Differential Sensitivity of Rice Cultivars to Salinity and Its Relation to Ion Accumulation and Root Tip Structure

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Abstract: Effects of NaCl on the growth, ion content, root cap structure and Casparian band development were examined in four rice (Oryza sativa L.) cultivars with different salt resistance (salt-sensitive indica-type IR 24 and japonica-type Nipponbare and salt-resistant indica-type Nona Bokra and Pokkali). Experiments were conducted to find the differences in salinity resistance during early seedling and developed seedling stages among the cultivars. For salinity treatment, sodium chloride (NaCl) was added to nutrient solution at concentrations of 0, 25 and 50 mM for 7 days from germination to the 7th day (early seedling stage) or from the 7th day to 14th day (developed seedling stage). Growth inhibition by salinity was more prominent in the early seedling stage than in the developed seedling stage. Based on the growth, the order of the sensitivity was IR24>Nipponbare>Nona Bokra>Pokkali. The growth of NaCl-treated rice cultivars relative to control was significantly and negatively correlated with the Na\(^+\) content and Na\(^+\)/K\(^+\) ratio in roots and shoots in both stages. Scanning electron microscopic observation revealed that the root cap tissues proliferated and extended to the basal part of the root tip by salinity. The length of root cap was, however, reduced by 50 mM NaCl in sensitive cultivars due to peeling off. An endodermal Casparian band was formed in the basal region of the root tip. Development of the Casparian band was more prominent in sensitive cultivars than in tolerant cultivars. Root cap proliferation might be related to Na\(^+\) exclusion in rice seedlings, but the Casparian band may not function efficiently in Na\(^+\) exclusion. Essentially the present results suggest that exclusion of Na\(^+\) from roots plays a critical role in expression of Na\(^+\) resistance in rice seedlings and the root cap is important for Na\(^+\) exclusion.

Key words: Casparian band, Ion accumulation, Rice (Oryza sativa L.), Root tip, Salinity resistance, Sodium chloride (NaCl).

Rice (Oryza sativa L.) is one of the most important cereals in the tropics and subtropics, but is sensitive to salinity (Francois and Maas, 1994; Flowers et al., 1997). Salinity stress is caused by excessive uptake of toxic ions from soil solution and it decreases plant growth and yield (Boyer, 1982). Grain yield of rice plants is reduced about 70% to 100% of its maximal yield performance by salinity (Heenan et al., 1988). The barriers for increase of crop production could be overcome by reducing the effects of salt stress on rice plants. Therefore, the effects of salinity stress on rice plants and the mechanism of resistance need to be studied to cope with the salinity stress problems.

There are marked differences in salinity resistance among rice cultivars (Munns and Termaat, 1986; Yeo and Flowers, 1986; Lee et al., 2003). Comparison of varietal differences in rice plants will be helpful in studying the mechanism of resistance. The varietal difference is recognized with respect to various parameters such as growth in length and weight, survival and physiological features (Yeo et al., 1990) in addition to ion exclusion capacity (Noble and Rogers, 1992). It has been reported that rice cultivars acquire salt resistance by excluding Na\(^+\) ion from the roots (Akita and Cabuslay, 1990; Naito et al., 1994).

However, previous authors did not investigate the mechanism of Na\(^+\) ion exclusion in roots.

Salinity resistance also varies with the growth stage. Previous studies have revealed that rice plants are highly sensitive to salinity especially at young seedling stages and less so at developed stages (Maas and Hoffman, 1977; Flowers and Yeo, 1981; Yoshida, 1981; Heenan et al., 1988; Maas, 1990; Yeo et al., 1990; Lutts et al., 1995; Shannon, 1998). Comparison of salinity resistance in different growth stages may present clues to the mechanism of resistance. However, the factors that cause the difference in salinity resistance with the growth stage have not been explored.

Accumulation of Na\(^+\) ion in plant tissues at excessive levels is one of the major factors causing salinity damage (Flowers and Hajibagheri, 2001; Mitsuwa et al., 2003a, 2003b). The plant tissues must prevent excessive accumulation of toxic ions in their cytoplasm by maintaining an exclusion, excretion and dilution mechanism (Hamza, 1980; Ben Rais et al., 1993). Munns and James (2003) found Na\(^+\) exclusion ability to be significantly correlated with salinity resistance in plants. We need to study how Na\(^+\) ion uptake or...
exclusion is controlled in plant tissues to clarify the mechanism of resistance.

