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Research Paper

Variation in antioxidant isozymes contributes to variation in salt tolerance in rice genotypes

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Abstract

Rice is a salt-sensitive crop and degree of salt sensitivity varies genotype to genotype. This is a result of existence of combination of mechanisms among genotypes. Salt-induced oxidative stress leads to the loss of membrane integrity and this may affect efficiency of mechanisms which contribute to salt tolerance. Antioxidant enzymes such as superoxide dismutase (SOD) and peroxidases (POXs) are known to be a part of oxidative stress tolerance mechanism. Their contribution in conferring salt tolerance and their individual isoform response was analyzed among genotypes with varying salt tolerance. The total antioxidant enzyme activity of SOD and POX was found to be genotype specific rather than salt tolerance level. Some of the isozymes of Cu/Zn SOD were found to be salt stress induced whereas chloroplastic and cytosolic Cu/Zn SOD isozyme activity found to be affected in most of the salt sensitive genotypes. Affected Cu/Zn SOD isozyme's activity might have enhanced salt sensitivity of genotypes. Transcript profiling of individual SOD isoforms revealed the variation in basal level transcript abundance and differential response of each isoform even among genotypes with similar STG.

Keywords: Salt tolerance, membrane damage, superoxide dismutase, peroxidases, isozyme, zymogram.

Introduction

Salt stress is one of the leading challenges in agriculture to keep up the productivity. Plant breeders rely on the salt tolerant genotypes available in a particular crop to use them in a breeding program. Plants have various mechanisms to cope with salt stress such as avoidance, tolerance by localizing ions into vacuoles and neutralizing oxidative stress induced by salt. The increase of Na^+ concentration within the cell lead to increase in the reactive oxygen species (ROS) such as singlet oxygen, superoxide anions, hydroxyl radicals, and hydrogen peroxide. Under stress conditions, antioxidant systems are induced to maintain ROS homeostasis and in case, this fails, it leads to a condition referred to as oxidative stress. Under oxidative stress, major damage is through the reaction of ROS with the lipid components of membrane and form lipid hydroperoxides that affect membrane properties[1]. In general protecting cell membrane from oxidative stress damage contributes significantly to salt tolerance [2].

Among antioxidant enzymes Superoxide dismutase (SOD)catalyzes the dismutation of superoxide anion and yields hydrogen peroxide. Hydrogen peroxide a byproduct of the SOD catalyzed reaction is further converted to water and oxygen by Catalases (CAT) and Peroxidase (POX). Within the cell hydrogen peroxide can react with Fe^{2+} to yield hydroxyl ions radical through Fenton reaction which are highly reactive species. However, Membrane phospholipids are impermeable to charged O_2^- molecules [3]. Therefore, SODs are crucial for the removal of O_2^- in the compartments where they are formed [3].

Based on cofactor SODs are classified into three groups viz. Cu-Zn SODs, Fe-SOD and Mn SODs Namely Cu-Zn SODs are localized in chloroplast as well as cytosol[4], Fe-SOD in mitochondria and Mn SODs in chloroplast and peroxisome[4].The effects of salt stress on the antioxidant responses have been studied in a number of plant species including wheat, rice, sorghum and pea[2,5,6]. The antioxidant enzyme response of the seedlings under salt stress is correlated with a salt tolerance of the genotype. In rice, under salt stress degree of increase in activities of SOD and POX differ with developmental stages of genotypes[8]. An enhancement in the POX activity in response to salt stress suggests that this enzyme serves as an intrinsic defense tool to resist stress-induced oxidative damage in plants[9,10]. Rice is known to be a salt-sensitive crop. In our previous paper[11] we reported a set of genotypes for early seedling stage salt tolerance with their salt tolerance grades (STG). In this manuscript, we present data on the correlation between MDA and STG and the genotype-specific changes in the antioxidant enzyme activity as well as their isoforms profiles under salt stress.

Salt tolerance in plants is a result of contribution of multiple mechanisms. One of the mechanisms is an antioxidant response which deals with the oxidative stress induced by an increase in salt concentration, the extent of tolerance depends on the mechanisms that are operative. In the previous paper[11], we reported early seedling stage salt tolerance of genetically diverse genotypes (included in present study). Here, we have analyzed whether the membrane damage (as measured by MDA) is correlated with the salt tolerance level and also looked for whether the total activity of an antioxidant enzyme or their specific isozymes activity is associated with the salt tolerance level of genotype .

Materials and Methods

Plant material and growth codition

A total of 10 rice genotypes used in the studies is listed in Table 1. These genotypes were selected based on their Salt Tolerance Grade (STG) assessed as described in[11].Rice seeds were surface sterilized with 0.1% $HgCl_2$, washed with distilled water several times and imbibed in distilled water overnight. Imbibed seeds were kept on a wet paper in glass petri plates for germination. Two days old seedlings were transferred to 96-well PCR microplates cut from the bottom and were kept in Hoagland's media. Where mentioned Six days old seedlings were transferred into Hoagland's basal media supplemented with 150 mM NaCl to study the effect of salt stress on growth. Nutrient solution was replaced every 48h. The seedlings were grown under controlled conditions [13 hrs light (28°C) / 11 hrs dark (26°C)]. The tissue samples were harvested after 0, 2 and 4 days in stress (DIS). Harvested tissues were immediately used or frozen in liquid nitrogen and stored at -70°C until further use.

