Differential Sensitivity of Chloroplasts in Mesophyll and Bundle Sheath Cells in Maize, an NADP-Malic Enzyme-Type C\textsubscript{4} Plant, to Salinity Stress

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Abstract: The changes in chloroplast ultrastructure and the contents of chlorophyll, Na and K in response to salinity stress were investigated in leaves of maize, an NADP-malic enzyme-type C\textsubscript{4} plant species possessing dimorphic chloroplasts. The seedlings were treated with 0, 1, 2 or 3\% NaCl for three or five days under a light or dark condition. In both light and dark conditions, the dry weight of salt-treated plants decreased as NaCl concentration increased. Chlorophyll and K contents of the second leaf blade decreased as NaCl concentration increased under the light condition but not under the dark condition. Na content of the second leaf blade was significantly higher at high NaCl concentrations under both light and dark conditions. However, Na content was much lower under the dark condition than light condition. Higher concentrations (2 and 3\%) of NaCl significantly increased the size of plastoglobules, decreased the number and size of starch granules and altered the chloroplast ultrastructure. Under the light condition, mesophyll cell (MC) chloroplasts appeared more sensitive to the damaging effect of salinity than the bundle sheath cell (BSC) chloroplasts. MC chloroplasts became more globular in shape and showed swollen and disorganized thylakoids and reduced thickness of grana by salinity. BSC chloroplasts were less affected by salinity than MC chloroplasts. Although chloroplast size and number and size of starch granules were reduced, there was no structural distortion in the thylakoids of BSC chloroplasts. However, the thickness of grana was increased by salinity. Under the dark condition, the chloroplast structure was less affected by salinity. Though the envelope of BSC chloroplasts was occasionally damaged, the thylakoids in both MC and BSC chloroplasts were preserved under salinity stress. The present study suggests that the chloroplast damage caused by salinity is light-dependent and MC chloroplasts are more sensitive to salinity than BSC chloroplasts.

Key words: Bundle Sheath, C\textsubscript{4} Plant, Chloroplast, Maize, Mesophyll, Salinity.

Salinity has been recognized as one of the most serious environmental problems in agriculture, especially in arid and semi-arid regions and land areas with mismanagement and irrigation. Salinity affects about 7\% of the land surface of the world and 50\% of irrigated land (McWilliam, 1986; Flowers et al., 1997). Plants suffer from composite stresses caused by salinity, which elicits complex effects on plant metabolism due to ion toxicity, ion imbalance and water deficit (Bohnert et al., 1995; Hasegawa et al., 2000). The plant growth is severely reduced by salinity although different plant species or parts of a plant have different responses based on their tolerance to salinity stress (Munns and Termaat, 1986).

Most commonly, the stress is caused by high Na and Cl concentrations in the soil, which lower photosynthetic efficiency and may cause oxidative stress that involves an accumulation of reactive oxygen species (ROS) such as superoxide (O\textsubscript{2}^-) and hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}). In plant cells, chloroplast is a potential source of ROS production (Dat et al., 2000) and the effect of salinity on important metabolic processes in this organelle has been well documented (Hernandez et al., 1995; Sairam and Srivastava, 2002; Meloni et al., 2003; Gomes et al., 2004). Salinity-induced structural changes in chloroplasts of C\textsubscript{3} plants such as rice (Rahman et al., 2000; Mitsuwa et al., 2003) and sweet potato (Mitsuwa et al., 2000) have also been reported. However, little is known about the salinity effect on the structure and biochemistry of chloroplasts in C\textsubscript{4} plant species.

