Pretreatment with a Low Concentration of Methyl Viologen Decreases the Effects of Salt Stress on Chloroplast Ultrastructure in Rice Leaves (Oryza sativa L.)

Koji Yamane*, Md. Shahidur Rahman**, Michio Kawasaki*, Mitsutaka Taniguchi* and Hiroshi Miyake*

(*Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan; **Department of Crop Botany, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh)

Abstract: We investigated the effects of pretreatment with a low concentration of methyl viologen (MV) on the salinity-induced chloroplast degeneration in rice seedlings. The seedlings grown in hydroponic culture containing nutrient solution for 3 wks were treated with 100 nM MV mixed in the hydroponic culture for 3 days, and then with 200 mM NaCl without MV for 3 days. In the plants without MV pretreatment, the chlorophyll content drastically decreased during the NaCl treatment accompanied by swelling of thylakoids and destruction of thylakoid membranes. These damages were alleviated by the pretreatment with MV. The activities of CuZn-SOD and Fe-SOD, which localize in chloroplasts, increased under salt stress in both plants with and without MV pretreatment. In the plants under salt stress without MV pretreatment, ascorbate peroxidase (APX) activity did not differ from that of control. However, in MV-pretreated plants, APX activity under salt stress was about 1.2- to 1.3-fold higher than that of the control. Catalase (CAT) activity in NaCl treated plants was decreased to 52% of the control and the reduction in CAT activity was suppressed by MV pretreatment. These results suggest that MV reduced the damages by salt stress in chloroplasts by increasing APX activity and preventing the decrease in CAT activity.

Key words: Ascorbate peroxidase, Catalase, Methyl viologen, Oryza sativa, Salt stress, Superoxide dismutase, Thylakoids, Ultrastructure.

Active oxygen species (AOS) such as singlet oxygen (\(1^O_2\)), superoxide anion (\(O_2^-\)), hydrogen peroxide (\(H_2O_2\)) and hydroxyl radical (\(\cdot OH\)) are inevitable by-products of the interaction between \(O_2\) and electrons leaked from the electron transport chains in chloroplasts and mitochondria during normal aerobic metabolism (Asada, 1999; Möller, 2001). All AOS can react with DNA, proteins and lipids (Fridovich, 1986), and thus, plants have protective mechanisms to escape from oxidative damages. The non-enzymatic AOS scavenging compounds include carotenoids, ascorbic acid (AsA), glutathione and tocophorols. The antioxidant enzymes include superoxide dismutase (SOD, EC 1.11.1.1), catalase (CAT, EC 1.11.1.6) and ascorbate peroxidase (APX, EC 1.11.1.1). SOD is a major scavenger of \(O_2^-\) and converts \(O_2^-\) to \(H_2O_2\) and \(O_2\). \(H_2O_2\) is scavenged by CAT and a variety of peroxidases. Especially in chloroplasts, \(H_2O_2\) is scavenged by APX using AsA as an electron donor in the ascorbate-gluthatione cycle (Asada, 1999). In normal conditions, plants effectively scavenge AOS, but, the balance between the production of AOS and antioxidant systems is upset by senescence and abiotic stresses such as drought, salt, low- and high-temperature, air pollutants and heavy metals, which often results in oxidative damage (Moran et al., 1994; Hernández et al., 1995; Saruyama and Tanida, 1995; Chang and Kao, 1998; Cho and Park, 2000; Sairam et al., 2000; Langebartels et al., 2002).

Accumulation of salts in irrigated soil is one of the major abiotic stresses limiting yield in crop production, because the crops including rice are mostly non-halophytes (Francois and Maas, 1994; Volkmar et al., 1998). When plants suffer from salt stress, rapid stomatal closure triggered by abscisic acid is induced to decrease water loss from the leaves (Fedina et al., 1994). However, such stomatal closure limits CO\(_2\) supply to the leaves and decreases the availability of CO\(_2\) to the photosynthetic apparatus (Chaves et al., 2002), which leads to an overreduction of the photosynthetic electron transport chain (Osmond and Grace, 1995), and induces generation of excess AOS, leading to oxidative stress (Hernández et al., 1995). Mitsuya et al. (2003) suggested that the salinity-
induced chloroplast damages are induced by light and not directly correlated with salt content in the tissue.