Table 1: List of *Oryza sativa indica* accessions

S. No.	Genotype	Tolerance	STG	Pedigree
1	*Cherivirappu**	T	3	Landrace
2	*Delhi rice **	T	3.5	-
3	*Panvel03***	MT	5	*Damodar / Pankaj*
4	*PSBRc50**	MT	5.5	*IR5657 / IR 4630-22-2-5-1-3*
5	*PSBRc84**	MT	7	*CSR10 / TCCP266-3B-10-3-1*
6	*Nonabokra**	MT	7	-
7	*Karjat03***	S	7.5	*IR36 / KJT 35-3*
8	*Gham **	S	8	Landrace
9	*RDN local **	S	9	-
10	*IR29***	S	9.5	*IR833 / IR2040*

Note: ***:** Accessions obtained from IRRI, Philippines, **: Accession obtained from the University of Pune, India. T: Tolerant, MT: Moderate Tolerant, S: Sensitive, STG (Tolerance Grade), #: From the reference Samant and Jawali (2016) [11].

Biochemical analysis

All the biochemical analyses were carried out from tissues obtained at 0, 2 and 4 DIS. The experiments were repeated three times and the results are presented as Mean ± SE.

Estimation of Malondialdehyde (MDA) content
Shoot tissue (0.4 gm) was ground in liquid nitrogen with mortar and pestle and was suspended in 2 ml of 80% ethanol. An aliquot of 0.5 ml of the homogenate was transferred to a 5ml plastic tube and 2 ml of thiobarbituric acid reagent [20%] trichloroacetic acid (TCA, Sigma, USA) and 0.5% TBA (Sigma-Aldrich, WI, USA. After incubating the mixture in a water bath at 95°C for 25 min the tube was cooled on ice. The solution was centrifuged at 4000 rpm for 12 min. The absorbance of the supernatant was measured at 600, 532 and 440nm on SHIMADZU UV-Visible spectrophotometer[12]. Reference blank contains 80% ethanol and TBA reagent whereas correction blank contains homogeneous and 20% TCA.

Enzyme activity measurements
Estimation of protein and antioxidant enzyme activities were carried out on an infinite 200 micro plate spectrophotometer (TECAN, UK) equipped with an internal temperature controlled incubator and shaker for kinetic analysis. All enzyme assays are performed in a final volume of 0.2 ml in the UV-micro plate well at 28-30°C. Samples and blanks were analyzed in triplicate.

Preparation of crude extract
Preparation of the crude extract from the shoot tissue was carried out at 4-8°C. The shoot tissue (~2 g) was ground in liquid nitrogen using a mortar and pestle and further homogenized in 12 ml of an ice-cold extraction buffer pH 7.8 containing 100 mm potassium phosphate buffer, 0.1 mm ethylenediaminetetraaceticacid (EDTA), 0.1% (w/v) polyvinyl pyrrolidone. The homogenate was centrifuged at 14,000 g for 15 min at 4°C and the supernatant (referred as crude extract) was used forenzyme assays or otherwise stored at -25 °C for further use. Protein concentration was estimated by Bradford's method[13].

Total Superoxide Dismutase activity
Total SOD activity was measured essentially by the method of Beyer and Fridovich[14], with some modifications. The reaction mixture contained 50mM potassium phosphate (pH 7.8), 13 mM methionine, 75 µM NBT, 1.3µM riboflavin and the crude extract. The reaction mixture was exposed to light for 2 min and further incubated in the dark for 3 min. Absorbance was measured at 560 nm. One unit of SOD is defined as the amount of enzyme that causes 50% inhibition of NBT reduction.

Peroxidase activity
Peroxidase (POX) activity was measured byamethod adapted from R. Murshed et al [14]. The reaction mixturecontained 0.1 mM EDTA and 8 mM Guaiacol (Sigma-Aldrich, USA) in 50 mM potassium phosphate buffer (pH 7.0), 2.5 mM H_2O_2 (Calbiochem, Germany) and 10 µl crude extracts. The reaction was initiated bythe addition of 5 µl of 100 mmH_2O_2. The microplate was shaken for 5s on the plate reader and activity is determined by monitoring the oxidation of Guaiacol at 470 nm up to 3 min. Specific activity was calculated using the extinction coefficient 26.6 mM^{-1} cm^{1}. One unit of enzyme activity was defined as that which oxidized 1 mM of Guaiacol per minute.

Analyses of SOD isozymes
Crude protein extract (total protein~70 µg) of was mixed with 6x sample loading dye and separated on native PAGE (15 %) essentially following the method of Laemmli[16]. Electrophoretic separation was performed at 4 °C for 45 min with a constant current of 20 mA and later for 3 hrs at 40 mA per gel. In-gel activity was assayed by following the method of Beauchamp and Fridovich (1971)[16], with some modifications. After electrophoresis, the gel was transferred to a tray containing 50 mM phosphate buffer (pH 7.8) and incubated at room temperature (28°C) for 10 min. At the end of 10 min, the gel was transferred to a solution containing 2.5 mM NBT and incubated at room temperature (28°C) for 25 min, followed by incubation in 50 mM potassium phosphate buffer (pH 7.8) containing 30 µM riboflavin and 30 mM TEMED under dark conditions. Subsequently, the gel was exposed to light (170 µmol m^2 s^{-1}) for 15 min at room temperature. Isozyme activity of SOD determined using inhibition assay. Before the activity staining, gel was incubated for 30 min in 50 mM phosphate buffer (pH 7.8) containing DETC (5 mM) and 50 mM phosphate buffer (pH 7.8) with H_2O_2 (5 mM) for discriminating Cu/Zn SOD isozymes and Cu/Zn, Fe-SOD isozymes respectively.

