*Agric. Chem. Biotechnol.* **48**(1), 1-6 (2005)

**Article**

# Expression of Antioxidant Isoenzyme Genes in Rice under Salt Stress and Effects of Jasmonic Acid and γ-Radiation

**Jin-Hong Kim, Byung Yeoup Chung*, Myung-Hwa Baek, Seung Gon Wi, Dae Hwa Yang, Myung Chul Lee[1] and Jae-Sung Kim**

*Division of Radiation Application Research, Korea Atomic Energy Research Institute (KAERI), Daejeon 305-353, Korea*
*[1]National Institute of Agricultural Biotechnology, R.D.A., Suwon 441-707, Korea*

Received November 18, 2004; Accepted March 4, 2005

**Analysis of chlorophyll (Chl) fluorescence implicated treatment of 40 mM NaCl decreased maximal photochemical efficiency of photosystem II (PSII) (Fv/Fm), actual quantum yield of PSII ($\Phi_{PSII}$), and photochemical quenching (qP) in rice, but increased non-photochemical quenching (NPQ). Decreases in Fv/Fm, $\Phi_{PSII}$, and qP were significantly alleviated by 30 μM jasmonic acid (JA), while NPQ increase was enhanced. Transcription levels of antioxidant isoenzyme genes were differentially modulated by NaCl treatment. Expression of cCuZn-SOD2 gene increased, while those of cAPXb, CATb, and CATc genes decreased. JA prevented salt-induced decrease of pCuZn-SOD gene expression, but caused greater decrease in mRNA levels of cAPXa and Chl_tAPX genes. Investigation of vacuolar $Na^+/H^+$ exchanger (NHX2) and 1-pyrroline-5-carboxylate synthetase (P5CS) gene expressions revealed transcription level of NHX2 gene was increased by JA, regardless of NaCl presence, while that of P5CS gene slightly increased only in co-presence of JA and NaCl. Unlike JA, γ-radiation rarely affected expressions of antioxidant isoenzyme, NHX2, and P5CS genes, except for increase in mRNA level of Chl_tAPX and decrease in that of pCuZn-SOD. These results demonstrate enhanced salt-tolerance in JA-treated rice seedlings may be partly due to high transcription levels of pCuZn-SOD, NHX2, and P5CS genes under salt stress.**

**Key words:** *rice, salt stress, antioxidant enzyme, γ-radiation, jasmonic acid*

The ionic and osmotic stresses imposed by high salinity on plants may create secondary stresses. These secondary or derived stresses include the accumulation of toxic or unwanted compounds, perturbance in the cellular metabolism, and nutritional disorders,[1] among which the oxidative stress is an important constraint for salt-tolerance.

Studies implicate that salt stress could generate reactive oxygen species (ROS) in plants, e.g., hydrogen peroxide ($H_2O_2$), superoxide anion ($\cdot O_2^-$), singlet oxygen ($^1O_2$), and hydroxyl radical ($\cdot OH$),[2-5] thereby causing damages to plants.[1,6] Superoxide dismutase (SOD) converts $\cdot O_2^-$ into $H_2O_2$, while ascorbate peroxidase (APX) and catalase (CAT) catalyze the breakdown of $H_2O_2$. These enzymes, which detoxify plants by scavenging the ROS, were found to be involved in salt-tolerance through studies of transgenic and mutant plants.[1,6,7] Recently, several reports showed that salt stress affects activities of SOD, APX, and CAT differing in the salt-sensitive and salt-tolerant varieties of a plant species.[8,9,10] However, the gene expression of the enzymes at the level of isoenzyme has been rarely investigated in plants under salt stress.

The vacuolar $Na^+/H^+$ exchanger (NHX) helps to maintain high concentration of $K^+$ and low concentration of $Na^+$ in the cytosol by sequestering $Na^+$ in the vacuole. This physiological role of NHX has been reported to contribute to the salt-tolerance in Arabidopsis and rice.[11-14] In contrast, proline and glycyinebetaine increase by salinity,[15] and their accumulation could contribute to the enhanced salt-tolerance by reducing the osmotic potential of the cytosol to facilitate water uptake, thereby protecting proteins from misfolding and alleviating the toxic effects of ROS generated by the salt stress.[5,16-18] In fact, studies showed an increase in the expression of 1-pyrroline-5-carboxylate synthetase (P5CS), which controls the level of proline, resulted in the enhanced salt-tolerance.[5,16]

Jasmonic acid (JA) and methyl jasmonate (JA-Me) have been demonstrated to elevate the salt-tolerance by increasing the proline content and by protecting against reduction in the ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity and the photosynthetic $CO_2$ fixation.[19-21] According to our preliminary data, JA treatment could partly mitigate growth inhibition in rice plants hydroponically cultured under salt stress, thus reducing electrolyte leakage, loss of relative water content, and lipid peroxidation. Similarly, these phenomena could be induced by γ-irradiation.

