Can aluminium resistance and nitrogen utilization of rice be enhanced simultaneously in acidic soils?

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Abstract
There are many factors limiting plant growth in acidic soils. Aluminium toxicity and ammonium-nitrogen often coexist in acidic soils due to low pH and poor nitrification. Understanding the mechanisms responsible for aluminium-ammonium interaction can help improve agricultural production and maintain ecological stability in acidic soils. The effects of ammonium and nitrate on Al resistance, of aluminium on ammonium uptake, and the correlation between aluminium resistance and nitrogen utilization were investigated here. The results indicated that ammonium enhances aluminium resistance of rice compared with nitrate. Aluminium does not inhibit ammonium uptake of Al-resistant rice cultivar but does that of the Al-sensitive rice cultivar, and Al-resistant rice cultivar exhibits higher ammonium uptake rate under Al stress compared with the Al-sensitive cultivar. Al resistance in rice is positively correlated with ammonium utilization or negatively correlated with nitrate utilization. Based on these results, it is possible to increase aluminium resistance and nitrogen utilization at the same time in acidic soils through the development of Al-resistant and ammonium-preferring rice cultivars and the application of suitable types of nitrogen fertilizers.

Key Words
Ammonium, nitrate, preference, toxicity, indica, japonica.

Introduction
Soil acidity is one of the most important limitations to agricultural production worldwide (Kochian *et al.* 2004). Approximately 30% of the world's total land area consists of acidic soils, and as much as 50% of the world's potentially arable lands are acidic (von Uexküll and Mutert 1995). In China, acidic soils cover about 2.18 million km^2, accounting for 22.7% of the total land (Zhao *et al.* 2002). At soil pH values at or below 5, dissolution of Al-bearing minerals results in toxic aluminium (Al) forms, inhibiting root growth and function, and thus reducing crop yields (Kochian *et al.* 2005). Except for aluminium toxicity, there are several other factors limiting crop production in acidic soils such as proton toxicity, manganese toxicity, and low phosphorus stress, but nitrogen (N) problems are generally not included in these limiting factors.

N is one of plant essential macronutrients and plays an important role in plant growth. If plants grown in acidic soils can not effectively utilize nitrogen, agricultural production and ecological safety of acidic soils can not be guaranteed even if other limiting factors were overcome. In fact, N utilization of plants and N transformation in acidic soils are apparently different from those in neutral to calcareous soils. Ammonium and nitrate are two main inorganic N forms available for plant uptake in soils. In the field, inorganic nitrogen occurs predominantly as NH_4^+ in soils of pH 4.0 to 6.0 and as NO_3^- in soils of pH 6.0 to 8.0 (McGrath and Rorison 1982). Another critical factor for the survival of plants in acidic soils is the presence of NH_4^+, which is a predominant N-source, since nitrification is depressed in these soils (Watanabe *et al.* 1998).

Thus, acidic soils may be dominated chemically not only by Al^{3+} but also by NH_4^+, and neutral to calcareous soils, although lacking toxic concentrations of Al^{3+}, do have higher concentrations of NO_3^- (Rorison 1985). Therefore, it is of interest and significance to investigate plant Al resistance and N utilization as a whole.

Rice prefers NH_4^+ as a major inorganic N-source since NH_4^+ is the predominant N species in anaerobic agricultural soils, in particular in paddy fields. In addition, the degree of Al resistance among small cereal crops usually follows the order rice ≥ rye > wheat > barley although genotypic variation also exists in each species (Ma *et al.* 2002). Our previous results have demonstrated that ammonium can alleviate Al toxicity in rice and reduce Al accumulation in roots compared with nitrate (Zhao *et al.* 2009). The present study is to further investigate N-Al interaction and the relationship between Al resistance and N utilization in rice, which are expected to provide experimental evidence for the hypothesis that Al resistance and N utilization of rice can be increased at the same time in acidic soils.