Analyses of POX isozymes
A three step Gradient (a top layer of 10%, middle layer of 12.5% and bottom layer of 17.5% PAGE) PAGE was used for resolving POX isoforms. Electrophoretic separation of proteins was performed at 4 °C for 45 min at a constant voltage of 50V and later for 14 hrs at a constant 100V. POX activity was

performed as per Lee et al., 1995[26] with some modification. After completion of electrophoresis, the gel was incubated in 50mM phosphate buffer (pH 6.4) for 10 min. Later the gel was incubated in a buffer (pH 6.4) containing 20 mM guaiacol for 10 min. POX activity was initiated by incubation in 50 mM potassium phosphate buffer (pH 6.4) containing 5 mM H_2O_2 under dark condition.

Transcript profile analysis

Isolation of Total RNA and reverse transcription: Total RNA was isolated from shoot tissue by using Trizol (Invitrogen, USA) and treated with *RNAse*-free *DNAse* I (Roche Diagnostics, Germany) (Saini et al 2010). Subsequent to the heat inactivation of *DNAse* I, total RNA (10 µg) reverse transcribed using *SuperScript* II reverse transcriptase (Invitrogen, USA) and oligo$(dT)_{23}$ anchored (Sigma-Aldrich, USA) as per the manufacturers protocol.

Quantitative Real-time PCR analysis

Expression analysis of (Cytosolic Cu/Zn SODs: Os3g22810 and Os07g46990, Chloroplastic Cu/Zn SOD: Os08g44770 and Mitochondrial Mn-SOD: Os05g25850) at transcript level was carried out by real-time PCR using the gene specific primers [Os-22810-F: 5'-ATCATTGGCAGAGCCGTCGTTG-3' and R: 5'-TCAGCCTTGAAGTCCGATGATCC-3', Os-46990-F: 5'-GAGCACACTCCATCATTGGCCG-3' and R: 5'-GTCCGATGATTCCGCAAGCAACT-3', Os-44770-F: 5'-AAGAAGGCCGTCGCCGTGCTCAA-3' and R: 5'-CACGGACATTCACTGTTGTAGGAC-3', Os-25850-F: 5'-CAGTGGAAACAACTGCTAACCAGG-3' and R: 5'-GTTGCTCAGGTAGTCTGGCCTGAC -3']. Real-time PCR analysis was performed on a Mastercycler ep *realplex* PCR machine (eppendorf, Germany) using SYBR Green Jumpstart *Taq* Ready mix (Sigma-Aldrich, USA). Following PCR conditions were used: initial denaturation at 95°C for 5 min, 45 cycles of 94°C for 15 s, 60°C for 20 s and 68°C for 25 s. The specificity of the amplification was assessed by melting curve analysis at the end of the PCR and confirmed the same by analyzing the PCR products on 2 % agarose gel. The real-time PCR analysis was carried out in two independent RNA preparations and the samples were analyzed in triplicates. Appropriate controls including no template control were included for each set of analysis. The basal normalized expression (NE) level using $2^{-\Delta Ct}$ method and the relative expression ratio (RER) using $2^{-\Delta\Delta Ct}$ method were calculated as per Livak and Schmittgen (2001) and Schmittgen and Livak (2008) using rice two reference genes [glyceraldehyde 3-phosphate dehydrogenase (*Osgapdh*, Locus: LOC_Os08g03290, For-P: 5′-GTG-ACAGCAGGTCGAGCATCTTCG-3′ and Rev-P: 5′-GTCGATGACACGGTTGCTGTAACC-3′) and Tubulin (*Ostubulin*, Locus: LOC_Os12g39650, For-P: 5′-TGCCCCTTCTTTGACAGATAGA-GATGC-3′ and Rev-P: 5′-CGCCTCTATCAAGAGCTCCATGAACC-3′)] were used as internal controls for analysis.

Results and Discussion

Analysis of salt stress-induced membrane damage and their correlation with STG

Among 10 rice genotypes, a wide difference in MDA content was observed even in the control condition [Table 1, Figure 1]. Similarly, Dionisio-Sese and Tobita, (1998) have reported the differences in the MDA content among four rice genotypes. Rice genotype Cherivirappu (tolerant) and Panvel03 (moderate tolerant) showed low (~7.2 more g^{-1}FW shoot) MDA content, whereas salt sensitive genotype RDN local showed relatively high (~26.1 nmole g^{-1}FW shoot) MDA content. Plants under different stress conditions including salt stress, elevation in MDA content are shown to be a measure of membrane damage[18,19]. Overall the MDA level at 2 and 4 DIS, a measure of salt stress-induced membrane damage was found to be positively correlated with STG of the genotypes (R= $0.57_{2\ DIS}$ and $0.73_{4\ DIS}$) (Figure 2). A good correlation between the STG and increase in MDA level under salt stress confirms that the method adopted for assigning the STG to the genotypes reflects well with their salt tolerance. Unsaturated fatty acids are prone to get oxidized and the degree of unsaturation of fatty acids in membrane lipids was associated as one of the mechanism of adaptation to salt stress [21,22]. However, a few genotypes were found to be exceptions for the correlation: salt sensitive genotype Gham (STG 8), moderate tolerant genotypes Nonabokra (STG 7) and Panvel03 (STG 5), where elevated MDA content was lower than the genotypes with relatively better salt tolerance. Knipper 1984, Mansour (1995) has linked the degree of unsaturated fatty acid with membrane fluidity and its functioning to salt tolerance. Such genotype-specific differences in degree of unsaturated fatty acids might be the reason of lack of perfect positive correlation between elevated MDA content and salt tolerance grade at the early seedling stage.