Chloroplasts are considered to be the most powerful source of AOS production (Foyer et al., 1994). Therefore, it is thought that chloroplasts are prone to effect of AOS under salinity. In fact, when plants are affected by salt stress prominent swelling of thylakoids, which is a typical phenomenon of oxidative damage (Hernández et al., 1995), is induced at the early stage of the damage (Mitsuya et al., 2003; Yamane et al., 2003). Therefore, it is necessary to suppress the oxidative damage of chloroplasts to reduce the adverse effects of salinity.

Though high concentrations of AOS in cells and organelles can lead to oxidative damage, there are some recent reports indicating that AOS are not simply toxic by-products of metabolism but function as signaling molecules (Vam Camp et al., 1998). Especially, low levels of H$_2$O$_2$ and nitric oxide may play an important role in signal transduction and pretreatment with these oxides enhances the adaptive plant response against abiotic stress (Liu and Shono, 1999; Lee et al., 2000; Jagendorf and Takabe, 2001; Hung et al., 2002; Yu et al., 2003).

Methyl viologen (MV), also known as paraquat, is a herbicide widely used in agriculture. It has long been known to exert its toxic effects by catalyzing the transfer of electrons from photosystem I to oxygen, which produces O$_2$$_{-}$ (Chu and McRae, 1982). It was reported that MV induces ultrastructural changes of photosynthetic cells (Harvey and Fraser, 1980) and decreases the non-enzymatic AOS scavenging compounds such as AsA and carotenoids (Li and van Staden, 1998) and the activity of antioxidant enzymes such as SOD, APX and CAT (Kraus and Fletcher, 1994). In the present study, we report that although MV may have deleterious effects at a high concentration, pretreatment with a low concentration of MV can suppress the ultrastructural damages in chloroplasts under salt stress.

### Materials and Methods

1. **Plant materials and stress treatment**

   Seeds of rice (*Oryza sativa* L. cv. Nipponbare) were surface sterilized with a 5% sodium hypochlorite solution for 5 minutes. After washing several times with distilled water, seeds were imbibed in a beaker containing distilled water in a culture room at 24 ± 2°C until the appearance of the white tip of the coleoptile.

   After imbibition, the seeds were sown on hydroponic culture containing nutrient solution according to Mae and Ohira (1981) and grown in a growth chamber under a 12-hr photoperiod at 400-500 μmol m$^{-2}$s$^{-1}$ and 28/25°C (day/night) for 3 wks. The 3-week-old plants were treated with MV mixed in the hydroponic culture for 3 days, and then with 200 mM NaCl for 3 days without MV.

2. **Measurement of chlorophyll content**

   The chlorophyll content of fully expanded most upper leaves (5th leaves) was determined in 100 % ethanol according to Knudson et al. (1977).

3. **Enzyme extraction and assays**

   All operations were carried out at 0-4°C. For the determination of SOD activity, fully expanded uppermost leaves were frozen with liquid nitrogen and homogenized with 50 mM HEPES buffer (pH 7.6) and 0.1 mM Na$_2$EDTA. The homogenate was centrifuged at 12000 g for 15 min at 4°C to obtain a crude extract. The crude extract was diazylized against 5 mM HEPES buffer to remove low-molecular-weight compounds interfering with SOD activity determination.

   SOD activity was assayed by monitoring the inhibition of photochemical reduction of nitro blue tetrazolium (NBT) (Beyer and Fridovich, 1987). For determination of total SOD, a 3 ml reaction mixture containing 50 mM HEPES (pH 7.6), 0.1 mM Na$_2$EDTA, 50 mM Na$_2$CO$_3$ (pH 10.4), 13 mM methionine, 0.025% (w/v) Triton X-100, 75 μM NBT, 2 μM riboflavin and an appropriate aliquot of enzyme extract was prepared. The reaction mixtures were illuminated for 10 min at light intensity of 40 μmol m$^{-2}$s$^{-1}$. One unit SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT as monitored at 560 nm. Identical reaction mixtures containing enzyme extracts that had not been illuminated were used to estimate background absorbance.

