Salinity Induces Granal Development in Bundle Sheath Chloroplasts of NADP-Malic Enzyme Type C_4 Plants

Eiji Omoto, Michio Kawasaki, Mitsutaka Taniguchi and Hiroshi Miyake

(Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan)

Abstract: In NADP-malic enzyme (NADP-ME) type C_4 plants, MC chloroplasts have well-developed grana, whereas BSC chloroplasts are generally characterized by highly reduced grana. In the previous study, salt treatment induced granal development in BSC chloroplasts of Zea mays, an NADP-ME type C_4 plant. Therefore, we examined the effects of salinity stress on the granal structure of BSC chloroplasts in seven other C_4 species belonging to the NADP-ME type. The plants were grown in soil and after a certain period of time, they were treated with 3% NaCl for 5 d. Ultrastructure and quantitative properties of chloroplasts at the middle part of leaf tissues were investigated. In BSC chloroplasts of all the C_4 species, almost no structural damage was observed, but the development of granal stacking was induced under salinity condition. Granal indices and appressed thylakoid density of BSC chloroplasts in the salt-treated plants were higher than those in the control plants. In all the species, the structure of MC chloroplasts was more or less damaged by salt stress; thylakoids were swollen and chloroplast envelope was disorganized. These results suggest that the granal development in BSC chloroplasts and the high damage of MC chloroplasts are common features of NADP-ME type C_4 plants under salinity stress.

Key words: Bundle sheath, C_4 plants, Chloroplast, Grana, Mesophyll, Salinity.

C_4 plants are assumed to have evolved from C_3 plants in response to the dramatic decrease of atmospheric CO_2 concentrations (Ehleringer et al., 1991, 1993; Edwards et al., 2001). These plants adapt to high light, arid and warm environments (Black, 1973) and show higher photosynthetic efficiency and lower photorespiration rate in comparison with C_3 plants by having a mechanism to concentrate CO_2 (Hatch, 1987). Moreover, it has been indicated that C_4 plants have high resistance to salinity stress (Osmond et al., 1982; Stepien and Klobus, 2005) and air pollution (Winner and Mooney, 1980). Thus, C_4 plants have many attractive traits for crops.

Leaves of C_4 plants contain two distinct types of photosynthetic cells, mesophyll (MC) and bundle sheath cells (BSC), which are quite different not only in structure but also in function. In these plants, CO_2 is initially fixed into C_4 acids in the MC, subsequently these compounds are transported to the inner BSC, where they are decarboxylated by decarboxylation enzymes, and the released CO_2 is refixed by Rubisco in the C_3 pathway (Hatch, 1987). Based on the difference of major decarboxylation enzyme in BSC, C_4 plants are classified into three biochemical subtypes: NADP-malic enzyme (NADP-ME), NAD-malic enzyme (NAD-ME) and phosphoenolpyruvate carboxykinase (PEP-CK) types (Gutierrez et al., 1974; Hatch et al., 1975). Most of the C_4 crop species belong to the NADP-ME type which possesses structurally dimorphic chloroplasts. In this subtype, MC chloroplasts have well-developed grana, whereas BSC chloroplasts generally lack grana and have extensive stroma-exposed thylakoids (Ghirardi and Melis, 1983; Taniguchi et al., 2003).

High salt level in soil is one of the most serious environmental problems in agriculture throughout the world (Dhaliwal and Arora, 1999). The most common salt composition of saline soil is NaCl (Cumming and Elliot, 1991). When plants are subjected to high NaCl, their growth is suppressed as a consequence of several physiological changes including ion balance, water status, mineral nutrition, osmotic pressure, stomatal behavior, photosynthetic efficiency and carbon allocation and utilization (Flowers et al., 1977; Greenway and Munns, 1980; Munns and Termaat, 1986; Niu et al., 1995; Hasegawa et al., 2000; Munns, 2002, 2005) although the salt sensitivity is different among species (Bor et al., 2003).