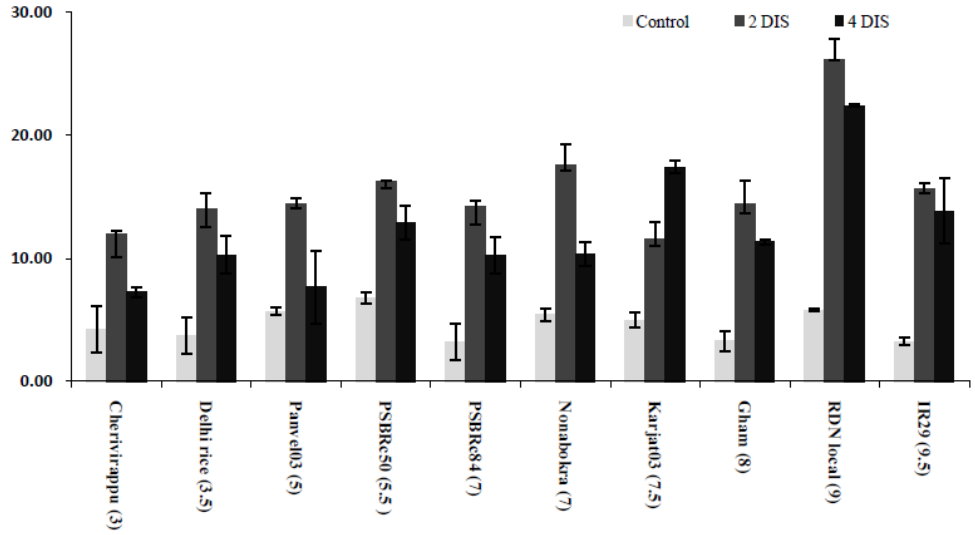
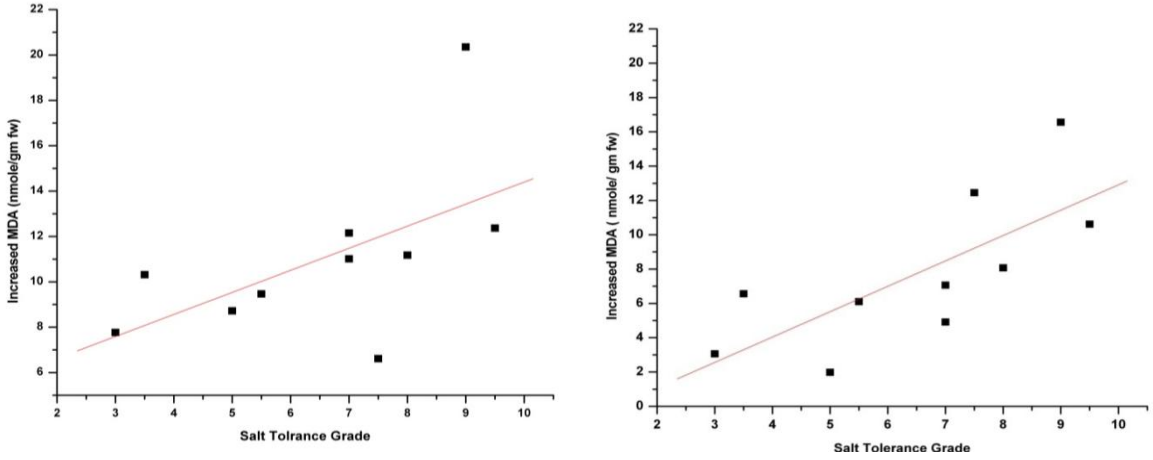


Figure 1: Effect of salinity on the membrane damage (MDA level) in rice genotypes



	Regression Value	Standard Deviation	Probability
2DIS	0.56	3.3	0.08
4DIS	0.73	3.1	0.01

Figure 2: Relationship between increased MDA content under salinity and tolerance grade of rice genotypes. Seedlings were grown for 4 days in 150 mM NaCl stress and MDA level was measured after 2 DIS and 4 DIS

Effect of salt stress on antioxidant enzyme levels

Salt tolerance in plants is a result of contribution of multiple mechanisms. One of the mechanisms is an antioxidant response which deals with the oxidative stress induced by salinity.The activity of SOD and POX antioxidant enzymes was analyzed quantitatively as well as qualitatively (Zymogram among 10 rice genotypes).

Total SOD activity

Ten rice genotypes showed a wide variation in the basal level of SOD activity (~20-52 U mg^{-1} protein min^{-1}) (Table 2). All the salt moderate tolerant (except Nonabokra) and salt sensitive genotypes Karjat03 and Gham showed low SOD activity (8-16 U mg^{-1} protein min^{-1}) after 4 DIS compared to 2 DIS (37-49 U mg^{-1} protein min^{-1}). Though at 4 DIS salt sensitive genotypes RDN local and IR29 showed SOD activity (59-90 U mg^{-1} protein min^{-1}) similar to tolerant genotypes (68-81 U mg^{-1} protein min^{-1}), in early stress period (at 2 DIS) SOD activity was found to be down-regulated in sensitive genotypes (RDN local and IR29).

Total POX activity

Ten rice genotypes showed nearly 10 fold difference in the basal level of POX activity (109-1167 U mg^{-1} protein min^{-1}) (Figure 3). Gham (sensitive) showed low (~109 U mg^{-1} protein min^{-1}) basal activity of POX whereas moderately salt tolerant genotypes viz., Panvel03 and Noanbokra showed a higher basal level (> 1000 U mg^{-1} protein min^{-1}) (Figure 4). In response to salt stress moderate tolerant genotypes (Panvel03, PSBRc84 and Nonabokra) and sensitive genotype (IR29) at 2 DIS showed high POX activity (> 1200 U mg^{-1} protein min^{-1}) whereas sensitive genotype Gham showed low POX activity(~ 150 U mg^{-1} protein min^{-1}). Compare to 2 DIS, POX activity at 4 DIS was found to be 2-8 folds lower in most of the genotypes. In general, no correlation was observed between the STG and the basal level of SOD and POX activity among 10 rice genotypes.