   For the determination of APX and CAT activities, fully-expanded uppermost leaves were frozen in liquid nitrogen and homogenized with 50 mM
Fig. 2. Ultrastructure of a chloroplast in a plant grown for 3 weeks (control). Bar = 1 µm.
Fig. 3. Ultrastructure of a chloroplast in a plant treated with MV for 3 days. Bar = 1 µm.
Fig. 4. Ultrastructure of chloroplasts in plants treated with 200 mM NaCl for 2 days (A) and 3 days (B). Bar = 1 µm.
Fig. 5. Ultrastructure of chloroplasts in the plants treated with 200 mM NaCl for 2 days (A) and 3 days (B) after treatment with 100 nM MV for 3 days. Bar = 1 µm.
potassium phosphate buffer (pH 7.8) containing 1 mM EDTA, 7 mM mercaptoethanol, 0.1% (w/v) Triton X-100, 5 mM sodium ascorbate and 1.0% (w/v) polyvinylpyrrolidone. The homogenate was centrifuged at 12000 g for 10 min and the supernatant was used for assays. The activities of APX and CAT were measured following Nakano and Asada (1981) and Aebi (1974), respectively.

Protein in the supernatant was quantified by the Coomassie brilliant blue-dye binding method according to Bradford (1976).

4. Electron microscopy

Electron microscopic studies were made using the middle section of fully-expanded uppermost leaves. Small pieces of leaves were fixed in Karnovsky’s fixative (mixture of 4% paraformaldehyde and 5% glutaraldehyde in 50 mM phosphate buffer (pH 7.2)) and post fixed in 2% osmium tetroxide in the same buffer. Samples were dehydrated in a series of graded acetone and propylene oxide and embedded in Spurr’s resin.

Ultrathin sections (70-90 nm in thickness) were cut with a diamond knife and placed on 150 mesh copper grid. The grids were stained with 2% uranyl acetate for 25 min followed by lead citrate for 5 min. Then the sections were examined on a Hitachi H600 transmission electron microscope at 100 kV. Photographs were taken at three or more random sites in at least 2 to 4 leaf segments and representative pictures were presented.

Results

1. Changes in chlorophyll content and ultrastructure

Fig. 1 shows the changes in chlorophyll content during stress treatment. Chlorophyll content decreased rapidly after the start of treatment with 200 mM NaCl. However, the pretreatment with 100 nM MV for 3 days suppressed the reduction of chlorophyll content. Pretreatment with MV at higher than 100 nM reduced the chlorophyll content, but that at lower than 100 nM did not (data not shown).

Fig. 2A shows a typical chloroplast of a mesophyll cell in the control plant. The chloroplast possessed well-developed granal and stromal thylakoids. Fig. 3 shows a chloroplast of a mesophyll cell treated with 100 nM MV for 3 days. Treatment with 100 nM MV alone did not induce any ultrastructural changes.

Figs. 4A and 4B show the chloroplasts treated with 200 mM NaCl for 2 and 3 days, respectively. NaCl treatment for 2 days caused swelling of thylakoids (Fig. 4A), and that for 3 days caused severe damage (Fig. 4B). Thylakoid membrane was scarcely observed after the 3-days treatment.

On the other hand, in the chloroplasts pretreated with MV, the distortion of thylakoid membrane was effectively suppressed under NaCl for 2 days (Fig. 5A). Though the arrangement of thylakoids was slightly disturbed under NaCl for 3 days in spite of pretreatment with MV, severe damage was suppressed (Fig. 5B).

2. Changes in antioxidant enzyme activity

Figs. 6A and 6B show the changes in CuZn-SOD and Fe-SOD activities during the 3-day treatment with 200 mM NaCl with and without MV pretreatment, respectively. During the treatment with 200 mM NaCl without MV pretreatment, CuZn-SOD activity increased rapidly and Fe-SOD activity increased gradually.

The activities of CuZn-SOD and Fe-SOD were
enhanced 1.1- and 1.5-fold, respectively by the 3-day treatment with MV (at day 0 in Fig. 6), and both SOD activities increased gradually during the following salt-stress period.

The changes in APX and CAT activity in the plants with and without MV pretreatment were monitored during growth in 200 mM NaCl for 3 days (Fig. 7A-B). APX activity did not differ from the control during the NaCl treatment, but it was enhanced about 1.1-fold by the 3-day treatment with MV (day 0). The APX activity after the 3-day treatment with NaCl in the plants pretreated with MV was about 1.2–1.3-fold higher than that of the control.

CAT activity was reduced to 52% of the control by 3-day treatment with NaCl (Fig. 7B). The reduction was slightly suppressed by MV pretreatment, but the activity was reduced to 58% of the control by the treatment with NaCl for 3 days even after MV pretreatment (Fig. 7B).

