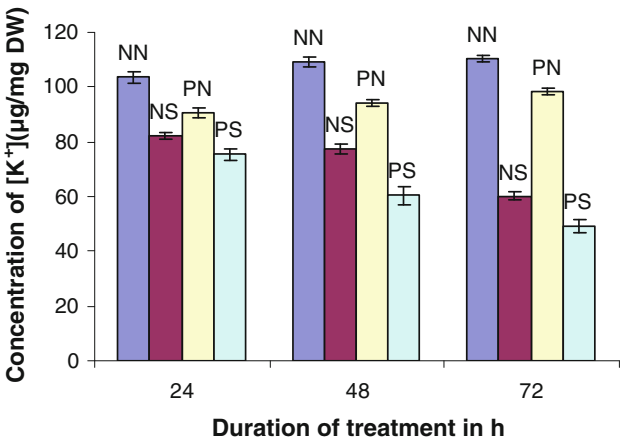

Fig. 2 Concentration of K^+ in the leaf of Pokkali and Nonabokra under normal and 200 mM salinity at different durations of treatment (24, 48 and 72 h). *NN* Nonabokra under normal condition, *NS* Nonabokra under salinity (200 mM), *PN* Pokkali under normal condition, *PS* Pokkali under salinity (200 mM)

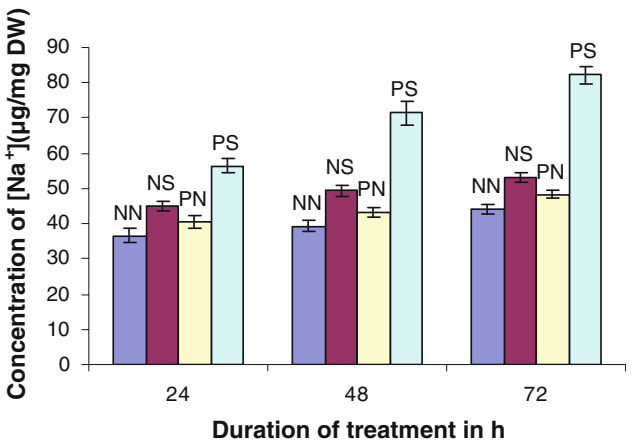

Fig. 1 Concentration of Na^+ in leaves of Pokkali and Nonabokra under normal and 200 mM salinity at different durations of treatment (24, 48 and 72 h). *NN* Nonabokra under normal condition, *NS* Nonabokra under salinity (200 mM), *PN* Pokkali under normal condition, *PS* Pokkali under salinity (200 mM)

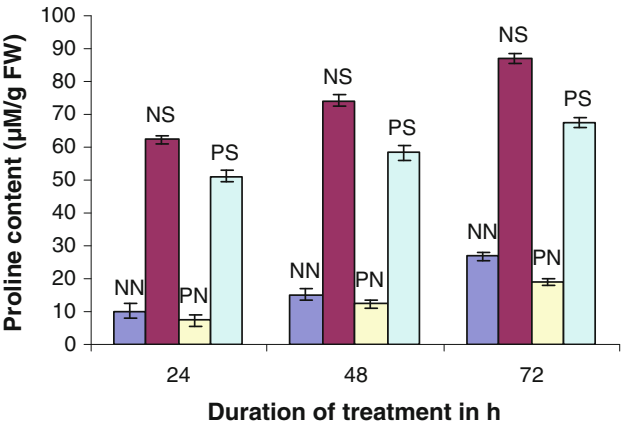

Fig. 3 Proline content of Pokkali and Nonabokra under normal and 200 mM salinity at different durations of treatment (24, 48 and 72 h). *NN* Nonabokra under normal condition, *NS* Nonabokra under salinity (200 mM), *PN* Pokkali under normal condition, *PS* Pokkali under salinity (200 mM)

differences were non-significant in proline content, even so, on average, Nonabokra recorded higher values in proline content than did Pokkali during the stress period.

Admittedly, both the varieties had higher proline concentration under stress, Nonabokra (3.2 times) and Pokkali, (3.5 times), when both were compared with the control condition at 72 h (Fig. 3). Salinity otherwise is impacted with the generation of free oxygen radicals which target the cellular membrane. So, when lipid peroxidation, an effect of salinity exposure, was considered in terms of MDA content, a significant ($P \leq 0.05$) variation of the plants was recorded under stress compared with the control throughout the stress period. However, in Pokkali, an almost steady increase of MDA content was recorded which reached a peak at 72 h duration. However, in Nonabokra, the lipid peroxidation was more moderate with a lower value of MDA content recorded at least up to 48 h, while it reached a peak thereafter (Fig. 4). However, maximum MDA content was recorded at 72 h in Nonabokra (2.4 times) and in Pokkali (1.5 times) as compared to the control (Fig. 4).

Denaturing the native structure of membranes or increasing their permeability often induces loss of chlorophyll content in salt-affected plant tissues. Thus, it was found that the chlorophyll content was also reduced significantly irrespective of the varieties when exposed to salinity at different durations. Both the varieties sustained their chlorophyll content at 24 h, i.e. no significant ($P \leq 0.05$) loss of chlorophyll was detected, while maximum loss of chlorophyll content was recorded in Pokkali (2.5 times) as well as in Nonabokra (2.6 times) at 72 h, as compared to control (Fig. 5).

An increase in total phenolics irrespective of the varieties of the stressed plants over the control was another interesting observation of the response of plants to salinity. A consistent trend in phenol accumulation was recorded from 48 h onward reaching a peak at 72 h (Fig. 6). The increase in total phenolics content in all varieties was significant as indicated by a two-sided t test at $P \leq 0.05$, and was maintained as an inductive response to salinity. As recorded, the maximum accumulation of phenolics under salinity was in Nonabokra (1.6 times) while it was much less in Pokkali (1.0 times) compared to the control. On the other hand, the plants apparently recorded some variations in protein content irrespective of variety even under control conditions, though it was not significant ($P \leq 0.05$). This could simply be interpreted as varietal differences. However, this was more pronounced, as it revealed a significant loss in protein content when the plants were exposed to

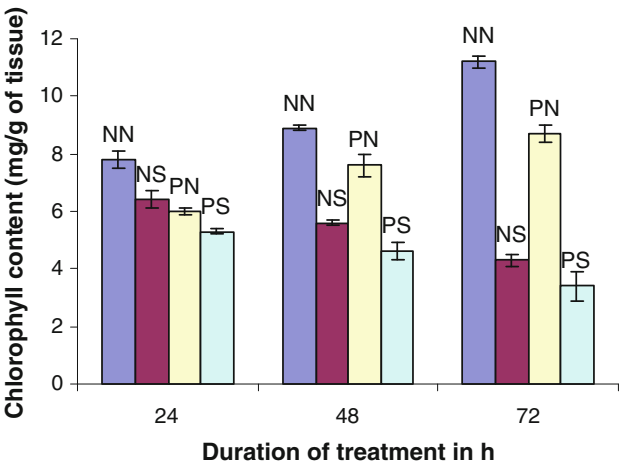

Fig. 5 Chlorophyll content of the leaf of Pokkali and Nonabokra under normal and 200 mM salinity at different durations of treatment (24, 48 and 72 h). *NN* Nonabokra under normal condition, *NS* Nonabokra under salinity (200 mM), *PN* Pokkali under normal condition, *PS* Pokkali under salinity (200 mM)