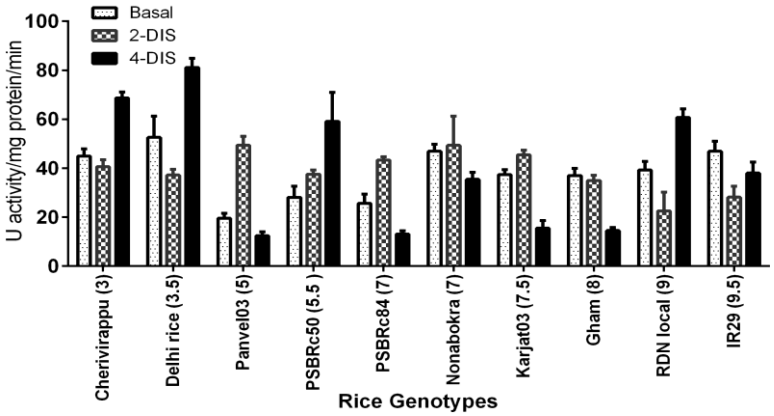


Figure 3: Total superoxide dismutase activity after 2 and 4 DIS in shoot of 10 rice genotypes

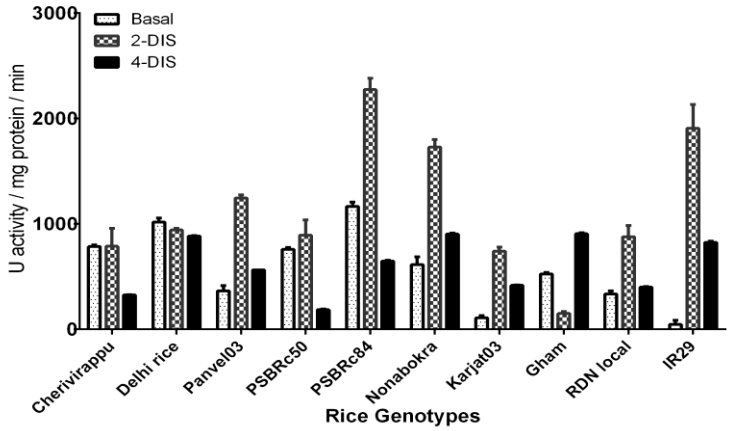


Figure 4: Total Peroxidase (POX) activity after 2 and 4 DIS in shoot of 10 rice genotypes

6^{th} day after germination, the seedlings were transferred to medium containing 150 mM sodium chloride. The experiments were repeated at least thrice and the values are presented as Mean ± SE.

SOD and POX zymogram analysis: Zymogram analysis was carried out to reveal if there is any change in the individual SOD and POX isoforms in response to salt stress and their contribution to total SOD and POX activity. Firstly, zymogram analysis of SOD isoforms was performed in presence and absence of DETC (a selective Cu chelator) to identify the isoforms of Cu/Zn SOD and Mn SOD (Figure 5). Zymogram showed11 bands of SOD isoforms among which 2 bands were distinct (Figure 6). The faster migrating band represents chloroplasts Cu/Zn-SOD (8^{th} isoform) and the slower one cytosolic Cu/Zn-SOD (7^{th} isoform) [23]. In general, Mn-SOD isoforms activity was found to be more distinct after 2 DIS compared to 4DIS. Among Mn-SOD isoforms, 2^{nd} isoform activity was more prominent in all genotypes whereas 1^{st} isoforms activity found to be genotype specific. In salt-sensitive genotypes (except Karjat03) after 2 DIS, decline in activity was observed in Cu/Zn-SOD isoforms 5, 6 but not in Mn-SOD isoforms activity. Among Cu/Zn-SOD isoforms, slow migrating isoforms 1-4 appeared after 4 DIS in most of the genotypes (except RDN local and IR29). Among fast

migrating Cu/Zn-SOD isoforms, the activity of 7th,8th and 9thisoform was found in all genotypes after 4 DIS whereas in the control condition only 7th isoform was found to be genotype specific.

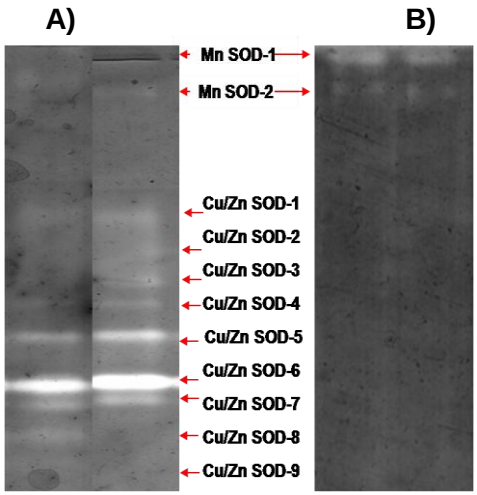


Figure 5: Isoforms of superoxide dismutase separated in non-denaturating 12.5% gel electrophoresis. A: without DETC (inhibitor), B: DETC

Cu/Zn SOD isoforms 5th and 6thof superoxide dismutase (major activity) are chloroplastic and cytosolic respectively [22].