Maize is an NADP-malic enzyme (NADP-ME) type of C\textsubscript{4} plant, which possesses two distinct types of chloroplasts, i.e., mesophyll cell (MC) and bundle sheath cell (BSC) chloroplasts with different structures and functions. MC chloroplasts have well-stacked thylakoids and both photosystem I (PS I) and photosystem II (PS II) activities, whereas BSC chloroplasts have few if any stacked thylakoids and low PS II activity (Malkins and Niyogy, 2000). ROS are produced in the chloroplast through non-cyclic photosynthetic electron transport which requires...
both PS I and PS II activities (Robinson, 1988). Due to deficiency in PS II activity, BSC chloroplasts in maize are specialized for cyclic electron transport and restricted for non-cyclic electron transport which is involved in generating NADPH and ROS (Doulis et al., 1997). Therefore, BSC chloroplasts may lack or show little ROS production and may be less sensitive to oxidative stress than MC chloroplasts. Comparison of damages caused by salinity between MC and BSC chloroplasts would provide insight into the involvement of ROS in salinity stress. In the present study, we examined the ultrastructural changes of MC and BSC chloroplasts in maize plants exposed to salinity stress under light and dark conditions. We also examined plant growth and the contents of chlorophyll, Na and K in leaves.

Materials and Methods

1. Plant materials
Maize (Zea mays L. ‘Golden Bantam’) seeds were washed with distilled water and allowed to imbibe in a beaker at 25°C for 24 h. Five seeds were planted in a 300 ml plastic pot filled with soil in a growth room and watered every two days. The growth room was controlled at 30/25°C (light/dark), relative humidity of about 70%, a 12-h photoperiod (6:00 - 18:00) and light intensity of 600 µm m⁻² s⁻¹. At the fifth day, three seedlings in each pot were selected and used for experiment, which was considered as one replicate. Each treatment was replicated five times. The salt treatment was started when the second leaf blades of the plants were fully developed (coleoptile segments were dried at 70°C for 48 h and weighed. Chloroplast structure was evaluated on the middle portion of the second leaf blade, which was compared with the control by Dunnett’s multiple comparison test. Data means were separated using F test and compared with the control by Dunnett’s multiple comparison test. Significant difference was analyzed based on P-value ≤ 0.05.

2. Transmission electron microscopy
For microscopic studies, the middle part of the second leaf blade of the plants was used. Sampling was done at 12:00, after six hours of illumination. Small portions (1×2 mm) of the leaf tissue were fixed in 5% glutaraldehyde in 0.05 M phosphate buffer (pH 7.2), post-fixed in 2% osmium tetroxide in the same buffer, dehydrated with a graded acetone series and propylene oxide, embedded in Spurr’s resin and polymerized at 70°C for 24 h. Chloroplast structure was evaluated on transverse ultrathin sections cut with a diamond knife on an Ultracut-N microtome (Reichert-Nissei, Austria). Sections were mounted on a 200 mesh grid, stained with uranyl acetate followed by lead citrate solution and examined with a transmission electron microscope (Hitachi H-7500, Tokyo, Japan) at 100 kV.

3. Na and K contents
A segment one cm in length was cut from the middle portion of the second leaf blade, which corresponds to the part for TEM observation. The segments were dried at 70°C for 48 h and weighed. Na and K were extracted with purified distilled water (Millipore SA 67120, Molshem, France) by shaking at room temperature for 70 h. Samples were centrifuged for 10 min, then Na and K contents of the extract were estimated with an atomic absorption flame spectrometer (Shimadzu AA 6400F, Shimadzu Co. Ltd.). The data were expressed on a dry weight basis.

4. Chlorophyll content
Chlorophyll content was analyzed at the same part of the leaves as for Na and K analyses. Chlorophyll was extracted with 100% ethanol by shaking at room temperature for 24 h in the dark and absorbance of the extract was read at 649 and 665 nm with a spectrophotometer (Ubest-50, Japan Spectroscopic Co. Ltd.). Thereafter, the leaf segments were dried at 70°C for 48 h and weighed. Chlorophyll content was calculated according to the formula of Knudson et al. (1977) and expressed on a dry weight basis.

5. Data analysis
Data on the plant growth and the contents of chlorophyll, Na and K were statistically analyzed with ANOVA. Data means were separated using F test and compared with the control by Dunnett’s multiple comparison test. Significant difference was analyzed based on P-value ≤ 0.05.