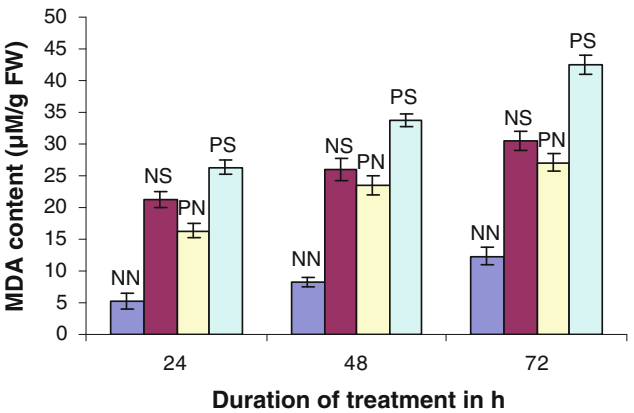

Fig. 4 MDA content of Pokkali and Nonabokra under normal and 200 mM salinity at different durations of treatment (24, 48 and 72 h). *NN* Nonabokra under normal condition, *NS* Nonabokra under salinity (200 mM), *PN* Pokkali under normal condition, *PS* Pokkali under salinity (200 mM)

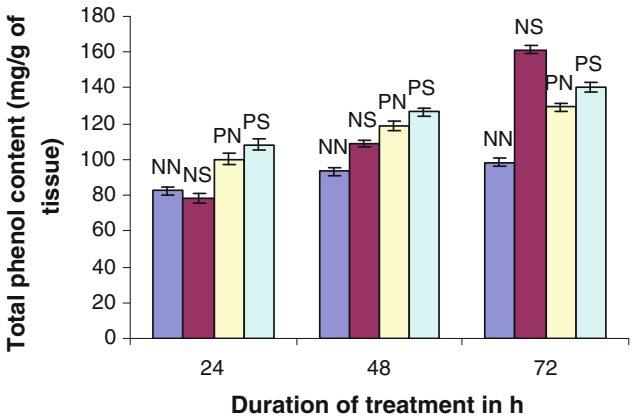

Fig. 6 Total phenol content of Pokkali and Nonabokra under normal and 200 mM salinity at different durations of treatment (24, 48 and 72 h). *NN* Nonabokra under normal condition, *NS* Nonabokra under salinity (200 mM), *PN* Pokkali under normal condition, *PS* Pokkali under salinity (200 mM)

salinity through 24, 48 and 72 h, being maximum in Pokkali and minimum in Nonabokra. Interestingly, Pokkali recorded a steady decline in protein content from 24 h onward, while it was at a maximum at 72 h (2.8 times) compared with control. Where Pokkali showed its higher sensitivity to salinity, Nonabokra tended to be less vulnerable since no significant loss of protein was found even at 48 h, but thereafter it was at a maximum (1.3 times) at 72 h (Fig. 7).

When buffer soluble protein from plants under both control and treatment was run on a denaturing SDS-polyacrylamide gel electrophoresis (PAGE), it showed some variations in polypeptides as band numbers and intensities (Fig. 8). The larger differences were in the intensities of bands of low molecular weights in the treated plants compared to those of the control.

Since salinity induces oxidative stress, the activity of one of the anti-oxidizing enzymes like GPX showed significant ($P \leq 0.05$) variations in both of the varieties as compared to the control plants. The increase in activity of GPX is proportionate to the duration of the stress, with the maximum activity recorded in Nonabokra, which was greater than in Pokkali, compared to the control, both of them at 72 h (Fig. 9). This result is also supported by the varietal difference in enzyme activity when measured under varying concentrations of substrate (i.e. hydrogen peroxide) for GPX (Fig. 10). From the kinetics of GPX

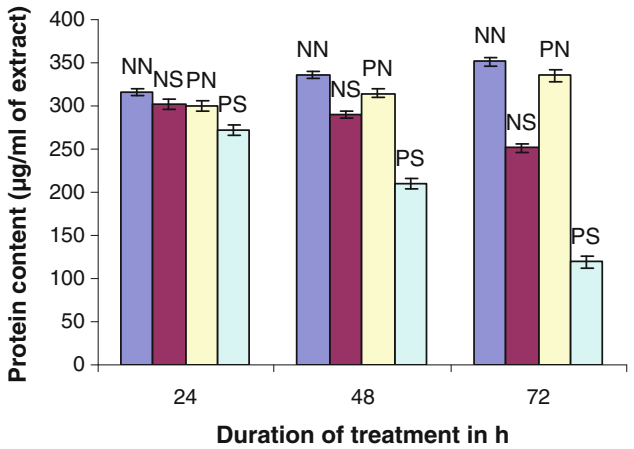

Fig. 7 Total buffer soluble protein content of the leaves of Pokkali and Nonabokra under normal and 200 mM salinity at different durations of treatment (24, 48 and 72 h). *NN* Nonabokra under normal condition, *NS* Nonabokra under salinity (200 mM), *PN* Pokkali under normal condition, *PS* Pokkali under salinity (200 mM)

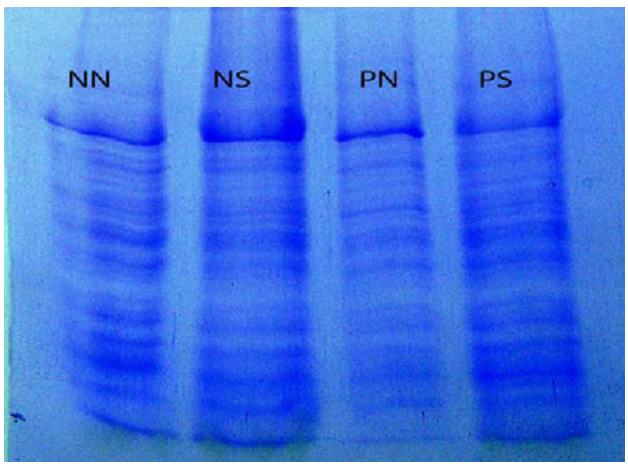

Fig. 8 Separation of proteins by PAGE from leaves of Pokkali and Nonabokra under normal and 200 mM salinity at different durations of treatment (24, 48 and 72 h). *NN* Nonabokra under normal condition, *NS* Nonabokra under salinity (200 mM), *PN* Pokkali under normal condition, *PS* Pokkali under salinity (200 mM)