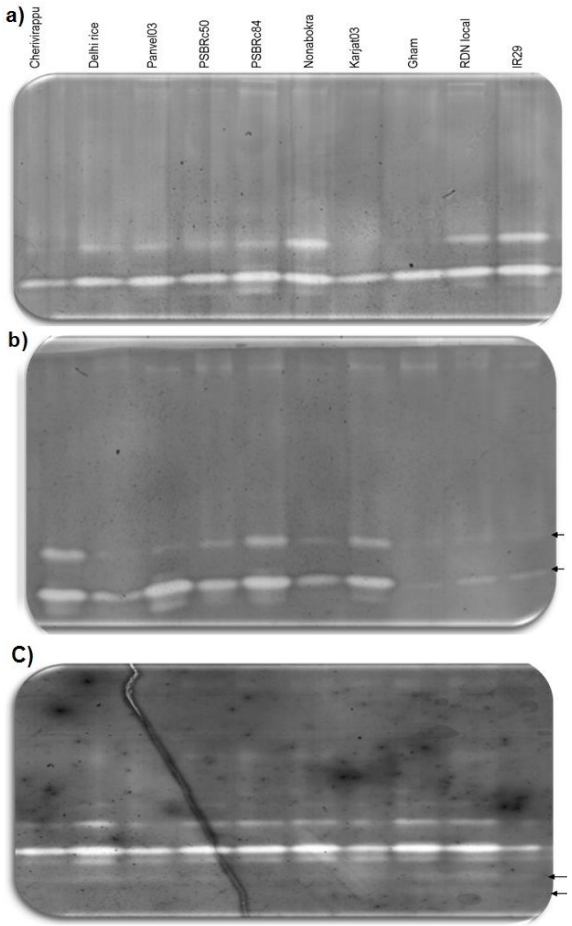


Figure 6: SOD isozyme profiles of shoot tissue of rice seedlings treated with 150 mM NaCl for 2-4 days. The arrow indicates the affected isoforms. Zymogram: a) 0-Day, b) 2-day stress, c) 4-daystress

Zymogram analysis revealed the presence of 9 isoforms of POX and was differentially affected by salt stress (Figure 7). POX isoforms 8 and 9 were found to be salt stress responsive, though activity pattern was found to be genotype specific. Intolerant genotypes, Cherivirappu and Delhi rice, 8th isoform was found to be induced after 2 DIS. Similarly, among sensitive genotypes, RDN local showed enhanced activity of 8th isoform, after 2 and 4 DIS whereas IR29 showed enhanced activity in 2 DIS only. Among moderate tolerant genotypes, only PSBRc50 showed enhanced activity in 8th isoform after 2 DIS.

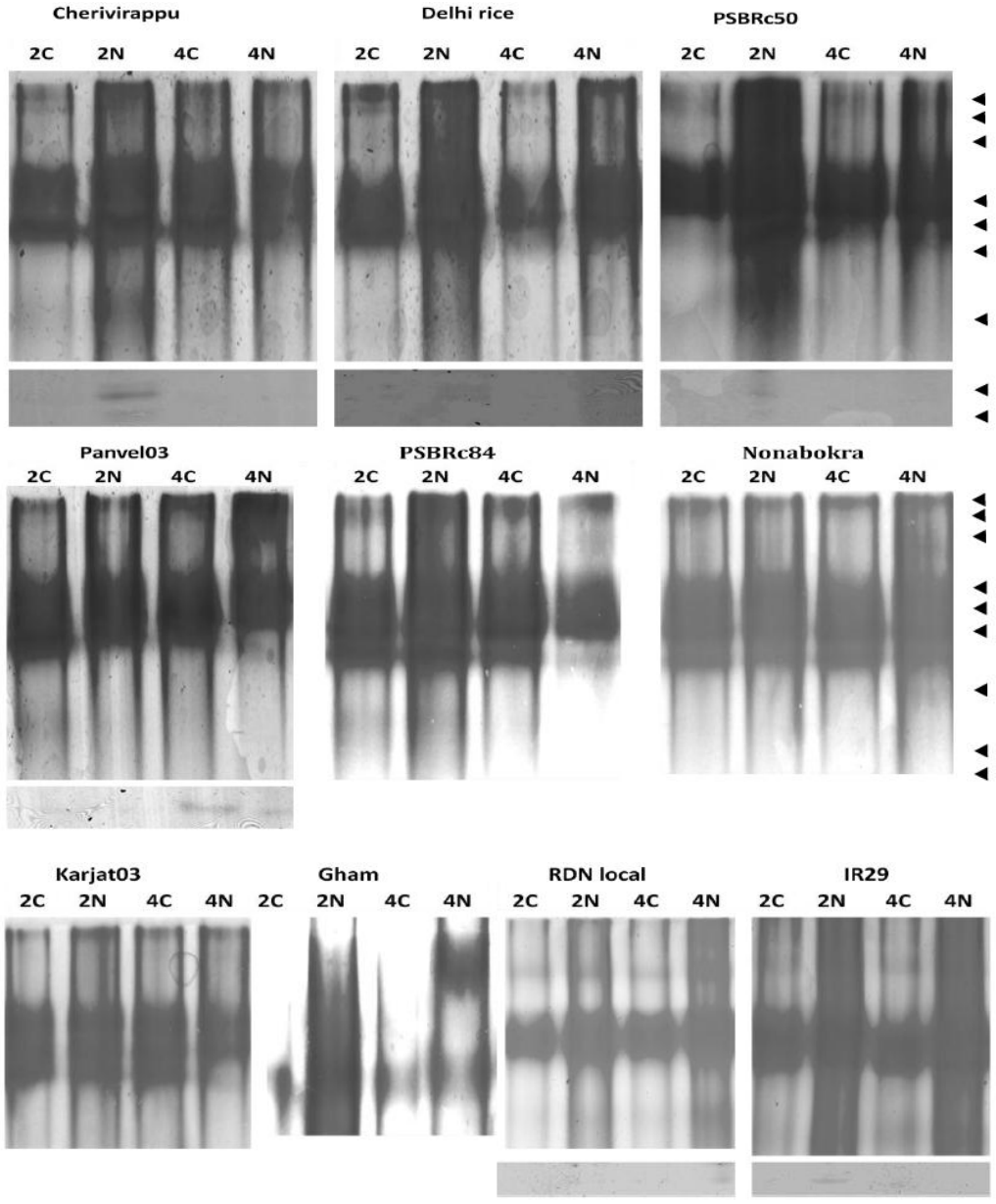


Figure 7: POX isozyme profiles of shoot tissue of rice seedlings treated with 150 mM NaCl for 2-4 days. The arrow indicates the affected isoforms