Chloroplasts have been known as an organelle which is one of the most sensitive to various stress factors. Several studies with regard to the effects of excessive NaCl on the structure of the chloroplasts have been reported in some C_4 plants such as rice (Rahman et al., 2000; Mitsuwa et al., 2003; Yamane et al., 2003) and sweet potato (Mitsuwa et al., 2000), and according to these reports the main damage to the chloroplasts caused by salt stress were swelling of thylakoids and...
destruction of chloroplast envelope. Meanwhile, only a few studies have dealt with C₄ plants. Hasan et al. (2005) and Barhoumi et al. (2007) reported that MC chloroplasts were more sensitive to salinity stress than BSC. Moreover, Hasan et al. (2005, 2006) revealed that salinity stress induced granal development in BSC chloroplasts of Zea mays, an NADP-ME type plant. However, this finding is reported only in Z. mays and it is not known whether other NADP-ME type C₄ species also show the same response.

It is important to know the structural changes of chloroplasts caused by salinity because chloroplasts have crucial influence on the crop productivity through the photosynthetic function. Therefore, in the present study we examined the effects of salinity stress on the granal structure of BSC chloroplasts in seven C₄ species belonging to NADP-ME type.

Materials and Methods

1. Plant materials

Seven C₄ species were used; Echinochloa utilis Ohwi et Yabuno cv. King Millet, Eleusine coracana L. var. L. Hack, Erianthus aristideae F. Muell, Paspalum notatum Flugge and Sorghum bicolor (L.) Moench belonging to Poaceae (monocots), and Gomphrena globosa L. and Portulaca grandiflora Hook, which are dicots. All these species belong to NADP-ME type (Sage et al., 1999). Seeds were surface sterilized with 5% sodium hypochlorite solution for 3 min. After washing with distilled water, seeds were imbibed in petri dish containing a little distilled water in a culture room at 25°C until the tip of coleoptile or cotyledon appears. Following germination, seedlings were planted in 300 mL plastic pots filled with soil and irrigated with tap water in a growth chamber. The growth chamber was controlled at 30/25°C (light/dark), relative humidity of 70%, a 12-h photoperiod and light intensity of 600 µmol m⁻² s⁻¹. In the monocot species, the salt treatment was started when the fourth leaf blades were fully developed by supplying 50ml of 3% NaCl solution every day. The dicot species were also treated with 3% NaCl from 5 wk after sowing. Control plants were supplied with tap water. After salt treatment for 5 d, the plants were harvested.

2. Measurement of growth and chlorophyll content

Growth was analyzed by measuring the dry weight of shoots. For measurement of chlorophyll content, the middle part of fourth leaf blades in monocots and the middle part of the fully developed uppermost leaves at the start of salt treatment in dicots were used. Chlorophyll content was assayed according to Kundson et al. (1977).

3. Transmission electron microscopy (TEM)

Electron microscopic studies were made using the same part of the leaves as that for chlorophyll content analysis. Small segments (about 1 x 2 mm) of the leaf tissues were fixed in 5% glutaraldehyde in 0.05 M phosphate buffer (pH 7.2) and post-fixed in 2% osmium tetroxide in the same buffer. Specimens were dehydrated in a graded acetone series (30%, 50%, 70%, 90%, 99% and 100%) and propylene oxide, and embedded in Spurr’s resin (Spurr, 1969). Chloroplast structure was evaluated on transverse ultrathin sections (75–90 nm) cut with a diamond knife on an ultramicrotome. Sections were mounted on a 200 mesh copper grid and double stained with 2% uranyl

---

**Fig. 1.** Effect of 3% NaCl treatment for 5 d on the relative shoot dry weight of seven C₄ species belonging to NADP-ME type. Control values were 282.8±13.1 (Echinochloa utilis), 162.5±10.3 (Eleusine coracana var. L. Hack, Erianthus aristideae F. Muell, Paspalum notatum Flugge and Sorghum bicolor (L.) Moench belonging to Poaceae (monocots)), 113.5±9.5 (Gomphrena globosa L. and Portulaca grandiflora Hook, which are dicots). Values are means ±SE from 7 samples. ** indicates significant difference from control at P<0.01.

