Vertical Distribution of Sodium in Roots of Rice Plants Exposed to Salinity as Analyzed by Cryo Time-of-Flight Secondary Ion Mass Spectrometry

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**Abstract:** Distribution of Na\(^+\) along the root axis under salinity stress was analyzed in two rice (Oryza sativa L.) cultivars with different salt resistance (salt-sensitive IR 24 and salt-resistant Pokkali). Rice plants were grown hydroponically and NaCl was applied with nutrient solution at concentrations of 0, 25 and 50 mM for 7 d after germination. The distribution of Na\(^+\) in roots under salinity was analyzed by the cryo time-of-flight secondary ion mass spectrometry (cryo TOF-SIMS). The Na\(^+\) content in the root was higher in salt-sensitive IR 24 than in salt-resistant Pokkali under NaCl stress. The content was highest at the root tip and was decreased basipetally along the root axis. The difference in Na\(^+\) content between the cultivars was apparent in all regions from the root tip.

**Key words:** Cryo time-of-flight secondary ion mass spectrometry (cryo TOF-SIMS), Rice (Oryza sativa L.), Salinity stress, Sodium.

Salinity stress is one of the most serious environmental factors limiting the growth and yield of crop plants (Hillel, 2000). Rice (Oryza sativa L.) is one of the most important cereals in the tropics and subtropics, and its yield is very sensitive to salinity (Khatun et al., 1995). Considerable efforts have been made to select salt resistant rice cultivars for sustained crop production (Lee et al., 2003), but progress has been slow due to inadequate understanding of the mechanism of resistance.

The resistance mechanism consists of avoidance and tolerance (Levitt, 1972). Previously we found that avoidance of Na\(^+\) by the root is important for resistance (Ferdose et al., 2009).

To further understand the mechanism of resistance, we examined the Na\(^+\) distribution in the roots under salinity in two rice cultivars differing in salinity resistance. For cryogenic analysis, we used cryo time-of-flight secondary ion mass spectrometry (cryo TOF-SIMS), a highly sensitive method (Hagenhoff, 2000) for quantification of soluble ions on the surface of the specimen (Metzner et al., 2008).

**Materials and Methods**

1. **NaCl treatment of hydroponically cultured rice cultivars**

Two indica type rice (Oryza sativa L.) cultivars, salt-sensitive IR 24 and salt-resistant Pokkali, were used. The cultivars were hydroponically cultured according to the method described previously (Ferdose et al., 2009). Seeds of each cultivar were surface-sterilized and imbibed. Uniformly germinated seeds were grown 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 for 7 d. The NaCl concentration in the nutrient solution was 0, 25 or 50 mM. The lower part of the beakers was covered with aluminum foil and plastic nets were shaded with black plastic sheets, to illuminate the shoot but not the root. The culture solutions were renewed every day.

2. **Growth response measurement**

The growth response was analyzed by measuring the lengths of seminal roots and expressed as the relative value of control. The data were expressed as the means of five independent experiments.

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3. Cryo time-of-flight secondary ion mass spectrometry

The seminal roots, 0 to 30 mm from the root tip, were quickly excised with a sharp razor blade and immediately frozen in liquid nitrogen and stored at −80°C. The samples were taken from the freezing device and cross sections of roots, 60 μm in thickness, were made with a cryostat (Cryocut 1800, Leica, Germany). The sections were mounted with adhesive carbon tape, layered with a silicon plate to place in horizontal orientation in the interior of stainless mesh. The specimens were immediately placed in liquid nitrogen with a liquid-nitrogen-cooled holder and subsequently transferred to the cryo TOF-SIMS, TRIFT III NDF model (ULVAC-PHI, inc. Japan) spectrometer. Positive secondary ions were generated using Ga ion. The recording time for imaging was 20 min. The total ion count per 20 min was adjusted to two million, to compare the relative abundance of Na⁺ in different sections. The relative content of Na⁺ was estimated in three roots. Cross sections for light microscopy were made with a microslicer (DTK-3000W, Dosaka), stained with 0.05% (w/v) toluidine blue O for 0.5−3 min, rinsed thoroughly with distilled water and were immediately observed under a light microscope (Olympus BX51).

4. Statistical analysis

The numerical data were subjected to analysis of variance and the difference from control and that between cultivars were statistically analyzed by Tukey’s HSD test.