Results

1. Plant growth
The growth of plants exposed to salinity was severely suppressed under the light condition. After 5 d of treatment, entire blades of the second leaf blades of control and 1% NaCl-treated plants were still green, but the leaf tip of 2 and 3% NaCl-treated plants showed yellowing (data not shown). Fig. 1 shows the growth of the plant treated with four NaCl concentrations under light and dark conditions. Both shoot and root dry weights decreased with elevation of the concentration and duration of salt treatment in light. Shoot dry weight of the NaCl-treated plants was significantly lighter than that of the control plants under the light condition. Treatment with 2 and 3% NaCl for 3d (Fig. 1A) decreased the shoot dry weight by 39 and 46%, respectively, and that for 5 d by 45 and
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56%, respectively (Fig. 1B).

The effect of salinity on root dry weight was not as strong as that on the shoot. Although root dry weight of the plants grown in the light decreased with the increase of salt concentration, there were no significant differences between the plants treated with NaCl for 3 d and the control plants (Fig. 1C). The root dry weight of the plants after exposure for 5 d to 2 and 3% NaCl was 26 and 41% lighter than that of the control plants, respectively (Fig. 1D).

In the plants exposed to salinity in the dark, the basal portion of the youngest fully expanded leaf of control and 1% NaCl-treated plants showed etiolation. However, no significant differences were observed in the shoot dry weight between the plants treated with NaCl for 3 d and the control plants. The shoot growth was reduced by about 23 and 20% by treatment with 2 and 3% NaCl in the dark for 5 d, respectively. In contrast, salinity promoted the growth of root with the increase of salt concentration under the dark condition. Root dry weight of the plants treated with 3% NaCl for 3 and 5 d was about 20 and 15% heavier, respectively, than that of the control plants. Root growth seems to be promoted by endosperm consumption at the expense of shoot growth.

2. Na and K contents

Fig. 2 shows the effect of salinity on Na contents in
the second leaf blades of the plants treated with NaCl for 3 and 5 d under light and dark conditions. The Na contents of the leaves increased dramatically in the plants treated with 2 or 3% NaCl in the light, especially for 5 d. On the other hand, there were no significant differences in Na contents between plants treated with NaCl under the dark condition and the control plants, except in the plants treated with 3% NaCl for 5 d. These findings suggest that transpiration plays a role in the transport of Na to the shoot.

Fig. 3 shows the effect of salinity on K contents of the second leaf blades of the plants treated with NaCl for 3 and 5 d under light and dark conditions. K contents of the second leaf blades of the plants exposed to salinity under the light condition declined considerably with the increase of salt concentration. However, it was almost constant throughout the treatment under the dark condition.

3. Chlorophyll content

Fig. 4 shows the chlorophyll content of the second leaf blades of plants exposed to various concentrations of NaCl. After 3 d of treatment, salinity had no effect on the chlorophyll contents either under the light or dark condition. After 5 d of treatment under light condition, chlorophyll content significantly decreased with the elevation of salt concentration. However, under the dark condition, the chlorophyll content was relatively constant throughout the period of NaCl treatment.

4. Ultrastructure

High accumulation of toxic ions in the leaf tissue caused cytosolic degradation in the cells of the plants exposed to salinity under both the light and dark conditions. Cytosolic damage increased as the NaCl concentration increased, and was severer in MC (Fig. 5D) than in BSC (Fig. 6D) and under the light (Fig 5D) than under the dark (Fig. 7D) condition. Under
the light condition plasmolysis appeared in the BSC (Fig. 6B). In contrast, under the dark condition, both MC (Fig. 7D) and BSC (Figs. 8A-D) showed plasmolysis. In addition, under the dark condition, plasmolysis also appeared in the cells of control plant (Fig 8A), but was more noticeable with the increase of salt concentration and more prominent in BSC (Fig 8D) than in MC (Fig 7D).