**Fig. 2.** Effect of 3% NaCl treatment for 5 d on the relative chlorophyll content of the middle part of leaf blades in seven C₄ species belonging to NADP-ME type. Chlorophyll content was measured as mg g⁻¹ DW and converted to relative value. Control values were 37.1±0.6 (Echinochloa utilis), 26.1±0.8 (Eleusine coracana var. L. Hack, Erianthus aristideae F. Muell, Paspalum notatum Flugge and Sorghum bicolor (L.) Moench belonging to Poaceae (monocots)), 34.9±0.7 (Gomphrena globosa L. and Portulaca grandiflora Hook, which are dicots). Values are means ±SE from 7 samples. ** indicates significant difference from control at P<0.01.
acetate for 20 min followed by lead citrate solution for 4 min. Then the sections were examined with a transmission electron microscope (Hitachi H-7500) at 100kV.

4. Quantitative parameters of BSC chloroplast properties

The length of appressed and non-appressed thylakoids, the number of thylakoids per granum, stromal and starch area were estimated with an ImageJ program, a free, Java-based image-processing package (Rasband, 1997-2007; Tsutsumi et al., 2008). Granal index represents a percentage of the length of all appressed thylakoid membranes to the total length of all thylakoid membranes. The frequency distribution of thylakoid number per granum in BS chloroplasts was investigated according to Hasan et al. (2006). Stromal area and appressed thylakoid density were calculated as the total area of the chloroplast minus the area of starch granules and the length of appressed thylakoid membranes per stromal area, respectively. In each species, at least 7 BSC chloroplasts from three different plants were investigated.

5. Statistical analysis

Data obtained from the experiments were statistically analyzed by ANOVA and Scheffe’s test. All values were expressed as mean ± SE.

Results

1. Plant growth

Fig. 1 shows the growth responses to salt stress of seven plant species. These data clearly indicate that the shoot dry weight was severely suppressed by salinity although salt sensitivity was different among the species. After 5 d of salt treatment, the most severely inhibited species was P. notatum, whereas the most insusceptible species was E. aristidea of which shoot dry weights were decreased by 71 and 29%, respectively. In the other species, the shoot dry weights decreased by 40-60%. In all the species except P. grandiflora, leaf chlorosis was caused by salt treatment especially in older leaves (data not shown). The leaves and stems of P. grandiflora exhibited dark green, bluish green or purplish colors under salt treatment (data not shown).

2. Chlorophyll content

Fig. 2 shows the effect of salt stress on chlorophyll content of the middle part of leaf tissues. After 5 d of salt treatment, the relative chlorophyll content significantly decreased in E. utilis, E. ophiuroides, E. aristidea, G. globosa, P. notatum and S. bicolor, while it increased by 14% in P. grandiflora.

3. Chloroplast ultrastructure

Changes in chloroplast structure in response to salinity were analyzed by TEM. In all the C₄ species, the chloroplasts of MC in control plants showed an elliptical shape and possessed typical well-developed grana which were aligned orderly (Figs. 3–9, A). On the other hand, structural damage was observed in MC chloroplasts of salt-treated plants although the degree of damage differed with the species (Figs. 3–9, B). The thylakoids were often swollen (Figs. 3B, 5B, 7B, 8B and 9B) and the chloroplast-envelope was more or less disrupted (Figs. 4–7, B). In addition, undulating thylakoids were observed in many species (Figs. 3B, 5B, 7B, 8B and 9B). In E. ophiuroides (Fig. 4B) and P. notatum (Fig. 6B), the structure of thylakoid membranes was less damaged by salinity than in the other species.

In all the C₄ species, the chloroplasts of BSC in control plants showed a typical structure of NADP-ME type whose thylakoids were scarcely appressed and grana were rudimentary (Figs. 3–9, C). In BSC chloroplasts in the salt-treated plants, almost no structural damage was observed (Figs. 5–9, D) although some unusual structures were seen in S. bicolor (Fig. 7D) and P. grandiflora (Fig. 9D). The stromal region without thylakoids was increased in S. bicolor (Fig. 7D). Whorling of thylakoids was observed in P. grandiflora (Fig. 9D). In addition, the development of granal stacking was induced by salinity especially in E. ophiuroides, P. notatum, S. bicolor, G. globosa and P. grandiflora (Figs. 4D, 6D, 7D, 8D and 9D).

