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Electric Conductivity, Na^+ Content and Photosynthetic Activity in Leaves of Salt–Stressed Rice Plants, and Their Cultivaral Difference

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The electric conductivity of a leaf (LEC) was determined with an insulation resistance tester, and discussed its relationship with Na^+ content in a leaf (LNC) and the photosynthetic activity in salt–stressed rice cultivars. The measurements were carried out using three cultivars of *O. sativa* and *O. glaberrima* grown at the middle vegetative growth stage. The plants showed a clear response to the salt treatment within 24 hours after its application. Both LEC and LNC in these cultivars increased with an increase in NaCl concentration in culture solution from 0 to 0.1 μmol L^{-1}. LEC had a close linear relationship with LNC in each cultivar. A negative relationship was found between LEC and gross photosynthetic rate (P_g). The stomatal conductance (G_s) and mesophyll conductance (G_m) decreased with an increase in LNC. A clear cultivaral difference was recognized in responses to salt treatments. LEC was regarded as a useful indicator for the estimation or diagnosis of LNC and photosynthetic activity in salt–stressed rice cultivars.

INTRODUCTION

The growth of plants is frequently restricted in soil environments having a high salt–concentration. In many cases, the growth restriction likely occurs when NaCl concentration in soil–water has exceeded about 0.1%, and a serious damage is found in growth at salt concentrations over 0.3%. Even under such a condition, some plants keep a high tolerance and continue their normal growth (Fageria, 1985; Gorham *et al.*, 1985). Rice species belong to a plant group sensitive to salt (Mass and Hoffman, 1977; Flowers *et al.*, 1985; Yeo *et al.*, 1985; Shannon, 1997), but a considerable difference has been known in the sensitivity among species and cultivars (Dionisio–Sese and Tobita, 2000; Khan *et al.*, 1997; Bohra and Doerffling, 1993; Cho *et al.*, 1995).

The difference in salt sensitivity in rice cultivars chiefly depends on two steps: one is the cultivaral difference in NaCl accumulation rate in leaves (Nakamura *et al.*, 2002), and the other is the cultivaral difference in photosynthetic response to NaCl content in leaves (Makihara *et al.*, 2001). To make clear cultivaral differences in response to salt, three

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rice cultivars with a genetically remote relationship were selected here from *O. sativa* and *O. glaberrima*, and used as experimental materials.

As the fundamental information for studying on salt sensitivities of plants, it is necessary to determine NaCl content in leaves (LNC). Usually chemical analyses have been used for this purpose, but it takes many hours. In the practical use, a newly devised measurement method is required for a quick and easy diagnosing of salt sensitivities of plants. It may be assumed that the electric conductance of a plant is higher as the plant accumulates more NaCl inside. In this study we determined electric conductivity of a leaf (LEC) using an electric insulation resistance tester partially improved, and examined its relationships with LNC and photosynthetic activity.

MATERIALS AND METHODS

Materials

Three rice cultivars used here were Nipponbare (NPB), a Japanese leading cultivar of *O. sativa*, and Shanyou–63 (S–63), a Chinese high–yield hybrid of *O. sativa*; and G174 (an African cultivar of *O. glaberrima*) introduced from Senegal.

Plant culture and salt treatments

The three cultivars were grown in nursery boxes for about 25 days before transplanting. After that, the plants were solution–cultured in containers including the standard Yoshida–solution of 400L in a greenhouse during July to August in 2003. The solution was renewed at one–week intervals and its pH was kept at about 5.0. The plants grown for 30 days after transplanting were re–moved into a small pot (2L in capacity) containing NaCl water. According to Yamamoto *et al.* (2000), when NaCl concentration in the soil water was 0.02 to 0.12 mol L^{-1}, rice plants were subjected to salt damages. Based on this information, NaCl concentrations in the treatment solutions were adjusted at three levels of 0, 0.05 and 0.1 mol L^{-1}. The treatment was begun in the early morning in a clear day, and changes in LEC, LNC and photosynthetic parameters were surveyed for six days. Na^+ content in culms and leaves in rice plants were known to greatly increase by root–cutting treatments (Tsuchiya, 1996). We also tried a root–tip cutting treatment and observed its effects. Three plants were tested in each cultivar and in each treatment.