**Discussion**

Salt-tolerant plants are better protected from oxidative stress by antioxidant enzymes (Shalata and Tal, 1998; Meneguzzo et al., 1998), therefore, it is thought that increasing the antioxidant enzyme levels under salinity is an effective strategy to confer salt tolerance in salt-sensitive plants. MV is a herbicide widely used in agriculture, and is toxic to the plants at a high concentration (Harvey and Fraser, 1980; Kraus and Fletcher, 1994; Hung et al., 2002). In this study, however, pretreatment with a low concentration of MV enhanced antioxidant-enzyme activities and suppressed the decrease in chlorophyll content and distortion of thylakoid membranes by salinity. MV is probably a useful chemical to decrease the damage caused by salt stress. The present result could be of importance from both biological and practical points of view.

Higher plants have three different molecular forms of SOD, namely CuZn-SOD, Mn-SOD and Fe-SOD. These metalloenzymes are localized in different compartments of plant cells including chloroplasts, mitochondria, peroxisomes and cytosol (Scandalios, 1993). The chloroplasts of rice plants (Oryza sativa L.) have Fe-SOD and CuZn-SOD (Kaminaka et al., 1997; 1999), and the cytosol has CuZn-SOD. MnSOD has been identified in chloroplasts as thylakoid-bound isozyme in spinach (Hayakawa et al., 1985), but, no genes for chloroplastic Mn-SOD have been characterized in rice plants.

Treatment with MV enhanced the activities of CuZn- and Fe-SOD isozymes (Fig. 6A-B). Treatment with NaCl also increased the activity of CuZn-SOD, even in the absence of pretreatment with MV (Fig. 6A-B). Thus salt stress itself seemed to cause excess generation of O$_2^-$.

SOD catalyzes the dismutation of O$_2^-$ to H$_2$O$_2$ and O$_2$ (Fridovich, 1986). H$_2$O$_2$ have to be removed as soon as possible to avoid the risk of the formation of ·OH by a metal-catalyzed site specific Haber-Weiss reaction (Halliwell and Gutteridge 2000). However, APX activity did not increase and CAT activity decreased in the plants without MV pretreatment under salt stress. On the other hand, APX activity in the plants treated with MV was generally higher than that in the control, and the decrease in CAT activity under stress was suppressed by MV pretreatment (Fig. 7). We previously suggested that excess H$_2$O$_2$ and H$_2$O$_2$-derived ·OH are responsible for the deleterious effects of salt stress on chlorophyll content and chloroplast ultrastructure (Yamane et al. 2004). The present experiments suggested that in the plants pretreated with MV, the
concentration of H$_2$O$_2$ was lower than that of the plants without MV pretreatment under salt stress due to the maintenance of a higher APX activity and the suppression of the decrease in CAT activity. This may be why MV suppressed the reduction of chlorophyll content and ultrastructural changes under stress condition.

In the plants without MV pretreatment, CAT activity rapidly decreased during salt stress (Fig. 7B). CAT activity decreased not only under salt stress (Lee et al., 2001) but also under low temperature and heat shock (Omran, 1980). Such reduction in CAT activity was suggested as a signal for antioxidant defenses (Hertwig et al., 1992), but, further studies are needed to confirm this idea (Dat et al., 2000).

The decrease in CAT activity up to 2 days of salt stress was suppressed by pretreatment with MV (Fig. 7B). However, at day 3 of NaCl treatment, CAT activity decreased, and chlorophyll content decreased and thylakoids slightly distorted even in the plants pretreated with MV, though APX activity was maintained at about 1.2-fold that of the control. Because CAT is mainly involved in the removal of H$_2$O$_2$ produced in the process of glycolate pathway (Noctor et al., 2002), decrease in CAT activity induces an increase in peroxisomal H$_2$O$_2$ concentration and H$_2$O$_2$ might diffuse from the peroxisomes into the cytosol. The diffused H$_2$O$_2$ is harmful to other organelles (Corpas et al., 1993). CAT can remove the bulk of H$_2$O$_2$ and is critical for maintaining the redox balance during oxidative stress (Willekens et al., 1997). Therefore, these results suggest that though H$_2$O$_2$ in chloroplasts is effectively scavenged by APX in the plants pretreated with MV (Asada, 1999), CAT activity is not sufficient to scavenge H$_2$O$_2$ derived from other cellular compartments, which might induce chlorophyll degradation and chloroplast damages under prolonged NaCl treatment. Therefore, CAT is also important to reduce the adverse effects of salt stress on chloroplasts, and will be another target for conferring salinity tolerance in crop plants.