The treatment with NaCl affected the chloroplast structure. Table 1 shows the data of quantitative analysis. Fig. 5 and 6 show the ultrastructure of MC and BSC chloroplasts in the second leaf blades in the plants treated with various NaCl concentrations in the light. The chloroplasts in MC and BSC of control plants were characterized by well-organized thylakoid membranes. MC chloroplasts possessed well-developed granal and stromal thylakoids and some starch granules (Fig. 5A). Meanwhile, the thylakoids of BSC chloroplasts were not appressed and the size and number of starch granules were large (Fig. 6A). There was no structural distortion in either MC or BSC chloroplasts in control plants. MC chloroplasts in 1% NaCl-treated plants were structurally similar to those in the control plants, but the thylakoid was slightly swollen (Fig. 5B, arrows). On the other hand, the structural damage was observed in MC chloroplasts of the plants treated with 2 and 3% NaCl. MC chloroplasts were more globular in shape and had swollen and disorganized
thylakoids with grana showing with reduced stacking thickness (Fig. 5C and 5D). The size of plastoglobules increased with the increase of salt concentration (Table 1). Meanwhile, BSC chloroplasts were less affected by salinity than MC chloroplasts in the light. Although the chloroplasts and starch granules were dramatically reduced in size with the increase of salt concentration (Table 1), the thylakoids and the envelope in BSC chloroplasts showed almost no structural distortion (Fig. 6D). Decrease in size of the BSC chloroplast is due to reduction of stromal area in addition to reduction of starch granules (Table 1).
1). Interestingly, salinity induced formation of granal stacking in BSC chloroplasts of the plants treated with 2 or 3% NaCl for 5 d (Fig. 6C and 6D). In the control plants, BSC chloroplasts commonly lack grana or have undeveloped grana which consist of less than four granal thylakoids. However, in the BSC chloroplasts of the plants treated with 3% NaCl, grana contained up to 16 granal thylakoids (Fig. 6D and Table 1).

Fig. 7 and 8 show the structures of MC and BSC chloroplasts in the second leaf blades of the plants treated with various concentrations of NaCl under the dark condition. There were no starch granules in either the MC or BSC chloroplasts of either the control or NaCl-treated plants. However, salinity does not seem to affect the entire structure of either MC (Fig. 7) or BSC (Fig. 8) chloroplasts, although the envelope of BSC chloroplasts was occasionally damaged (Fig. 8D). The thylakoids in MC chloroplasts were preserved even in the plants treated with 3% NaCl for 5 d in the dark (Fig. 7D) which accumulated even a higher concentration of Na in the leaf blades than did the plants treated with 1% NaCl for 5 d in the light (Fig. 2),

Fig. 7. MC chloroplasts in the middle part of mature second leaf blades in maize after treatment with NaCl at various concentrations for 5 d under dark condition. (A–D) MC chloroplasts of 0, 1, 2 and 3% NaCl-treated plants, respectively. Bars=1 μm.
Discussion

The effect of salt stress was more prominent in the light than in the dark. Na content of the shoot was higher in light probably due to transpiration, which leads to a high Na accumulation. The decrease in the chlorophyll content of the leaf blades due to a high accumulation of Na have been well recognized in many non-halophytes exposed to salinity (Hernandez et al., 1995; Singh and Dubai, 1995; Randjbarforduei et al., 2002). The present study, however, showed that salinity did not affect the chlorophyll content of leaf in the dark. A similar result was also obtained by Mitsuya et al. (2003) who assumed that the decrease in chlorophyll content caused by salinity stress was light dependent.

Salinity induces osmotic stress and ion toxicity (Munns and Termaat, 1986). It might cause the

in which MC chloroplasts had swollen thylakoids (Fig. 5B).