Measurement of electric conductivity in leaves

The LEC measurement tool is outlined in Fig. 1. Both positive and negative electrodes of an insulation resistance tester (PDM–507, SANWA, Japan) were placed 40 to 50 cm apart and tightly touched on a young expanded leaf on the main or a sub–main culm of a plant. The aluminum electrode was coiled around with a cotton thread. The area of the electrode was 1 cm^2. On the measurement, the electrode plate was moistened with NaCl solution to make complete the electric connection between the electrodes and a leaf. The electric resistivity (Ω) per 1 cm of leaf length was determined, and its inverse value was shown as LEC (S cm^{-1}).

Measurement of photosynthetic parameters

The gross photosynthetic rate (P_g), stomatal conductance (G_s) and mesophyll

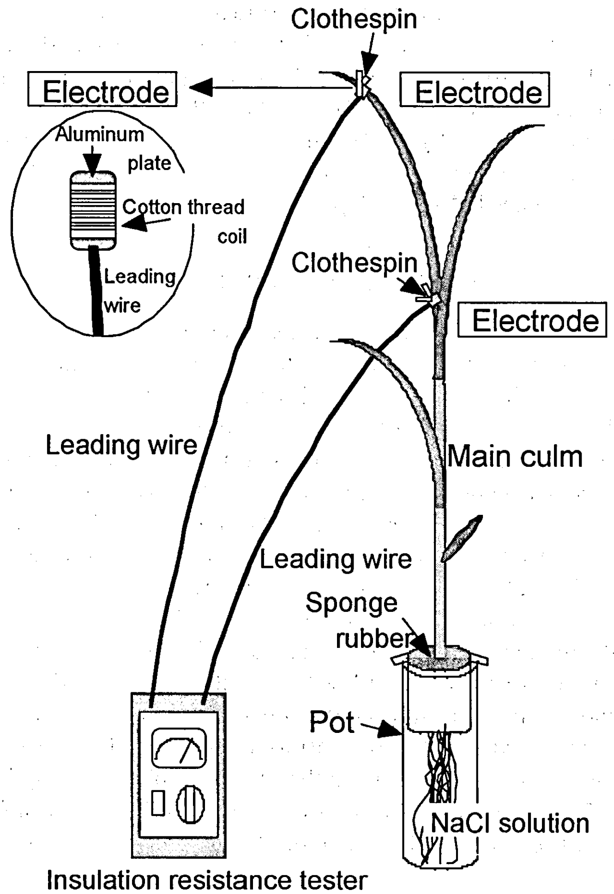

Fig. 1. The measurement of electric resistance in a rice leaf.

conductance (G_m) of a leaf were measured using a photosynthesis–evaporation measurement system (LI–6262, LI–COR, USA), and concurrently LEC was monitored. The environmental conditions for measurement were 500 μmol $m^{-2}s^{-1}$ in light intensity; 30 °C in leaf temperature; and 370 μmol mol^{-1} in CO_2 concentration, 21% in O_2 concentration and 50% in relative humidity of the reference air. The leaf area used for measurements was 4.4 cm^2 in the central part of a leaf.

Determination of Na^+ content in a leaf

After the measurements of LEC and photosynthetic parameters, LNC was determined just before and one day after the beginning of NaCl treatment. Leaf blades were sampled from plants and dried at 80 °C. The dried materials were powdered with an electric grinder. The powder of 1g was put in a centrifugation cell of 50 ml capacity, and 1 N hydrochloric acid of 10mL was added in. Then the cell was capped and shaken for one minute, and placed at the room temperature for one night. The extracted solution in the cell was passed through a filter paper (Cat No 1002 125, Whatman,USA), and re–filtered with a filter (DISMIC–13HP 0.20μm, Advantec, USA). The obtained solution was adequately diluted and the concentration of Na^+ was determined with an atomic spectrophotometer (AA–6200, SHIMADZU, Japan).