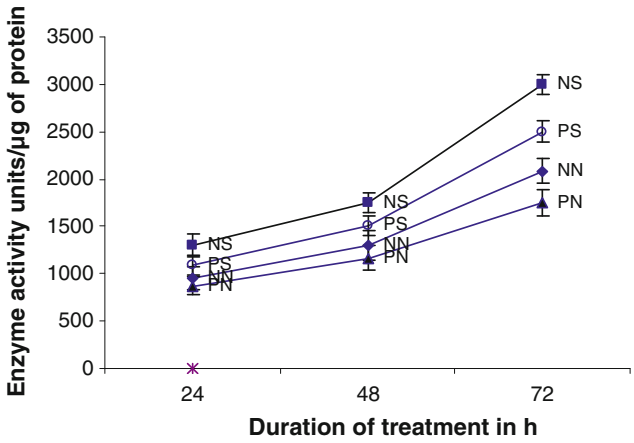

Fig. 9 Activity of GPX from the leaves of Pokkali and Nonabokra under normal and 200 mM salinity at different durations of treatment (24, 48 and 72 h). *NN* Nonabokra under normal condition, *NS* Nonabokra under salinity (200 mM), *PN* Pokkali under normal condition, *PS* Pokkali under salinity (200 mM)

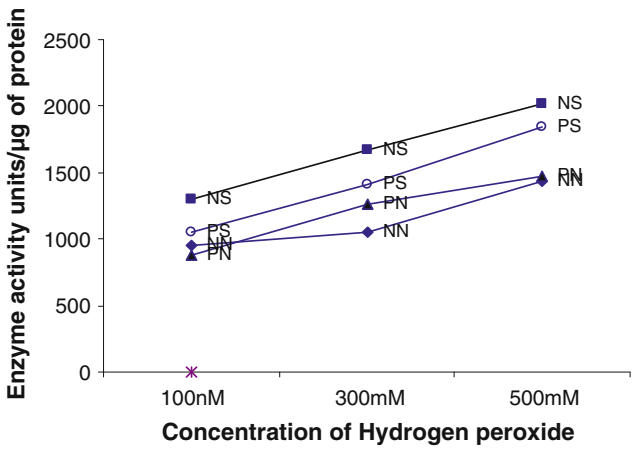

Fig. 10 Enzyme kinetics of GPX with increasing concentration of substrate H_2O_2 (100, 300 and 500 mM) from the leaves of Pokkali and Nonabokra under normal and 200 mM salinity at different durations of treatment (24, 48 and 72 h). *NN* Nonabokra under normal condition, *NS* Nonabokra under salinity (200 mM), *PN* Pokkali under normal condition, *PS* Pokkali under salinity (200 mM)

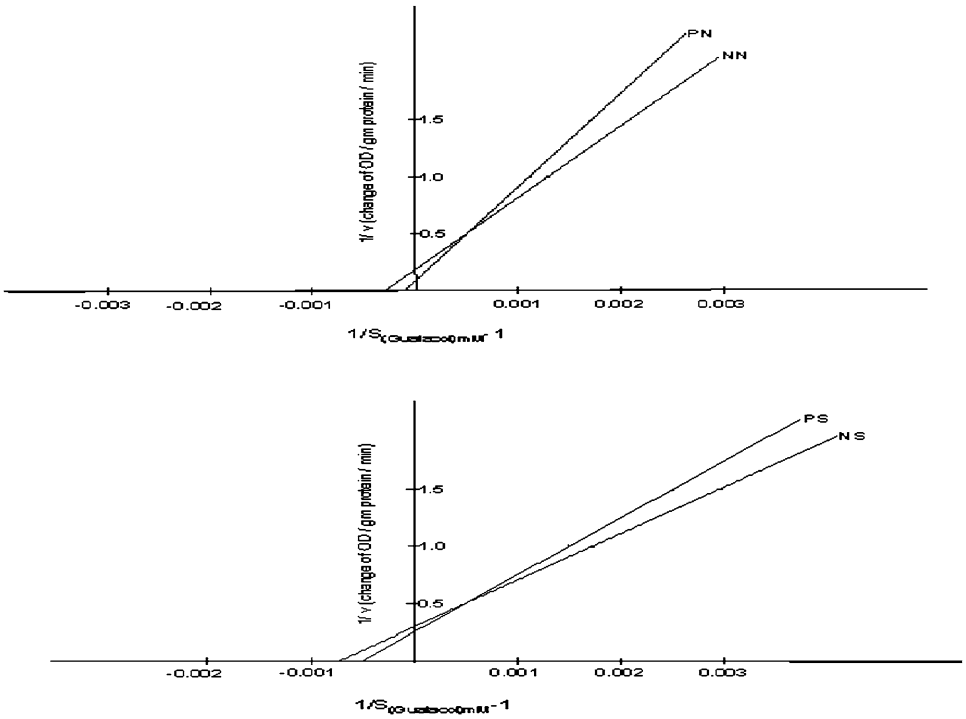

Fig. 11 The [V_{max}] and [K_M] values for Pokkali and Nonabokra under normal and 200 mM salinity at different durations of treatment (24, 48 and 72 h). *NN* Nonabokra under normal condition, *NS* Nonabokra under salinity (200 mM), *PN* Pokkali under normal condition, *PS* Pokkali under salinity (200 mM)

Table 1 Effect of salinity on the kinetic behaviour of GPX activity in Nonabokra and Pokkali

Variety	K_M (mM^{-1})	V_{max} (μM/min/mg protein^{-1})	Catalytic efficiency V_{max}/K_M (mg/protein)
NN	300	8.4	0.0280
NS	750	14.4	0.0192
PN	100	3.6	0.0360
PS	500	12	0.0240

Values are mean (±SE) ($n = 3$)

NN Nonabokra under normal condition, *NS* Nonabokra under salinity (200 mM), *PN* Pokkali under normal condition, *PS* Pokkali under salinity (200 mM)

activity of both the varieties, it was shown that [V_{max}] and [K_M] both are affected under salinity (Fig. 11). A comparison of [V_{max}] and [K_M] values as well as the catalytic efficiency of the two varieties are documented in Table 1. Interestingly, the [K_M] values was more liable to change significantly ($P \geq 0.05$) in both the varieties under salinity compared to the control. On the other hand, catalytic efficiency (which otherwise ensures the maximum activity per unit amount of protein) of GPX became more affected in both the varieties under stress conditions. Still, the change in [V_{max}] remained non-significant. Thus, Nonabokra appeared more efficient in sustaining the enzyme activity even under stress conditions. For detection of isoenzymic profiles of plants under both control and salt stress, equal amounts of protein were run in a non-denaturing/native polyacrylamide gel followed by incubation in a reaction

