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Differential Response in Scavenging of Reactive Oxygen Species in Rice var. IR 20 Exposed to Sublethal and Lethal Level of NaCl Stress

M. Djanaguiraman, J.A. Sheeba, D. DurgaDevi and U. Bangarusamy

Department of Crop Physiology
Tamil Nadu Agricultural University
Coimbatore, India

ABSTRACT. *The effects of NaCl on plant growth, oxidants production, antioxidant activity and ion accumulation were analysed in both pretreated [plants treated with sublethal (50mM) dose of NaCl before subjecting to lethal dose (100mM) NaCl]and non-pretreated [plants directly subjected to lethal dose (100mM) NaCl] rice seedlings. A significant reduction in shoot length, root length, leaf area and total dry matter production was observed in non-pretreated plants than pretreated plants. The pretreated plants had reduced contents of superoxide radical ($O_2^{\cdot}$), hydrogen peroxide (H_2O_2), malondialdehyde (TBARS contents) coupled with lipoxygenase (LOX) activity over non-pretreated plants. The antioxidant enzymes viz., superoxide dismutase (SOD), catalase (CAT), peroxidase (POX) and ascorbate peroxidase (APOX) had shown a significantly higher activity in pretreated plants over non-pretreated plants. The pretreated plants showed improved ionic regulation than non-pretreated plants as evidenced from lower Na^+, Cl^- and higher K^+ contents. The roots of both pretreated and non-pretreated plants had lower Na^+ and Cl^- contents than leaves. Our results suggest that pretreatment of rice seedlings with sublethal dose of NaCl enabled the plants to acclimatize to lethal NaCl stress by enhanced antioxidant enzyme activity and reduced oxidants production, membrane deterioration, sodium and chloride contents.*

INTRODUCTION

Rice is a main staple crop around the world, feeding and providing the necessary daily calories for millions of people. Major environmental limitations on rice production are salinity and drought. Soil salinity is a complex and harmful threat faced by plants, due to disruption of ionic, osmotic and cell-water homeostasis. Further consequences are disturbance of membrane integrity, nutrient imbalance, altered levels of growth regulators, and disturbances on general metabolic activities.

Aerobic oxidative metabolism *i.e.*, the use of molecular oxygen as the final electron acceptor posed a risk of oxidative damage due to production of partially reduced intermediates, known as reactive oxygen species (ROS). ROS have the potential to interact with many cellular components, triggering peroxidative reactions and causing significant damage to proteins, lipids and nucleic acids. Therefore, their levels must be carefully monitored and controlled in cells. To cope with, plants have evolved both enzymatic and non-enzymatic mechanisms for ROS scavenging (Asada, 1994). For instance, they use a

diverse array of enzymes like superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) to scavenge different types of ROS.

Researchers have made numerous attempts to clarify plant response to salt stress. However, most of these studies have been based on the assumption that salt tolerance is due only to the expression of 'preexisting genetic information' that counteracts the effect of the stress. It is a general understanding that response of plants to stress agents is particularly of adaptive nature when the stress is sublethal. On the other hand, response shown may be biased towards senescence or cell death if the stress is lethal. Application of sublethal stress regimes in experimentation has thus emerged as an effective approach for unveiling the fundamentals of stress responses (Grover *et al.*, 2001). Strognov (1964) conducted a pioneering experiment in which he showed that salt tolerance of plants increased by presowing salt treatment. Amzallag *et al.* (1993) also showed that sorghum treated with 150 mM NaCl at the seedling stage for over 20 days could survive and produce seeds even in the presence of 300 mM NaCl a concentration that was lethal for normal plants. Same type of result was also observed by Umezawa *et al.* (2000) in soybean. Analysis of the physiological changes associated with such acclimation may be useful for advancing the study of plant tolerance to environmental stresses, in that it may suggest the strategies by which plants acquire stress tolerance.