Results

1. Effect of NaCl on the growth

Figure 1 shows the effects of NaCl on the relative seminal root length of two rice cultivars. Relative growth of roots in both cultivars was decreased with the increase of NaCl concentrations, but the salinity stress in the present condition was not so detrimental. IR 24 was more sensitive than Pokkali.

2. Effect of NaCl treatment on the distribution of Na⁺

Figure 2 shows the effects of NaCl on the distribution of Na⁺ in the basal part of the root tip (7−12 mm from the tip) in two rice cultivars treated with NaCl at three concentrations. The signal of Na⁺ was scarcely detected in the control (Fig. 2, top), but was higher in salt-sensitive IR 24 (Fig. 2A, second and third row) than in salt-resistant Pokkali (Fig. 2B, second and third row) under salinity. Na⁺ was evenly distributed within a cross section. The bright field light microscopic photographs show the root tissues of rice cultivars as reference (Fig. 2, bottom).

3. Vertical distribution of Na⁺ content along the root axis

Figure 3 shows the Na⁺ content at different distances from the root tip in two rice cultivars. Na⁺ count of both cultivars was much higher at the root tip, abruptly decreased in the next region and gradually decreased basipetally along the root axis. The Na⁺ content was significantly higher in salt-sensitive IR 24 than in salt-resistant Pokkali.

Discussion

Previously, we found a significant negative correlation between the growth and Na⁺ content of roots irrespective of cultivars and growth stages (Ferdose et al. 2009). Thus, avoidance of Na⁺ from roots seems to be involved in the difference in the expression of Na⁺ resistance among rice cultivars.

In this study, we investigated the distribution of Na⁺ in rice roots under salinity stress by cryo TOF-SIMS to gain insight into the root function in Na⁺ avoidance. TOF-SIMS is a useful tool to examine the distribution of soluble materials prepared cryogenically (Belu et al., 2003; Martin et al., 2004; Nygren and Malmberg, 2007; Kuroda et al., 2008; Metzner et al., 2008). In the present study, the cryo TOF-SIMS image revealed that the distribution of Na⁺ was higher under NaCl treatments, and the difference was more prominent in IR 24 than in Pokkali (Fig. 2).

Storey et al. (2003) reported that Na⁺ content analyzed by X-ray microanalysis is higher in the pericycle cells than other cell types in salinized grapevines. Lauchli et al. (2008) also reported that Na⁺ content is higher in the pericycle in a low salt-transport wheat genotype and is higher in xylem parenchyma in a high salt-transport wheat genotype. However, localized distribution of Na⁺ was not detected in the present observation (Fig. 2). This is probably due to the limit of resolution of TOF-SIMS and technical difficulty of tissue preservation in cryo-sections of rice. However, cryo-TOF-SIMS clearly revealed the vertical
Fig. 3. Vertical distribution of Na⁺ content along the seminal root axis in two rice cultivars (A: IR 24, B: Pokkali) treated with 50 mM NaCl. Na⁺ content in sections from different regions was estimated with cryo TOF-SIMS as the count of Na⁺ signals in 20 min. Each value is the mean ± standard error of three independent experiments. ** represents difference from the tip (0–1 mm) region and ++ represents significant difference between cultivars at P < 0.01 as analyzed by Tukey’s HSD test.
distribution of Na⁺ content along the root axis of salinity-treated rice by quantification of ions on the sections (Fig. 3).

In the present study we measured the count of Na⁺ signals from the cross sections of roots during 20 min of analysis. The counts of total ion signals were adjusted to two million to compare the relative abundance of Na⁺ in different sections. The count of Na⁺ was highest at the root tip region and was abruptly decreased in the next region but reduced gradually in the further basal regions along the root axis (Fig. 3). In addition the count of Na⁺ was lower in resistant Pokkali than in sensitive IR 24 in all regions from the root tip to the root base.

The present observation clarified the vertical distribution of Na⁺ content and showed that the Na⁺ content was highest at the root tip. This suggests that the root function is responsible for the cultivar difference in Na⁺ incorporation. However, the cultivar difference in Na⁺ content of the root (0−30 mm) was at most 20% (Fig. 3) while the cultivar difference in the whole root system is about 50% (Ferdose et al., 2009). Therefore, other root characteristics such as branching pattern of root system (Faiyue et al., 2010 a, b) may be involved in the cultivar difference in Na⁺ incorporation.

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