In the present study, we attempted to reveal a relationship between the expression of antioxidant isoenzyme gene and salt-tolerance. Accordingly, the transcription levels of SOD, APX, and CAT isoenzyme genes were investigated in rice

*Corresponding author
Phone: +82-42-868-8691; Fax: +82-42-868-8061
E-mail: bychung@kaeri.re.kr

seedlings under salt stress in the absence or presence of JA and/or γ-radiation. Moreover, the expressions of NHX2 and P5CS were also investigated.

## Materials and Methods

**Plant materials, γ-irradiation, and treatments of NaCl and JA.** Rice (*Oryza sativa* L. cv. Ilpoombyeo) plants were hydroponically cultivated in one-half-strength Murashige and Skoog (MS) nutrient solution under greenhouse conditions for 2 weeks during the late spring season (May to June). The plants were then irradiated with γ-rays at 4 Gy using a γ-irradiator ($^{60}Co$, ca. 150 TBq of capacity, Atomic Energy of Canada Limited) in Korea Atomic Energy Research Institute (KAERI). The control and irradiated plants were allowed to further grow in an MS-NaCl solution supplemented with 40 mM NaCl. One day later, JA was treated to half of these plants by directly adding to the MS-NaCl solution to a final concentration of 30 μM. All plants were cultivated for 7 days after the treatment of NaCl.

**Chlorophyll fluorescence analysis.** Chlorophyll (Chl) fluorescence was measured using a Chl fluorometer (IMAGING-PAM, Walz, Germany) as described in the operation manual. Leaf samples were excised (0.5 cm in diameter) from the fully expanded leaves detached 7 days after the treatment of NaCl. Readings were taken after the samples were dark-adapted for 15 min at room temperature. The variable fluorescence (Fv) was obtained by subtracting the initial Chl fluorescence (Fo) from the maximum yield of fluorescence (Fm). The ratio of Fv/Fm served as a measure of the maximal photochemical efficiency of photosystem II (PSII).[22]

The parameters for photochemical (qP) and non-photochemical quenching (NPQ) were obtained by analysis of Chl fluorescence quenching using the same fluorometer. The calculations of the qP and NPQ parameters were based on the equations of van Kooten and Snel[23] as follows: $qP = (Fm' - Ft)/(Fm' - Fo')$ and $NPQ = (Fm - Fm')/Fm'$, where Fm' is the maximum yield of fluorescence at the steady-state level reached during the application of a saturation pulse onto the light-acclimated leaves, Ft is the steady-state fluorescence level under the continuous actinic illumination, and Fo' is $Fo/(Fv/Fm + Fo/Fm')$ estimated using the approximation of Oxborough and Baker.[24] The actual quantum yield of PSII ($\Phi_{PSII}$) was calculated according to the equation set by Genty *et al.*[25] as follows: $\Phi_{PSII} = (Fm' - Ft)/Fm'$.

