Relationship between Salinity-Induced Damages and Aging in Rice Leaf Tissues

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Abstract: Segments of rice leaves at different nodal positions were incubated in NaCl solutions for various periods, and the chlorophyll content, Na content, Cl content, Na/K ratio and the ultrastructure of excised leaf tissues were examined. The chlorophyll content of the leaf tissues decreased with increasing NaCl concentration and incubation period. Na and Cl contents of the leaf tissues also increased with increasing NaCl concentration, but the decrease in chlorophyll content by salt stress was greater in old than in young tissues even when both tissues contained comparable amounts of Na and Cl. The presence of benzylaminopurine (BAP) alleviated the salt stress-induced decrease in chlorophyll content, but did not significantly affect the element contents. Ultrastructural damages were apparent in the chloroplasts of the leaf tissues subjected to salt stress. In 0.1% NaCl-treated old leaf tissues, the thylakoids were swollen, the envelope was partly destroyed causing leakage of the chloroplast contents. However, these damages were alleviated by the addition of BAP to the NaCl solution. In young leaf tissues, the thylakoids were swollen by incubation in 1.0% NaCl solution, but no structural distortion was observed in a 0.1% NaCl solution even without BAP added. The present study suggests that the leaf tissues were damaged by an increasing salt content and became more sensitive to salt stress with advancing leaf age. BAP seemed to alleviate the damages by salt stress through retardation of leaf aging.

Key words: Aging, Chlorophyll, Cl content, Na content, Na/K ratio, Rice, Salt stress, Ultrastructure.

Salinity affects 7% of the world's land surface, which amounts to 930 million ha (Szabolcs, 1994). In those areas, plant growth is severely affected by salt. The most common salt composition of saline soils is NaCl (Cumming and Elliot, 1991). When NaCl is applied to media, most crop plants decreased 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).

In recent years, many researchers have been trying to increase salt tolerance of plants. For this purpose, we need to understand how the plants are damaged by salinity. When the plants are exposed to salt, the concentrations of Na and Cl and the Na/K ratio in the tissue increase (Gregorio and Senadhira, 1993). It is reported that the salt carried in the transpiration stream is deposited in leaves as the water evaporates and the salt gradually accumulates with time (Munns and Termaat, 1986). In addition, in non-halophytes, salinity-induced damages appear in old leaves that have been transpiring for a long time than in young leaves. Therefore, Munns and Termaat (1986) concluded that salt injury is due to salt accumulation to the levels exceeding the ability of the cells to compartmentalize the salt into the vacuole.

In our previous study, the damages induced by salinity in rice leaves were correlated with age rather than Na content of the leaf tissue (Mitsuya et al., 2002). This observation indicates that, even in the same plant, salt sensitivity differs among tissues. Often the response to salinity of a salt-sensitive species is compared with that of a tolerant species to examine how the tolerant species survives in saline environments. The salt tolerance mechanisms include synthesis of osmoprotectants and late embryogenesis-abundant proteins (Moons et al., 1995), extrusion and compartmentalization of Na (Matoh et al., 1987) and adjustment of ion homeostasis (Durand and Lacan, 1994). However, the difference in salt tolerance among the tissues in the same plant has been poorly studied.

In the present study, we examined the effects of salinity on the chlorophyll content, element contents (Na, Cl and K) and ultrastructure of the tissues excised from rice leaves at different ages. In addition, we discuss the correlation of the salinity-induced damages with the element content and the age of the leaf tissue.

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. After imbibition, the seeds were planted in 1/5000 a
plastic pots filled with soil and grown with tap water for 2 months until the 10th leaf emerged. The plants were cultured in a glasshouse. Environmental condition was naturally controlled.

2. Preparation and NaCl treatment of leaf tissues

After the emergence of the 10th leaf, the 6th (old) and 9th (young) leaves (counting from the bottom of the plant) were used for experiments. The central part of the leaf blades without the main vein was cut off to make 60 mm² segments with a fresh razor blade. The leaf tissues were placed into petri dishes containing various concentrations of NaCl in 10 mM MES buffer (pH 6.5 adjusted by NaOH) supplemented with or without 5 µM benzylaminopurine (BAP). They were incubated under continuous white fluorescent light (about 600 μmol m⁻² s⁻¹) at 28°C.