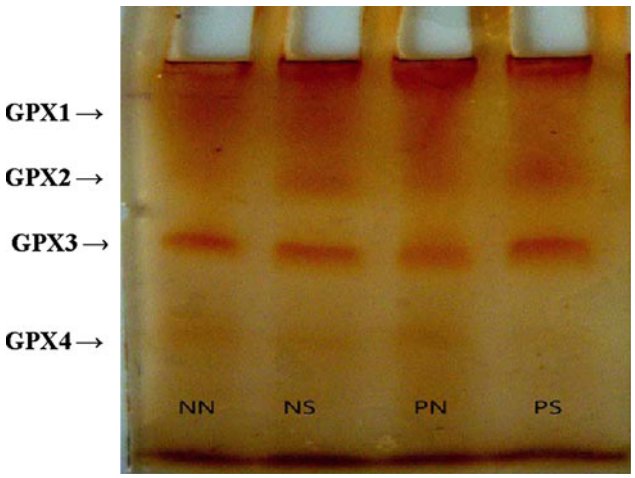

Fig. 12 Separation of peroxidase on a non-denaturing gel from the leaves of Pokkali and Nonabokra under normal and 200 mM salinity at different durations of treatment (24, 48 and 72 h). *NN* Nonabokra under normal condition, *NS* Nonabokra under salinity (200 mM), *PN* Pokkali under normal condition, *PS* Pokkali under salinity (200 mM)

mixture for GPX activity. Four distinct isoenzymic bands for GPX (GPX 1, GPX 2, GPX 3 and GPX 4) were resolved from the samples concerned (Fig. 12). The most stable forms were GPX 1 and GPX 3; however, intensities of other bands also varied for the two varieties in response to salinity. It is interesting to note that in comparison to the control, the activity of the forms GPX 2 and GPX 3 was notably enhanced under stress conditions.

Total polyamine content showed significant variations in both the cultivars, particularly when exposed to salinity

throughout the stress period (Fig. 13). Both the varieties recorded over-folded polyamine activity under each duration of treatment, and became maximum at 72 h. However, polyamine content under normal conditions was not significantly variable ($P \leq 0.05$) between the two cultivars. Under stress, it was significantly variable and Nonabokra recorded a higher total polyamine content than normal whereas Pokkali recorded a comparatively smaller amount.

It is interesting to note that individual polyamine content was not consistent in accumulation.

Likewise, in Nonabokra, Put and Spm, for example, were recorded to be the highest at 48 h, but for Spd it was 72 h (Table 2), whereas in Pokkali, Put and Spm were highest at 72 h and Spd at 48 h (Table 3). So it appears, at least from the present experiment, that polyamine profiles are a function of the duration of salt treatment to the tissue as well as of the genotypic variability of plants concerned.

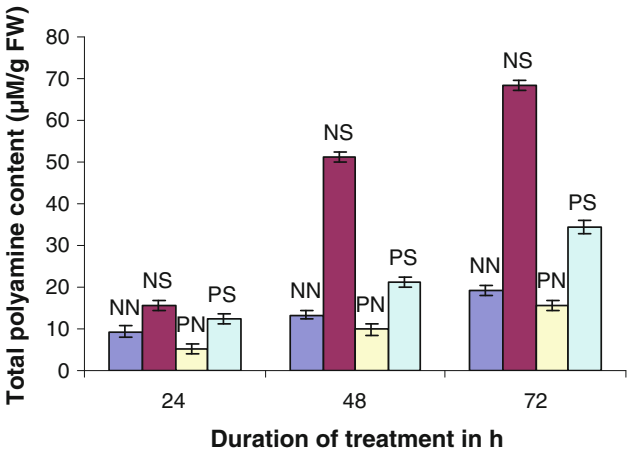

Fig. 13 Total polyamine content of the leaves of Pokkali and Nonabokra under normal and 200 mM salinity at different durations of treatment (24, 48 and 72 h). *NN* Nonabokra under normal condition, *NS* Nonabokra under salinity (200 mM), *PN* Pokkali under normal condition, *PS* Pokkali under salinity (200 mM)

Hydrogen peroxide is one of the potent and common reactive oxygen intermediates and occurs in significant amounts in plant tissues under stressful conditions. The accumulation of H_2O_2 content is more interesting to note,as it reveals that there was a consistent rise of peroxide content in the plants ($P \leq 0.05$) in accordance with the duration of salinity exposure (Fig. 14). Furthermore, comparing the two varieties, Pokkali registered as being more sensitive to producing H_2O_2 at different durations which peaked at 72 h (1.2 times) as compared to the control. Unlike Pokkali, however, the rise in H_2O_2 was less pronounced in Nonabokra (1.0 times) under salinity than the control at 72 h (Fig. 14).

Discussion

Abundant salt accumulation in soil creates a bottleneck for plants' normal growth and development in two ways: primarily, by perturbing the osmotic relationship of tissues and, secondarily, by specific ion effects. Specifically, in rice, a semi-aquatic plant usually cultivated in turbid swampy lowland, particularly close to coastal areas or even in freshwater lowland conditions, salt deposition in different amounts and durations limits the normal growth and development of the plants and consequently leads to substantial losses of average yield in many areas of South-east

Table 2 Content (μmol/g FW) of different polyamine in Nonabokra

Polyamine types	24 h		48 h		72 h	
	Control	Stress	Control	Stress	Control	Stress
Putrescine	31 ± 0.11	50 ± 0.10	24 ± 0.13	72 ± 0.26	30 ± 0.13	61 ± 0.36
Spermine	26 ± 0.13	40 ± 0.11	31 ± 0.10	97 ± 0.35	47 ± 0.10	70 ± 0.26
Spermidine	36 ± 0.15	49 ± 0.21	39 ± 0.11	89 ± 0.21	40 ± 0.12	131 ± 0.15

Values are mean (±SE) ($n = 3$)

Table 3 Content (μmol/g FW) of different polyamine in Pokkali

Polyamine types	24 h		48 h		72 h	
	Control	Stress	Control	Stress	Control	Stress
Putrescine	41 ± 0.12	114 ± 0.16	59 ± 0.14	156 ± 0.36	58 ± 0.21	190 ± 0.32
Spermine	40 ± 0.15	73 ± 0.36	55 ± 0.13	123 ± 0.41	55 ± 0.15	135 ± 0.26
Spermidine	26 ± 0.10	50 ± 0.26	40 ± 0.11	110 ± 0.52	39 ± 0.11	90 ± 0.21

Values are mean (±SE) ($n = 3$)

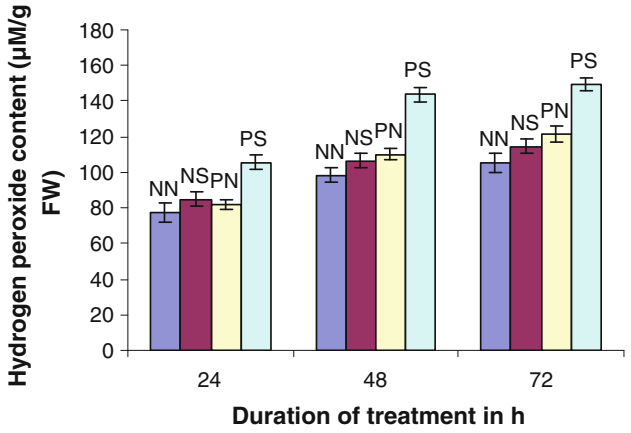

Fig. 14 Endogenous H_2O_2 concentration of the leaves of Pokkali and Nonabokra under normal and 200 mM salinity at different durations of treatment (24, 48 and 72 h). *NN* Nonabokra under normal condition, *NS* Nonabokra under salinity (200 mM), *PN* Pokkali under normal condition, *PS* Pokkali under salinity (200 mM)