**RNA extraction and reverse transcription (RT)-PCR.** Total RNA was extracted from the whole seedlings except roots 7 days after the treatment of NaCl using RNeasy Plant Mini Kit (Qiagen, Chatsworth, CA, USA) according to the supplier's recommendation. One microgram total RNA was reverse-transcribed in an RT system, AccuPower RT Premix (Bioneer, Daejeon, Korea) for 60 min at 42ºC using 0.5 μg anchored oligo$(dT)_{18}$V primers. Gene-specific primers for the genes of SOD, APX, and CAT isoforms previously designed by Kim *et al.* were used.[26] The sense/antisense primers for NHX2 (accession number: AY360145) and P5CS (accession number: AY574031), 5'-CTCGTTCTCAAATCCGGCGGGCTGT-3'/5'-TGAACGCTAGTATAACAAGGCTATC-3' and 5'-CCAGACAAGATGGAAGATTGGCTTT-3'/5'-GAGTACTAACATCCTTAGATGATTC-3', respectively, were generous gifts from Dr. In-Seok Lee (Radiation Mutant Breeding Lab., KAERI, Daejeon, Korea). The primers for actin and rRNA, used as internal controls, were 5'-TCCATCTTGGCATCTCTCAG-3'/5'-GTACCCTCATCAGGCATCTG-3' [27] and 5'-CTTCGGGATCGGAGTAATGA-3'/5'-AACTAAGAACGGCCATGCAC-3',[26] respectively, while those for LIP5 (accession number: AB011368) and DHN1 (accession number: AY786415), used as positive controls, were 5'-CGAGCACAAGGAGAAGAAGG-3'/5'-ATCATCTGCAGCATCACCTG-3' and 5'-GAAGCATGAGGAGGAGCTTG-3'/5'-CTCCTTGAGCCCCTTCTTCT-3'. The subsequent PCR was carried out with 3 μL of a total 20 μL RT reaction mixture in a PCR system, Perfect Premix ver. 2.0 (Takara Korea Biomedical Inc., Seoul, Korea) as follows: denaturation at 94ºC for 5 min, 24 or 35 cycles of 94ºC (45 s) - 58ºC (45 s) - 72ºC (1.5 min), and an extension at 72ºC for 7 min[26] in all genes except for NHX2, P5CS, LIP5 and DHN1, which were subjected to denaturation at 95ºC for 5 min, 30 cycles of 95ºC (80 s) - 57ºC (1 min) - 72ºC (1.5 min), and an extension at 72ºC for 10 min. The resultant RT-PCR products were electrophoresed and analyzed on a 2.0% (w/v) agarose gel after staining with ethidium bromide (EtBr).

## Results and Discussion

**Changes in Chl fluorescence parameters.** Chl fluorescence analysis can be used to assess the salt-tolerance in crop plants *in vivo*.[28] Dionisio-Sese and Tobita[29] have suggested that the salt-sensitivity in rice is associated with increased shoot sodium levels, decreased photosynthetic efficiency of PSII, and enhanced NPQ using Chl fluorescence parameters. One of these parameters, the maximal photochemical efficiency of PSII or Fv/Fm, has been reported to decrease with increasing salt stress in pea, rice, and sorghum.[15,20,30] Similarly, our results showed that the Fv/Fm values of rice were slightly decreased by the treatment of 40 mM NaCl, and this decrease was alleviated in the presence of JA (Fig. 1A). The alleviatory effect of JA on the salt stress-induced decrease of the photosynthetic efficiency was also confirmed by the actual quantum yield of PSII, $\Phi_{PSII}$, and the parameter for photochemical quenching, qP. Studies have revealed that salinity decreases these parameters in rice, sorghum, and sorrel.[15,30,31] However, the $\Phi_{PSII}$ and qP values were significantly higher in the JA-treated plants under salt stress than in the control ones (Figs. 1B and C). In contrast, the parameter for non-photochemical quenching, NPQ, was slightly increased by the treatment of NaCl, and this increase was significantly enhanced in the presence of JA (Fig. 1D). Although Oh *et al.*[15] reported a simultaneous decrease in the Fv/Fm, qP, and NPQ in rice, NPQ appears to generally

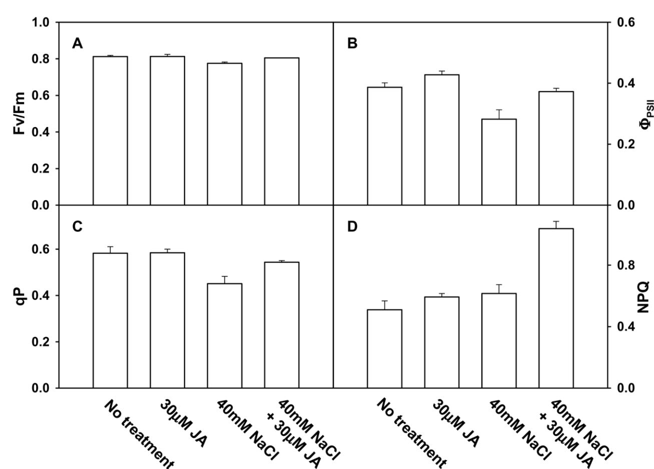


**Fig. 1. Changes of Chl fluorescence parameters in rice leaves.** The treatments of NaCl and JA were performed in rice seedlings under hydroponic growth conditions as stated in 'Materials and Methods'. Fv/Fm, the maximum photochemical efficiency of PSII; $\Phi_{PSII}$, the actual quantum yield of PSII; qP and NPQ, the photochemical and non-photochemical quenching. Error bars represent the S.E. (n=4-5).