3. Measurement of chlorophyll content

After incubation, the leaf tissues were blotted dry on a filter paper and the fresh weight measured. Chlorophyll was extracted from the leaf tissues by grinding with 80% acetone and absorbance of the supernatant was read at 663.1 and 646.2 nm using a spectrophotometer (Japan Spectroscopic Co., LTD., Ubest-50). Chlorophyll content was calculated according to the formulae rendered by Barnes et al. (1992) and converted to µg g⁻¹ FW.

4. Measurement of element (Na, K, Cl) contents

The leaf tissues were rinsed with distilled water and blotted dry. The dry weights were determined after the leaf tissues 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. The Na and K contents were measured by atomic absorption spectrometry (Shimadzu Co., Ltd., AA-6400F) in the emission mode. The Cl content was measured with a liquid chromatograph (Shimadzu Co., Ltd., LC-10AD).
Fig. 4. Effect of salinity on the relative chlorophyll contents of the old and young leaf tissues incubated for 24 h in solutions containing NaCl at various concentrations with or without BAP added (old leaf: 100% = 3.85 ± 0.08, old leaf + BAP: 100% = 4.31 ± 0.18, young leaf: 100% = 4.54 ± 0.14, young leaf + BAP: 100% = 4.55 ± 0.15 μg g⁻¹ FW). Data are means ± SE (n = 5). * and **, significantly different from control at P < 0.05 and 0.01, respectively.

The data are expressed on a unit-dry-weight basis.

Data were statistically analyzed according to Fisher's PLSD.

5. Transmission electron microscopy

For microscopic studies, the small samples of the old and young leaf tissues incubated in 0, 0.1 and 1.0% NaCl solution with or without BAP for 24 h were fixed in Karnovsky's fixative (mixture of 4% paraformaldehyde and 2% glutaraldehyde in 0.05 mol L⁻¹ 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

Figure 1 shows the effect of salinity on the chlorophyll content of the young leaf tissues incubated in 0 and 1.0% NaCl solution for up to 60 h. The chlorophyll content was decreased by salinity after the incubation for 36 hr or more. Figure 2 shows the effect of salinity on the chlorophyll content of the young leaf tissues incubated for 48 h in a solution containing NaCl at various concentrations. The chlorophyll content decreased with increasing salt concentration.

Figure 3 shows the relationship between the relative chlorophyll content (% of control) and element contents of the young leaf tissues incubated for 48 h in solutions containing NaCl at various concentrations. The relative chlorophyll content decreased with increasing Na and Cl contents and Na/K ratio (Fig. 3 a, b and c, respectively) of the young leaf tissues.

Figure 4 shows the effect of salinity on the relative chlorophyll contents of the old and young leaf tissues incubated for 24 h in solutions containing NaCl at various concentrations with or without BAP added. The chlorophyll content of the old leaf tissues markedly decreased in the solution with NaCl at a 0.1% or higher concentration. In the presence of BAP, the chlorophyll content of the old leaf tissues was significantly decreased by salinity but the degree was less than without BAP. The chlorophyll content of the young leaf tissues tended to decrease with increasing salinity but the decrease was not statistically significant at 0.1 and 0.3% NaCl. Thus, the salinity-induced decrease in chlorophyll content was markedly alleviated by BAP.

Figure 5 shows the correlation of the relative chlorophyll content with element contents and Na/K ratio in the old and young leaf tissues incubated for 24 h in solutions containing NaCl at various concentrations with or without BAP added. Data are means (n = 5). Vertical bars at the lower left represent the least significant difference (LSD) of the relative chlorophyll content. Lateral bars at the lower left represent the LSD of the element contents or Na/K ratio. (a) Na content. (b) Cl content. (c) Na/K ratio.
Fig. 6 to 8. Ultrastructure of chloroplasts in mesophyll cells of the old leaf tissues incubated in water (Fig. 6), 0.1% NaCl (Fig. 7) and 0.1% NaCl + BAP (Fig. 8) for 24 h. Bar = 1 μm. P, plastoglobuli; S, starch grain.

Fig. 9 to 11. Ultrastructure of chloroplasts in mesophyll cells of the young leaf tissues incubated in water (Fig. 9), 1.0% NaCl (Fig. 10) and 1.0% NaCl + BAP (Fig. 11) for 24 h. Bar = 1 μm. S, starch grain.
phyl content with Na content, CI content and Na/K ratio in the old and young leaf tissues. The leaf tissues were incubated for 24 h in solutions of NaCl at various concentrations with or without BAP added. In both old and young leaf tissues, the relative chlorophyll content decreased with increasing Na and CI contents and Na/K ratio in the tissues. In addition, the old tissues showed a marked decrease in chlorophyll content as compared with the young tissues at comparable Na and CI contents and Na/K ratio. The marked decrease in chlorophyll content in old leaf tissue was greatly alleviated by the presence of BAP in the solution.