Fig. 3. Ultrastructure of chloroplasts in the middle part of mature fourth leaf blades in Echinochloa utilis after 3% NaCl treatment for 5 d. (A) MC chloroplast of control plant. (B) MC chloroplast of 3% NaCl-treated plant. (C) BSC chloroplast of control plant. (D) BSC chloroplast of 3% NaCl-treated plant. Solid arrows show swelling of thylakoids. Bars = 0.5 μm.
Fig. 4. Ultrastructure of chloroplasts in the middle part of mature fourth leaf blades in *Eremochloa ophiuroides* after 3% NaCl treatment for 5 d. (A) MC chloroplast of control plant. (B) MC chloroplast of 3% NaCl-treated plant. (C) BSC chloroplast of control plant. (D) BSC chloroplast of 3% NaCl-treated plant. Dashed arrow shows disruption of envelope. Bars = 0.5 μm.

Fig. 5. Ultrastructure of Chloroplasts in the middle part of mature fourth leaf blades in *Eriachne aristidea* after 3% NaCl treatment for 5 d. (A) MC chloroplast of control plant. (B) MC chloroplast of 3% NaCl-treated plant. (C) BSC chloroplast of control plant. (D) BSC chloroplast of 3% NaCl-treated plant. Solid and dashed arrows show swelling of thylakoids and disruption of envelope, respectively. Bars = 0.5 μm.

Fig. 6. Ultrastructure of chloroplasts in the middle part of mature fourth leaf blades in *Paspalum notatum* after 3% NaCl treatment for 5 d. (A) MC chloroplast of control plant. (B) MC chloroplast of 3% NaCl-treated plant. (C) BSC chloroplast of control plant. (D) BSC chloroplast of 3% NaCl-treated plant. Dashed arrows show disruption of envelope. Bars = 0.5 μm.

Fig. 7. Ultrastructure of chloroplasts in the middle part of mature fourth leaf blades in *Sorghum bicolor* after 3% NaCl treatment for 5 d. (A) MC chloroplast of control plant. (B) MC chloroplast of 3% NaCl-treated plant. (C) BSC chloroplast of control plant. (D) BSC chloroplast of 3% NaCl-treated plant. Solid and dashed arrows show swelling of thylakoids and disruption of envelope, respectively. Bars = 0.5 μm.
4. Quantitative properties of BSC chloroplasts

We examined the quantitative properties to evaluate the granal development in BSC chloroplasts (Fig. 10 and Table 1). Fig. 10 shows the relative distribution of thylakoid number per granum in BSC chloroplasts of seven C₄ species. In control chloroplasts of all the species, most grana had two thylakoids and the percentage of grana tended to decrease with increasing number of thylakoids. Meanwhile, the percentage of grana with two thylakoids was significantly reduced and the percentage with more than four thylakoids was markedly increased in salt-treated plants as compared with control.

In all species, the number of thylakoids per granum in salt-treated plants was significantly higher than that in the control. However, the degree of increase of granal stacking by salinity varied with the species. The granal stacking of *E. ophiuroides*, *P. notatum* and *S. bicolor* was markedly increased, while that of *E. utilis* was hardly increased (Table 1). Granal indices and appressed thylakoid density of BSC chloroplasts were also higher in salt-treated plants than in the control (Table 1). In control chloroplasts, the granal indices in two species, *E. ophiuroides* and *S. bicolor*, were considerably lower than those in the other *C₄* species. However, the granal indices in these species were dramatically increased by salt treatment (approximately fourteen and twelve times, respectively) (Table 1). On the other hand, the granal indices in *E. utilis*, *E. aristidea*, *G. globosa*, *P. notatum* and *P. grandiflora* were also significantly increased by salinity although the degree of increase was not so much as *E. ophiuroides* or *S. bicolor* (Table 1). Appressed thylakoid density of *E. ophiuroides* was conspicuously increased by salinity, whereas that in *E. utilis* was hardly increased. Stromal and starch areas in the salt-treated plants basically decreased in all the species, but the stromal area in *E. utilis* slightly increased although no significant difference was shown.