The root is an important plant organ that is directly exposed to the soil, therefore, the effects of salinity stress are more pronounced in roots (Hajibagheri et al., 1985). According to Jeschke and Wolf (1988) salt tolerance of the plant depends decisively on the tolerance of roots. Ultrastructural changes of root cells in response to salt stress have been studied in wheat (Udovenko et al., 1970), maize (Yeo et al., 1977), barley (Huang and Stevenick, 1990), bean (Nassery and Jones, 1976), sorghum (Koyro, 1997), rice (Rahman et al., 2001, 2002) and other species. Rahman et al. (2001) observed the expansion of root cap cells in rice plants under salinity stress and suggested it to be an adaptive response to salinity. Root cap cells may function in Na⁺ exclusion under salinity stress. In rice roots, the Casparian band also functions as a barrier to water and ion movement through the apoplast (Morita et al., 1996; Ranathunge et al., 2005; Watanabe et al., 2006). However, the root function in Na⁺ exclusion has not been studied in detail.

Accordingly, the present study was conducted to examine the relationship between growth inhibition, ion accumulation and root structure under salinity stress at two different growth stages in four rice cultivars differing in salinity resistance to obtain insight into the mechanism of resistance.

Materials and Methods

1. NaCl treatment of hydroponically cultured rice cultivars

A salt-sensitive japonica-type rice (*Oryza sativa* L.) cultivar Nipponbare, a salt-sensitive indica-type rice cultivar IR 24, and two salt-resistant indica-type cultivars Nona Bokra and Pokkali were used (Akita and Cabuslay, 1990; Dionisio-Sese and Tobita, 1998; Sahi et al., 2006). Seeds of each cultivar were surface sterilized with a 5% sodium hypochlorite solution for 5 min and washed several times with distilled water. The seeds were then imbibed in distilled water in a small beaker in a growth chamber at 28ºC until the appearance of the white tip of the coleoptiles. After imbibition, uniformly germinated seeds (7 seeds) were sown on a plastic net placed on the surface of a 300 mL solution of full strength Kimura’s nutrient solution B (pH 5.5) with or without NaCl. Seedlings were grown in a culture room at 28ºC under a continuous light intensity of 70 μmole m⁻² s⁻¹. Salinity treatment was given for 7 d after germination (early seedling stage) or the succeeding 7 d (developed seedling stage). The NaCl concentration in the nutrient solution was 0, 25 or 50 mM. The culture solutions were changed everyday.

2. Growth response measurements

The growth response to salinity was shown by ‘relative growth’, which is the growth in dry weight of the NaCl-treated seedlings relative to that in the control (%). The increase in dry weight of roots and shoots during the 7-day treatment with NaCl in the early and developed seedling stages was measured to determine the relative growth. Dry weight was determined after drying at 70°C for 48 h. All the values for each parameter were means of 5 independent replications.

3. Determination of Na⁺ and K⁺ contents

The content of Na⁺ and K⁺ in materials used for dry weight measurement was determined by extracting 8
mg dry matter in 10 mL of 1N HCl with shaking for 3 d. The extracts of roots and shoots were analyzed by atomic absorption flame spectrophotometer (Model AA-6400F, Shimadzu Corporation, Japan) in the emission mode. The Na⁺ and K⁺ contents were measured and expressed on a unit-dry-weight basis. The data were expressed as the means of three independent experiments.

4. Scanning electron microscopy

The seminal root tip after the application of NaCl was observed by scanning electron microscopy. Root tip sections (2 mm in length) were fixed with 2% glutaraldehyde in 0.05 M phosphate buffer, pH 7.2, for 4 h at room temperature (about 25°C). The samples were washed with buffer several times and post fixed in 1% OsO₄ in the buffer for 12 h at 4°C. They were then rinsed with the buffer and distilled water, dehydrated in an ethanol series (30, 50, 70, 90, 95 and 100%), treated with isoamyl acetate and critical point dried in a Hitachi HCP-2 apparatus. The specimens were mounted on a stub with adhesive carbon tape to place in horizontal orientation, gold coated with an ion sputtering apparatus, Eiko IB-3, and examined with a Hitachi S-4200 scanning electron microscope. Root cap length was estimated as the median length of root cap from the root tip (Fig. 5). For comparison of the length of root cap, the data were expressed as the means of three independent experiments.