Djanaguiraman *et al.* (2003), earlier showed that rice plants could also acclimate to salt stress by pretreatment with sublethal dose of NaCl which recorded an increased yield (18%) over non-pretreated plants. Therefore, better understanding of biochemical mechanisms involved in adaptation will help in development of transgenics or better breeding program. In rice, salt tolerance is correlated with antioxidant enzymes activity (Davenport *et al.*, 2003). Modulation of the activities of antioxidant enzymes might be important in the resistance of a plant to salt stress. However, it remains to be clarified, whether pretreated rice acclimates to salt stress by enhanced antioxidant enzyme activity. Our objective was to determine, whether salt stress acclimation during exposure to sublethal NaCl concentration in rice is by altered oxidants production, antioxidant enzymes activity and membrane damage.

MATERIALS AND METHODS

Plant material

Rice (*Oryza sativa* L.) cv. IR 20 seeds were obtained from the Department of Rice, Tamil Nadu Agricultural University, Coimbatore, India.

Growth conditions and salinity treatment

Rice seeds were sterilized with 70% of ethanol for 5 min and with 10% H_2O_2 for 10 min. They were then washed thoroughly, soaked in distilled water for 48 h and then incubated for 24 h at 30° C under moist, dark conditions. The germinated seeds were grown hydrophonically; four plants per container of 8 L aerated half strength Hoagland nutrient solution containing (mM): 2.4 $Ca(NO_3)_2$, 1.0 KH_2PO_4, 3.0 KNO_3, 1.0 $MgSO_4$ and (µM) 23.1 H_3BO_3, 4.6 $MnCl_2$, 0.38 $ZnSO_4$, 0.16 $CuSO_4$, 0.052 H_2MoO_4 and 44.8 $FeSO_4$ (as ferric sodium ethylene diamine tetra acetate (EDTA) complex) in three sets

in completely randomized block design up to 40 days. In each set, a minimum of 20 containers were maintained.

One set served as control. In the second set, salinity treatment was started on 22nd day after sowing (DAS). Uniform seedlings that had complete third leaf and fourth leaf of 1-2 cm length were used. Plants were exposed to sublethal dose of NaCl (50 mM) added to the half strength Hoagland solution for one week. This constitutes pretreatment (Djanaguiraman *et al.*, 2003). After one week of treatment, the plants were transferred to half strength Hoagland solution containing 100 mM NaCl and maintained for one week. In the third set, plants of 29 days old were directly transferred to half strength Hoagland solution containing 100 mM NaCl, and maintained for one week. The nutrient solution was renewed every two days. The photoperiod was 14 h and the day/night temperatures were 28/22°C. The light source was fluorescent incandescent lamps with a PAR of 450 $\mu M\ m^{-2}\ s^{-1}$ and the relative humidity was 70%. The plants of all three sets were analysed for growth, lipid peroxidation, ROS assay, lipoxygenase and antioxidant enzymes activity at 29th DAS and 36th DAS in 2nd and 3rd leaves. A minimum of ten plants per treatment were analysed.

Growth parameters

Shoot length (cm), root length (cm) and leaf area ($cm^2\ plant^{-1}$) was measured and then samples were oven dried at 80°C for 24 h and weighted for determining total dry matter production ($g\ plant^{-1}$) from all three sets.

Lipid peroxidation and ROS assay

The lipid peroxidation was determined by malondialdehyde (MDA) content produced by thiobarbituric acid (TBA) reaction at low pH as described by Behra *et al.* (1999). Lipid peroxidation was expressed as malondialdehyde content in $\mu M\ g^{-1}$ dry weight (DW). The H_2O_2 level was colorimetrically measured as described by Okuda *et al.* (1991) and expressed in $nM\ g^{-1}$ DW. Superoxide anion was estimated according to Chaitanya and Naithani (1994) and expressed as $\Delta OD\ 540\ nm\ min^{-1}\ g^{-1}$ DW.