Fig. 8. BSC chloroplasts in the middle part of mature second leaf blades in maize after treatment with NaCl at various concentrations for 5-d under dark condition. A, B, C and D show BSC chloroplasts of 0, 1, 2 and 3% NaCl-treated plants, respectively. Bars = 1 µm.
Chloroplast damage caused by salinity was also prominent in light. Leaf blade of the plants treated with 3% NaCl for 5 d in the dark accumulated a comparable or larger amount of Na than the plants treated with 1% NaCl for 5 d in light (Fig. 2). However, the swelling of thylakoids appeared only in MC chloroplasts of the latter plants (Fig. 5B). The structure of both MC and BSC chloroplasts was preserved in the plants treated with NaCl in the dark (Fig. 7 and 8). The swelling of thylakoids in MC chloroplasts of the plants treated with NaCl in the light was accompanied by reducing the thickness of granal stacking, increasing the number and size of plastoglobules and decreasing the number and size of starch granules (Table 1). The increase in the number and size of plastoglobules is a typical indicator of leaf senescence and is connected with the degradation of thylakoids (Bondada and Oosterhuis, 1998). However, the decrease of starch granules in the chloroplasts was associated with various stresses and advancement of senescence (Kutik, et al. 2001). The present results suggest that the damages in MC chloroplasts caused by salt stress are light dependent and not due to direct effects of an excessive accumulation of Na in the leaf tissue. Mitsuya et al. (2003) also reported the light dependency and suggested the involvement of ROS in chloroplast damages induced by salinity in rice. Yamane et al. (2004) reported ameliorative effects of antioxidants and suggested the involvement of H$_2$O$_2$ and hydroxyl radical in the chloroplast damage.

Involvement of ROS in the damage of chloroplast is connected with the function of chloroplasts, which are considered as a powerful site of ROS production. Molecular oxygen can serve as an electron acceptor at the reducing site of PS I in the non-cyclic electron transport for producing ROS (Foyer, 1996; Polle, 1996; Robinson, 1998). In addition, ROS may also be formed at the acceptor site of PS II, as the result of passing electrons from quinone to O$_2$ (Ananyev et al., 1994). However, the mechanism of ROS generation under salt stress is not well understood. Under salt stress, plants reduce transpiration by closing the stomata, which consequently decreases the CO$_2$ concentration inside the chloroplasts (Hernandez et al., 1995). The lower concentration of CO$_2$ inside the chloroplasts reduces NADP$^+$ generation by the Calvin cycle (Biehler and Fock, 1996) and allows electrons from ferredoxin to reduce O$_2$ as an alternative acceptor (Navari-
Izzo et al., 1996) with the concomitant initiation of superoxide (\(O_2^-\)) formation in the chloroplasts (Hippeli et al., 1999; Dat et al., 2000). Thereafter, \(O_2^-\) can spontaneously initiate a chain reaction to produce hydrogen peroxide (\(H_2O_2\)) and hydroxyl radical (\(OH\)), a more toxic ROS (Hippeli et al., 1999; Vranova et al., 2000). These toxic compounds are suggested to induce oxidative stress, which causes the damage in chloroplasts in the plants exposed to salinity in light. However, the damage in chloroplasts is much more prominent under salinity stress than under drought stress although both stresses may cause stomatal closure (Yamane et al., 2003a; Yamane et al., 2003b).

Maize is an NADP-ME type \(C_4\) plant, which possesses two distinct types of chloroplasts, MC and BSC chloroplasts. MC chloroplasts have well-developed grana and both PS I and PS II activities. In contrast, BSC chloroplasts lack grana or possess grana with greatly reduced thickness (Dengler and Nelson, 1999; Taniguchi et al., 2003), which is correlated with a deficiency of PS II activity (Meierhoff and Westhoff, 1993; Malkin and Niyogy, 2000). Hence, the BSC chloroplasts show limited non-cyclic electron flow and reduced conversion of NADP\(^+\) to NADPH (Douilis et al., 1997) and therefore may have limited production of ROS. In addition, a spatial isolation from oxygen evolved in MC chloroplasts and its ROS may contribute to the lower sensitivity of BSC chloroplasts to salinity stress.

Development of granal stacking in BSC chloroplasts in the plants treated with 2 and 3% NaCl in light (Fig. 6C and 6D) may be an adaptation to conduct \(C_3\) photosynthesis when \(C_4\) photosynthesis is distorted due to severe damage in MC chloroplasts. It is known that maize have potency to conduct \(C_3\) photosynthesis (Lavergue et al., 1992). Another possibility is that salinity prevents the suppression of photosynthesis (Lavergue at al., 1992). Another possibility is that salinity prevents the suppression of photosynthesis (Lavergue at al., 1992). Another possibility is that salinity prevents the suppression of photosynthesis (Lavergue at al., 1992). Another possibility is that salinity prevents the suppression of photosynthesis (Lavergue at al., 1992). Another possibility is that salinity prevents the suppression of photosynthesis (Lavergue at al., 1992).