RESULTS AND DISCUSSION

The change in electric resistance of a leaf blade was monitored using the measurement tool illustrated in Fig. 1, and from the observed value, LEC was calculated. In order to know an adequate time and period for measurement of LEC, the time course changes in LEC, P_g and G_s of salt–treated cultivars were surveyed, and are shown in Table 1. The effect of NaCl application on the photosynthetic parameters and its cultivaral difference were sufficiently recognized within one day after treatments. All the parameters listed did not show a recovering trend within the limited six days. *O. glaberrima* has been known to have a trait of keeping stomatal openness in a lower leaf–water–potential (Furuya *et al.*, 1994), and G–174 has been also reported to have such a specific feature (Tagawa *et al.*, 2000). But as shown in Table 1, G_s and P_g of G–174 have a larger depression under the salt treatment. Of the three cultivars, NPB is less sensitive to NaCl.

The relationship between LEC and NaCl concentration in solution is shown in Fig. 2. LEC was determined with plants subjected to one–day treatments. LEC of the three cultivars increased linearly with NaCl concentration from 0 to 0.1 mol L^{-1}. LEC in plants under the root–cut treatment were higher than those of plants having intact roots. This means that a physical or physiological barrier against NaCl solution inflow considerably reduced by root cutting. It may be considered that the transport of Na^+ is varied by

Table 1. Effects of NaCl solution (0.1 mol L^{-1}) treatment on LEC and photosynthetic parameters.

Cultivar	Days after treatment	LEC (μS cm^{-1})	P_g (μmol m^{-2} s^{-1})	G_s (mol m^{-2} s^{-1})	G_m (mol m^{-2} s^{-1})
NPB	0	3.60 (100)	16.8 (100)	0.19 (100)	0.084 (100)
	1	4.26 (118)	14.0 (83)	0.16 (84)	0.073 (87)
	6	4.90 (136)	11.8 (70)	0.14 (74)	0.054 (64)
S–63	0	4.80 (100)	17.5 (100)	0.27 (100)	0.064 (100)
	1	5.73 (119)	13.7 (78)	0.18 (67)	0.058 (91)
	6	5.76 (120)	12.3 (70)	0.17 (63)	0.055 (86)
G–174	0	5.00 (100)	19.5 (100)	0.32 (100)	0.074 (100)
	1	6.58 (132)	13.2 (68)	0.17 (53)	0.059 (80)
	6	7.04 (141)	12.0 (62)	0.16 (50)	0.051 (69)

Values between parentheses indicate the ratios. LEC, leaf electric conductance; P_g, gross photosynthetic rate; G_s, stomatal conductance; G_m, mesophyll conductance, NPB, Nipponbare; S–63, Shan you–63; G–174, G174 of *O. glaberrima*.

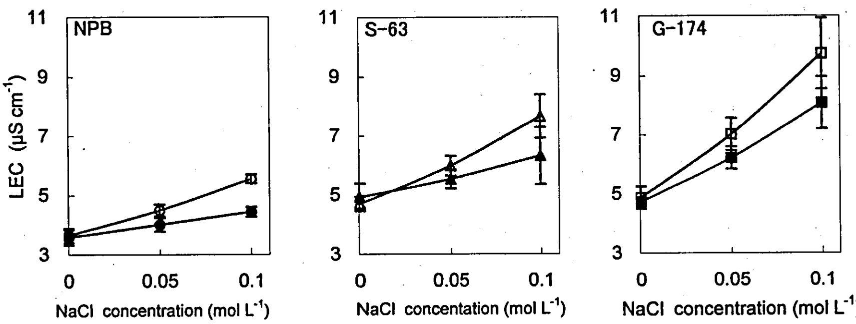

Fig. 2. Effects of NaCl concentrations in solution on LEC of rice cultivars: ●, NPB; ▲, S-63; ■, G-174; ○, Root-cut NPB; △, Root-cut S-63; □, Root–cut G–174. Bars indicate standard errors.