**Discussion**

The 3% NaCl treatment for 5 d reduced the shoot growth in all the *C₄* species (Fig. 1). A previous study showed that the shoot dry weight of *Z. mays* belonging to NADP-ME type *C₄* plant decreased roughly 50% under the same salt condition as in the present study (Hasan et al., 2005). In all the species except for *P. grandiflora*, the chlorophyll content of the leaf tissues was decreased by salt treatment (Fig. 2). The decrease of chlorophyll content by salt stress has been well recognized in many plants (Hernandez et al., 1995; Mitsuya et al., 2002; Hasan et al., 2005), and was considered as one of the indicators of salinity stress (Chen et al., 1998). However, the chlorophyll content of *P. grandiflora* was significantly higher in salt-treated plants than in the control (Fig. 2). We suggest that the
chlorophyll in this plant was possibly less affected by salt stress than biomass, hence the salt-treated plant might have an apparently higher chlorophyll content per biomass than the control.

The chloroplasts of MC were more sensitive to salt stress than those of BSC. Treatment with NaCl induced severe damage such as the swelling of thylakoids and the disruption of envelopes in MC chloroplasts (Figs. 3–9, B), whereas almost no structural damage was observed in BSC chloroplasts (Figs. 3–9, D). Since similar responses have also been observed in Z. mays (Hasan et al., 2005), BSC chloroplasts of the NADP-ME type C₄ plant were considered to be less sensitive to salt stress than MC chloroplasts. However, whorling of thylakoids was seen in BSC chloroplasts of P. grandiflora subjected to salt stress (Fig. 9D).
Similar images have also been reported in bean plants subjected to chilling stress under light condition (Wise et al., 1983). With respect to the damage of MC chloroplasts under salt stress, Hasan et al. (2005) showed that the disorganization of MC chloroplasts of *Z. mays* was induced under the light condition, but was less induced under the dark condition. In addition, Mitsuya et al. (2003) reported that the structure of chloroplasts in rice treated with salinity was almost intact under the dark condition even if the leaf tissues accumulated a larger amount of Na and Cl than under the light condition. These authors suggested that the damage of chloroplasts was not due to direct effects of an excessive accumulation of Na in the leaf tissues. Therefore, the damage of chloroplasts caused by salinity is considered to be induced by reactive oxygen species (ROS).

ROS which include the superoxide anion (O$_2^-$), hydroxyl radical (·OH), singlet oxygen (¹O$_2$) and hydrogen peroxide (H$_2$O$_2$) are toxic substances, and their generation is promoted by salinity stress (Hernandez et al., 1995, 2001; Rios-Gonzales et al., 2002; Yamane et al., 2004). Moreover, Yamane et al. (2004) suggested that H$_2$O$_2$ and ·OH are responsible for the deleterious effects of salt stress on chloroplast ultrastructure in rice. Chloroplasts are one of the major sites for ROS production in plant cell (Dat et al., 2000; La Rocca et al., 2001; Pechova et al., 2003); and, therefore especially sensitive to ROS damage (Foyer et al., 1994). In chloroplasts, H$_2$O$_2$ and ·OH are suggested to be mainly generated around photosystem II(PSII) (Yamane et al., 2004), which is predominantly located in the stacked region of grana. The grana of BSC chloroplasts in NADP-ME type C$_4$ plants are generally reduced (Ghirardi and Melis, 1983; Taniguchi et al., 2003), and the PSII activity of BSC chloroplasts is low (Schuster et al., 1985; Malkin and Niyogy, 2000; Romanowska and Drozak, 2006). Therefore, BSC chloroplasts seem to be less active in the production of H$_2$O$_2$ and ·OH and less affected by salinity than MC chloroplasts.

Quantitative data showed that salinity induced the granal development of BSC chloroplasts in all the species examined (Fig. 10 and Table 1). Since a similar tendency has also been reported in *Z. mays* by Hasan et al. (2005, 2006), the granal development of BSC chloroplasts under salt condition may be a common response in NADP-ME type C$_4$ plant species in both