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References

Ananyev, G., Renger, G., Walker, U. and Klímov, V. 1994. The photoproduction of superoxide radicals and the superoxide-dismutase activity of Photosystem II – The possible involvment of cytochrome B559. Photosynth. Res. 41 : 327-338.

Beher, K. and Fock, H. 1996. Evidence for the contribution of the Mehler-peroxidase reaction in dissipating excess electrons in drought-stressed wheat. Plant Physiol. 112 : 265-272.

Bohnert, H.J., Nelson, D.E. and Jensen, R.G. 1995. Adaptations to environmental stresses. Plant Cell, 7 : 1099-1111.

Bondada, B. R. and Oosterhuis, D. M. 1998. Decline in photosynthesis as related to alterations in chloroplast ultrastructure of a cotton leaf during ontogeny. Photosynthetica 35 : 467-471.

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

Dengler, N.G. and Nelson, T. 1999. Leaf structure and development in \(C_4\) plants. In R.F. Sage and R.K. Monson eds., \(C_4\), Plant Biology. Academic Press, Sandiego. 153-164.

Douilis, A.G., Debia, N., Kingston-Smith, A.H. and Foyer, C.H. 1997. Differential localization of antioxidants in maize leaves. Plant Physiol. 114 : 1031-1037.

Flowers, T.J. and Yeo A.R. 1986. Ion relations of plant under drought and salinity. Aust. J. Plant Physiol. 13 : 75-91.

Flowers, T.J., Garcia, A, Koyama, M. and Yeo, A.R. 1997. Breeding for salt tolerance in crop plants. The role of molecular biology. Acta Physiol. Plant. 19 : 427-433.

Foyer, C.H. 1996. Oxygen processing in photosynthesis. Bioch. Soc. Trans. 24 : 427-432.

Gomez, J.M., Jimenez, A., Olmos, E and Sevilla, F., 2004. Location and effects of long-term NaCl stress on superoxide dismutase and ascorbate peroxidase isoenzymes of pea (\(Pisum sativum\) cv Puget) chloroplasts. J. Exp. Bot. 55 : 119-130.

Hasegawa, P.M., Bressan, R.A., Zhu, J.K. and Bohnert, H.J. 2000. Plant cellular and molecular responses to high salinity. Annu. Rev. Plant Physiol. Plant Mol. Biol. 51 : 463-499.

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

Hippeli, S., Heiser, I. and Elsner, E. 1999. Activated oxygen and free oxygen radicals in pathology: New insights and analogies between animals and plants. Plant Physiol. Biochem. 37 : 163-178.

Knudson, L.L., Tibbits, T.W. and Edward, G.E. 1977. Measurement of ozone injury by determination of leaf chlorophyll concentration. Plant Physiol. 60 : 606-608.

Kutik, J., Hola, D., Vicankova, A, Smidova, M., Kocova, M., Kornerova, M. and Kubirova, L. 2001. The heterogeneity of structural and functional photosynthetic characteristics of mesophyll chloroplast in various parts of mature and senescing leaf blade of two maize (\(Zea mays\) L.) genotypes.

Lavergue, D., Nato, A., Dupuis, J.-M., Péan, M. and Chagvardieff, P. 1992. Evidence for the expression of morphological and biochemical characteristics of \(C_4\)-photosynthesis in chlorophyllous callus cultures of \(Zea mays\). Plant Physiol. 84 : 292-300.

Malkin, R. and Niyogy, K.2000. Photosynthesis. In B. Buchanan, W. Grussrem and R. Jones eds., Biochemistry and Molecular Biology of The Plants. American Society on Plant Physiologists. Rockville, Maryland. 568-627.
McWilliam, J. R. 1986. The national and international importance of drought and salinity effects on agriculture production. Aust. J. Plant Physiol. 13 : 1-13.