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References

Aebi, H. 1974. Catalases. In H.U. Bergmeyer ed., Methods of Enzymatic Analysis. Vol. 2. Academic Press, New York. 675-684.

Asada, K. 1999. The water-water cycle in chloroplasts: Scavenging of active oxygens and dissipation of excess photons. Annu. Rev. Plant Physiol. Plant Mol. Biol. 50 : 601-639.

Beyer, W.F. and Fridovich, I. 1987. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Anal. Biochem. 161 : 559-566.

Bradford, M.M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein using the principle of protein dye-binding. Anal. Biochem. 72 : 248-254.

Cha, L.S. and McRae, D.G. 1982. Light-dependence of paraquat-initiated membrane deterioration in bean plants. Evidence for the involvement of superoxide. Physiol. Plant. 56 : 492-499.

Chang, C.J. and Kao, C.H. 1998. H$_2$O$_2$ metabolism during senescence of rice leaves: changes in enzyme activities in light and darkness. Plant Growth Regul. 25 : 11-15.

Chaves, M.M., Pereira, J.S., Maroco, J., Rodrigues, M.L., Ricardo, C.P.P., Osório, M.L., Carvalho, I., Faria, T. and Pinheiro, C. 2002. How plants cope with water stress in the field. Photosynthesis and growth. Ann. Bot. 89 : 907-916.

Cho, U-H and Park, J-O. 2000. Mercury-induced oxidative stress in tomato seedlings. Plant Sci. 156 : 1-9.

Corpas, F.J., Gómez, M., Hernández, J.A. and del Río, L.A. 1993. Metabolism of activated oxygen in peroxisomes from two Pisum sativum L. cultivars with different sensitivity to sodium chloride. J. Plant Physiol. 141 : 160-163.

Dat, J., Vandenabeele, S., Vranova, E., Van Montagu, M., Inzé, D. and Van Breusegem, F. 2000. Dual action of the active oxygen species during plant stress responses. Cell Mol. Life Sci. 57 : 779-795.

Fedina, I.S., Tsonev, T.D. and Guleva, E.I. 1994. ABA as a modulator of the response of Pisum sativum to salt stress. J. Plant Physiol. 143 : 245-249.

Foyer, C.H., Descourvèrres, P. and Kumert, K.J. 1994. Protection against oxygen radicals: an important defense mechanism studied in transgenic plants. Plant Cell Environ. 17 : 507-523.

Francois, L.E. and Maas, E.V. 1994. Crop response and management on salt-affected soils. In M. Pessarakli ed., Handbook of Plant and Crop Stress. Marcel Dekker, New York. 149-181.

Fridovich, I. 1986. Biological effects of the superoxide radical. Arch. Biochem. Biophys. 247 : 1-11.

Halliwell, B. and Gutteridge, J.M.C. 2000. Free radicals in biology and medicine. Third edition. Oxford University Press, London. 1-936.

Harvey, B.M.R and Fraser, T.W. 1980. Paraquat tolerant and susceptible perennial ryegrasses: effects of paraquat treatment on carbon dioxide uptake and ultrastructure of photosynthetic cells. Plant Cell Environ. 3 : 159-166.

Hayakawa, T., Kanematsu, S. and Asada, K. 1985. Purification and characterization of thylakoid-bound Mn-superoxide dismutase in spinach chloroplasts. Planta 166 : 111-116.

Hernández, J.A., Olmos, E., Corpas, F.J., Sevilla, F. and del Río, L.A. 1995. Salt-induced oxidative stress in chloroplasts of pea plants. Plant Sci. 105 : 151-167.

Hertwig, B., Streb, P. and Feierabend, J. 1992. Light dependence of catalase synthesis and degradation in leaves and the influence of interfering stress conditions. Plant Physiol. 100 : 1547-1553.

