Formation of Organic Acids in the Root Apices of Rice Plants (Oryza sativa L.) Grown in Acidic Nutrient Solution Containing Aluminum

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Aluminum (Al) is a major phytotoxic mineral in the soil at low pH conditions (Jones and Kochian, 1996). Pellet et al. (1995) reported that Al would damage the roots by making the plants more sensitive to other abiotic stresses such as deficiency of water and nutrients, and eventually reduce the crop yield.

One of the Al-tolerance mechanisms in plants would be Al exclusion from the root apices. In several upland plants, Al tolerance is closely associated with an increased ability of roots to release organic acids, such as citrate (de la Fuente et al., 1997; Ma et al., 1997a; Pineros et al., 2002), malate (Osawa and Matsumoto, 2001; Tesfaye et al., 2001), and oxalate (Ma et al., 1997b; Wenzl et al., 2001), which may prevent $\text{Al}^{3+}$ uptake by chelating Al. Rice, a lowland plant, has been identified as a high Al-excluder at root apex (Osaki et al., 1997), but the mechanism of Al exclusion in rice plants has not been fully elucidated.

The purpose of the present study is to confirm that the varietal difference of the Al tolerance in rice is associated with organic acid formation in the intact root apices when Al was added to the low pH nutrient solution, and furthermore, to identify the sort of organic acid formed in the root exposed to Al.

Materials and Methods

Seven acid-tolerant rice varieties, that is, GP15, Luk-daeng (L-daeng), KDML105, Suphanburi 90 (S-buri...
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(90) and IR53650 of Indica-type, and Norin 30 (N-30) and Hatsusakumochi (H-mochi) of Japonica-type, and three acid-intolerant rice varieties, that is, Kinangdang Puti (KP), IR36 of Indica-type, and Nipponbare (N-bare) of Japonica-type identified in our previous study (Kang and Ishii, 2003), were used in the present study. The pre-germinated seeds were sown on a nursery bed of sand, and the seedlings were grown for three weeks in Yoshida nutrient solution, adjusted to pH 5.3 (Yoshida et al., 1976). The solution was renewed every 2 days. The seedlings were transplanted at 3-cm intervals on a styrofoam plate floated on culture solution containing 0.2 mM NH4NO3 and 0.1 mM CaCl2 • 2H2O, with either 0 (−Al), or 0.15 mM (+Al) AlCl3 • 6H2O in a plastic box (60 × 40 × 20 cm). The culture solution was adjusted to pH 3.8 every day. The experiment was conducted with three replicates each for −Al, and +Al condition. The plants were grown for 10 days in a growth chamber controlled at 25°C/20 °C in a 16-h day/8-h night regime. The light intensity was provided from cool-white fluorescent and halogen lamps at 1,000 µmol m−2 s−1 at the level of the top of the plants.

For organic acid analysis, the root apices 10 mm in length were sampled from three plants of each variety. They were sampled from all the roots in each plant at 10 days after transplanting, and frozen immediately in liquid nitrogen. Frozen samples were stored at −80°C in a deep freezer, and served for the analysis of organic acids. The concentration of organic acids was measured by the method by Ma et al. (1997a), using an HPLC system (HIC-6A type, Shimadzu Ltd.).

The aboveground parts of the plants whose root apices were used for the determination of organic acid content, were harvested and dried at 80°C for 72 h in an oven to determine their dry weight. All data were subjected to a one-way analysis of variance (ANOVA) with Tukey’s multiple range test between the mean values.

Results and Discussion

To confirm the varietal difference of Al tolerance reported previously (Kang and Ishii, 2003), we determined the dry weight of the aboveground part of the plants grown in the −Al, and +Al condition at 10 days after transplanting (Table 1). Dry weight under the +Al condition was as light as 17~38% of that under the −Al condition in the acid-intolerant varieties, although it was 45~84% in the acid-tolerant varieties, confirming the results reported previously.

Figure 1 shows the HPLC profiles of the five organic acids; citrate, malate, succinate, formate and acetate extract from the root apices of the rice grown in the −Al, and +Al condition. Only three organic acids, citrate, malate, and succinate, were detected in the root apices. We compared the concentration of these three organic acids in the roots between the plants grown in the −Al, and +Al condition for 10 days (Fig. 2). The concentration of succinate was the highest among the three organic acids in both −Al and +Al conditions, but the difference between the two conditions was not great. On the other hand, the concentrations of malate and citrate, particularly citrate were extremely low in the −Al condition, while they were markedly high in the +Al condition. This suggests that malate...
and citrate were formed in the root apices in the +Al condition. Moreover, the citrate concentrations in the +Al condition tended to be lower in the acid-intolerant varieties than in the tolerant varieties, but no obvious varietal difference was observed for malate. Previously, we reported that the main mechanism of Al detoxification in the two tolerant varieties, L-daeng and KDML105, might be slow-translocation of Al from the root to the leaf (Kang and Ishii, 2003). If we exclude the two varieties, L-daeng and KDML105, from the tolerant varieties in the present experiment, the citrate concentration in the +Al condition was significantly higher in the tolerant varieties than in the intolerant varieties (Fig. 2).

Pellet et al. (1995) reported that in maize roots Al was detoxified in the symplasm where organic acids such as citrate and malate could chelate with Al. Hue et al. (1986) investigated the Al-detoxifying ability of various organic acids, and classified them into three categories; 1) the strong detoxifiers of Al, i.e., citric, oxalic, and tartaric acids, 2) the moderate detoxifiers, malic, malonic, and salicylic acid, and 3) succinic, lactic, formic and acetic acids as weak detoxifiers.

Ma et al. (2002) reported that the release of citrate induced by Al was unlikely to be the mechanism of Al tolerance in rice plants. However, in the present study, the acid-tolerant rice varieties, in the +Al conditions tended to accumulate a larger amounts of citrate in
the root apices than the intolerant varieties. Since we measured the concentration of organic acids in the root instead of the release of organic acids from the root, we could not confirm the release of organic acids from the root apices as the mechanism of Al tolerance. The present results, however, clearly showed a significant increase in the amounts of malate and citrate in the roots when the plants were grown in +Al condition, and also a higher level of citrate formation in the acid tolerant varieties, than in the intolerant varieties. Therefore, the formation of malate and citrate, particularly citrate in the root apices was considered to be associated with Al tolerance in rice plants.

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