Light Dependency of Salinity-Induced Chloroplast Degradation

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Abstract: The contents of Na, K, Cl, chlorophyll and the foliar ultrastructure of rice seedlings grown in NaCl solution at various concentrations were investigated under light and dark conditions. The seedlings were first grown in water for 7 d under a light condition and then in NaCl solutions at various concentrations for 24 h under a light or dark condition. The Na and Cl contents in the 3rd leaves increased as the concentration of NaCl in the culture solution increased, and were significantly higher under a light condition than under a dark condition. The K content was scarcely influenced by the NaCl concentration under both conditions. The chlorophyll content in the 3rd leaves of the seedlings decreased as the NaCl concentrations of the culture solution increased under a light condition but not under a dark condition. In the 3rd leaves of the seedlings grown in the NaCl solution under a light condition, the thylakoids of chloroplasts in mesophyll cells were swollen and showed a wavy configuration. Under a dark condition, however, the thylakoids appeared intact under saline conditions although the leaves accumulated a large amount of Na and Cl than in a light condition. The present study suggests that the damages in the chloroplasts, such as a decrease in the chlorophyll content and the degradation of thylakoids, were caused by a light-dependent reaction and not directly by accumulation of excess salt.

Key words: Chlorophyll, Chloroplast, Cl content, Na content, Oxidative reaction, Rice, Salt stress, Ultrastructure.

Salinity affects 7% of the world's land surface (Szabolcs, 1994) and is one of the main limiting constraints to global agriculture. In those areas, plant growth was severely affected by salinity through water deficit, salt-specific damages (Munns and Termaat, 1986) or oxidative stress (Hernandez et al., 1995; Zhu, 2001). The salinity-induced decrease in photosynthetic activity (Sakamoto et al., 1998; Wang and Nii, 2000), inhibition of foliar growth (Hu and Schmidhalter, 1998) and ultrastructural changes (Mitsuya et al., 2000; Rahman et al., 2000) have been reported.

It is important to understand how the plants are damaged by salinity to increase their salt tolerance. Munns and Termaat (1986) concluded that salt injury is due to salt accumulation in transpiring leaves to the levels exceeding the ability of the cells to compartmentalize the salt into the vacuole. On the other hand, Tattini et al. (1995) reported that the salinity-induced decrease of photosynthesis in olive trees was due to the osmotic effect of the salt outside the roots, not to a specific effect of the salt in the leaves. In addition, in our previous study, the damages caused by salinity were not correlated with Na content in the tissue (Mitsuya et al., 2002). Therefore, it is still unclear how the plant cell is affected by salt stress and whether the damage is due to accumulation of excess salt or salt-induced secondary stress.

One of the notable effects of salinity is the degradation of the membranes of cell organelles (Mitsuya et al., 2000; Rahman et al., 2000). In particular, the thylakoids of the chloroplasts degrade rapidly under salt stress (Mitsuya et al., 2000). However, the membranes of etioplasts are scarcely damaged by salinity under dark conditions (Mitsuya et al., 2000). In the present study, to examine whether the degradation of the chloroplasts was caused by accumulation of excess salt, we cultured rice seedlings in solutions containing NaCl at various concentrations under a light or dark condition and examined the contents of some elements (Na, Cl and K) and chlorophyll and the ultrastructure of the 3rd leaves. We show that the degradation of the chloroplasts was caused by a light-dependent oxidative reaction and not directly by accumulation of excess salt.

Materials and Methods

1. Plant material

The seeds of rice (Oryza sativa L. cv. Nipponbare) were surface sterilized with 5% sodium hypochlorite solution for 5 min and thoroughly washed with distilled water. Then the seeds were allowed to imbibe in petri dishes containing distilled water in an incubator kept at about 28°C until the white coleoptile tips appeared. The uniformly germinated seeds were sown on plastic nets placed on the surface of 300 mL distilled water. After 7 d, the seedlings were transferred to the supporting net on the surface of 300 mL of 0, 0.4, 0.6, 0.8, 1.0 or 1.2 M NaCl solution (light condition) or 0, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2 or 2.4 M NaCl solution (dark condition) in tall beakers (500 mL). Then they were incubated in a growth chamber at 28°C under a light (under fluorescent white lamps at a light intensity of about 450 μmol m⁻² s⁻¹) at the plant...
Fig. 1. Na, Cl and K contents in the 3rd leaf blades of the seedlings cultured for 24 h in a solution containing NaCl at various concentrations under a light or dark condition. Data are means±SE (n=5). Vertical bars on each symbol represent SE. (a) Na content. (b) Cl content. Na and Cl contents in the NaCl-treated seedlings were significantly higher than the control (P<0.01). (c) K content. NaCl treatment under light (except at 0.6 M (P<0.05)) and dark conditions did not change the K content significantly.