Lipoxygenase (LOX) (EC 1.13.11.12) and membrane stability index (MSI)

LOX (1.13.11.12) was measured spectrophotometrically at 234 nm (Gallego *et al.*, 1996) and expressed as the absorbance increase per mg of protein per minute. Membrane stability index (MSI) was determined by recording the electrical conductivity of leaf leachates in double distilled water at 40 and 100°C (Chaudhuri and Choudhuri, 1992) and expressed as percentage.

Antioxidant enzymes activity

Extraction and assay

For superoxide dismutase, catalase and peroxidase enzymes activity estimations, frozen tissue was homogenized in ice-cold 0.1M Tris–HCl buffer at pH 7.8 containing 1mM EDTA, 1mM dithiotreitol and 5ml of 4% polyvinyl pyrrolidone per gram fresh weight. For the measurement of ascorbate peroxidase (APOX) activity, 2 mM ascorbate was added

(Vaidyanathan *et al.*, 2003). The homogenate was filtered through a nylon mesh and centrifuged at 20,000x*g* at 4°C. The supernatant was used for measuring enzyme activity.

SOD (EC 1.15.1.1) was determined by nitroblue tetrazolium (NBT) method of Beyer and Fridovich, (1987) by measuring the photoreduction of NBT at 560 nm. One unit of SOD activity equaled to the amount required to inhibit photoreduction of NBT by 50%. CAT (EC 1.11.3.6) was estimated according to Teranishi *et al.* (1974) and the enzyme activity was expressed as μM H_2O_2 reduced min^{-1} mg^{-1} protein. POX (1.11.1.7) activity was determined in the homogenate by measuring the increase in absorption at 470 nm due to formation of tetraguaiacol (Racusen and Foote, 1965). APOX (EC 1.11.1.11) enzyme activity was measured according to Gerbling *et al.* (1984), following the oxidation of ascorbate to dehydroascorbate spectrophotometrically. Activity was expressed in μM ascorbate oxidised min^{-1} mg^{-1} protein.

Ion analysis

After collecting the plant samples, they were dissected into roots, and leaves for ion analysis. Na^+ and K^+ were quantified by atomic absorption spectrometry (Perkin-Elmer, 5500), and Cl^- was quantified by titration method, according to Ross, (1984), and expressed as mmole g^{-1} DW. Leaf nitrogen content was determined by microkjeldhal method according to Humphries (1956) and expressed in percentage on dry weight basis.

Statistical analysis

Duplicate sample from at least six replications were taken for all the enzyme assays and non-enzymatic analysis (n=12). The mean values ±S.E. are given in all tables. The data were analysed statistically using a general linear model for analysis of variance (repeated measures Anova). Significance between control and treatment was compared at 0.05 probability level by Duncan's Multiple range Test.

RESULTS

The effect of NaCl pretreatment on growth, oxidants production, lipoxygenase enzyme activity, membrane stability, antioxidant enzymes activity, ion analysis results are presented hereunder.

Growth

The effect of NaCl pretreatment on shoot and root length were found to be non-significant at 29th DAS over control, whereas leaf area and total dry matter production were significantly decreased in pretreatment, by 16 and 13.4% respectively, over control. However, at 36th DAS both pretreated and non pretreated plants showed a significant reduction in shoot length, leaf area and total dry matter production over control (Table 1).