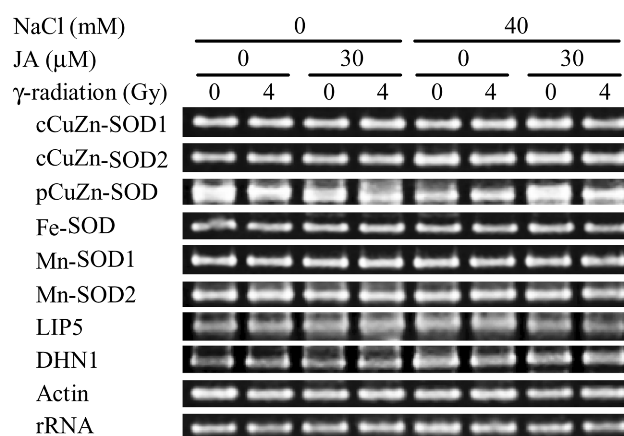


**Fig. 2. Expression of SOD isoenzyme genes.** The RT-PCR conditions are described in 'Materials and Methods'. For the RT, one microgram total RNA was reverse transcribed for 60 min at 42°C using 0.5 μg anchored oligo$(dT)_{18}$V primers. The subsequent PCR was performed as follows: denaturation at 94°C for 5 min, cycle reactions of 94°C (45 s) - 58°C (45 s) - 72°C (1.5 min) and an extension at 72°C for 7 min. Alternatively, the PCR conditions for LIP5 and DHN1 were denaturation at 95°C for 5 min, cycle reactions of 95°C (80 s) - 57°C (1 min) - 72°C (1.5 min), and an extension at 72°C for 10 min. All genes had 24 cycle reactions except 35 in pCuZn-SOD and Mn-SOD2 and 30 in LIP5 and DHN1. LIP5 and DHN1 were used as positive controls inducible by salt stress,[33,34] while Actin and rRNA were amplified as internal controls.

increase under salt stress as a defense mechanism.[30,31,32] Therefore, the JA treatment could help reduce the excitation pressure on photosystems through an increase in the thermal energy dissipation under salt stress and maintain the photosynthetic efficiency.

**Expression of genes of SOD isoforms.** Expression of SOD isoenzyme genes was analyzed in the JA-treated and/or γ-irradiated rice seedlings under salt stress. Transcription levels of two cytosolic isoforms, cCuZn-SOD1 and cCuZn-SOD2, one plastidic isoform of CuZn-SOD, pCuZn-SOD, one isoform of Fe-SOD, Fe-SOD, and two isoforms of Mn-SOD, Mn-SOD1 and Mn-SOD2, used in the report of Kim *et al.*[26] were investigated by RT-PCR analysis. This methodological approach was adopted to reveal expression of antioxidant isoenzyme genes difficult to be subjected to Northern blot and quantitative PCR analyses due to the high sequence homology among genes and the number of genes to be analyzed.

The treatment of NaCl increased the expression of cCuZn-SOD2 gene; however, this increase was less noticeable in the presence of JA (Fig. 2). Hernandez *et al.*[35] reported that in pea (*Pisum sativum* cv. Puget) higher concentrations of NaCl (110-130 mM) enhance the activity of cCuZn-SOD2. According to our preliminary data, 50 mM NaCl could also induce an increase in the total activity of SOD in rice (*O. sativa* L. cv. Ilpoombyeo) seedlings (data not shown). Thus, the increased expression of cCuZn-SOD2 gene might contribute to the high activity of SOD in the salt-treated rice seedlings. In contrast, the transcripts of pCuZn-SOD gene were markedly decreased by the treatment of NaCl, and this decrease could be protected by the JA treatment only, and not by γ-irradiation. This effect of JA appears to be associated with the protective functions of JA-Me as described by previous studies.[19-21] Interestingly, the transcription level of pCuZn-SOD gene was synergistically decreased by both the JA treatment and γ-irradiation in the absence of NaCl. The genes of cCuZn-SOD1, Fe-SOD, Mn-SOD1, and Mn-SOD2 were rarely affected by JA, NaCl, and γ-radiation. However, Parida *et al.*[36] reported that salinity induces a preferential enhancement of Mn-SOD in the mangrove, Bruguiera parviflora. This discrepancy can be explained by the difference in the NaCl concentration and plant species used.