| Table 1. Parameters of granal development in BSC chloroplasts of seven C$_4$ species belonging to NADP-ME type. |
| --- |
| **Species** | **No. of thylakoids per granum** | **Granal index (%)** | **Appressed thylakoid density (µm µm$^{-2}$)** | **Stromal area (µm$^2$)** | **Starch area (µm$^2$)** |
| **Control** |
| Monocot | | | | | |
| *Echinochloa utilis* | 3.3±0.1 | 16.3±0.7 | 3.4±0.3 | 30.2±4.3 | 0.8±0.5 |
| *Eremochloa atheroides* | 2.6±0.1 | 1.5±0.1 | 0.3±0.0 | 20.5±1.3 | 1.5±0.2 |
| *Eriachne aristidea* | 3.2±0.1 | 21.1±0.9 | 2.3±0.4 | 33.8±1.3 | 5.2±0.7 |
| *Paspalum notatum* | 3.4±0.1 | 19.1±1.0 | 4.7±0.7 | 23.2±0.7 | 3.1±0.3 |
| *Sorghum bicolor* | 2.2±0.0 | 1.1±0.1 | 0.2±0.0 | 26.9±1.5 | 2.1±0.7 |
| Dicot | | | | | |
| *Gomphrena globosa* | 2.3±0.1 | 19.2±0.8 | 3.5±0.4 | 13.4±0.8 | 0.8±0.2 |
| *Portulaca grandiflora* | 2.4±0.0 | 16.5±0.8 | 2.6±0.4 | 20.5±1.4 | 0.6±0.3 |
| **3%NaCl** |
| Monocot | | | | | |
| *Echinochloa utilis* | 3.7±0.1** | 24.9±2.0* | 3.9±0.2 | 32.4±0.9 | 0.0±0.0** |
| *Eremochloa atheroides* | 4.7±0.1** | 21.5±0.8** | 3.5±0.2** | 11.9±0.7** | 0.0±0.0** |
| *Eriachne aristidea* | 4.4±0.1** | 29.8±2.5* | 5.0±0.5** | 19.7±1.5** | 0.0±0.0** |
| *Paspalum notatum* | 5.6±0.1** | 36.1±1.0** | 6.4±0.6* | 17.0±1.2** | 0.6±0.3** |
| *Sorghum bicolor* | 4.8±0.2** | 12.7±1.9** | 1.7±0.5** | 13.0±0.9** | 0.0±0.0** |
| Dicot | | | | | |
| *Gomphrena globosa* | 3.6±0.1** | 30.9±1.9** | 4.6±0.3* | 8.8±0.3** | 0.6±0.1 |
| *Portulaca grandiflora* | 3.4±0.1** | 28.0±0.6** | 4.3±0.5* | 10.5±0.7** | 0.2±0.1 |

Granal index, the percentage of the length of all appressed thylakoid membranes to the total length of all thylakoid membranes in BSC chloroplasts. Appressed thylakoid density, the length of appressed thylakoid membranes per stromal area. Stromal and starch areas represent mean area per chloroplast. Values are means ± SE from 7 BSC chloroplasts. * and ** indicate significant differences from control at P<0.05 and P<0.01, respectively.
monocots and dicots. However, there was no relation between the degree of increase in granal stacking of BSC chloroplasts (Table 1) and the degree of decrease in shoot dry weight (Fig. 1) or chlorophyll content (Fig. 2) by salinity. Consequently, these findings indicated that the difference in salt tolerance was not a factor of interspecific difference in the granal development of BSC chloroplasts under salt condition. Garab and Mustárdy (1999) described that grana possess significant flexibility, which is essential for optimizing the photosynthetic machinery under various environmental conditions. Many reports described the development of granal stacking in C₃ plants by various environmental factors, such as NaCl treatment of isolated chloroplasts (Barber, 1982), desiccation (Navari-Izzo et al., 2000), low light intensity (Rozak et al., 2002), excess Cu (Bernal et al., 2006) and heat shock treatment (Kislyuk et al., 2007). In this study, the granal development in BSC chloroplasts was accompanied by the damages of MC chloroplasts. Hasan et al. (2006) considered that the granal development in BSC chloroplasts might contribute to increase PSII activity of BSC in salt-treated plants because the photosynthetic activity in MC may be diminished due to the structural damages, and might be an adaptive response to salinity stress. Hasan et al. (2006) also proposed that salinity stress might remove the suppression of granal development in BSC chloroplasts of NADP-ME type C₄ plants.