Table 1. Effect of NaCl pretreatment on shoot length, root length, leaf area and total dry matter production in rice variety IR 20

Parameters	29th DAS		36th DAS		
	0 mM	50 mM	0 mM	50/100 mM	100 mM
Shoot length (cm)	14.9[a] (±0.80)	14.4[a] (±0.98)	29.0[a] (±0.86)	19.6[b] (±0.98)	15.5[c] (±1.02)
Root length (cm)	4.53[a] (±0.18)	4.18[a] (±0.19)	4.65[a] (±0.14)	5.17[b] (±0.23)	4.9[c] (±0.13)
Leaf area (cm^2 $plant^{-1}$)	14.3[a] (±1.0)	12.0[b] (±0.95)	30.2[a] (±1.6)	24.1[b] (±1.8)	16.7[c] (±1.5)
Total dry matter production (g $plant^{-1}$)	3.63[a] (±.12)	3.14[b] (±0.19)	5.45[a] (±0.14)	4.05[b] (±0.18)	3.8[c] (±0.10)

Within a row, means followed by a same letter are not significantly different by the Duncan's Multiple range Test at p=0.05. Values in parenthesis indicate S.E.M

Oxidant production, LOX activity and membrane stability

NaCl pretreatment at 29th DAS significantly increased oxidants namely superoxide radical and hydrogen peroxide production (Table 2). Increased $O_2^{\cdot}$ and H_2O_2 contents resulted in momentous increase of lipoxygenase activity and TBARS content (Table 2). NaCl pretreated and nonpretreated plants at 36th DAS showed a significant increase in LOX activity and TBARS content over control. However, the pretreatment reduced the $O^{\cdot}_2$ and H_2O_2 production by 18 and 20% respectively, over non-pretreatment. Higher lipoxygenase enzyme activity and malondialdehyde formation (TBARS content) was observed in nonpretreated plants than pretreated plants. Under non-pretreatment, lipoxygenase and malondialdehyde recorded significant increase of 1.9 and 2.5 change in OD min^{-1} mg^{-1} protein and 7.8 and 9.1 nM g^{-1} DW over pretreatment and control, respectively. The membrane stability analysis indicated that at both 29th and 36th DAS the pretreated and non-pretreated plants had a lower stability than control plants (Table 2). Maximum reduction in MSI due to non-pretreatment was observed at 36th DAS.

Antioxidant enzymes

NaCl pretreatment at 29th DAS and 36th DAS significantly increased the antioxidant enzyme activity *viz.*, superoxide dismutase, catalase, peroxidase and ascorbate peroxidase activity (Table 3). Pretreatment at 29th DAS, recorded a maximum increase in peroxidase activity (80.6%) followed by catalase (65.5%), ascorbate peroxidase (61.1%) and superoxide dismutase (50%) over control, respectively. Though an increase in antioxidant enzymes activity over control was observed in nonpretreated plants at 36th DAS, maximum increase was observed in pretreated plants. The pretreatment at 36th DAS increased the activity of peroxidase, catalase, superoxide dismutase and ascorbate peroxidase by 73.1, 70.3, 69.1, and 61.1% over non-pretreatment, respectively.

Table 2. Effect of NaCl pretreatment on superoxide radical, hydrogen peroxide contents, LOX activity, TBARS content and membrane stability index in rice variety IR 20

Parameters	29th DAS		36th DAS		
	0 mM	50 mM	0 mM	50/100 mM	100 mM
Superoxide radical (ΔOD 540 nm min^{-1} g^{-1} DW)	0.74^a (±0.023)	1.02^b (±0.029)	0.98^a (±0.024)	1.24^b (±0.035)	1.52^c (±0.029)
Hydrogen peroxide (μM g^{-1} DW)	0.84^a (±0.042)	1.24^b (±0.039)	0.93^a (±0.052)	1.34^b (±0.062)	1.68^c (±0.035)
Lipoxygenase activity (ΔOD 234 min^{-1} mg^{-1} protein)	3.7^a (±0.12)	4.1^b (±0.15)	4.2^a (±.0.18)	4.8^b (±0.16)	6.7^c (±0.15)
TBARS content (nM g^{-1}DW)	18.5^a (±1.2)	21.8^b (±1.4)	24.9^a (±1.0)	29.0^b (±1.1)	34.5^c (±1.9)
Membrane stability index (%)	93.0^a (±2.1)	81.0^b (±1.6)	92.0^a (±2.6)	84.0^b (±2.1)	70.0^c (±1.8)