**Expression of genes of APX isoforms.** Our preliminary data also indicated that the treatment of NaCl up to 100 mM induces an increase in the activity of APX in rice plants (data not shown). Similarly, many studies have revealed that the salinity enhances the activity of APX in various plant species, e.g., pea, rice, and beet.[9,10,35] Therefore, we investigated mRNA levels of two cytosolic and two chloroplastic (stromal and thylakoid) isoforms of APX, cAPXa, cAPXb, Chl_sAPX, and Chl_tAPX, respectively.[26] The expression of cAPXb gene was noticeably decreased by the treatment of NaCl, and the mRNA levels of cAPXa and Chl_tAPX were slightly lower in the JA-treated groups under salt stress (Fig. 3). Interestingly, the expression of Chl_tAPX gene was slightly elevated by γ-irradiation without salt stress, while that of Chl_sAPX gene remained unaffected by JA, NaCl, and γ-radiation. Yoshimura *et al.*[37] reported that the cytosolic APX activity of spinach increased in parallel with the transcript abundance during high-light stress, while the protein level was not altered. Accordingly, the present data may indicate no direct correlation between the gene expression and enzyme activity

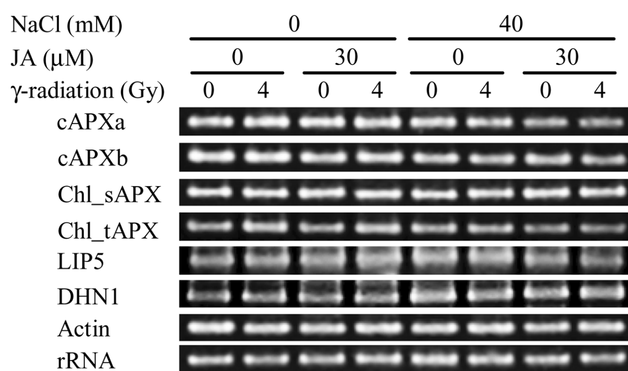


**Fig. 3. Expression of APX isoenzyme genes.** The RT-PCR conditions are described in 'Materials and Methods' and Fig. 2. All genes except LIP5 and DHN1 had 24 cycle reactions. LIP5 and DHN1 were used as positive controls inducible by salt stress,[33,34] while Actin and rRNA were amplified as internal controls.

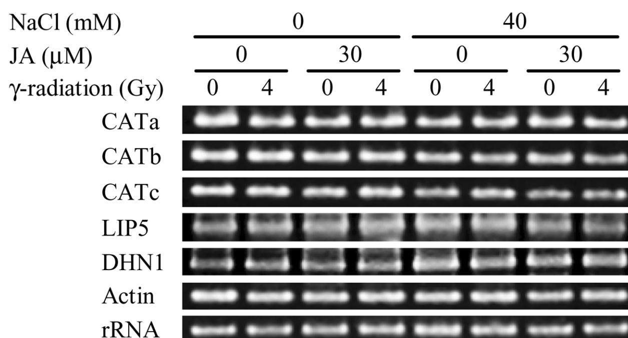


**Fig. 4. Expression of CAT isoenzyme genes.** The RT-PCR conditions are described in 'Materials and Methods' and Fig. 2. All genes except LIP5 and DHN1 had 24 cycle reactions. LIP5 and DHN1 were used as positive controls inducible by salt stress,[33,34] while Actin and rRNA were amplified as internal controls.

of APX under salt stress.

Expression of genes of CAT isoforms. Together with APX, CAT plays an important role in the breakdown of $H_2O_2$ in cells. Many experimental evidences have shown that salt stress induces a significant increase in the activity of CAT in salt-tolerant rice cultivars.[9,10] However, in the salt-sensitive cultivars or other plant species, the activity of CAT was almost unaffected or decreased with increasing concentration of NaCl.[9,10,38] In the present study, the transcription levels of two CAT isoforms, CATb and CATc, were decreased by the treatment of NaCl, while that of CATa remained unaffected (Fig. 4). These results indicate that CATb and CATc may contribute to the salt-induced decrease in total transcripts and activities of CAT. However, neither JA nor γ-radiation affected the expression of CAT isoenzyme gene.