Methods

Plant materials and grown conditions

Two rice cultivars, indica cultivar kasalath (Al-sensitive) and japonica cultivar koshihikari (Al-resistant) (Ma *et al.* 2002) were used to investigate the effects of N forms on Al resistance and of Al on ammonium uptake. Thirty rice cultivars were used to study the correlation between N utilization and Al resistance. Rice seedlings were cultured in a growth chamber as described previously (Zhao *et al.* 2009). Al, NH_4^+, and NO_3^- were applied as $AlCl_3 \cdot 6H_2O$, NH_4Cl, and $NaNO_3$, respectively. Each experiment was conducted with three replicates.

Effects of ammonium and nitrate on Al resistance

Ten-day-old seedlings were treated in a full-strength Kimura B nutrient solution (pH 4.5) containing 1mM N as NH_4^+ or NO_3^- without (-Al) or with (+Al) 50 µM Al for 28 days. The pH of the culture solutions was initially adjusted to 4.5 by addition of 0.1 M HCl or NaOH and the solutions were renewed daily.

Ammonium uptake

Twenty eight-day-old seedlings were firstly treated in an N free full-strength Kimura B nutrient solution (pH 4.5) for 24 h, and then in an N free full-strength Kimura B nutrient solution (pH 4.5) without (-Al) or with (+Al) 50 µM Al for another 24 h. The treated rice seedlings (two seedlings each) were placed in a 150-mL black cup containing full-strength Kimura B solution (pH 4.5) of 1 mM NH_4^+ for 3 h. The initial and final concentrations of NH_4^+ in solution were determined colorimetrically at 610 nm. Transpiration (water loss) was also recorded. At the end of the experiment, the roots were separated and its dry weight was measured. NH_4^+ uptake rate was estimated via the depletion of NH_4^+ in the absorption solution during the experiments.

Evaluation of Al resistance and nitrogen utilization

Relative root dry weight (root dry weight with ammonium / root dry weight with nitrate X 100%) was used to express ammonium utilization ability of rice cultivars. Root dry weights were measured after different rice cultivars were grown in a full-strength Kimura B nutrient solution (pH 4.5) containing 2 mM NH_4^+ or NO_3^- for 24 days. Relative root elongation (root elongation with +Al / root elongation with -Al X 100%) was measured before and after rice seedlings were grown in 0.5 mM $CaCl_2$ solution (pH 4.5) without (-Al) or with 50 µM Al (+Al) for 24 h.

Results

Effects of ammonium and nitrate on Al resistance

After rice seedlings were treated with 50 µM Al in nutrient solutions containing different N forms for 28 days, almost no inhibiting effects of Al on rice growth were observed under ammonium nutrition irrespective of Al-resistant or Al-sensitive cultivar, but huge inhibiting effects of Al on rice growth of two cultivars exhibited under nitrate nutrition (Figure 1).

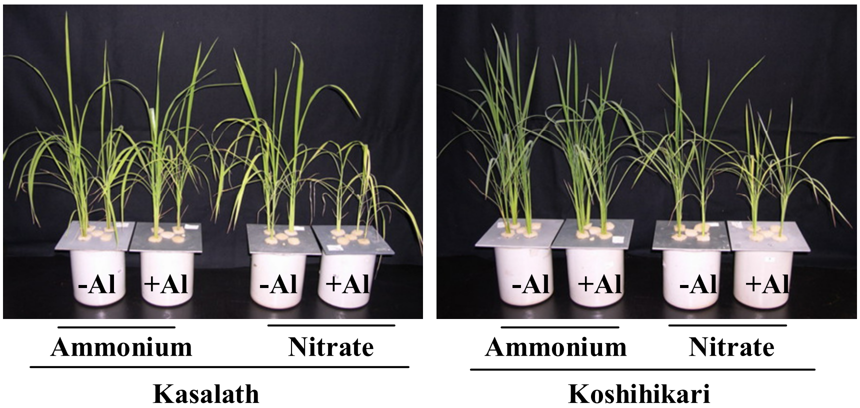

Figure 1. The growth appearance of Al-resistant cultivar koshihikari and Al-sensitive cultivar kasalath under Al stress with different nitrogen form supply.