Within a row, means followed by a same letter are not significantly different by the Duncan's Multiple range Test at p=0.05. Values in parenthesis indicate S.E.M

Ion analysis

Pretreatment with NaCl at 29th DAS, the leaf and root Na^+ contents increased significantly (Table 4), when compared to root, leaf accumulated more of Na^+. In leaf, Na^+ accounted for a raise of 85% to that of control, while root showed a boost of 27.7% only. At 36th DAS, the pretreatment had a lower percent increase (85%) than non-pretreatment (141%) in leaf Na^+ content, over control. The reverse trend was followed in potassium *i.e.*, nonpretreated recorded a lower K^+ content than pretreated and control plants.

Table 3. Effect of NaCl pretreatment on SOD, CAT, POX and APOX activity in rice variety IR 20.

Parameters	29th DAS		36th DAS		
	0 mM	50 mM	0 mM	50/100 mM	100 mM
SOD (Enzyme units min^{-1} mg^{-1} protein),	3.2^a (±0.21)	4.8^b (±0.31)	3.5^a (±0.41)	6.8^b (±0.35)	4.7^c (±0.29)
CAT (μM H_2O_2 reduced min^{-1} mg^{-1} protein),	2.9^a (±0.10)	4.8^b (±0.15)	3.2^a (±0.18)	5.8^b (±0.19)	4.1^c (±0.14)
POX (nM tetraguaiacol formed min^{-1} mg^{-1} protein)	0.93^a (±0.04)	1.68^b (±0.02)	1.20^a (±.0.03)	1.88^b (±0.04)	1.38^c (±0.04)
APOX (μM ascorbate oxidised min^{-1} mg^{-1}.protein)	0.18^a (±0.12)	0.29^b (±0.016)	0.21^a (±0.018)	0.38^b (±0.15)	0.27^c (±0.18)

Within a row, means followed by a same letter are not significantly different by the Duncan's Multiple range Test at p=0.05. Values in parenthesis indicate S.E.M

Table 4. Effect of NaCl pretreatment on leaf sodium, root sodium, leaf potassium, root potassium contents, leaf chloride, root chloride, leaf nitrogen and root nitrogen contents in rice variety IR 20.

Parameters	29th DAS		36th DAS		
	0 mM	50 mM	0 mM	50/100 mM	100 mM
Leaf sodium (mmole g^{-1} DW)	0.21^a (±0.02)	0.39^b (±0.03)	0.28^a (±0.02)	0.52^b (±0.03)	0.68^c (±0.03)
Root sodium (mmole g^{-1} DW)	0.18^a (±0.012)	0.23^b (±0.016)	0.21^a (±0.015)	0.38^b (±0.016)	0.57^c (±0.013)
Leaf potassium (mmole g^{-1} DW)	5.2^a (±0.12)	3.6^b (±0.13)	5.4^a (±.0.18)	3.8^b (±0.16)	3.1^c (±0.15)
Root potassium (mmole g^{-1} DW)	3.6^a (±0.12)	2.3^b (±0.20)	3.3^a (±0.18)	2.8^b (±0.11)	2.0^c (±0.14)
Leaf chloride (mmole g^{-1} DW)	0.98^a (±0.15)	1.42^b (±0.19)	1.2^a (±0.20)	2.1^b (±0.17)	3.8^c (±0.13)
Root chloride (mmole g^{-1} DW)	0.71^a (±0.10)	1.03^b (±0.15)	0.80^a (±0.20)	1.30^b (±0.16)	2.90^c (±0.15)
Leaf nitrogen (mmole g^{-1} DW)	1.13^a (±0.01)	1.02^b (±0.02)	1.24^a (±.0.02)	1.16^b (±0.02)	1.04^c (±0.01)
Root nitrogen (mmole g^{-1} DW)	1.04^a (±0.01)	0.95^b (±0.02)	1.15^a (±0.01)	1.02^b (±0.02)	0.93^c (±0.02)