**Expression of genes of NHX2 and P5CS.** Salt-tolerance is a multigenic trait, and a number of genes categorized into different functional groups are responsible for the acquisition of salt-tolerance in plants: genes for (i) photosynthesis, (ii) ROS-scavenging, (iii) vacuolar-sequestering, and (iv) synthesis

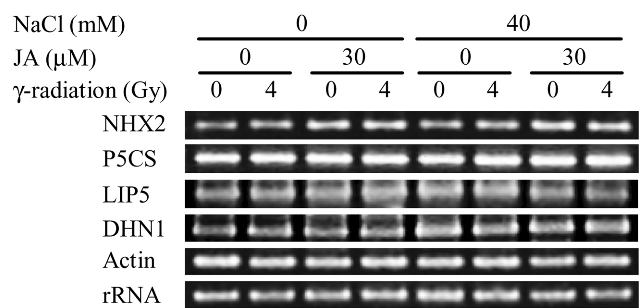


**Fig. 5. Expression of NHX1 and P5CS genes.** The RT-PCR conditions are described in 'Materials and Methods' and Fig. 2. The subsequent PCR was performed as follows: denaturation at 95°C for 5 min, 30 cycle reactions of 95°C (80 s) - 57°C (1 min) - 72°C (1.5 min) and an extension at 72°C for 10 min. LIP5 and DHN1 were used as positive controls inducible by salt stress,[33,34] while Actin and rRNA were amplified as internal controls.

of compatible osmolytes.[39] Until now, we have demonstrated that the expressions of SOD, APX, and CAT isoenzyme genes are differentially regulated in response to JA, NaCl, and γ-radiation. However, besides pCuZn-SOD, none of the isoenzyme genes tested could be positively associated with the higher salt-tolerance in the JA-treated seedlings (Fig. 1). Therefore, we attempted to assess the expressions of NHX2 and P5CS genes belonging to the third and fourth categories, respectively. Like NHX1, NHX2 is localized to the vacuole in plant cells, facilitating $Na^+$ compartmentalization and maintaining intracellular $K^+$ status as an $Na^+/H^+$ exchanger.[11,13,14,40] JA treatment increased the expression of NHX2 gene, regardless of the presence of NaCl and γ-irradiation, while that of P5CS gene appeared to slightly increase only in the co-presence of NaCl and JA. These results are in good agreement with our preliminary data, indicating that the JA treatment helps to elevate the proline content, and partially protect against electrolyte leakage and reduction of relative water content under salt stress (data not shown).

In conclusion, the present study clarified that JA can induce enhanced salt-tolerance in rice under salt stress using Chl fluorescence parameters. The transcription levels of SOD, APX, and CAT isoenzyme genes were differentially modulated under salt stress. Furthermore, our results suggest that the enhanced salt-tolerance in the JA-treated rice seedlings may be partly due to the high transcription levels of pCuZn-SOD, NHX2, and P5CS genes under salt stress.

**Acknowledgments.** This research was supported by the Nuclear R&D Program of the Ministry of Science and Technology, Korea.

## References

1. Xiong, L. and Zhu, J.-K. (2002) Salt tolerance. In *The Arabidopsis Book*, Somerville, C. R. Meyerowitz, E. M. (eds.), American Society of Plant Biologists, Rockville, MD, doi/

