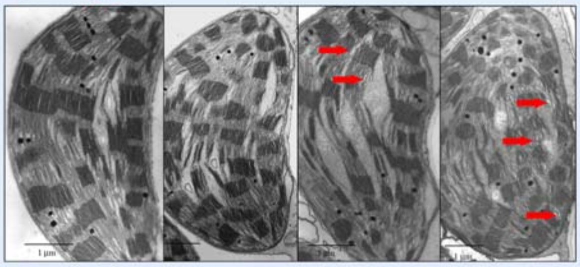
EFFECTS OF SALINITY STRESS ON THE ULTRASTRUCTURE OF DIMORPHIC CHLOROPLASTS IN MAIZE

(Rusdi Hasan, Michio Kawasaki, Mitsutaka Taniguchi and Hiroshi Miyake)

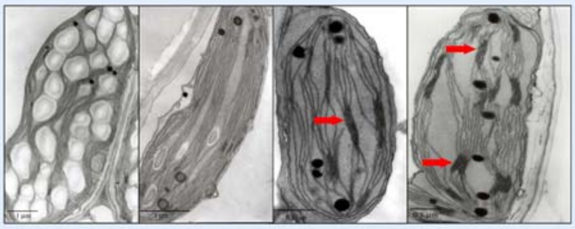
Effects of salinity on the ultrastructure of dimorphic chloroplasts in maize, an NADP-ME type of C_4 plants were studied. The study was undertaken into three sections. In the first study, effects of NaCl on the ultrastructure of chloroplast alteration, the plant growth and the contents of chlorophyll, Na and K were investigated. The maize plants 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. In the second study, the effects of salinity on granal development in BSC chloroplasts of maize and chlorophyll fluorescence parameter were investigated. The plants were grown in the soil media and after the second leaf was fully developed they were irrigated with four different concentrations (0, 1, 2 and 3%) of NaCl for 5 d. Ultrastructure, quantitative properties of chloroplasts and chlorophyll fluorescence parameters were evaluated. Granal stacking in BSC chloroplasts was induced by 2 and 3 % NaCl treatments. In contrast, granal stacking in mesophyll cell (MC) chloroplasts was reduced and disorganized due to swelling of thylakoid. In control plants, only 2% of grana in BSC chloroplasts contained more than three thylakiods. Meanwhile, in the plants treated with 3% NaCl, 66% of grana in BSC chloroplasts contained more than three thylakoids. Maximum number of thylakoids in grana of BSC chloroplasts was 4 and 16 for control and 3% NaCl-treated plants, respectively. Granal index in BSC chloroplasts of 3% NaCl-treated plants was more than three times higher than that of control. Chlorophyll fluorescence parameter analysis showed that the maximal quantum yield (Fv/Fm), the effective quantum yield of PSII (Φ_{PSII}) and PSII-driven electron transport rate (ETR) were decreased with the increase of salinity stress. These results suggest that the suppression mechanism of granal development in BSC chloroplasts of maize is influenced by salinity. In the third study, cellular localization of chloride and hydrogen peroxide (H_2O_2) in the leaf blades of maize plants exposed to 3% NaCl were investigated. This study was aimed to investigate whether differential sensitivity to salinity between MC and BSC in maize associated with differential accumulation of Cl and H_2O_2 in MC and BSC. Cl distribution was examined with X-ray microanalysis, whereas H_2O_2 was analyzed histochemicaly and cytochemicaly using DAB and $CeCl_3$. The present study shows that Cl accumulation in MC and BSC of the plants treated with NaCl was relatively comparable.

However, salinity-induced H_2O_2 formation in MC chloroplasts was higher compared with that in BSC chloroplasts. In addition, H_2O_2 accumulation was also detected in apoplast of MC and BSC with the greatest accumulation was detected in cell walls of MC facing to intercellular space. These results suggest that differential sensitivity between MC and BSC chloroplasts to salinity are not caused by differential cellular Cl accumulation, but may be associated with differential contents of H_2O_2 in those chloroplasts which are generated in response to comparable cellular accumulation of Cl in MC and BSC.

TEM micrographs of MC chloroplasts in the second leaf blade of maize plants after treatment with NaCl for 3 d in light condition.



TEM micrographs of BSC chloroplasts in the second leaf blade of maize plants after treatment with NaCl for 5 d in light condition.