Meierhoff, K. and Westhoff, P. 1993. Differential biogenesis of photosystem II in mesophyll and bundle-sheath cells of monocotyledonous NADP-malic enzyme-type C₄ plants: the non-stochiometric abundance of subunits of photosystem II in bundle sheath chloroplasts and the translational activity of the plastome-encode genes. Planta 191 : 23-33.

Meloni, D.A., Oliva, M.A., Martinez, C.A. and Cambaria, J. 2003. Photosynthesis and activity of superoxide dismutase, peroxide and glutathione reductase in cotton under salt stress. Environ. Exp. Bot. 49 : 69-76.

Mitsuya, S., Takeoka, Y. and Miyake, H. 2000. Effects of sodium chloride on foliar ultrastructure of sweet potato (*Ipomoea batatas* Lam.) plantlets grown under light and dark condition in vitro. J. Plant Physiol. 157 : 661-667.

Mitsuya, S., Kawasaki, M. Taniguchi, M. and Miyake, H. 2003. Light dependency of salinity-induced chloroplast degradation. Plant Prod. Sci. 6 : 219-223.

Munns, R. 2002. Comparative physiology of salt and water stress. Plant Cell Environ. 25 : 239-250.

Munns, R. and Termaat, A. 1986. Whole plant responses to salinity. Aust. J. Plant Physiol. 13 : 145-160.

Navari-Izzo, F., Quartacci, M.F. and Sgherri, C.M.L. 1996. Superoxide generation in relation to dehydration and rehydration. Biochem. Soc. Trans. 24 : 447-451.

Polle, A. 1996. Mehler reaction: Friend or foe in photosynthesis? Bot. Acta 109 : 84-89.

Randjbarforduei, A., Samson, R., Lemeur, R. and Van Damme, P., 2002. Effects of osmotic drought stress induced by a combination of NaCl and polyethylene glycol on leaf water status, photosynthesis gas exchange, and water use efficiency of *Pistacia khinjuk* and *P. mutica*. Photosynthetica 40 : 165-169.

Rahman, M.S., Matsumuro, T., Miyake, H. and Takeoka, Y. 2000. Salinity-induced ultrastructural alterations in leaf cell of rice (*Oryza sativa* L.). Plant Prod. Sci. 3 : 422-429.

Robinson, J.M. 1988. Does O₂ photoreduction occur within chloroplasts in vivo? Plant Physiol. 72 : 666-680.

Sairam, R.K. and Srivastava, G.C. 2002. Changes in antioxidant activity in sub-cellular fractions of tolerant and susceptible wheat genotypes in response to long term salt stress. Plant Sci. 162 : 897-904.

Singh, A.K. and Dubai, R.S. 1995. Changes in chlorophyll *a* and *b* contents and activities of photosystem I and 2 in rice seedlings induced by NaCl. Photosynthetica 31 : 489-499.

Taniguchi, Y., Taniguchi, M., Kawasaki, M. and Miyake, H. 2003. Strictness of the centrifugal location of bundle sheath chloroplasts in different NADP-ME type C₄ grasses. Plant Prod. Sci. 6 : 274-280.

Yamane, K., Kawasaki, M., Taniguchi, M. and Miyake, H. 2003a. Differential effect of NaCl and polyethylene glycol on the ultrastructure of chloroplasts in rice seedlings. J. Plant Physiol. 160 : 573-575.

Yamane, K., Hayakawa, K., Kawasaki, M., Taniguchi, M. and Miyake, H. 2003b. Bundle sheath chloroplasts of rice are more sensitive to drought stress than mesophyll chloroplasts. J. Plant Physiol. 160 : 1319-1327.

Yamane, K., Rahman, M.S., Kawasaki, M., Taniguchi, M. and Miyake, H. 2004. Pretreatment with a low concentration of methyl viologen decreases the effects of salt stress on chloroplast ultrastructure in rice. Plant Prod. Sci. 7 : 435-449.

Vranova, E., Inze, D. and Van Breusegem, F. 2002. Signal transduction during oxidative stress. J. Exp. Bot. 53 : 1227-1136.