10.1199/tab.0048, http://www.aspb.org/publications/arabidopsis/

2. Burdon, R. H., O'Kane, D., Fadzillah, N., Gill, V., Boyd, P. A. and Finch, R. R. (1996) Oxidative stress and responses in *Arabidopsis thaliana* and *Oryza sativa* subjected to chilling and salinity stress. *Biochem. Soc. Trans*. **24**, 469-472.
3. Shen, B., Jensen, R. G. and Bohnert, H. (1997) Increased resistance to oxidative stress in transgenic plants by targeting mannitol biosynthesis to chloroplasts. *Plant Physiol.* **113**, 1177-1183.
4. Tsugane, K., Kobayashi, K., Niwa, Y., Ohba, Y., Wada, K. and Kobayashi, H. (1999) A recessive Arabidopsis mutant that grows photoautotrophically under salt stress shows enhanced active oxygen detoxification. *Plant Cell* **11**, 1195-1206.
5. Hong, Z., Lakkineni, K., Zhang, Z. and Verma, D. P. S. (2000) Removal of feedback inhibition of D1-pyrroline-5-carboxylate synthetase results in increased proline accumulation and protection of plants from osmotic stress. *Plant Physiol.* **122**, 1129-1136.
6. Zhu, J.-K. (2001) Plant salt tolerance. *Trends Plant Sci.* **6**, 66-71.
7. Bohnert, H. J. and Sheveleva, E. (1998) Plant stress adaptations, making metabolism move. *Curr. Opin. Plant Biol.* **1**, 267-274.
8. Dionisio-Sese, M. L. and Tobita, S. (1998) Antioxidant responses of rice seedlings to salinity stress. *Plant Sci*. **135**, 1-9.
9. Vaidyanathan, H., Sivakumar, P., Chakrabarty, R. and Thomas, G. (2003) Scavenging of reactive oxygen species in NaCl-stressed rice (*Oryza sativa* L.) - differential response in salt-tolerant and sensitive varieties. *Plant Sci.* **165**, 1411-1418.
10. Bor, M., Özdemir, F. and Turkan, I. (2003) The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet *Beta vulgaris* L. and wild beet *Beta maritime* L. *Plant Sci.* **164**, 77-84.
11. Apse, M. P., Aharon, G. S., Snedden, W. A. and Blumwald, E. (1999) Salt tolerance conferred by overexpression of a vacuolar $Na^+/H^+$ antiport in Arabidopsis. *Science* **285**, 1256-1258.
12. Fukuda, A., Nakamura, A. and Tanaka, Y. (1999) Molecular cloning and expression of the $Na^+/H^+$ exchanger gene in *Oryza sativa. Biochim. Biophys. Acta* **1446**, 149-155.
13. Yokoi, S., Quintero, F. J., Cubero, B., Ruiz, M. T. Bressan, R. A. Hasegawa, P. M. and Pardo, J. M. (2002) Differential expression and function of Arabidopsis thaliana NHX $Na^+/H^+$ antiporters in the salt stress response. *Plant J.* **30**, 529-539.
14. Aharon, G. S., Apse, M. P., Duan, S., Hua, X. and Blumwald, E. (2003) Characterization of a family of vacuolar $Na^+/H^+$ antiporters in *Arabidopsis thaliana. Plant Soil* **253**, 245-256.
15. Oh, M. J., Chun, H. S. and Lee, C. B. (2003) Differences in photosynthetic characterization of salt tolerance for two rice (*Oryza sativa*) cultivars. *J. Plant Biol.* **46**, 17-22.
16. Kishor, P. B. K., Hong, Z., Miao, G., Hu, C. A. and Verma, D. P. S. (1995) Overexpression of Δ-pyrroline-5-carboxylate synthase increases proline production and confers osmotolerance in transgenic plants. *Plant Physiol.* **108**, 1387-1394.
17. Hayashi, H., Alia, Mustardy, L., Deshnium, P., Ida, M. and Murata, N. (1997) Transformation of *Arabidopsis thaliana* with the coda gene for choline oxidase; accumulation of glycinebetaine and enhanced tolerance to salt and cold stress. *Plant J.* **12**, 133-142.
18. Nuccio, M. L., Rhodes, D., McNeil, S. D. and Hanson, A. D. (1999) Metabolic engineering of plants for osmotic sress resistance. *Curr. Opin. Plant Biol.* **2**, 128-134.
19. Fedina, I. S. and Tsonev, T. D. (1997) Effect of pretreatment with methyl jasmonate on the response of Pisum sativum to salt stress. *J. Plant Physiol.* **151**, 735-740.
20. Velitchkova, M. and Fedina, I. (1998) Response of photosynthesis of *Pisum sativum* to salt stress as affected by methyl jasmonate. *Photosynthetica* **35**, 89-97.
21. Fedina, I. S. and Benderliev, K. M. (2000) Response of *Scenedesmus incrassatulus* to salt stress as affected by methyl jasmonate. *Biol. Plant.* **43**, 625-627.
22. Krause, G. H. and Weis E. (1991) Chlorophyll fluorescence and photosynthesis: the basics. *Annu. Rev. Plant Physiol. Plant Mol. Biol*. **42**, 313-349.
23. van Kooten, O. and Snel, F. H. (1990) The use of chlorophyll fluorescence nomenclature in plant stress physiology. *Photosynth. Res.* **25**, 147-150.
24. Oxborough, K. and Baker, N. R. (1997) Resolving chlorophyll a fluorescence images of photosynthetic efficiency into photochemical and non-photochemical components-Calculation of qP and Fv'/ Fm' without measuring Fo'. *Photosynth. Res.* **54**, 135-142.
25. Genty, B., Briantais, J. M. and Baker, N. R. (1989) Relationship between the quantum yield of photosynthetic electron transport and the quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta* **990**, 87-92.
26. Kim, J.-H., Chung, B. Y., Kim, J.-S., Wi, S. G., Yang, D. H., Lee, C.-H. and Lee, M.-C. (2004) Construction of gene-specific primers of various antioxidant isoenzyme genes and their expressions in rice (*Oryza sativa* L.) seedlings obtained from gamma-irradiated seeds. *J. Photosci*. **11**, 115-120.
27. Yamanouchi, U., Yano, M., Lin, H., Ashikari, M. and Yamada, K. (2002) A rice spotted leaf gene, *Spl7*, encodes a heat stress transcription factor protein. *Proc. Natl. Acad. Sci. USA* **99**, 7530-7535.
28. Smille, R. M. and Nott, R. (1982) Salt tolerance in crop plants monitored by chlorophyll fluorescence *in vivo. Plant Physiol.* **70**, 1049-1054.
29. Dionisio-Sese, M. L. and Tobita, S. (2000) Effects of salinity on sodium content and photosynthetic responses of rice seedlings differing in salt tolerance. *J. Plant Physiol.* **157**, 54-58.
30. Netondo, G. W., Onyango, J. C. and Beck, E. (2004) Sorghum and salinity II. Gas exchange and chlorophyll fluores-

