Long Term Salinity Stress Reveals Variety Specific Differences in Root Oxidative Stress Response

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Abstract: Salinity stress induces oxidative stress caused by reactive oxygen species (ROS): superoxide radicals, hydrogen peroxide (H2O2) and hydroxyl radicals. Activities of both enzymatic and non-enzymatic components of the antioxidant system and related growth parameters were studied in the roots of the salt tolerant rice variety FL478 and the sensitive variety IR29 in response to long term stress (12 d) induced by 50 mmol/L NaCl. The comparative study showed that FL478 maintained higher relative growth rate and lower Na+/K+ in the roots than IR29 due to a higher membrane stability index that effectively exclude Na+. Lower TBARS (thiobarbituric acid reactive substance) content in FL478 roots indicated that its membrane was relatively unaffected by ROS despite high H2O2 content recorded under the salinity stress. Relatively higher superoxide dismutase activity along with a parallel increase in transcript level of superoxide dismutase (Os07g46990) in FL478 indicated that this protein might make a vital contribution to salt stress tolerance. Although the content of ascorbic acid remained unchanged in FL478, the activity of ascorbic peroxidases (APOXs) was reduced comparably in the both varieties. Transcriptomic data showed that a larger number of peroxidase genes were upregulated in FL478 compared to IR29 and several of which might provide engineering targets to improve rice salt tolerance.

Key words: root membrane stability; salinity stress; transcriptome; superoxide dismutate; ascorbic peroxidase; rice

Rice (Oryza sativa), the staple crop of more than 60% of the world population, is relatively salt sensitive with a threshold tolerance capacity of around 1.9 to 3.0 dS/m (Grattan et al, 2002). Rice sensitivity to salinity is dependent on growth stage with plants being most susceptible at the seedling and panicle induction stages (Akbar et al, 1972).

In general, salinity stress induces an initial osmotic stress and subsequent toxicity due to the accumulation of ions. However, damage can also ensue due to reactive oxygen species (ROS) are produced which negatively affect cellular metabolism and physiology (Munns et al, 2006). Salt tolerance in rice, like in other glyophytes, is predominantly associated with the maintenance of ion homeostasis, particularly a low Na/K ion ratio, through exclusion, compartmentation and partitioning of Na+ (Blumwald, 2000). Nevertheless, salinity tolerance varies considerably across rice varieties since the above strategies can be employed at different levels. Indeed, comparative studies of tolerant and sensitive varieties have revealed different mechanisms adopted by tolerant varieties to better sustain their growth and development under salt stress (Walia et al, 2005; Senadheera et al, 2009).

As is the case for other abiotic stress, ROS such as superoxide radicals (O2·−), hydrogen peroxide (H2O2) and hydroxyl radicals (·OH) are produced at a high rate in plants during salt stress. ROS accumulation leads to lipid oxidation and thus adversely affects the membrane integrity. In addition, ROS can harm other essential macromolecules including photosynthetic pigments, proteins and DNA (Hernandez et al, 1995; Fadzilla et al, 1997; Asada, 1999). Chloroplasts, mitochondria and peroxisomes are the primary sites of ROS production and they also contain most of the machinery to detoxify ROS. The anti-oxidant system that scavenges ROS is based on both enzymatic and non-enzymatic components. The enzyme superoxide dismutase (SOD, EC 1.15.1.1) is a key component of the anti-oxidant system and converts the superoxide radicals to H2O2 (Bowler et al, 1994). Further, detoxification is carried out by both catalases (CAT, EC 1.11.1.6) and ascorbate peroxidases (APOX, EC 1.11.1.11) that break down H2O2 to water although APOX is more efficient than CAT (Dhindsa et al, 1981; Anderson et al, 1995). CAT has been found predominantly in leaf peroxisomes or in glyoxysomes to remove H2O2 formed in photosynthesis and β-oxidation of fatty acids (Dat et al, 2000).