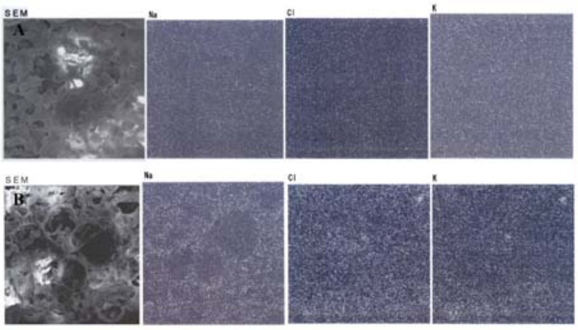


Figure [redacted] Images of Na, Cl and K distributions on transverse sections of leaf blade in maize. A. Control. B. 3% NaCl.

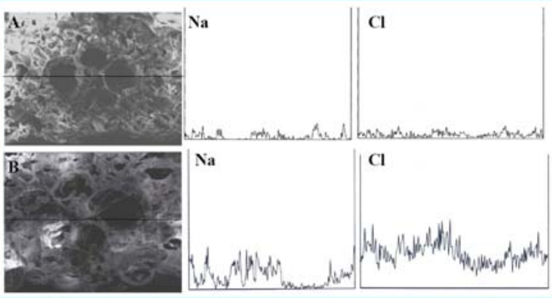


Figure [redacted] Distributions of Na, and Cl from line scanning on transverse sections of leaf blade in maize. A. Control. B. 3% NaCl.

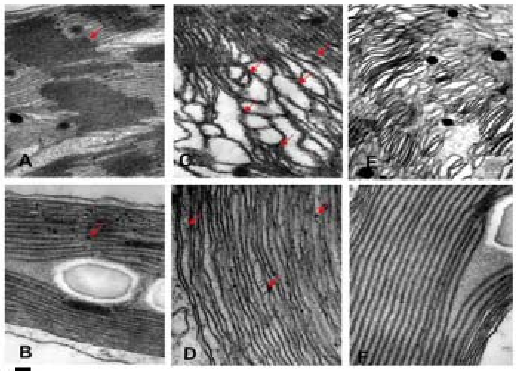


Figure [redacted]
H_2O_2 localization (arrows) in the chloroplasts. A. Mesophyll chloroplast of control plant. B. Bundle sheath chloroplast of control plant. C. Mesophyll chloroplast of 3% NaCl-treated plant. D. Bundle sheath chloroplast of 3% NaCl-treated plant. E,F. Mesophyll and bundle sheath chloroplasts of 3% NaCl-treated plant without $CeCl_3$ staining.

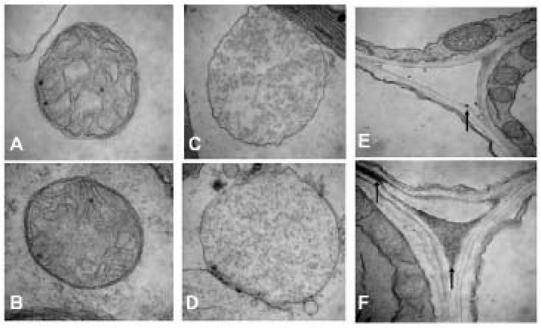


Figure [redacted]
A. Mitochondria of bundle sheath cell from 3% NaCl-treated plant. B. Mitochondria of mesophyll cell from 3% NaCl-treated plant. C. Peroxisome of bundle sheath cell from 3% NaCl-treated plant. Mesophyll chloroplast of 3% NaCl-treated plant. D. Peroxisome of mesophyll cell from 3% NaCl-treated plant. E. Cell wall and intercellular space of control plant. F. Cell wall and intercellular spaces of 3% NaCl-treated plant. Arrows, H_2O_2 accumulation.

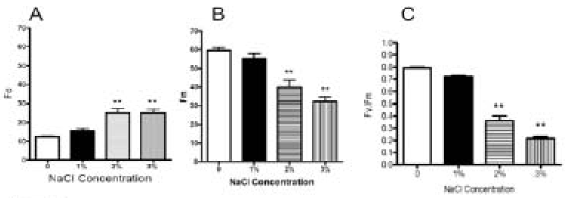


Figure [redacted]
Chlorophyll fluorescence parameters of control and NaCl-treated plant show Fo, Fm and Fv/Fm. Fo = Dark-adapted yield of chlorophyll fluorescence. Fm = Maximum yield of chlorophyll fluorescence. Fv = Fm - Fo. Asterisks = significantly different with control (p<0.01).

Na content in the second leaf of maize plants after treatment with various concentrations of NaCl for 3 and 5 d under light and dark conditions.