SOD and POX enzyme response and salt tolerance

The total antioxidant enzyme activity of POX and SOD as well as their individual isoforms activity was found to be varied among 10 rice genotypes under control and salt condition (Table 1 and Figure 3, 4, 6 and 7). Similarly,genotype-specific variation in the total activity of SOD and POX has been reported in rice[2,8]. Sairam et al[6] in wheat and Chawla et al [24] in rice has associated antioxidant enzyme level withsalt tolerance of genotypes. In our investigation, genotypes with higher STG (except Karjat03 and RDN local) showed very high POX activity in response to salt stress.

Change in levels of particular isozymes rather than changes in their total activity may be more important [25,26]. Irrespective of genotypes salt tolerance 6th isoform of (cytosolic) Cu/Zn-SOD found to be a prominent SOD. Under salt stress, contribution of Mn-SOD isoforms in total SOD activity seems to be more than in control condition, especially in salt-sensitive genotypes (Figure 6). Similarly, in rice leaves, salt stress-induced expression of Mn-SOD isoform 2 has been reporte[27]. Under salt stress, enhancement in the activity of Cu/Zn-SOD isoforms 7,8 and 9 suggest that these isoforms might be involved in regulation of ROS generated under salt stress (Figure 6).In few genotypes (PSBRc50 and (RDN local) under stress, change in isozymes activity in zymogram did not correlate with the change in total SOD activity. Such difference in zymogram and total activity of antioxidant isozymes has also been reported (28). Our finding on total SOD and POX activity among genotypes with varying salt tolerance is agreement with earlier reports[29] that their level is not liable marker for salt tolerance of genotype. Earlier investigation on salt stress caused change in Na^+ and K^+ content among10 rice genotypes have shown that Delhi rice (salt tolerant) and Gham (salt sensitive) are genotypes with relatively low shoot Na^+ content, whereas salt, moderate tolerant genotypes (PSBRc84 and Nonabokra) and salt sensitive genotypes (Karjat03 and RDN local) are among very high shoot Na^+ under stress[11]. Decrease in activity of cytosolic and chloroplasts Cu/Zn-SOD isoforms might have enhanced susceptibility to O_2^- generated in chloroplast. And hence increase in lipid peroxidation under stress. The role of Cu/Zn SOD in conferring salt tolerance to rice [29] and Chloroplast Cu/Zn SOD-mediated salt tolerance in Pea [30] has been reported. Similarly,Tanaka and coworkers [31] findings suggested that high level of chloroplastic SOD is an important factor for salt tolerance in rice.

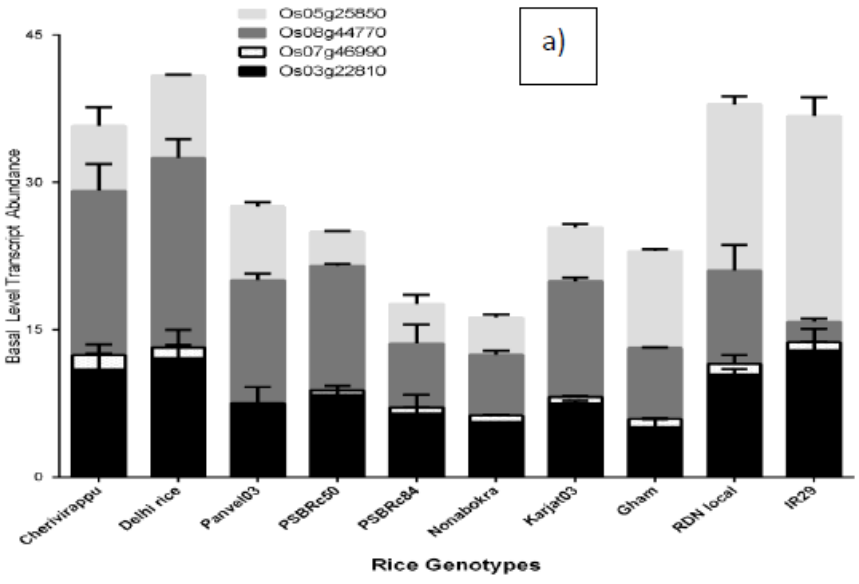
SOD isozymes activity highlighted that individual isoforms contribution in total SOD activity varies even among genotypes with similar salt tolerance. To make best utilization of revealed information for germplasm development, it is important to know whether this differential expression is regulated at transcript level and or activity level. To address this we have carried out transcript profile analysis of four SOD isoform coding genes viz., (Cytosolic Cu/Zn SODs: Os3g22810 andOs07g46990, Chloroplastic Cu/Zn SOD: Os08g44770 and Mitochondrial Mn-SOD: Os05g25850).

Cu/Zn and MnSOD's basal transcript abundance

Basal transcript abundance of all 3 Cu/Zn SOD (Os3g22810, Os07g46990 and Os08g44770) found to be higher in tolerant genotypes Cherivirappu and Delhi rice (exception Viz., Os3g22810 in RDN local and IR 29). This high transcript abundance might be encouraging these tolerant genotypes to quickly cope up with oxidative stress caused by salinity. Most of the moderately salt sensitive genotypes showed low basal level of Mn-SOD transcript (Os05g25850) (Figure 8a).