Abiotic stress activates the antioxidant system, in part through up-regulated transcription of genes that are directly responsive to ROS or through ROS signalling mediated by mitogen activated protein kinases (Chen and Sigh, 1999; Samuel et al, 2000; Garreton et al, 2002). Previous studies that followed
the changes in antioxidant activity in response to salinity stress showed that tolerant varieties tended to maintain relatively high levels of ROS scavenging activity, which played an important role in minimizing salinity-induced toxicity (Dionisio-Sese and Tobita, 1998; Khan et al, 2002; Sairam and Srivastava, 2002; Singh et al, 2007). However, such studies were based exclusively on relatively short term observation (up to one week for shoots and 24 h for roots) and did not assess whether these mechanisms are also relevant for physiological conditions such as exposure to prolonged moderate salinity. To test this hypothesis, we studied the response of the antioxidant system in rice roots during long term exposure to salinity. Specifically, we recorded growth parameters, the activities of SOD and APOX, levels of ascobic acid, membrane lipid peroxidation, H2O2 production and the expression profiles of genes that are likely to encode these enzymes. To further assess the significance of these parameters on salt tolerance, we also determined whether they showed different responses in the salt tolerant variety FL478 in comparison with the salt sensitive variety IR29.

**MATERIALS AND METHODS**

**Rice cultivation**

*Rice (Oryza sativa L.)* seeds of FL478 and IR29 were obtained from the International Rice Research Institute. Seeds were germinated on washed sand and watered with distilled water. Ten days after sowing (DAS), seedlings were transplanted into hydroponic medium [1.25 mmol/L KNO3, 0.5 mmol/L Ca(NO3)2·4H2O, 0.5 mmol/L MgSO4·7H2O, 42.5 μmol/L FeNaEDTA, 0.625 mmol/L K2HPO4, 0.1 μmol/L of each of Cu2+, Zn2+, Mn2+, B3+, Mo2+ and Co2+, and 3 mmol/L Si in the form of Na2O2SiO3 (Arteca and Arteca, 2000) and grown under the following conditions: 32 °C/27 °C day and night temperatures, 1000 μmol/(m2·s) of mean irradiance for 12 h per day and 90% relative humidity (in the semi-controlled plant growth facility of the Open University of Sri Lanka). Seedlings were exposed to salinity stress by adding 50 mmol/L NaCl to the hydroponic solution at 15 DAS. Hydroponic solution was renewed every 3 d. Plants were harvested at 12 d after the treatment for analysis. All experiments were repeated at least twice with three replications.

**Measurement of relative growth rate**

Fresh and dry weights of the shoots and roots of the seedlings were obtained at the beginning and end of the treatment to calculate the relative growth rate (RGR) using the equation: \( RGR = (\ln W_2 - \ln W_1) / (t_2 - t_1) \) (Hoffmann and Poorter, 2002), where ‘\( W \)’ is the tissue weight, and ‘\( t \)’ is the time between the initial and final sampling.

**Membrane stability index (MSI)**

Root tissue (100 mg) was cut into small pieces and immersed in 10 mL of distilled water. Conductivity of the solvent was measured after incubating the tubes at 40 °C for 30 min (\( C_1 \)) and at 100 °C for 10 min (\( C_2 \)). MSI was calculated by the equation: \( MSI = [1 - (C_1/C_2)] \times 100 \) (Sairam and Srivastava, 2002).

**Determination of Na and K ion concentrations**

\( Na^+ \) and \( K^+ \) concentrations of the rice roots were measured using flame photometry. Harvested plants were washed with cold 20 mmol/L LaCl3 solution for 10 min. Dry weights of the samples were noted after drying at 80 °C for 3 d. Dried samples were incubated in 5 mL of 20 mmol/L LaCl3 for 24 h and measurements were recorded using a flame photometer (Sherwood flame photometer-410, Cambridge, UK).

**Measurement of chlorophyll content**

Chlorophyll content was measured using an SPAD (soil-plant-analysis-development) system (Konika Minolta SPAD-502, Osaka, Japan). Readings were obtained at the tip, base and middle of the leaf blades of the 1st and 2nd most expanded younger leaves of six plants with three replicates. SPAD value for each plant was computed by averaging the leaf SPAD values.

**Measurement of hydrogen peroxide content**

The titanium-hydro peroxide complex method was used to determine the hydrogen peroxide content in the rice roots (Mukherjee and Choudhari, 1983). Root tissue (0.1 g) was macerated in 10 mL of ice cold acetone and 4 mL of titanium reagent and 5 mL of ammonium solution were added to the filtrate of the extract. Then the reaction mixture was centrifuged at 10 000 × g for 10 min. Titanium-hydro peroxide precipitate was dissolved in 10 mL of 2 mol/L H2SO4 and then re-centrifuged. Absorbance of the supernatant was recorded at 415 nm using a Labomed UVD 3500 spectrophotometer (Labomed Inc, Culver City, CA, USA).