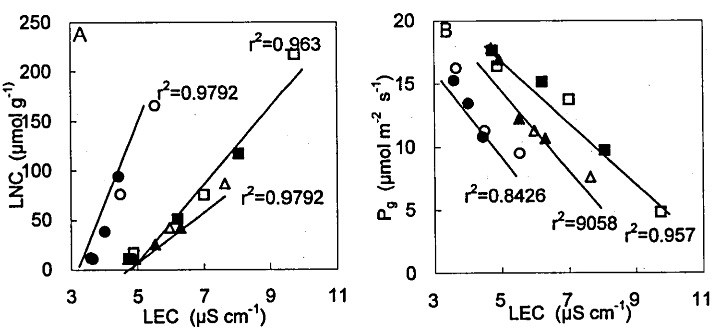

Fig. 3. Relationships between LEC and LNC (A), and P_g (B). LNC, NaCl content in a leaf. See Fig. 2 for the symbols.

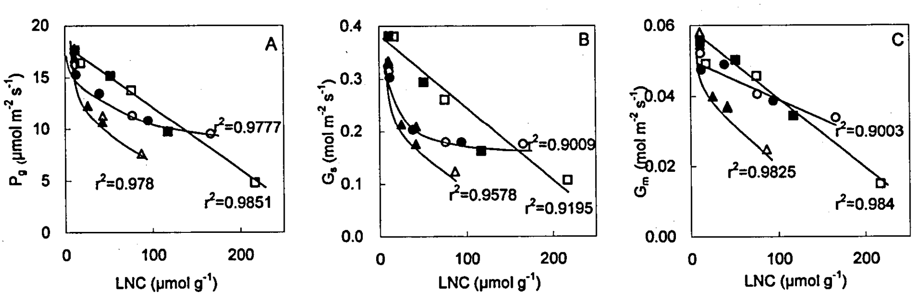

Fig. 4. Relationships between LNC and P_g (A), G_s (B) and G_m (C). See Fig. 2 for symbols.

evaporation rate and also affected by the balance between Na^+ intake and Na^+ exclusion functions in roots (Tsuchiya *et al.*, 1992; Naito *et al.*, 1994).

As shown in Fig. 3–A, LEC and LNC in each cultivar showed a highly significant positive relationship, which means that LNC of rice cultivars can be estimated from LEC. A negative linear relationship of a high significance was found between LEC and P_g as shown in Fig. 3–B. LEC is also useful as an indicator for photosynthetic activity in salt–stressed plants.

The relationship between LNC and P_g is shown in Fig. 4–A. In each cultivar, P_g decreased as LNC increased, and a large cultivaral difference was found in this relationship. G–174 showed a linear depression in P_g, and the other cultivars showed an exponential decreasing curve. In the range of LNC below 100μmol g^{-1}, the highest value of P_g was observed in G–174 and the lowest was in S–63. S–63 is regarded as a cultivar more sensitive in photosynthesis to LNC among the materials used here. In this cultivar, Na^+ accumulation in leaves was efficiently restricted, while the photosynthetic activity was quickly depressed by a small accumulation of Na^+ in a leaf. On the other hand, G–174 accumulated a larger amount of NaCl in leaves; according to this, the P_g was considerably depressed.

Makihara *et al.* (2001) reported that below 3–5 mg g^{-1} (51.3–85.5μmol g^{-1}) in NaCl accumulation in rice leaves, the photosynthetic depression was chiefly caused by stomatal closure, but in a higher concentration beyond this level the photosynthesis was decreased by any other factors than stomatal closure. However, such a distinct phenomenon was not observed in our experiment, and the stomatal response, or G_s to salt considerably differed among cultivars. As shown in Fig. 4–B, G_s of both NPB and S–63 represented a sharp drop at a low LNC, but in the range of LNC over 50μmol g^{-1} G_s of NPB did not show further depression. On the other hand, G_s of G–174 deceased linearly down to a considerably lower level with an increase in LNC. G_m also decreased with an increasing LNC, and a clear cultivaral difference was observed as shown in Fig. 4–C.

In this study it has been confirmed that LEC can be easily measured using the simple electric tool, and this parameter is an effective indicator for estimation and diagnosis of Na^+ accumulation in a leaf and photosynthetic activity in salt–stressed rice cultivars.

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