Fig. 2. The relative chlorophyll content (µg g⁻¹ DW) in the 3rd leaf blades of the seedlings cultured for 24 h in a solution containing NaCl at various concentrations under light and dark conditions (100% = 2.03 ± 0.03 (light), 1.72 ± 0.03 (dark)). Data are means±SE (n=5). Vertical bars on each symbol represent SE. **, significantly different from the control at P<0.01.

3. Measurement of element (Na, K, Cl) contents in the 3rd leaves

The 3rd leaf blades of the seedlings were rinsed with distilled water and blotted dry. The dry weight was determined after they were dried at 70°C for 48 h and cooled in a desiccation chamber. They were extracted with distilled water at room temperature for 70 h. Contents of Na and K were measured with an atomic absorption spectrometer (Shimadzu Co., Ltd., AA-6400F) in the emission mode. Cl content was measured with a liquid chromatograph (Shimadzu Co., Ltd., LC-10AD). The data were expressed on a unit-dry-weight basis.

Data for chlorophyll content and element contents were statistically analyzed according to Duncan's multiple range test.

4. Transmission electron microscopy

For microscopic studies, small samples of the middle part of the 3rd leaf blades grown under light and dark conditions were fixed in Karnovsky's fixative (mixture of 4% paraformaldehyde and 2% glutaraldehyde in 0.05 M phosphate buffer (pH 7.2)) and post-fixed in 2% osmium tetroxide in the same buffer. The samples were dehydrated in a series of graded acetone and propylene oxide and embedded in Spurr's resin. Ultrathin sections were cut with a diamond knife on an Ultracut-N microtome (Reichert, Nissei) and mounted on grids. Then the sections were stained with uranyl acetate, followed by lead citrate and examined under a transmission electron microscope (Hitachi, H-600). At least 3 samples from each condition were examined with a transmission electron microscope.

Results

In the rice seedlings used in the present experiments, the 3rd leaf was developing at sampling. In the control seedlings, all the leaves were green. In the salt-treated seedlings, the 2nd leaf blades and the tip of the 3rd leaf
Fig. 3 to 5. Ultrastructure of the chloroplasts in mesophyll of the 3rd leaf blades of rice seedlings cultured in a NaCl solution under a light condition. Fig. 3: Control. Figs. 4 and 5: seedlings cultured in 0.6 and 0.8 M NaCl solutions, respectively. Bar = 1 μm. G, grana thylakoids; St, stroma thylakoids; arrows show swollen thylakoids.

Fig. 6 to 8. Ultrastructure of the chloroplasts in mesophyll of the 3rd leaf blades of rice seedlings cultured in an NaCl solution under a dark condition. Fig. 6: Control. Figs. 7 and 8: seedlings cultured in 1.8 and 2.4 M NaCl solutions, respectively. Bar = 1 μm.
blades were slightly yellow under the light condition, although all the leaves were green under the dark condition.

1. Salinity effect on Na, Cl, K and chlorophyll contents

Figure 1 shows the effect of salinity on Na, Cl and K contents in the 3rd leaf blades of the seedlings cultured in a solution containing NaCl at various concentrations under a light or dark condition for 24 h. Under both light and dark conditions, Na and Cl contents increased as the NaCl concentration in the culture solution increased. However, at the same concentration of NaCl in the solution, the leaves under the light condition contained larger amounts of Na and Cl than in the dark condition. Therefore, we observed the ultrastructure of the 3rd leaf blades of the seedlings cultured in 0, 0.6 and 0.8 M NaCl solutions under a light condition and those cultured in 0, 1.8 and 2.4 M NaCl solutions under a dark condition for 24 h. Among these samples, Na and Cl contents were the highest in the seedling cultured in a 2.4 M NaCl solution under a dark condition. Salt treatment under light (except at 0.6 M) and dark conditions did not change the K content significantly.