Some investigators presented possible interpretations about the mechanisms of granal formation. Barber (1982) reported that the salt-induced grana formation in isolated chloroplasts was caused by electrostatic screening which weakened repulsive forces between the membrane surfaces. Navari-Izzo et al. (2000) suggested that the rise in the protein-to-lipid ratio may play a role in the increase of granal stacking. However, the mechanism of granal development induced by various stresses is not fully understood. Detailed analyses such as investigation on the change in gene expression involved in granal constitution are needed to clarify the mechanism of granal development.

References

Barber, J. 1982. Influence of surface changes on thylakoid structure and function. Annu. Rev. Plant Physiol. 33 : 261-295.

Barhoumi, Z., Djebari, W., Chaibi, W., Abdelly, C. and Smaoui, A. 2007. Salt impact on photosynthesis and leaf ultrastructure of Aeluropus littoralis. J. Plant Res. 120 : 529-537.

Bernal, M., Ramiro, M.V., Cases, R., Picorel, R. and Yruela, I. 2006. Excess copper effect on growth, chloroplast ultrastructure, oxygen-evolution activity and chlorophyll fluorescence in Glycine max cell suspensions. Physiol. Plant. 127 : 312-325.

Black, C.C., Jr. 1973. Photosynthetic carbon fixation in relation to net CO₂ uptake. Annu. Rev. Plant Physiol. 24 : 253-286.

Bor, M., Ozdemir, F. and Türkân, I. 2003. The effects of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet Beta vulgaris L. and wild beet Beta maritima L. Plant Sci. 164 : 77-84.

Chen, D.M., Keiper, F.J. and De Filippis, L.F. 1998. Physiological changes accompanying the induction of salt tolerance in Eucalyptus microcorys shoots in tissue culture. J. Plant Physiol. 152 : 555-563.

Cumming, R.W. and Elliot, G.L. 1991. Soil chemical properties. In P.E.V. Charman and B.W. Murphy eds., Soils: Their Properties and Management. Sydney University Press, Melbourne. 193-205.

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

Dhalwal, G.S. and Arora, R. 1999. Stresses in agroecosystems: concepts and approaches. In G.S. Dhalwal and R. Arora eds., Environmental Stress in Crop Plants. Ajay Verma Commonwealth Publishers, New Delhi. 1-18.

Edwards, G.E., Furbank, R.T., Hatch, M.D. and Osmond, C.B. 2001. What does it take to be C₂ lessons from the evolution of C₃ photosynthesis. Plant Physiol. 125 : 46-49.

Ehleringer, J.R., Sage, R.F., Flanagan, L.B. and Pearcy, R.W. 1991. Climate change and the evolution of C₄ photosynthesis. Trends Ecol. Evolut. 6 : 95-99.

Ehleringer, J.R. and Monson, R.K. 1993. Evolutionary and ecological aspects of photosynthetic pathway variation. Annu. Rev. Ecol. System. 24 : 411-439.

Flowers, T.J., Troke, P.F. and Yeo, A.R. 1977. The mechanisms of salt tolerance in halophytes. Annu. Rev. Plant Physiol. 28 : 89-121.

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

Garab, G. and Mustárdy, L. 1999. Role of LHCII-containing macromdomains in the structure, function and dynamics of grana. Aust. J. Plant Physiol. 26 : 649-658.

Ghirardi, M.L. and Melis, A. 1983. Localization of photosynthethic electron transport components in mesophyll and bundle sheath chloroplasts of Zea mays. Arch. Biochem. Biophys. 224 : 19-28.

Greenway, H. and Munnis, R. 1980. Mechanisms of salt tolerance in non-halophytes. Annu. Rev. Plant Physiol. 31 : 149-190.

Gutierrez, M., Gracen, V.E. and Edwards, G.E. 1974. Biochemical and cytological relationships in C₄ plants. Planta 119 : 279-300.

Hasan, R., Ohnuki, Y., Kawasaki, M., Taniguchi, M. and Miyake, H. 2005. Differential sensitivity of chloroplasts in mesophyll and bundle sheath cells in maize, an NADP-malic enzyme-type C₄ plant, to salinity stress. Plant Prod. Sci. 8 : 567-577.