5. Fluorescence microscopy

Light microscopic studies were made using the

Fig. 2. Effect of NaCl on the contents of Na⁺ (a) and K⁺ (b) in roots and shoots of four rice cultivars in the early seedling (ES) and developed seedling (DS) stages. The cultivars are IR 24 (IR 24), Nipponbare (Nip), Nona Bokra (NB) and Pokkali (Pok). Each value is the mean ± standard error from three independent experiments. * and ** represent difference from the control at P<0.05 and P<0.01, respectively by Tukey’s HSD test. DW, dry weight.
Fresh cross sections (up to 35 mm from the root tip) of the seedlings were prepared. Cross sections of 100 μm in thickness were made using a razor blade with a microslicer (DTK-3000W, Dosaka). Sections were stained with 0.1% (w/v) berberine hemisulfate for 0.5−1 h and rinsed thoroughly with distilled water. They were counterstained with 0.05% (w/v) toluidine blue O for 0.5−3 min and again rinsed with distilled water (Watanabe et al., 2006). Cover slips were sealed to prevent evaporation of the mountant and the specimens were immediately observed with a fluorescence microscope (Olympus BX51) fitted with a 100-W high pressure mercury burner (HBO105W/2) and a ultraviolet filter assembly (U-MWU2) and photographed.

6. Statistical analysis
The numerical data were subjected to analysis of variance at P <0.05 and the difference from control were statistically analyzed by Tukey's HSD test.

Results

1. Effect of NaCl on the growth
Fig. 1 shows the effect of NaCl on the relative growth of roots and shoots in four cultivars treated with NaCl at three concentrations in the early seedling (ES) and developed seedling (DS) stages. The cultivars are IR 24 (IR), Nipponbare (Nip), Nona Bokra (NB) and Pokkali (Pok). Data are obtained from three independent experiments. r values followed by ** represent significance of correlation at P<0.01. DW, dry weight.

**Fig. 3. Correlation of the relative growth of roots and shoots with Na⁺ contents (a) and Na⁺/K⁺ ratio (b), in four rice cultivars in the early seedling (ES) and developed seedling (DS) stages. The cultivars are IR 24 (IR), Nipponbare (Nip), Nona Bokra (NB) and Pokkali (Pok). Data are obtained from three independent experiments. r values followed by ** represent significance of correlation at P<0.01. DW, dry weight.**
salinity more severely in the early seedling stage than in the developed seedling stage.

2. Effect of NaCl on Na\(^+\) and K\(^+\) contents

Fig. 2 shows the effect of NaCl on the contents of Na\(^+\) and K\(^+\) in roots and shoots in four cultivars treated with NaCl. With NaCl treatment, Na\(^+\) content of roots and shoots in all cultivars increased compared with control (Fig. 2a). The Na\(^+\) content of roots in all cultivars was higher than that of shoots, except for Nipponbare at the developed stage. The salt-sensitive cultivars IR 24 and Nipponbare showed higher Na\(^+\) contents than the salt-tolerant cultivars Nona Bokra and Pokkali. The K\(^+\) content of roots and shoots decreased with increasing concentration of NaCl in the nutrient solution (Fig. 2b).

3. Correlation of relative growth with Na\(^+\) content and Na\(^+\)/K\(^+\) ratio

Fig. 3a shows the correlation between relative growth and Na\(^+\) content in four cultivars at the early seedling and developed seedling stages. There was a significant negative correlation between the relative growth and Na\(^+\) content in roots and shoots regardless of cultivars and the growth stages. There was also a significant correlation between the relative growth and K\(^+\) content (data not shown). Fig. 3b shows the correlation between the relative growth and Na\(^+\)/K\(^+\) ratio. There was a significant negative correlation between the relative growth and Na\(^+\)/K\(^+\) ratio in roots and shoots regardless of cultivars and the growth stages.