**Measurement of lipid peroxidation**

The degree of lipid peroxidation was determined by measuring the formation of the thiobarbituric acid reactive substance (TBARS) malndialdehyde as described by Stewart and Bewley (1980). Crude enzyme extract was prepared by grinding 0.1 g of tissue in 1% trichloro acetic acid (TCA) and centrifuging at 15 000 × g for 15 min. A 100 μL of the supernatant was mixed
with 4 mL of 0.5% thiobarbituric acid (TBA) solution with 20% TCA. The mixture was heated to 95 °C for 30 min, cooled in an ice bath and centrifuged at 10 000 × g for 10 min. The absorbance of the supernatant was measured at 532 nm and the TBARS content calculated using an extinction coefficient of 155 mL/(mol·cm).

**Determination of ascorbic acid content**

To determine ascorbic acid (AA) content, fresh leaf sample (0.1 g) was extracted with 10 mL of 6% trichloroacetic acid. Subsequently, 2 mL of 2% acidic dinitrophenylhydrazine and one drop of thiourea (in 70% ethanol) were added to 4 mL of the extract. Then, the mixture was boiled for 15 min and 5 mL of 80% H2SO4 was added after cooling to room temperature. AA content was determined using the absorbance at 530 nm (Mukherjee and Choudhari, 1983).

**Assay of enzyme activity**

SOD activity was assayed by recording its ability to inhibit the photochemical reduction of nitro-blue tetrazolium by the procedure described by Dhindsa et al (1981). Root tissue (0.1 g) was extracted into 10 mL of 50 mmol/L potassium phosphate buffer. Then 100 μL of the above extract was mixed with 3 mL of reaction mixture I (13 mmol/L methionine, 25 mmol/L nitroblue tetrazolium chloride (NBT), 0.1 mmol/L EDTA, 50 mmol/L phosphate buffer (pH 7.8) and 50 mmol/L sodium carbonate). The reaction was started with addition of 2 μmol/L riboflavin and placing the reaction mixture under a 15 W fluorescent lamp. Absorbance was measured at 560 nm after 15 min of exposure to light. Non-irradiated reaction mixture served as the blank. A complete reaction mixture exposed to light without the enzyme showed the maximum colour development and served as the positive control. The unit of SOD activity was defined as the amount of enzyme that caused 50% reduction in optical density in comparison to the control.

APOX activity was measured through its capacity to reduce the optical density of AA according to Nakano and Asada (1981). Root tissue extract (0.1 g) in 10 mL of 50 mmol/L potassium phosphate buffer was mixed with 3 mL of reaction mixture II (50 mmol/L potassium phosphate buffer (pH 7.0), 0.5 mmol/L AA, 0.1 mmol/L EDTA, 1.5 mmol/L H2O2). The reaction was triggered by adding 1.5 mmol/L H2O2 and absorbance was measured at 290 nm.

**Transcriptomic assay**

Genome wide root transcriptomic assay was carried out in response to NaCl stress in two rice varieties FL478 and IR29, which exhibited different tolerance to salinity (Senadheera et al, 2009). Transcriptomic data were reanalyzed to identify the transcripts of the known anti-oxidant enzymes which showed different salt responsive expression in FL478 and IR29 (Mittler et al, 2004). Transcripts with the average of signal ratio values for treated and control transcripts differed more than 1.5-fold were taken as differentially regulated.

**RESULTS**

**Growth parameters and cationic concentrations**

Studies on the growth and physiological parameters of the salt tolerant rice variety FL478 and its salt sensitive counterpart IR29 in response to salt stress of 50 mmol/L NaCl for 12 d showed significant differences. Growth of both FL478 and IR29 was affected by salinity. There was a significant decrease in growth (approximately 40%) in the shoot and root tissues of IR29 grown in 50 mmol/L NaCl compared to FL478, which showed no significant growth reduction (Fig 1-A). Despite the observation that FL478 maintained relatively unaffected growth amidst salt stress, the leaf chlorophyll content was reduced (nearly by 20%) in response to salt stress in the manner similar to IR29 (Fig. 1-B).

On the contrary, two important cations, Na+ and K+, as reflected in the Na/K ion ratio, showed different accumulation in the roots of FL478 and IR29 after grown in 50 mmol/L NaCl for 12 d. FL478 was able to maintain significantly lower Na/K ion ratio in comparison to IR29, which had approximately 1.5-fold higher Na/K ion ratio in comparison to IR29, which had approximately 1.5-fold higher Na/K ion ratio in roots (Fig. 1-C). Root MSI of the salt tolerant variety FL478 remained unchanged in the 50 mmol/L NaCl treatment compared to the control condition (Fig. 1-D). However, MSI of IR29 roots showed significant reduction around 25% compared to FL478 in response to salt stress.