Figure 2 shows the effect of salinity on the chlorophyll content in the 3rd leaf blades of the seedlings cultured in a solution containing NaCl at various concentrations under a light or dark condition for 24 h. Under the light condition, the chlorophyll content decreased with increasing NaCl concentration, but was not changed under a dark condition.

2. Foliar ultrastructure

Figures 3 to 5 show the ultrastructure of the chloroplasts in the mesophyll of the 3rd leaf blades of the seedlings cultured in 0 (control), 0.6 and 0.8 M NaCl solutions under a light condition. The chloroplasts of the control seedlings showed no structural distortion and possessed typical well-developed grana and stroma thylakoids (Fig. 3). In the mesophyll of salt-treated seedling, however, the thylakoids of the chloroplasts were swollen (Fig. 4 and 5, arrow) and had a wavy shape. Figures 6 to 8 show the ultrastructure of the chloroplasts in mesophyll of the 3rd leaf blades of the seedlings cultured in 0 (control), 1.8 and 2.4 M NaCl solutions under a dark condition. The chloroplasts of the control and salt-treated seedlings showed no structural distortion and possessed typical well-developed grana and stroma thylakoids without swelling although the leaves contained a larger amount of Na and Cl than in a light condition.

Discussion

Salt carried in the transpiration stream is reported to deposit in leaves as the water evaporates and the salt gradually accumulates with time in mature plants (Munns and Termaat, 1986). In the present study with rice seedlings, Na and Cl contents in the leaves increased with increasing NaCl concentration in the culture solution under both light and dark conditions. However, under a dark condition, the Na and Cl contents in the leaves were lower than under the light condition, probably because the transpiration rate was lower in the dark.

In the salt-stressed leaves under light condition, the chlorophyll content decreased as the NaCl concentration in the culture solution increased (Fig. 2). Chen et al. (1998) suggested that the degradation of chlorophyll is an indicator of salt stress. In the salt-stressed leaves under a dark condition, however, the chlorophyll content did not decrease although the contents of Na and Cl were higher than in the light condition. These results indicated that the decrease of the chlorophyll content caused by salinity was light-dependent.

Under the light condition, the chlorophyll content of the chloroplasts were swollen by salinity and showed a wavy shape. Membranes are important cellular targets common to salt stress and the thylakoid is particularly susceptible to disturbance by salt (Baker, 1991). Salama et al. (1994) reported that the structural change and swelling of thylakoid might be due to a change in the ionic composition of the stroma. However, under the dark condition, the thylakoids of the chloroplasts appeared intact even when the leaf tissues accumulated a larger amount of Na and Cl than under the light condition. These results indicated that the degeneration of the thylakoids of the chloroplasts caused by salinity was not directly caused by accumulation of salt to the excessive level in the tissue. The change of the thylakoids has been reported as a typical symptom of oxidative stress (Hernandez et al., 1995; Bondada and Oosterhuis, 1998), and chloroplast is an important intracellular generator of activated oxygen. In chloroplasts, O$_2^-$ and O$_2$---derived H$_2$O$_2$ are mainly produced by the electron acceptor of photosystem I (Asada and Takahashi, 1987; Salin, 1991). Hernandez et al. (1995) suggested that salinity induced an overproduction of O$_2^-$ and O$_2$-- derived H$_2$O$_2$ in salt-sensitive plants, which was only partially counterbalanced by ascorbate, a-tocopherol plus the Cu/Zn-SOD II and ascorbate peroxidase activities in the chloroplasts. In the leaves of rice subjected to salt stress, SOD leads to the overproduction of H$_2$O$_2$ (Lee et al., 2001). In the present study, the degradation of the thylakoids was caused by salt treatment only under a light condition. This suggested that the damages in chloroplasts were induced by a photooxidative reaction caused by salt stress and not directly correlated with salt content in the tissue.

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