Hasan, R., Kawasaki, M., Taniguchi, M. and Miyake, H. 2006. Salinity stress induces granal development in bundle sheath chloroplasts of maize, an NADP-malic enzyme-type C₄ plant. Plant Prod. Sci. 9 : 256-265.

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.

Hatch, M.D., Kagawa, T. and Craig, S. 1975. Subdivision of C₄ photosynthesis. Plant Physiol. 51 : 111-128.
Hatch, M.D. 1987. C4 photosynthesis: a unique blend of modified biochemistry, anatomy, and ultrastructure. Biochim. Biophys. Acta 895 : 81-106.

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

Hernandez, J.A., Ferrer, M.A., Jimenez, A., Ros Barcelo, A. and Sevilla, F. 2001. Antioxidant systems and O2•/-H2O2 production in the apoplast of pea leaves. Its relation with salt-induced necrotic lesions in minor veins. Plant Physiol. 127 : 817-831.

Kislyuk, I.M., Bubolo, L.S., Kamentseva, I.E., Kotlova, E.R. and Pechova, R., Kutik, J., Dhola, D., Kocova, M., Haisel, D. and Vicankova, A. 2003. Ultrastructure of chloroplasts, content of photosynthetic pigments, and photochemical activity of maize (Zea mays L.) as influenced by different concentrations of the herbicide amitrole. Photosynthetica 41 : 127-136.

Rasband, W.S. 1997-2007. ImageJ. U.S. National Institutes of Health, Bethesda, Maryland, USA. http://rabi.info.nih.gov/ij/.

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

Rios-Gonzales, K., Erdei, L. and Lips, S.H. 2002. The activity of antioxidant enzymes in maize and sunflower seedlings as affected by salinity and different nitrogen sources. Plant Sci. 162 : 923-930.

Romanowska, E. and Drozak, A. 2006. Comparative analysis of biochemical properties of mesophyll and bundle sheath chloroplasts from various subtypes of C4 plants grown at moderate irradiance. Acta Biochim. Pol. 53 : 709-719.

Rozak, P.R., Seiser, R.M. Wacholtz, W.F. and Wise, R.R. 2002. Rapid, reversible alterations in spinach thylakoid appression upon changes in light intensity. Plant Cell Environ. 25 : 421-429.

Sage, R.F., Li, M. and Monson, R.K. 1999. The taxonomic distribution of C4 photosynthesis. In R.F. Sage and R.K. Monson eds., C4 Plant Biology. Academic Press, San Diego. 551-584.

Schuster, G., Ohad, I., Martineau, B. and Taylor, W.C. 1985. Differentiation and development of bundle sheath and mesophyll thylakoids in maize. Thylakoid polypeptide composition, phosphorylation, and organization of photosystemII. J. Biol. Chem. 260 : 11866-11873.

Spurr, A.R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26 : 31-43.

Stepien, P. and Klobus, G. 2005. Antioxidant defense in the leaves of C4 and C3 plants under salinity stress. Physiol. Plant. 125 : 31-40.

Tanimuchi, Y., Tanimuchi, 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.

Tsutsui, K., Kawasaki, M., Tanimuchi, M. and Miyake, H. 2008. Gene expression and accumulation of Rubisco in bundle sheath and mesophyll cells during leaf development and senescence in rice, a C3 plant. Plant Prod. Sci. 11 : 336-343.

Winner, W.E. and Mooney, H.A. 1980. Ecology of SO2 resistance: III. Metabolic changes of C3 and C4 Atriplex species due to SO2 fumigations. Oecologia 46 : 49-54.

Wise, R. R., McWilliam, J. and Naylor, A.W. 1983. A comparative study of low-temperature-induced alterations of three species with differing chilling sensitivities. Plant Cell Environ. 6 : 525-535.

Yamane, K., Kawasaki, M., Tanimuchi, 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., Rahman, M.S., Kawasaki, M., Tanimuchi, M. and Miyake, H. 2004. Pretreatment with antioxidants decreases the effects of salt stress on chloroplast ultrastructure in rice leaf segments (Oryza sativa L.). Plant Prod. Sci. 7 : 292-300.