4. Effect of NaCl treatment on the root cap structure

Fig. 4 shows the effects of NaCl on the structure of seminal root cap in rice cultivars in the early seedling stage. Upper row, A: IR 24 and lower row, B: Pokkali. Left: control, middle: 25 mM NaCl and right: 50 mM NaCl treatment. Arrow indicates root cap cells peeled off. Bar = 100 μm.

Fig. 5. Measurement of the root cap length. Root cap length = (L+S)/2. RC, root cap; RP, root proper; L, largest part of root cap; S, shortest part of root cap.

5. Effect of NaCl on the Casparian band development in root tip

Fig. 7 shows the effects of NaCl on the endodermal
Casparian band appearance in the root tip of IR 24 and Pokkali as observed by fluorescence microscopy. Casparian bands were observed as fluorescent bands (Fig. 7, arrowheads) on the radial walls in the endodermis in the basal part of the root tip (Fig. 7, En). Development of the Casparian band was more
prominent in the salt-sensitive IR 24 than in the salt-tolerant Pokkali. The Casparian band matured closer to the root tip under high NaCl treatment than in the control. Table 1 shows the effects of NaCl on the length of the root tip without the endodermal Casparian band in the early seedling stage. Both the length of seminal root and that of the root tip without the Casparian band in all cultivars decreased under NaCl treatment. The letter was comparatively shorter in salt-sensitive cultivars (IR 24, Nipponbare) than in salt-tolerant cultivars (Nona Bokra, Pokkali).

Discussion

Salinity stress variously affects the biochemical and anatomical processes in the plant development (Zhu, 2001; Yokoi et al., 2002). The present study demonstrated a significant variation among rice cultivars in growth responses to salinity stress (Fig. 1). Based on the effect of salinity on relative growth, the order of sensitivity in four rice cultivars was IR 24>Nipponbare>Nona bokra>Pokkali. Similar trends have been reported previously (Akita and Cabuslay, 1990; Dionisio-Sese and Tobita, 1998; Sahi et al., 2006).

Sensitivity of rice seedling to salinity stress varies with the growth stage. The relative growth of seedlings was reduced more severely in the early seedling stage than in the more developed stage (Fig. 1). The present study suggested that the young rice seedling tissues were more sensitive to salinity, which may be because they could not avoid NaCl accumulation as effectively as more developed seedlings. Another possibility of differential sensitivity was the difference in adaptive capacity to Na⁺ toxicity (Yousfi et al., 2007). These possibilities will be discussed later based on the correlation between Na⁺ accumulation and growth.

The present study showed that the sensitive cultivars accumulated a larger amount of Na⁺ and absorbed a smaller amount of K⁺ in roots and shoots than the tolerant cultivars (Fig. 2a, b). Roots accumulated a larger amount of Na⁺ and a smaller amount of K⁺ than shoots (Fig. 2a, b), which coincides with the results of previous studies (Akita and Cabuslay, 1990). Significant negative correlations were found between Na⁺ content or Na⁺/K⁺ ratio and the relative growth of roots and shoots (Fig. 3a, b). Thus, resistant cultivars had the ability to avoid Na⁺ accumulation and absorb K⁺ to maintain low Na⁺/K⁺ ratios, which could be a good tolerance determinant for NaCl stress (Dasgan et al., 2002; Lee et al., 2003; Ren et al., 2005; Colmer et al., 2006; Munns et al., 2006; Munns and Tester, 2008). In addition, the correlation between growth and Na⁺ content or Na⁺/K⁺ ratio was observed regardless of growth stages. This fact suggests that the apparent difference in sensitivity at different growth stages is due to difference in accumulation of Na⁺ and K⁺ and not due to difference in physiological mechanism of adaptation.

