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Physiological and genetic analyses of aluminium tolerance in rice, focusing on root growth during germination

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Abstract

Aluminium (Al) ion limits root growth of plants in acidic soils, and rice exhibits the highest level of Al-tolerance among graminous crops. To elucidate Al-tolerance mechanisms in rice, response to Al was compared between rice (*Oryza sativa* L., cv. Nipponbare) and wheat (*Triticum aestivum* L., cv. ET8), focusing on seminal root growth at seedling stage and germination stage. At seedling stage, rice and wheat were similarly sensitive to Al in both dose- and time-dependent manner during a 24-h Al exposure. On the contrary, at germination stage, rice was more tolerant to Al than wheat, and wheat roots displayed the loss of plasma membrane integrity more extensively than rice. A rice mutant exhibiting Al hypersensitivity at germination stage was obtained by screening 42,840 R_2 progeny derived from the regenerated R_0 plants of Nipponbare and thereafter confirmation of the mutant phenotype in R_3 progeny. At germination stage, root growth of the mutant was strongly inhibited in the presence of Al but not in the absence of Al. However, at seedling stage, root growth of the mutant and wild type was similarly tolerant to Al. Taken together, we conclude that rice possesses Al-tolerant function that is under genetic control and specifically operates for root growth at germination stage, making rice more tolerant to Al than wheat.

Keywords: Aluminium toxicity; Aluminium-sensitive rice mutant; Germination; Rice; Root growth; Wheat

1. Introduction

Phytotoxic Al ion (mainly Al^{3+}) restricts crop productivity in acidic soils that cover almost 40% of world's arable land [1–4]. The primary toxic symptom of Al ion is root growth inhibition. Several mechanisms of Al phytotoxicity [5–11] or tolerance (or resistance)[12–14] have been proposed in various plants. There are two distinct classes of Al-tolerance mechanisms. One class of mechanisms allows the plant to tolerate Al accumulation in the root and shoot symplasm, and the other class operates to exclude Al from the root apex, which is often related to the Al-triggered exudation of organic acids. Many of Al-tolerant plant species release organic acid anions such as citrate, malate or oxalate from root apices in response to Al-stress [15–17], and organic acid anions chelate Al cation and ameliorate Al-induced inhibition of root elongation [18,19]. A wheat gene, *ALMT1*, encoding a malate transporter has been recently isolated from root apices of Al-tolerant wheat cultivar ET8, and heterologous expression of the *ALMT1* gene enhanced Al-tolerance in cultured tobacco cells [20] and barley roots [21]. Thus, the efflux of organic acids from root apices under Al-stress seems to play a central role in Al-tolerance mechanism. However, a possible drawback of this mechanism involves loss of carbon source due to the exudation of

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organic acids. Moreover, organic acids released into rhyzosphere appear to get easily decomposed by bacteria [22] or yeast [23].

On the other hand, among graminous crops such as rice, maize, wheat and barley, rice exhibits the highest level of Al-tolerance, despite of its quite low efflux capacity for organic acids under Al-stress [24]. These results suggest that Al-tolerance mechanism in rice is seemingly not associated with the efflux of organic acids. Aluminum tolerance mechanisms in rice have not been elucidated.

In this paper, in order to reveal the mechanism associated with highest level of Al-tolerance of rice among graminous crops, Al-sensitivity of root growth was compared between rice (cv. Nipponbare) and wheat (cv. ET8), focusing on root growth at seedling stage where most previous studies of Al-toxicity in root system have been conducted, and also on root growth at germination stage, which seems to be the first critical step for survival of plants under Al-stress. Our physiological analyses demonstrate that rice exhibits much higher tolerance to Al than wheat especially at germination stage but not at seedling stage. Based on this finding, we isolated an Al-sensitive rice mutant at germination stage, indicating that rice has a unique Al-tolerant gene specifically operating during root growth at germination stage.

2. Materials and methods

2.1. Determination of Al-sensitivity of root growth at seedling stage

Seeds of rice (cv. Nipponbare) and wheat (cv. ET8, kindly provided by Dr. Z. Rengel) were surface sterilized in a 0.1% solution of sodium hypochlorite, rinsed twice for 2 h with distilled water, and then soaked in distilled water at 4 °C in the dark for 4 days. The pre-soaked seeds were then germinated on a mesh floating on 0.5 mM $CaCl_2$ solution at 30 °C at 65% relative humidity in the dark for 2 days, and then transferred to the illuminated condition (150 μmol photons m^{-2} s^{-1}, 14-h photoperiod) for 2 days. Four-day old seedlings were exposed to 0.5 mM $CaCl_2$ solution (200 mL per seedling) without (control) or with $AlCl_3$ at pH 4.5 for up to 24 h at 30 °C with aeration. Root growth of each plant was measured before and after the treatment, and the net root growth during the treatment was calculated. Since each wheat seed developed a few roots (see Fig. 2B), the length of the longest one was measured.

2.2. Determination of Al-sensitivity of root growth at germination stage

We made a device for observation and measurement of root growth from germination stage to seedling stage in hydroponic culture (see Fig. 2A). Two plastic plates (145 mm × 215 mm and 50 mm × 215 mm) were combined together with a 10 mm space. In this space, a plastic mesh was set. Then the sterilized and pre-soaked seeds as described above (see Section 2.2) were transferred on the mesh, and the device was placed on the top of a tank. Treatment solution (0.5 mM $CaCl_2$ containing with or without 50 μM $AlCl_3$ at pH 4.5) was filled till the mesh position (~6 L solution per 10 devices), then the seeds were germinated and grown on the mesh in the dark for 2 days at 30 °C with aeration, and then in the illuminated condition for 2 days. The Al solution was changed to fresh one every 2 days. Then, the germinated seeds were photographed and the length of roots was determined.

2.3. Assessment of the loss of integrity of the plasma membrane

The loss of plasma membrane integrity was evaluated by a spectrophotometric assay based on Evans Blue stain retained by root cells, as described previously with minor modifications [9]. In brief, after Al treatment with or without 50 μM $AlCl_3$ for up to 7 days as described above (see Section 2.2), roots were stained with a 0.025% [w/v] Evans blue solution for 10 min. Then, stained roots were washed with 0.5 mM $CaCl_2$ solution (pH 4.5) extensively, and were photographed. For quantification of the Evans blue retained in root apex, a 2-mm section from the root tip was removed and the trapped Evans blue was extracted by shaking with 0.5 mL of *N*,*N*-dimethylformamide. After extraction, optical density of the extracted solution was determined using spectrophotometer at 600 nm.

2.4. Screening and characterization of Al-sensitive rice mutant

To determine a suitable concentration of Al for screening Al-sensitive rice mutants during germination stage, seeds of Nipponbare were sterilized and soaked in distilled water as described above (see Section 2.2) and then the seeds were germinated in a well of 24-well plastic plate [three seeds per well of 15.5 mm (diameter) × 17.3 mm (height), Asahi technoglass, Japan, see Fig. 4A] containing 0.5 mL of 0.5 mM $CaCl_2$ solution with increasing concentrations of $AlCl_3$ up to 500 μM at pH 4.5 for 4 days in the dark. Fresh Al solution was provided every day. Under the condition, root growth was not significantly inhibited by Al up to 300 μM. Thus, we decided 150 μM of Al as a suitable concentration for screening mutants more sensitive to Al than Nipponbare (wild type).

The regenerated Nipponbare plants[25–27] were produced as described previously[25], and R_0 seeds were obtained from National Institute of Agrobiological