cence of Sorghum under salt stress. *Crop Sci.* **44**, 806-811.

31. Chen, H.-X., Gao, H.-Y., An, S.-Z. and Li, W.-J. (2004) Dissipation of excess energy in Mehler-peroxidase reaction in *Rumex* leaves during salt shock. *Photosynthetica* **42**, 117-122.
32. James, R. A., Rivelli, A. R., Munns, R. and von Caemmerer, S. (2002) Factors affecting $CO_2$ assimilation, leaf injury and growth in salt-stressed durum wheat. *Func. Plant Biol.* **29**, 1393-1403.
33. Tabaei-Aghdaei, S. R., Harrison, P. and Pearce, R. S. (2000) Expression of dehydration-stress-related genes in the crowns of wheatgrass species [*Lophopyrum elongatum* (Host) A. Love and *Agropyron desertorum* (Fisch. Ex Link.) Schult.] having contrasting acclimation to salt, cold and drought. *Plant Cell Environ.* **23**, 561-571.
34. Jang, I.-C., Oh, S.-J., Seo, J.-S., Choi, W.-B., Song, S. I., Kim, C. H., Kim Y. S., Seo, H.-S., Choi, Y. D., Nahm, B. H. and Kim, J.-K. (2003) Expression of a bifunctional fusion of the *Escherichia coli* genes for trehalose-6-phosphate synthase and trehalose-6-phosphate phosphatase in transgenic rice plants increases trehalose accumulation and abiotic stress tolerance without stunting growth. *Plant Physiol.* **131**, 516-524.
35. Hernandez, J. A., Campillo, A., Jimenez, A., Alacon, J. J. and Sevilla, F. (1999) Response of antioxidant systems and leaf water relations to NaCl stress in pea plants. *New Phytol.* **141**, 241-251.
36. Parida, A. K., Das, A. B. and Mohanty, P. (2004a) Defense potentials to NaCl in a mangrove, *Bruguiera parviflora*: differential changes of isoforms of some antioxidant enzymes. *J. Plant Physiol.* **161**, 531-542.
37. Yoshimura, K., Yabuta, Y., Ishikawa, T. and Shigeoka, S. (2000) Expression of spinach ascorbate peroxidase isoenzymes in response to oxidative stress. *Plant Physiol.* **123**, 223-233.
38. Parida, A. K., Das, A. B., Mittra, B. and Mohanty, P. (2004b) Salt-stress induced alterations in protein profile and protease activity in the mangrove, *Bruguiera parviflora. Z. Naturforsch.* **59c**, 408-414.
39. Parida, A. K. and Das, A. B. (2005) Salt tolerance and salinity effects on plants: a review. *Ecotox. Environ. Safe.* **60**, 324-349.
40. Qiu, Q.-S., Guo, Y., Quintero, F. J., Pardo, J. M., Schumaker, K. S. and Zhu, J.-K. (2004) Regulation of vacuolar $Na^+/H^+$ exchange in *Arabidopsis thaliana* by the Salt-Overly-Sensitive (SOS) pathway. *J. Biol. Chem.* **279**, 207-215.