Figures 6 to 8 show the ultrastructure of chloroplasts in the mesophyll cells of the old leaf tissues incubated in water and 0.1% NaCl with or without BAP added. The chloroplasts of the control (0% NaCl) showed many plastoglobules and possessed typical well-developed grana and stroma thylakoids (Fig. 6). In the chloroplasts of 0.1% NaCl-treated tissues, the thylakoids were swollen (Fig. 7) and a part of the envelope was destroyed resulting in leakage of the stromal content. However, the destruction of the thylakoids and envelope in 0.1% NaCl was alleviated by the addition of BAP to the solution (Fig. 8).

Figures 9 to 11 show the ultrastructure of chloroplasts in the mesophyll cells of the young leaf tissue incubated in water and 1% NaCl with or without BAP added. The chloroplasts of the control showed no structural distortion and possessed typical well-developed grana and stroma thylakoids (Fig. 9). In the mesophyll of 0.1% NaCl-treated tissues, the ultrastructure of the chloroplasts showed no differences from those in the control and their structure was not distorted (data not shown). In the chloroplasts of 1.0% NaCl-treated tissues, the thylakoids were swollen and showed a wavy configuration (Fig. 10). In mesophyll cells of 1% NaCl + BAP-treated tissues, however, the chloroplasts showed no structural distortion (Fig. 11).

Discussion

In the rice leaf tissues, the chlorophyll content was decreased and the ultrastructure of the chloroplasts damaged by the incubation in NaCl solution. The decrease in the chlorophyll content is a typical result of salt stress (Chen et al., 1998; Mitsuya et al., 2002) and is an indicator of salt stress (Chen et al., 1998). The thylakoids of the chloroplasts were swollen by salinity and showed a wavy configuration. 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 structural changes and swelling of the thylakoid might be due to a change in the ionic composition of the stroma. On the other hand, the change of thylakoid has been reported as a typical symptom of oxidative stress (Hernandez et al., 1995; Bondada and Oosterhuis, 1998). Chloroplasts are important intracellular generators 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 induces an overproduction of O$_2^-$ and O$_2^-$-derived H$_2$O$_2$ in salt-sensitive plants, which is only partially counterbalanced by ascorbate, α-tocopherol and the Cu/Zn-SOD II and ascorbate peroxidase 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 addition, the ultrastructural changes of the thylakoid are induced by salinity dependent on light (Mitsuya et al., 2000). Therefore, we suggested that the degradation of the thylakoids of the chloroplasts was caused by a salt-induced oxidative reaction (Mitsuya et al., 2000).

In leaf tissues of the same age, the chlorophyll content decreased with time and with increasing Na and CI contents. This result was consistent with the report of Munns and Termaat (1986) that the salinity-induced damages are mainly due to exposure to salt above a threshold level. Generally, in non–halophytes, the damages caused by salinity appear first on the old leaves. It was concluded that, in salt–stressed non–halophytes, old leaves are damaged long before young leaves simply because the salt content is always higher in old leaves (Greenway and Munns, 1980). In the present investigation, however, the old leaves showed severer damage than the young leaves even when they contained comparable amounts of Na and CI. This indicated that the old tissues had lower threshold values for Na and CI than the young tissues. This means that the tolerance to salt stress decreases with aging. This observation was consistent with the results of a previous study with intact rice leaves in which the salinity-induced damages were correlated with the age of the tissue rather than Na content in the tissue (Mitsuya et al., 2002). Therefore, we suggest that leaf tissues become more sensitive to oxidative stress by aging and that older tissues suffer severer damages by lower with less Na and CI contents than those in younger leaves. In addition, the salinity–induced damages could be alleviated by the presence of BAP in the solution. It is generally assumed that cytokinin is one of the major regulators of plant aging (Yang et al., 2002). Treatment with cytokinins has been shown to delay leaf aging in many plants (van Staden et al., 1988). In addition, Gidrol et al. (1994) reported that the abundant cytokinins can serve as antioxidants during germination in soybean and that cytokinins can protect cells against oxidative stress in Escherichia coli. Therefore, in the present study, treatment with BAP seemed to delay aging and decrease the sensitivity of old tissues to salt stress.

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