Hung, K.T., Chang, C.J. and Kao, C.H. 2002. Paraquat toxicity is reduced by nitric oxide in rice leaves. J. Plant Physiol. 159 : 159-166.
Moran, J.F., Becana, M., Iturbe-Ormaetxe, I., Frechilla, S., Møller, I.M. 2001. Plant mitochondria and oxidative stress: Mitsuya, S., Kawasaki, M., Taniguchi, M. and Miyake, H. Meneguzzo, S., Sgherri, C.L.M., Navari-Izzo, F. and Izzo, R. 1998. Mae, T. and Ohira, K. 1981. The remobilization of nitrogen related to leaf growth and senescence in rice plants (Oryza sativa L.). Plant Cell Physiol. 22 : 1067-1074. Meneguzzo, S., Sgherri, C.L.M., Navari-Izzo, F. and Izzo, R. 1998. Stromal and thylakoid-bound ascorbate peroxidases in NaCl-treated wheat. Physiol. Plant. 104 : 735-740. Mitsuya, S., Kawasaki, M., Taniguchi, M. and Miyake, H. 2003. Light dependency of salinity-induced chloroplast degradation. Plant Prod. Sci. 6 : 219-223. Möller, I.M. 2001. Plant mitochondria and oxidative stress: Electron transport, NADPH turnover, and metabolism of reactive oxygen species. Annu. Rev. Plant Physiol. Plant Mol. Biol. 52 : 561-591. Moran, J.F., Becana, M., Iturbe-Ormaetxe, I., Frechilla, S., Khucus, R.V. and Aparicio-Tejo, P. 1994. Drought induces oxidative stress in pea plants. Planta 194 : 346-352. Nakano, Y. and Asada, K. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 22 : 867-880. Noctor, G., Veljovic-Jovanovic, S., Driscoll, S., Novitskaya, L. and Foyer, C.H. 2002. Drought and oxidative load in the leaves of C3 plants: a predominant role for photorespiration? Ann. Bot. 89 : 841-850. Omran, R.G. 1980. Peroxide levels and the activities of catalase, peroxidase, and indolacetic acid oxidase during and after chilling cucumber seedlings. Plant Physiol. 65 : 407-408. Osmond, C.B. and Grace, S.C. 1995. Perspectives on photoinhibition and photorespiration in the field: quintessential inefficiencies of the light and dark reactions of photosynthesis? J. Exp. Bot. 46 : 1351-1362. Sairam, R.K., Srivastava, G.C. and Saxena, D.C. 2000. Increased antioxidant activity under elevated temperatures: a mechanism of heat stress tolerance in wheat genotypes. Biol. Plant. 43 : 245-251. Saruyama, H. and Tanida, M. 1995. Effects of chilling on activated oxygen-scavenging enzymes in low temperature-sensitive and -tolerant cultivars of rice. Plant Sci. 109 : 105-113. Scandalios, J.G. 1993. Oxygen stress and superoxide dismutases. Plant Physiol. 101 : 7-12. Shalata, A. and Tal, M. 1998. The effect of salt stress on lipid peroxidation and antioxidants in the leaf of the cultivated tomato and its wild salt-tolerant relative Lycopersicon pennellii. Physiol. Plant. 104 : 169-174. Van Camp W., Van Montagu, M. and Inzé, D. 1998. H2O2 and NO: redox signals in disease resistance. Trends Plant Sci. 3 : 330-334. Volkmar, K.M., Hu, Y. and Steppuhn, H. 1998. Physiological responses of plants to salinity: a review. Can. J. Plant Sci. 78 : 19-27. Willekens, H., Chamnongpol, S., Davey, M., Schraudner, M., Langebartels, C., Van Montagu, M., Inzé, D. and Van Camp, W. 1997. Catalase is a sink for H2O2 and is indispensable for stress defence in C3 plants. EMBO J. 16 : 4806-4816. Yamane, K., Kawasaki, M., Taniguchi, M. and Miyake, H. 2003. Differential effect of NaCl and polyethylene glycol on the ultrastructure of chloroplasts in rice seedlings. J. Plant Physiol. 160 : 573-575. Yamane, K., Kawasaki, M., Taniguchi, M. and Miyake, H. 2004. Treatment with antioxidants decreases the effects of salt stress on chloroplast ultrastructure in rice leaf segments (Oryza sativa L.). Plant Prod Sci. 7 : 292-300. Yu, C.W., Murphy, T.M. and Lin, C.H. 2003. Hydrogen peroxide-induced chilling tolerance in mung beans mediated through ABA-independent glutathione accumulation. Funct. Plant Biol. 30 : 955-963. Yu, Q. and Rengel, Z. 1999. Drought and salinity differentially influence activities of superoxide dismutases in narrow-leaved lupins. Plant Sci. 142 : 1-11.