Within a row, means followed by a same letter are not significantly different by the Duncan's Multiple range Test at p=0.05. Values in parenthesis indicate S.E.M

The leaf and root chloride content revealed that both pretreated and no-pretreated plants had a higher content at 29th and 36th DAS over control (Table 4). However, pretreated plants had lower chloride content than non-pretreated plants in both leaf and root. Leaves had more chloride content than roots. The leaf nitrogen content at 36th DAS recorded a decrease of 6.5 and 16.1% over control by pretreatment and non-pretreatment, respectively. The similar trend was also observed in root nitrogen content. Ion analysis revealed a significant difference in Na^+, K^+, Cl^- and nitrogen content in both leaf and root by NaCl treatment.

DISCUSSION

Growth

Salt stress has been generally considered to be made up of both osmotic and ionic effects. The accumulation of toxic ions, Na^+ and Cl^- in plants is often claimed to be toxic and the main cause of growth inhibition induced by salinity (Muscolo *et al.*, 2003). Improved growth rate under salinity is essential for salt acclimation (Amzallag *et al.*, 1993). This might be accomplished by maintenance of optimum level of Na^+ and K^+ concentrations under salinity (Boursier and Lauchli, 1990). Plants pretreated with sublethal level of NaCl showed a good degree of accumulation of K^+ in roots and shoots. Hence, the stimulation of growth under pretreatment than nonpretreated plants could have been due to enhanced K^+ accumulation. In addition, the decreased contents of Na^+ and Cl^- ions in pretreatment would definitely lessen the cellular toxicity, which further augments the growth under pretreatment condition. Increased concentration of Na^+ in the leaves of non-pretreated plants may be due to increased transport of this ion from root to shoot and/or high influx. The growth reduction

in non-pretreatment might be due to the toxic effect created by the presence of excess amount of salt coupled with low K^+ concentration.

Both pretreated and non-pretreated plants showed decreased shoot length, leaf area and total dry matter production over control at 36th DAS. However, enhanced growth was observed in NaCl pretreated plants over non-pretreated plants. This indicated that the low concentration of NaCl pretreatment had a stimulative effect in acclimation process as reported by Umezawa *et al.* (2000) in soybean. Salinity causes increase in root length of pretreated plants at 36th DAS, whereas, at 29th DAS the pretreated plants did not showed any variation over control, indicating that roots were more sensitive than the shoots to lethal dose of salinity as reported by Sharma (1996).

Active oxygen species, membrane stability and antioxidant enzymes activity

Salt stress could increase the oxygen-induced damage to cells by increased generation of reactive oxygen species. Reactive oxygen species (ROS) bring about peroxidation of membrane lipids, which lead to membrane damage. The resistance to environmental stress may therefore depend, atleast partially on the inhibition of ROS production or the enhancement of antioxidant enzymes activity. Salt tolerance has been correlated with an increased SOD, CAT, POX and APOX enzymes activity (Davenport *et al.*, 2003).

Salinity treatments caused significant increase in H_2O_2 and superoxide radical content, which were higher in non- pretreated plants than pretreated and control plants. Increased H_2O_2 accumulation and lipid peroxidation due to salt stress resulted in significant decrease in membrane stability index (MSI). MSI was higher in pretreated plants than non-pretreated plants. Lower H_2O_2 accumulation and lipid peroxidation and higher membrane stability have been reported in salt tolerant genotypes of wheat (Sairam *et al.*, 2002). Since, the pretreated plants had lower H_2O_2, O_2^-, MDA content, lipoxygenase activity and higher MSI indicates that, it has adapted well to higher concentration of NaCl than non-pretreated plants.