**Oxidative stress responses**

In this study, oxidative stress responses of FL478 and IR29 under salt stress were compared by the evaluation of activities/contents of TBARS, H2O2, SOD, APOX and AA in the root tissues. Salt sensitive IR29 showed about 20%−25% higher accumulation of TBARS in roots in comparison to the tolerant FL478 under salt stress (Fig. 2-A). There was no significant increase in TBARS content between the control and treated FL478 plants. Comparison of root H2O2 content revealed that salt stress induced H2O2 accumulation in the roots of both FL478 and IR29 (Fig. 2-B). However, there was a significantly higher accumulation of H2O2 in the FL478 roots compared to IR29 in response to moderate salt stress for 12 d. The degree of H2O2...
accumulation in salt-grown FL478 was as twice as that of the control, and nearly 1.3-fold of the IR29 plants grown under salt stress condition.

The activities of SOD decreased in both varieties in response to salt stress at significant proportions (Fig. 2-C). However, salt sensitive variety IR29 showed greater reduction in comparison to FL478 under salt stress. Similar trend was observed in case of APOX activity and AA content in FL478 and IR29 in response to salt stress (Fig. 2-D and 2-E).

Transcriptional responses of antioxidant enzymes

The supplementary Table 1 (see at http://www.sciencedirect.com/science/journal/16726308) lists around 120 rice genes that are involved in antioxidant responses. Out of these, very few showed significant regulation compared to the control condition (Table 1). Differentially regulated transcripts belong to the gene
families of superoxide dismutase, peroxidase and catalase.

**DISCUSSION**

**Growth and ionic parameters**

Comparative studies with the salinity tolerant FL478 and its sensitive counterpart IR29 revealed differences in mechanisms of salt tolerance between these varieties at the growth, physiological and transcript levels (Walia et al, 2005; Senadheera et al, 2009). Relatively higher growth rate in FL478 (Fig. 1-A), reflects its superior tolerance to salinity. The significant and comparable decrease in chlorophyll content in both FL478 and IR29 after the treatment with 50 mmol/L NaCl (Fig. 1-B), suggests that the difference in growth performance is not due to cellular levels of chlorophyll and hence photosynthetic capacity. In contrast, part of the growth advantage of FL478 is likely to be due to altered cation homeostasis which includes a lower rate of net uptake of Na⁺ (Senadheera et al, 2009) and a lower tissue Na/K ion ratio in this variety (Fig. 1-C). The approximately 1.5-fold higher Na/K ion ratio in IR29 observed in roots may also contribute to increased translocation of Na⁺ to shoot tissue in this variety (Senadheera et al, 2009). Altered cation homeostasis can occur due to many reasons including the activity of membrane transporters.

In addition, the overall integrity of the membrane affects the movement and compartmentation of ions. A stable non-permeable membrane is one of the major factors that curtail the intrusion of Na⁺ into the roots (Nedjimia and Daoud, 2009). Membrane stability index (MSI) points to the functional and structural integrity of the membranes that govern the intracellular homeostasis, which showed a considerable difference between varieties (Fig. 1-D).

<table>
<thead>
<tr>
<th>Locus</th>
<th>Annotation</th>
<th>Expression (fold)</th>
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<tbody>
<tr>
<td>Os07g46990</td>
<td>Superoxide dismutase 2</td>
<td>1.95 1.13</td>
</tr>
<tr>
<td>Os05g06970</td>
<td>Peroxidase family protein</td>
<td>3.06 1.31</td>
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<tr>
<td>Os03g55410</td>
<td>Peroxidase 51 precursor</td>
<td>2.90 1.28</td>
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<tr>
<td>Os07g01410</td>
<td>Peroxidase family protein</td>
<td>2.96 1.37</td>
</tr>
<tr>
<td>Os03g25300</td>
<td>Peroxidase family protein</td>
<td>1.53 0.73</td>
</tr>
<tr>
<td>Os05g04490</td>
<td>Peroxidase family protein</td>
<td>2.19 1.57</td>
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<td>Os07g48020</td>
<td>Peroxidase 2 precursor</td>
<td>2.15 1.84</td>
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<td>0.72 1.55</td>
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<td>0.93 2.05</td>
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<tr>
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<td>Catalase isozyme B</td>
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**Oxidative stress responses**

A low MSI is indicative of damage to the membrane due to lipid peroxidation, a phenomenon that occurs during oxidative stress (Borsani et al, 2001; Bor et al, 2003). The extent of the damage to membrane lipids is monitored by TBARS that are produced during the peroxidation of polyunsaturated fatty acids in the membrane. Increased TBARS content in IR29 after grown in 50 mmol/L salt but not in FL478 (Fig. 2-A) shows that FL478 is more capable withstanding the deleterious effects of oxidative stress. Vaidyanathan et al (2003) suggested that the relatively salt tolerant rice variety Pokkali also had lower levels of lipid peroxidation during salinity stress.