Asia (Chao et al. 2008). This is caused by the inherent sensitivity of rice plants to salt stress with substantial modulation of different phenological and physiological responses at sub-lethal levels of salinity. There exists a wider variability in the sensitivity of the rice genotypes to such conditions which is exploited in the selection of plant types resistant to such harsh conditions. Even so, salt stress appears to be a constraint on rice greater than on other crop species since both soil moisture stress and oxidative exposure are simultaneously operating under such conditions.

It is obvious from the results of the present experiment that salinity has a conspicuous effect, obviously detrimental in nature, regardless of the rice cultivars, i.e. Pokkali and Nonabokra. Furthermore, these effects are in proportion to the duration of salinity exposure under a particular concentration of NaCl (i.e. 200 mM). Since salt tolerance is the concerted action of some altered cellular and physiological events, varietal performances under such conditions predominantly depend upon the unfolding of the genetic potentials to up- and/or down-regulate them to alleviate the salt effects within threshold values (Cheeseman 2006). Thus, in Nonabokra, the local check of salt tolerance showed its performances to be well ahead of the other variety, i.e. Pokkali, in withstanding salinity as recorded using different physiological parameters. Primarily, the results indicate a lesser accumulation of sodium ion (Na^+) content in Nonabokra compared with Pokkali which may characterize the former as more resistant to Na^+ acquisition than the latter. It is also evident that Pokkali recorded a greater sensitivity to salinity by Na^+ accumulation in excess than did Nonabokra all through the duration of the salinity period. So, the results show the differential responses of the rice varieties for Na^+ content in the leaves and thus agrees with the view that there is an inverse relationship between Na^+ accumulation and salt tolerance. The exclusion of Na^+ thus becomes an essential function for a species regarded as tolerant (Reddy et al. 2004). Interestingly, the putative salt sensitive variety, Pokkali, recorded a rapid loss of potassium ion ($K^{+)}$ under salinity that might lead to its fall in osmotic turgidity (Lee et al. 2001). In fact, Na^+ may hinder the cellular ion homeostasis affecting the osmotic balance in several ways, most predominantly through binding the transporter protein for K^+ or through some other intercellular processes yet to be confirmed (Sairam and Tyagi 2004). The loss of K^+ from Pokkali also thus supports its limitation for sustaining K^+ under salinity, and thus suffering from inadequate ion balance for osmotic turgidity in tissues, particularly in photosynthetic organs or foliage. Quite naturally, plants have to frame strategies for replenishing the loss of K^+ by development of a number of compatible solutes, proline being one of those (Parveiz and Satyawati 2008).

Therefore, to combat the loss of K^+, over-accumulation of proline has possibly been a feature of both the varieties when exposed to salinity, as was also recorded in the present study. Moreover, Nonabokra had a several-fold increase in proline content, which was higher than in the other variety, Pokkali. This is in agreement or in conformity with one of the cellular responses to water-deficit stress from salinity which also indicates one of the osmotic adjustments by over-folded accumulation of compatible solutes like proline (Alia et al. 2001). Proline, the amino acid, is the most readily available moiety for maintaining the osmotic balance as accumulated in plants under diverse forms of abiotic stresses, particularly those involved in water-deficit stress (Liu et al. 2007). Interestingly, al though Pokkali could efficiently compete with Nonabokra in proline accumulation at least at the initial phases of salinity, Nonabokra was more efficient in maintaining osmotic balance even at prolonged periods of exposure (i.e. 72 h under salinity duration) and thus is likely to be more tolerant. Supposedly, the over-accumulation of proline in plants under stress might be the manifestation of some gene induction committed to the de novo synthesis of some enzymes at rate-limiting steps of its own biosynthetic pathways. This is an entirely genotypic adaptability or plasticity of the plants concerned and also depends on doses and duration of salinity (Lejocka and Kluk 2005). The impaired osmotic balance in the leaf tissues, although adjusted by plants, still leaves almost all the cellular and physiological responses affected or even modulated.

Chlorophyll content becomes a first indication of responses in different plants subjected to salinity stress (Roy Choudhury and Basu 2008). Thus, in the present experiment, the results revealed a considerable variation in