Cu/Zn and MnSOD's transcript response to salinity

Under salt stress, each Cu/Zn SOD isoform showed different trend of expression even among genotypes with similar salt tolerance. Among cytosolic Cu/Zn SOD isoforms, Os3g22810 coding isoform seems to be having high contribution in SOD activity in Cherivirappu (tolerant) and Panvel03 (moderate tolerant) throughout stress (Figure 8b). Other isoform (Os07g46990) found relatively more induced in genotypes with higher STG (Figure 8c).



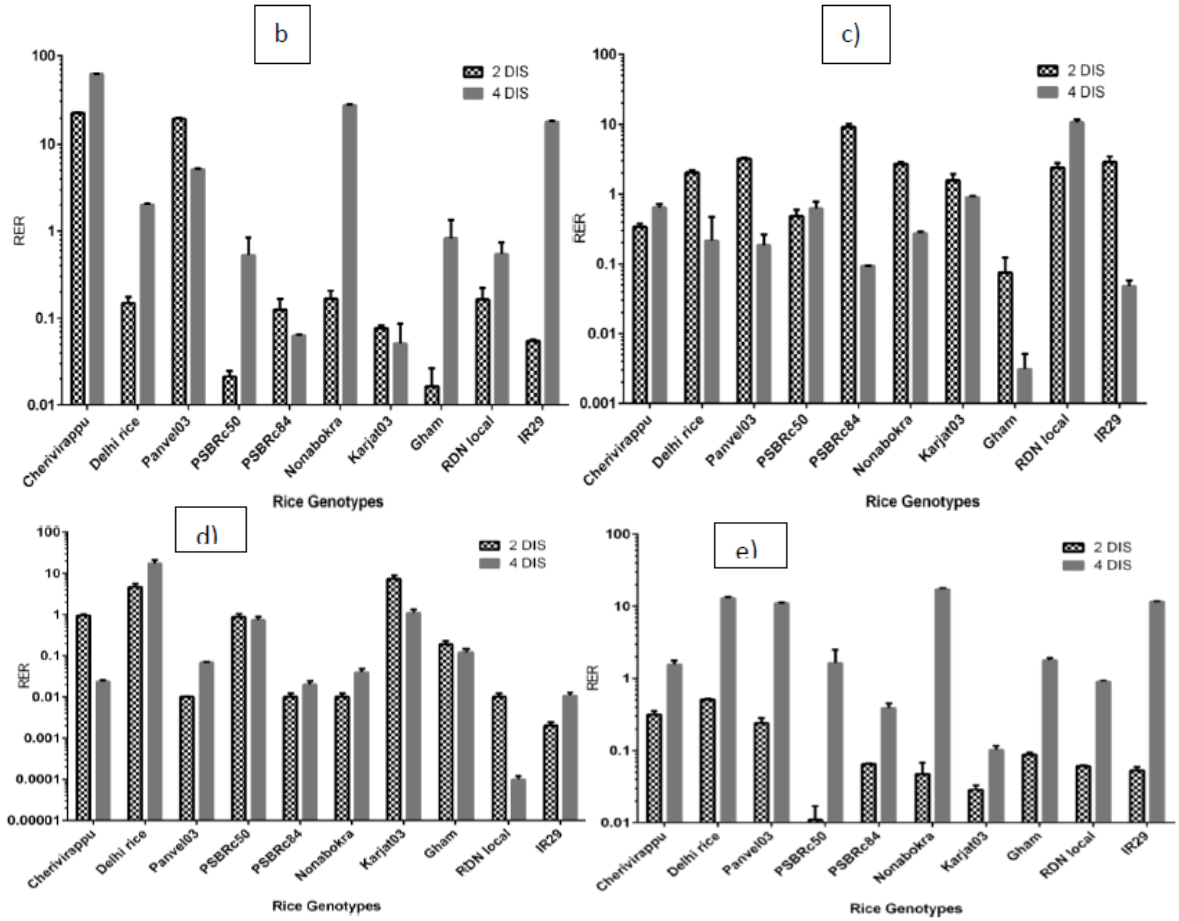


Figure 8: Transcript profile ofSOD isoforms in shoot tissue of rice seedlings (treated ± 150 mM NaCl for 2-4 days)
a) Transcript profile: Basal level of Os3g22810, Os07g46990, Os08g44770 and Os05g25850
b) Transcript profile of Os3g22810 (Cytosolic Cu/Zn SOD)
c) Transcript profile ofOs07g46990 (Cytosolic Cu/Zn SOD)
d)Transcript profile ofOs08g44770 (Chloroplastic Cu/Zn SOD)
e)Transcript profile ofOs05g25850 (Mitochondrial Mn-SOD)

On the other hand Chloroplastic, Cu/Zn SOD found to be upto100 fold down-regulated especially in genotypes with higher STG (Figure 8d). In-gel activity and transcript profile analysis, collectively suggest that poor expression of chloroplastic Cu/Zn SOD isoform might have enhanced these genotypes salt sensitivity. Also, in earlier study we have observed major visual salt injuries (pale yellow leaves and contracted shoot) in these genotypes which indicate affected photosynthesis system (chloroplast) (Figure 9). Mn-SOD (Os05g25850) transcript found to be induced in most of genotypes after 4 DIS.

Figure 9: Visual Salt Injury in shoot tissue of rice seedlings (treated ± 150 mM NaCl for 4 days)

Conclusion

By taking together the results of current investigation [Viz., elevated MDA content, change in antioxidant enzymes (SOD and POX) total activity and STG, suggest that the total activity may not be taken as marker of salt tolerance level of genotype at early seedling stage. On other hand, declined activity of chloroplastic and cytosolic Cu/Zn SOD isoform might be enhancing genotypes salt sensitivity at early seedling stage. Also by taking together transcript abundance in control and stress, Os3g22810 coding isoform seems to be having relatively higher contribution in total SOD activity.

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