Interestingly, although FL478 displayed a higher MSI and lower levels of lipid peroxidation, it showed a larger accumulation of hydrogen peroxide in its roots compared to IR29 (Fig. 2-B). This is contrary to what was previously reported for the peroxide content in shoots of salt tolerant varieties under salt stress (Mittal and Dubey, 1991; Dionisio-Sese and Tobita, 1998; Sairam and Srivastava, 2002). Similarly, the accumulation patterns of peroxides in rice roots in response to short term salt stress showed that tolerant varieties accumulate relatively less H₂O₂ than their sensitive counterparts (Khan and Panda, 2008). Thus, our data suggest that long term salinity stress brings about a change in these patterns and leads to higher H₂O₂ levels in the roots of tolerant varieties whilst maintaining a better overall growth performance. There may be several reasons why this seemingly counterintuitive phenomenon occurs: High growth rates, particularly during stressful conditions, often accompany high metabolic rates which lead to the generation of ROS (Asada, 1999; Karpinski et al, 1999). Such elevated levels of H₂O₂ may facilitate effective ROS dependent signalling necessary to withstand prolonged exposure to salt stress (Breusegem et al, 2001; DeYulia et al, 2005). Transient increase of H₂O₂ was observed in response to thermotolerance in mustard seedlings whereas treatment with H₂O₂ induced the thermotolarent proteins in wheat (Matsuda et al, 1994; Dat et al, 2000). Moreover, the relative reduction of H₂O₂ content in IR29 compared to FL478 can partly be attributed to diversion of H₂O₂ to generate highly toxic hydroxyl radicals through the Haber-Weiss reaction which is apparently being suppressed in FL478 (Haber and Weiss, 1934; Sairam and Tyagi, 2004). Higher TBARS content and lower MSI in IR29 in comparison to FL478 further indicates the effect of hydroxyl radicals which is more deleterious to the lipid membrane than other ROS.
and dismutates them to H$_2$O$_2$. Peroxide is subsequently broken down to water and oxygen by peroxidases such as APOX and CAT (Fridovich, 1986; Sudha and Ravishankar, 2002; Tsukamoto et al, 2005), although the latter is predominantly localized in leaf peroxisomes and therefore of limited efficacy in roots (Corpas et al, 1993). Total SOD and APOX activities were studied in the roots of FL478 and IR29. The SOD activity decreased in both FL478 and IR29 roots in long term saline conditions (Fig. 2-C) as was previously observed in short term exposure to salinity (Khan and Panda, 2008). However, the reduction in SOD activity was significantly greater in the salt sensitive IR29. The relatively higher SOD activity in FL478 may therefore lead to more effective detoxification of superoxide and a concomitant higher peroxide production. A relatively high SOD activity was also detected in the shoots of tolerant genotypes of both rice and wheat in response to salinity (Sairam and Srivastava, 2002; Singh et al, 2007), and a comparative proteomic study revealed that there was nearly 3-fold up-regulation of SOD protein in roots after 24 h exposure to salt compared to leaf sheath tissue (Abbasi and Komatsu, 2004).

The activity of APOX in FL478 and IR29 in response to long term salt stress (Fig. 2-D) was in contrast to short term salinity treatments after which increased APOX activity was recorded (Singh et al, 2007; Khan and Panda, 2008). However, there is a significantly greater decrease in the APOX activity in IR29 compared to FL478, and in the AA content in response to salt stress. Partly, this reduction of APOX activity may have contributed for the accumulation of H$_2$O$_2$ in FL478 under the long term salt stress condition. AA has a dual role in detoxification of H$_2$O$_2$, non-enzymatically and enzymatically with APOX. Although Khan and Panda (2008) recorded the decrease in AA content in the roots of both salt tolerant and susceptible varieties in response to short term salt stress, FL478 showed no significant reduction in AA content whereas IR29 showed a significant reduction in long term exposure to salt stress.