the total chlorophyll content and its different fractions in plants. Interestingly, both the varieties sustained their chlorophyll content with the threshold duration of salinity, but thereafter gradually declined. Nonabokra, however, recorded less loss of chlorophyll than Pokkali and this might be due to its genetic potential to accommodate the impact of salinity. The loss of chlorophyll in plants under salinity is better clarified with both the impairment or down-regulation of its biosynthetic pathways as well as hydrolysis of chlorophyll by enzymes like chlorophylase (Gill et al. 2003). However, degradation of chlorophyll under salinity irrespective of varieties could possibly be attributed at least partially by the sensitivity of the chloroplast membrane to oxidative stress. Since this situation induces exuberated reactive oxygen species (ROS), which are well recognized for lipid peroxidation on the membrane of the chloroplast, and thus disintegrating the thylakoid membrane might be obvious. Consequently, plants are severely devoid of adequate chlorophyll content under salinity (Ashraf et al. 2005). The cellular membrane is the first site of perception of signals as well as the primary defense against any abiotic stresses with salinity being no exception. The integration of membrane structure and its proportionate functioning both remain in a non-sustainable state under salt stress, particularly in a state of excessive fluidity (Mittler 2002). The salinity-induced ROS as mentioned is otherwise validated with its potential for lipid peroxidation of cellular and organellar membranes (cellular or of either chloroplastic). Thus, the variation in lipid peroxidation can be used to measure the extent of damage of membranes by the salt exposure. Since lipids (particularly of the unsaturated fatty acid layer) are the predominant structural moiety in varying forms and proportions constituting cellular/organellar membrane, they form the most vulnerable target for free radicals to oxidize (Chutipaijit et al. 2009). The volatile aldehyde like MDA content, the common marker for lipid peroxidation, is very much contextual for measuring the salinity- induced membrane damage. Thus, it is evident from the results that Nonabokra tended to consistently maintain a low content of MDA than did Pokkali throughout the stress period which measured its tolerance to lipid peroxidation under conditions of salinity. The aggravation of lipid peroxidation in plants as a function of salt concentration is more circumvented by the higher degrees of LOX activity, as was documented from other studies also in rice (Gama et al. 2007). In the present study, the sensitivity of oxidative stress from salinity by both the varieties became evident when substantial accumulations of H_2O_2 were recorded throughout the duration of the stress period. Thus, irrespective of varieties, accumulation of H_2O_2 in significant amounts under salinity might be ascertained as a preliminary indication of oxidative stress which, moreover, was significantly higher in Nonabokra than in Pokkali. The variability of H_2O_2 content in the tissues affected by salt or any other abiotic stress is involved in a wide array of metabolic reactions in the cells. H_2O_2 mostly becomes an impairment to cellular membranes due to its greater mobility through both apoplastic and symplastic paths in/within the tissues. Furthermore, it is not actually a free radical. However, it is quite reactive with those molecules containing transition metals (like $Fe^{+3/+2}$, $Cu^{+3/+2}$, etc.) mostly in the proteins and other macro-molecules (Bhattacharya 2005). In most abiotic stresses, as for example in salinity, the majority of the regular metabolic pathways are modified in hyper- or hypo-expression and some of those may circumvent the evoking of the anti-oxidation system in plants. Likewise, secondary metabolites, particularly phenolics and phenylpropanoid derivatives, are most important as they are highly required as non-enzymatic antioxidant moieties (Muthukumarasamy et al. 2000). So, mechanisms relating to the down-regulation of the oxidative injury of salinity might also have been considered as an index to justify the varietal performances in the present study. Thus, Pokkali was recorded as salt sensitive at least as is evident from its higher loss of chlorophyll, loss of protein, and higher values of MDA under salinity. Interestingly, Nonabokra recorded marginal and non-significant increases in H_2O_2 content than did Pokkali under salinity. In general, H_2O_2, the two electron reduction product of O_2^-, is regarded as a less potent ROS, but must not be the free radical. Moreover, in terms of oxidizing potential, H_2O_2 appears rather safe in plants as compared to super oxide, O_2^-, OH^-, etc. Thus, Nonabokra posed some confusing discrepancies when it showed some other activities antagonizing the salinity-induced oxidative stress. This suggests a differential attitude of H_2O_2 accumulation in tissues that may circumvent alternative paths related to stress adoption in a way that recognizes normal activities. This is supposedly supporting the turning of H_2O_2 as non-reactive, sensing the oxidative perception at its sub-optimum concentration which, otherwise, may also induce anti-oxidation systems in plants (Cheeseman 2006). Still, it is questionable what that means in terms of tissue level H_2O_2 concentration having an oxidative stress or acting as an inducer responding to salinity or to other abiotic stress. This is further compounded by the rapidity of H_2O_2 turnover both in vivo and also after tissue harvesting (Pearse et al. 2005). Hence, higher H_2O_2 content in Nonabokra (marginally greater than that of Pokkali) might be speculated as a smaller turnover of H_2O_2 in the former variety. It could be better understood that accumulation of H_2O_2 in Nonabokra was sub-adequate in concentration and thus sensed by plants as having a lesser oxidative potential. Rather, it might be involved in signaling and in some sort of downstream processes in stress response as reported

earlier (Sagi and Fluhr 2006). Salinity in this present experiment also regulated the variable accumulation of phenol content in Nonabokra and Pokkali. Interestingly, Pokkali maintained higher but non-significant phenol content than Nonabokra at least in the initial period of stress, but Nonabokra proved its efficiency to over-express the phenolics content more than Pokkali at extreme duration, i.e. 72 h. This is indeed interesting and could be hypothesized as an inherent property of Nonabokra for unfolding its full potential under maximum exposure or induction of salinity. This is in agreement with other reports, including in rice, where plant varieties had been variable in differential phenol accumulation as a function of degrees and duration of exposure to drought stress (Basu et al. 2009). In rice, reports on phenol accumulation under stress, at least as revealed from the present study, are in conformity with others showing that it can be used as a stress indicator. Thus, Nonabokra in the present experiment might be regarded as tolerant since it recorded significant amounts of total phenol content as compared to Pokkali. Additionally, other predominant pathways of antioxidation along with the phenolic compounds in plants under salinity remain operative which is accomplished by some antioxidative enzymes (Jayaprakasha et al. 2001). This was well supported when enzymatic antioxidation efficiency was recorded in both of the varieties. The increased capacity to catabolize H_2O_2 under salinity all through the period was evident from the observations as more induction in GPX activity in Nonabokra compared to Pokkali over the values of the control. The changes in buffer soluble protein both in quantitative and qualitative (banding pattern) are likely the most affected under salt treatment. Nonabokra as expected recorded significantly higher protein content than Pokkali under salinity. This seems to be an adaptive feature for Nonabokra to sustain the greater protein content or a lesser degradation of the same throughout the period. Supposedly, the reduction of protein concentration in Pokkali might be due to down-regulation of de novo synthesis of the protein or denaturing of the protein under salt conditions. Protein turnover is an irreversible oxidation process and encompasses almost all the proteins for cellular machineries being disrupted, even cell walls containing proteins once considered to be of lesser biological importance. Many of these become the target of oxidative injury from salinity; however, some proteins are also over-expressed under the same conditions (Zeid and Shedeed 2006). Protein carbonylation, an irreversible oxidation process leading to the loss of protein, is predominantly attributed to the generation of free radicals under salinity and thus it has also been documented as a reliable marker of oxidative stress in plants.

Furthermore, protein profiles from leaves of treated plants indicated a reduction in band intensities, particularly for those having low molecular weights; in Pokkali, the reduction is more than in Nonabokra. Therefore, there is a possible involvement of alternation in some sets of genes which might have been less expressed than others under salt induction and thus manifested into synthesis of proteins. The latter is also evident from the comparative protein profile on SDS-PAGE of the two varieties in the present study.

Plants becomes most vulnerable to salinity with the exposure of some highly reactive chemical species, commonly called ROS, being generated in the tissues, particularly when the stomata are closed under moisture deficit in soil and intense illumination.