Sairam and Srivastava (2002) reported the enhancement of lipid peroxidation and membrane damage in the leaves subjected to higher degree of salt stress than mild stress. Parallel to these observations, we also noticed an increase in lipid peroxidation in non-pretreated rice leaves. On the other hand, pretreated rice leaves exhibited a decrease in lipid peroxidation. The occurrence of lipid peroxidation is an indication of the prevalence of free radical reactions and a change in the balance between O_2^-/H_2O_2 in leaves (Hsu and Kao, 2003). Increased SOD activity was correlated with increased protection from damage associated with oxidative stress (Asada, 1994). It is generally expected that the SOD activity of salt-tolerant cultivars would be substantially higher as compared to the sensitive ones. It has been demonstrated that salt tolerant rice (Djanaguiraman *et al.*, 2003), wheat (Sairam and Srivastava, 2002) exhibited higher levels of SOD activity as compared to their salt sensitive counter parts.

A higher activity of SOD in NaCl pretreated plants as compared with non-pretreated plants was observed in the present study. The better protection in NaCl pretreated plants seems to depend on SOD activity for detoxification of O_2^- to H_2O_2. Apart from having higher SOD activity, pretreated plants also exhibited nearly steady state level of H_2O_2 under lethal dose. This does not mean that pretreated plant is experiencing little or no oxidative

stress, since it showed elevated activities of antioxidant enzymes activity like CAT, POX and APOX upon exposure to lethal stress. The enhanced activities of these antioxidant enzymes in pretreatment indicates *de novo* synthesis of SOD, POX, CAT and APOX proteins probably as an adaptive mechanism to protect the leaves against oxygen radical damage (Grover *et al.*, 2001). Hsu and Kao (2003) also observed the same phenomenon in rice under sorbitol induced antioxidant enzyme activity.

The enhanced activities of CAT and POX in pretreated plants helped in the rapid detoxification of H_2O_2 thus produced, so that a steady state level of this ROS is maintained even under high NaCl stress. High levels of H_2O_2 in non-pretreated plants could also accelerate processes like Haber-Weiss reaction, resulting in the formation of hydroxyl radicals that can cause lipid peroxidation. This is reflected in the greater extent of lipid peroxidation in non-pretreated plants upon exposure to lethal dose of NaCl.

Peroxidase decomposes H_2O_2 by oxidation of co-substrates such as phenolic compounds and/or antioxidants. The pretreated plants have higher POX activities than non-pretreated and control plants, showing that POX is involved in reducing the effects of oxidative stress. In plants, an alternative and more effective detoxification mechanism against H_2O_2 also exists through the operation of the ascorbate–glutathione cycle. In plant cells, APOX, which specifically makes use of ascorbate as a physiological reductant is considered a crucise component in the metabolic defense against oxidative stress. It acts through a series of coupled redox reactions, both in photosynthetic (Asada, 1994) and non-photosynthetic tissues (Arrigoni, 1994). In pretreated plants the increase in the activity of APOX over non-pretreated and control plants suggests that this enzyme has a higher ability to eliminate H_2O_2. This is in accordance with the finding of Muscolo *et al.* (2003) in kikuyu grass, that APOX activity is enhanced at 100 mM than 200 mM NaCl concentration.

Apart from causing peroxidation of membrane lipids, high levels of H_2O_2 have been reported to cause a net reduction in photosynthesis in plants by over 50% (Kaiser, 1979). By channeling most of the photoreductants for the detoxification of H_2O_2 produced under stress, CO_2 fixation is compromised (Vaidyanathan *et al.*, 2003) which leads to poor growth of the crop plant. This effect was highly evident in non-pretreated plants than pretreated plants.

CONCLUSIONS

Efficient detoxification of both $O_2^{\cdot}$ and H_2O_2 is required to combat salinity induced oxidative stress in rice. Thus, the adaptation of the plant to lethal dose of NaCl is modified by pretreatment with sublethal dose of NaCl through enhanced antioxidant enzymes activity and ion regulation.

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