**Transcriptional responses of antioxidant enzymes**

Many studies have revealed that the oxidative stress that accompanies salinity stress has adverse effects and therefore plants with adequate responses to deal with harmful ROS are more likely to withstand salt stress (Dionisio-Sese and Tobita, 1998; Singh et al, 2007). For the purpose of developing more tolerant crops, it is therefore imperative to identify the relevant genes and proteins that contribute to oxidative stress responses. The supplementary Table 1 lists around 120 rice genes that are involved in antioxidant responses containing superoxide dismutases, various types of peroxidases, peroxiredoxins, catalases and glutathion reductases. Out of these, very few showed significant regulation compared to the control condition (Table 1). Only one (Os07g46990) out of seven SOD isoforms showed up-regulation in FL478. It is therefore tempting to associate this gene with the higher SOD activity in FL478 roots. Overexpression of SOD isoforms in transgenic plants showed enhanced tolerance to abiotic stress. Transgenic rice over-expressing yeast Mn-SOD showed enhanced tolerance to salinity whereas *Medicago sativa* transformed with Mn-SOD cDNA showed 100% winter survival and herbage yield (Mckersie, 1999; Tanaka et al, 1999). Peroxidase transcript levels were increased in both varieties. In FL478, Os03g25300, Os03g55410, Os04g55740, Os05g04490, Os05g06970, Os07g01410 and Os07g48020 were significantly induced. Three of these peroxidases (Os04g55740, Os05g04490 and Os07g48020) were also up-regulated in IR29, which may be important in the rice antioxidant response.

Despite the fact that the experiment by Senadheera et al (2009) was carried out under different conditions, Cotsaftisa et al (2011) recorded the same trend of up-regulation of peroxidise genes (Os.32471.1.S1_at) in both salt sensitive and tolerant varieties. Some of these transcripts have shown to be differentially regulated under abiotic stress. Os07g01410 was down-regulated in shoots of both FL478 and IR29 whereas Os07g48020 was up-regulated in IR64 in response to salt stress (Walia et al, 2005). Similarly, cold and drought stress induced Os07g01410 and Os07g48020, respectively. Drought and cold stress suppressed the expression of transcripts Os04g55740 and Os03g55410 whereas cold stress induced Os05g06970 (Tyagi et al, 2007). Moreover, the transcripts of Os03g25300, Os07g48020, Os04g55740 and Os05g06970 were shown to be responsible to anoxia (Kudahettige et al, 2007).

The observation that several peroxidases are up-regulated in FL478 but not in IR29 may underlie the lower levels of TBARS and increased membrane integrity in FL478 (Figs. 2-A and 1-D). High activity levels of peroxidases have previously shown to be correlated with growth reduction due to the involvement of these enzymes in cell wall thickening (Zheng and van Huystee, 1992; Lee and Lin, 1995). However, our data do not support this notion: both lipid peroxidation...
levels (Fig. 2-A) and transcriptional regulation (Table 1) implied higher peroxidase activity in FL478 in spite of a larger relative growth rate (Fig. 1-A). One catalase isoform (isozyme B) was up-regulated in FL478 but none was up-regulated in IR29. The observation that the FL478 roots contained higher H₂O₂ content (Fig. 2-B) suggests that the up-regulation of catalase isozone B is not of great relevance in lowering tissue H₂O₂ content. None of the APX genes was up-regulated in FL478 or IR29, showing that their expression was insensitive to both the APX genes was up-regulated in FL478 or IR29, indicating two APX genes, OsAPX7 and OsAPX8, in rice roots whereas ABA only induced OsAPX8 (Teixeira et al., 2006; Hong et al., 2007).

This study on the long term effects of salinity on the oxidative stress tolerance in rice revealed that the superior of tolerant varieties may lie in the maintenance of membrane integrity by avoiding the effects of damaging ROS. The tolerant rice variety FL478, in this way maintained a lower root Na/K ion ratio than the sensitive variety IR29. At the same time, FL478 could endure a relatively high H₂O₂ content in its roots without compromising growth or membrane integrity. The activities of the antioxidant enzymes SOD and APOX, though reduced relative to the control condition, was maintained at relatively higher levels in FL478 than in IR29. This factor is also likely to contribute to greater salt tolerance. Concomitant observation showed that the specific up-regulation of transcripts encoding antioxidant enzymes such as SOD and peroxidises and as such identify prospective gene candidates to improve salt tolerance in rice.

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