Essentially, salinity reduces the water potential of tissues around the critical value and thereby stomatal closure is the most immediate response that subsequently leads to spillage of electrons from the normal path (i.e. from NADH/NADPH + H^+ to oxygen). Thus, the more reduced oxygen emerges in different forms (O^{2-}, $^{1}\!/_{2}O_2$, H_2O_2, OH^- and similar other free radicals) and becomes the most potent reactant agents for lipid peroxidation (Jung 2004). It is a well-established fact that the ability of plants to control or disintegrate ROS levels is highly correlated with salt/stress tolerance. The lysis of these free radicals is a sequential event with concerted action of some antioxidative enzymes which have the ability to reduce free anionic superoxides, and thereby terminate the free-radical reactions. Peroxidase has several isoforms contributng predominantly to the lysis of peroxide and allied free radicals. GPX, with its smaller number of isoforms brings out [OH^-] from H_2O_2 in the presence of [O_2^-] and NADH in salt-induced tissues and thus NADH/NADPH-GPX becomes a primary concern in contributing to oxidative burst for [O_2^-], [H_2O_2], and [OH^-]. Thus, significant variation was also recorded in GPX functioning in the leaf tissues of the two varieties in the present experiment. It showed over-folded activity of GPX regardless of variety in this experiment under salt stress as compared to normal. In general, the tolerant species recorded over-folded activity in a consistent manner as a function of stress which could catabolize the superoxide into less reactive moieties; thus, Nonabokra appeared to be more tolerant by significant over-expression in activity throughout the period than did Pokkali. The isozymic forms appearing as a function of salt and other stresses have also been reported frequently in rice (Maribel et al. 1998). Interestingly, in the present study, in-gel assay of GPX activity hardly recorded any significant changes in band numbers in either of the varieties when exposed to salt concentration. Still, the intensities of those bands, particularly those of low molecular weight, are variable in comparison to those of others under control conditions. It is logical to speculate that the salt induced an increase in GPX activity which was in conformity with the corresponding increase in some particular

mRNA level followed by synthesis of polypeptides for a specific isoenzyme. The salinity-induced changes are otherwise manifested into altered cellular pH and probably this leads to the alteration of the affinity of the enzyme to the substrate for binding and thus changing the [V_{max}] or and [K_M] as also recorded in the present experiment.

This also holds the possibility that ionic imbalance might be inducing the distortion of the native structure of each isozymic form so as to reduce the affinity of the enzyme as a whole for the substrate, and thus ensuring the possible shifting of [V_{max}] and [K_M] values as was the result in the present experiment.

Polyamine, the secondary metabolites in plant systems and its accumulation is involved as an integral component of plant responses to abiotic stress (Tang and Newton 2005). Nevertheless, a decrease in polyamine contents has also been frequently described, particularly in relation to the change of free putrescine, which might be hypothesized as salt stress influencing polyamine metabolism in various ways. It remains elusive for the occurrence of contradictory changes in polyamine content under stress, which is the function of plant species, types and developmental stages of tissue as well as duration of stress (Ding et al. 2010). Thus, we also find in the current work that salt induction had changed the nature of polyamine content differentially for Putrescine (Put), Spermidine (Spd) and Spermine (Spm). Still, a significant increase in total polyamine content was recorded from the present experiment regardless of variety under salinity as compared to that of control. Moreover, there was no consistency in the trends of concentrations of individual polyamines (like Put, Spm and Spd) in either of the varieties. Thus, Spd content was higher in Nonabokra at 72 h than for the other polyamines (i.e. Spm and Put) but in Pokkali the Put content was the highest. This trend was changed in the duration of 48 h, when Spm content was the highest in Nonabokra, while in Pokkali, it was Put for both control and salinity. Again this trend was changed in case of the duration of 24 h when Put content was the highest in both varieties compared with the control. So, it is rather inconsistent to characterize the response of plants in terms of an individual polyamine/polyamines accumulation and its abundance. Reports available so far to clarify this non-precise nature of polyamine under salt induction are based on the partial understanding so far revealed from the studies. In most cases, it is stated that polyamine fractions and their function are highly variable in the tissues beyond a threshold concentration and depend on the age and metabolic status of tissues (Kumaran and Karunakaran 2006). In general agreement, polyamines are highly protonated and can bind electrically to negatively charged functional groups of membranes, nucleic acid, amino acid residues of protein, etc., and thus probably influencing the stability of membranes and other organelles, which are more sensitive to free radicals or ROS attack (Lejocka and Kluk 2005). It could be worth mentioning in several cases that polyamine accumulation is detectable in early phases of salt treatment, thereafter sharply declining with the progress of the duration of stress, and this is highly variable for different polyamine profiles (Tonon et al. 2004). However, on average, we found an increase only in Put content under salt stress which was more pronounced in Pokkali than the Nonabokra. So, Nonabokra could be hypothesized as salt tolerant if Put appears to be linked with salt induction in any way.

In conclusion, this study has demomnstrated that there were substantial differences between the physiological and anti-oxidation responses of two varieties of rice to salinity exposure. During salt stress, the two varieties, Nonabokra and Pokkali, had unfolded their genetic plasticity to combat both salt and salt-induced oxidative stresses. With the elucidation of physiological responses to salt stress there is evident a clear demarcation between the two varieties in their sensitivity and tolerance. Pokkali was sensitive to salinity by characterization of high accumulation of Na^+ and maximum loss of K^+, more lipid peroxidation, rapid loss of chlorophyll, low content of phenols, smaller concentration of proline accumulation, etc. On the other hand, Nonabokra appeared as a better performer by warding off the irreversible damage of over-oxidation of tissues by defolding the physiological features as evidenced by a greater induction of phenolics and proline, low lipid peroxidation, moderate free radical accumulation and sustaining the high GPX activity, etc. And probably these confirmed the variety as less prone to oxidative damage by peroxide and free radicals. So, the differential responses of these two varieties in the present experiment could be deciphered as the tolerance to salinity depending upon its minimizing of salt accumulation coupled with up-regulation of an anti-oxidation system. In contrast, a declining or subdued fate of this system is linked to sensitivity or susceptibility to such oxidative impairment. Admittedly, it is important to note that a merely short-term exposure to salinity under simulated conditions and only at seedling stages might not necessarily be sufficient assess the full potential for tolerance when compared to a long-term salt exposure in practice in rice fields. However, on the plus side, the present study may actually be more informative for understanding the pathways or mechanisms of salinity tolerance in rice. Thus, this information could also be used to set the selective pressures or indices under salinity at least at initial phases of plant growth and also with brief periods of exposure to salt.

References

Alia, Mohanty P, Matysik J (2001) Effect of proline on the production of singlet oxygen. Amino Acids 21:195–200

Ammar WB, Nouairi I, Zarrouk M, Ghorbel MH, Jemal F (2008) Antioxidative response to cadmium in roots and leaves of tomato plants. Biol Plant 52:727–731

Arnon D (1949) Copper enzyme in isolated chloroplast and poly phenol oxidase in *Beta vulgaris*. Plant Physiol 24:1–7

Ashraf MY, Akhtar K, Sarwar G, Ashraf M (2005) Role of rooting system in salt tolerant potential in different Guar accessions. Agron Sustain Dev 25:243–249

Bascola PR, Menossi M, Jorge RA (2003) Aluminum-induced oxidative stress in plants. Photochemistry 62:181–189

Basu S, Roychowdhury A, Saha P, Sengupta DN (2009) Differential antioxidative responses of indica rice cultivars to drought stress. Plant Growth Regul 10:219–225

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water stress studies. Plant Soil 39:205–207

Bhattacharya S (2005) Reactive oxidative species and oxidative stress in plants: roles in stress, senescence and signal transduction in plants. Curr Sci 89:1113–1121

Botella MA, De Amor F, Amoros A, Serrano M, Marlinez V, Cerda A (2000) Polyamines, ethylene and other physico-chemical parameters in tomato fruits as affected by salinity. Plant Physiol 109:428–434