SEM observation revealed that root cap tissue increased and proliferated toward the basal part of the root tip under salinity (Fig. 4). Expansion of root cap cells may be an adaptive response of rice seedling to high salinity stress (Rahman et al., 2001). Root cap cells in sensitive cultivars tended to peel off at 50 mM NaCl treatment. The length of root cap was increased by NaCl at both 25 mM and 50 mM in all cultivars compared with the control (Fig. 6). However, the length of root cap in sensitive cultivars was reduced by NaCl at 50 mM compared with that at 25 mM. This reduction in root cap length was due to peeling off of root cap cells. The length of the root cap was shorter in the sensitive cultivars than in the tolerant cultivars and this trend was more prominent in the early seedling stage. Therefore, proliferation of root cap cells might be related to exclusion of Na⁺ or avoidance of Na⁺ entry to the root proper. In fact, the transcripts of salt-inducible OsPMP3 genes, which encode plasma membrane protein 3 (PMP3) and are proposed to function in regulating the plasma membrane potential and restrict Na⁺ uptake, are specifically located in root cap cells of salt-treated rice plants (Mitsuya et al., 2007).

The Casparian band is another candidate for controlling Na⁺ exclusion. The Casparian band was developed in endodermis and matured closer to the root tip under NaCl treatment (Fig. 7). The length of the root tip without an endodermal Casparian band was shorter in sensitive than in tolerant cultivars (Table 1). Development of Casparian band was more prominent in a sensitive cultivar than in a tolerant cultivar (Fig. 7). The Casparian band is considered

<table>
<thead>
<tr>
<th>Parameter (mm)</th>
<th>IR 24 Control</th>
<th>25 mM NaCl</th>
<th>50 mM NaCl</th>
<th>Nipponbare Control</th>
<th>25 mM NaCl</th>
<th>50 mM NaCl</th>
<th>Nona bokra Control</th>
<th>25 mM NaCl</th>
<th>50 mM NaCl</th>
<th>Pokkali Control</th>
<th>25 mM NaCl</th>
<th>50 mM NaCl</th>
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<tr>
<td>Length of root</td>
<td>50.8±2.2</td>
<td>45.8±1.5</td>
<td>40.3±1.4*</td>
<td>75.8±5.3</td>
<td>66.3±3.7*</td>
<td>56.4±4.1**</td>
<td>50.4±4.1*</td>
<td>45.3±2.4</td>
<td>35.6±1.9</td>
<td>25.8±1.7</td>
<td>23.8±1.5</td>
<td>20.5±1.1</td>
</tr>
<tr>
<td>Casparian band</td>
<td>26.4±11</td>
<td>20.5±11</td>
<td>18.7±0.7*</td>
<td>28.4±14</td>
<td>22.8±13</td>
<td>18.0±0.5*</td>
<td>32.3±3.2</td>
<td>28.6±13*</td>
<td>22.8±13*</td>
<td>33.0±1.5</td>
<td>29.5±1.7</td>
<td>23.8±15*</td>
</tr>
</tbody>
</table>

* and ** represent significant difference from the control at P<0.05 and P<0.01, respectively by Tukey’s HSD test.

In addition, the correlation between growth and Na⁺ content or Na⁺/K⁺ ratio was observed regardless of growth stages. This fact suggests that the apparent difference in sensitivity at different growth stages is due to difference in accumulation of Na⁺ and K⁺ and not due to difference in physiological mechanism of adaptation.

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to prevent the apoplastic passage of Na⁺ from cortex to stele due to barrier effects (Enstone et al., 2003; Karahara et al., 2004; Kosala et al., 2005; Zhu et al., 2006). However, Tsuchiya et al. (1994) reported that Na⁺ exclusion across the endodermis is more efficient in a sensitive cultivar than in a resistant cultivar, which coincides with our finding that the endodermal Casparian band developed more in sensitive cultivars than in resistant cultivars. These facts suggest that the Casparian band may not avoid Na⁺ incorporation efficiently to establish the varietal difference in salinity resistance. In addition development of the Casparian band may disturb K⁺ absorption, which may cause an adverse effect on salinity resistance. Therefore, development of the Casparian band may not be a determinant of salinity resistance.

In conclusion, Na⁺ exclusion from the root is important to reduce Na⁺ content and Na⁺/K⁺ ratio and contributes to salinity resistance of rice seedling. The root cap is suggested to play an important role in Na⁺ exclusion, while the Casparian band may not efficiently function in salinity resistance.

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* In French with English summary.