Bradford MM (1976) Rapid and sensitive method for quantitation of micro gram quantities of protein utilizing the principle of protein-binding dye. Ann Biochem 72:248–254

Chao YJ, Jae GS, Chang HS, Sheon A, Hwang, Young ML, Se Y, Jeong Y, Chung B (2008) Integrated nutrient management for environmental friendly rice production affected by salt deposition Saemangeum reclaimed land of South Korea. Paddy Water Environ 6:263–273

Cheeseman JM (2006) Hydrogen peroxide and plant stress: a challenging relationship. Plant Stress 1:4–11

Chutipaijit S, Cha Um S, Sompornpailin K (2009) Differential accumulation of proline and flavonoids in indica rice varieties against salinity. Pak J Bot 41:2496–2506

Ding C, Shi G, Xu X, Yang H, Xu Y (2010) Effects of exogenous spermidine on polyamine metabolism in water hyacinth leaves under mercury stress. Plant Growth Regul 60:61–67

Dionisio-Sese ML, Tobita S (1998) Antioxidant response of rice seedlings to salinity stress. Plant Sci 135:1–9

Gama PBS, Inanana S, Tanaka K, Nakazawa R (2007) Physiological response of common bean seedlings to salinity stress. Afr J Biotechnol 6:79–88

Gill PK, Sharma AD, Singh P, Bhullar SS (2003) Changes in germination, growth and soluble sugar contents of *Sorghum bicolour* (L.) Moench seeds under abiotic stress. Plant Growth Regul 40:157–162

Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125:189–198

Hoagland DR, Arnon DI (1950) The water culture method for growing plants without soil. Calif Agric Stn Circ 347:1–32

Hong TD, Ellis RH (2004) The survival and germinating orthodox seeds after desiccation and hermetic storage. J Exp Bot 43:239–247

Hu Y, Ge Y, Zang C, Zu T, Cheng W (2009) Cd toxicity and translocation in rice seedlings are reduced by hydrogen peroxide treatments. Plant Growth Regul 5:51–61

Jayaprakasha GK, Singh RP, Sakariah KK (2001) Antioxidant activity of grape seed extract on peroxidation models in vitro. Food Chem 73:285–290

Jung S (2004) Variation in antioxidation metabolism of young and mature leaves of *Arabidopsis thaliana* subjected to drought. Plant Sci 166:459–466

Kumaran A, Karunakaran RJ (2006) Antioxidant and free radical scavenging activity of an aqueous extract of *Coleus aromaticus*. Food Chem 97:109–114

Lee DH, Kim YS, Lee CB (2001) The inductive response of the antioxidant enzymes by salt stress in rice (*Oryza sativa* L.). J Plant Physiol 158:737–745

Lejocka J, Kluk A (2005) Effect of salt and osmotic stress on changes in polyamine content and argentine decarboxylase activity in *Lupinus luteus* seedlings. Plant Physiol 162:662–668

Liu J, Kitshiba H, Wang S, Ban Y, Moriguchi T (2007) Polyamines and their ability to provide environmental stress tolerance to plants. Plant Biotechnol 24:117–126

Liu JH, Inove H, Moriguchi T (2008) Salt stress mediated changes in free polyamine titers and expression of genes responsible for polyamine biosynthesis of apple in in vitro shoots. Environ Exp Bot 62:28–35

Loreto F, Velikova V (2001) Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. Plant Physiol 127:1781–1787

Mittler R (2002) Oxidative stress, antioxidant and stress tolerance. Trends Plant Sci 7:405–410

Mohsen S, Ammar ASM (2009) Total phenolic contents and antioxidant activity in maize tassel extract. Food Chem 112:595–598

Muthukumarasamy M, Gupta SD, Pannerselvam R (2000) Enhance of peroxidase, polyphenol oxidase and super oxide dismutase activities by triadimefon in NaCl stressed *Raphanus sativus* (L.). Biol Plant 43:317–320

Parveiz A, Satyawati S (2008) Salt stress and phyto-biochemical responses in plants. Plant Soil 54:89–99

Pearse I, Heath KD, Cheeeseman JM (2005) A partial characterization of peroxidase in *Rhizophora mangle*. Plant Cell Environ 28:612–622

Peng S, Cassman KG, Virmani SS, Sheehy J, Khush GS (1999) Yield potential trends of tropical rice since the release of IR8 and the challenge of increasing rice yield potential. Crop Sci 39:1552–1559

Reddy AR, Chaiytanya KV, Vivekanandan M (2004) Drought induced responses of photosynthesis and antioxidant metabolism in higher plants. J Plant Physiol 161:1189–1202

Roy Choudhury A, Basu S (2008) Over expression of an abiotic stress responsive plant protein in bacteria *E. coli*. Afr J Plant Biotechnol 7:3231–3234

Roy P, Niyogi K, Sengupta DN, Ghosh B (2005) Spermidine treatment to rice seedlings recovers the salinity stress induced damage of plasma membrane and PM-ATPase in salt tolerant and sensitive cultivars. Plant Sci 168:583–589

Roychowdhury A, Roy C, Sengupta DN (2007) Transgenic tobacco plants overexpressing the heterologous lea gene *Rab 16a* from rice during high salt and water deficit display enhanced tolerance to salinity stress. Plant Cell Rep 26:1839–1859

Sagi M, Fluhr R (2006) Production of reactive oxygen species by plants NADPH oxidase. Plant Physiol 14:336–340

Sairam RK, Tyagi A (2004) Physiology and molecular biology in salinity tolerance in plants. Curr Sci 86:407–420

Sambrook J, Russell DW (2001) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor

Tang W, Newton RJ (2005) Polyamine reduces salt induced oxidative damage by increasing the activity of antioxidant enzyme and decreasing lipid peroxidation. Plant Growth Regul 46:31–43

Tonon G, Kevers C, Faibre Rampant P, Graziani M, Czaspar T (2004) Effects of NaCl stress on proline and free polyamine levels in emerging *Fraxinus angustifolia* callus. Plant Physiol 161:701–708

Wang W, Kin Y-H, Haeng, Lee S, Deng W-P, Sookwak S (2009) Differential antioxidation activities in two alfa cultivars under chilling stress. Plant Biotechnol Rep 3:301–307

Yokoi S, Bressan RA, Hasegawa PM (2002) Salt stress tolerance of plants. JIRCAS working report 2002, pp 25–33

Zeid IM, Shedeed ZA (2006) Alterations in nitrogen metabolites after putrescine treatment in alfalfa under drought stress. Pak J Biol Sci 10:1513–1518

Zhao H, Yang Z (2008) Exogenous polyamines alleviate the lipid peroxidation induced by cadmium chloride stress in *Malus hupehensis* Rehd. Sci Hortic 116:442–447

Zhu JK (2001) Plant salt tolerance. Trends Plant Sci 6:66–71