Corresponding with the appearance of rice seedlings (Figure 1), stronger decrease in shoot and root dry weight by Al treatment was found with nitrate than with ammonium (Figure 2). These results further demonstrated that ammonium can alleviate Al toxicity of rice compared with nitrate, which is consistent with our previous reports (Zhao *et al.* 2009).

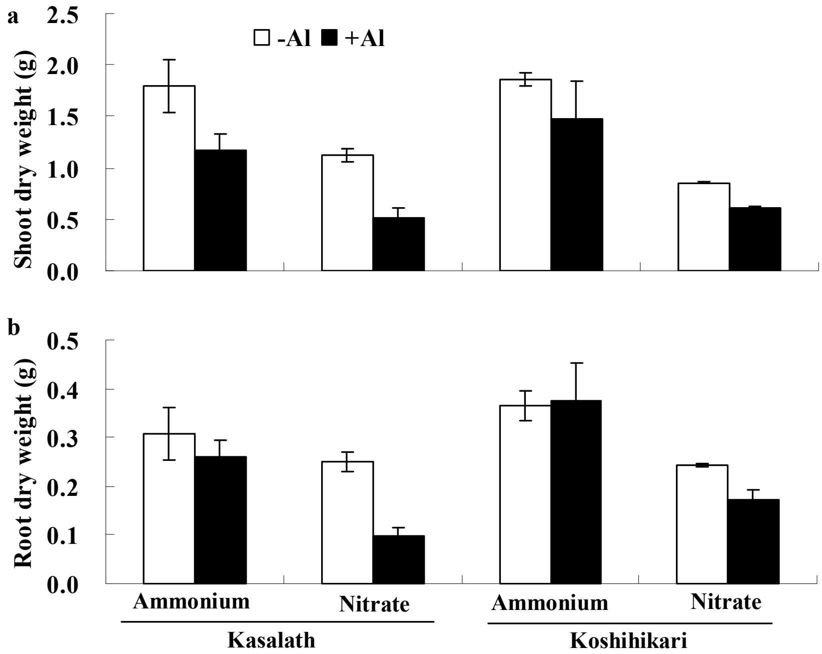

Figure 2. Effects of Al on shoot dry weight (a) and root dry weight (b) of Al-resistant cultivar koshihikari and Al-sensitive cultivar kasalath with different N form supply. Data are means ± SD (n = 3).

Effects of aluminium on ammonium uptake

Pre-treatment with 50 µM Al significantly decreased ammonium uptake of kasalath but not that of koshihikari (Figure 3). When no Al addition, koshihikari showed similar even lower ammonium uptake rate compared with kasalath, but when Al was added, ammonium uptake rate of koshihikari was higher than that of kasalath (Figure 3). These results suggested that Al did not inhibit ammonium uptake of Al-resistant rice cultivar which obtained higher ammonium uptake rate under Al stress.

Correlated relationship between rice Al resistance and N utilization

The correlation analyse indicated that there was a positive correlation between Al resistance and ammonium utilization ability in rice (Figure 4). It can be said that: if one rice cultivar can preferentially utilize ammonium over nitrate, it will be more Al-resistant, or, if one rice cultivar can preferentially utilize nitrate over ammonium, it will be more Al-sensitive. The two genetic traits of inorganic N utilization and Al resistance in rice often come along with each other.

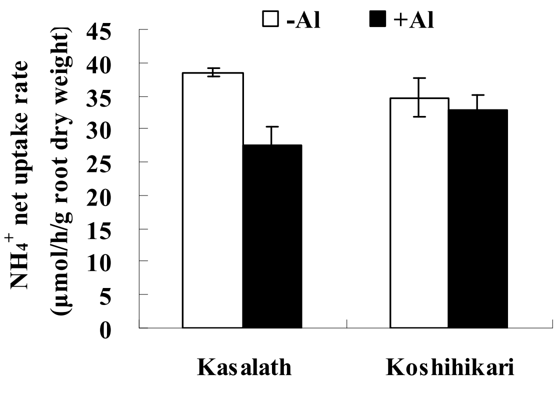

Figure 3. Effects of aluminium on ammonium uptake by Al-resistant cultivar koshihikari and Al-sensitive cultivar kasalath. Data are means ± SD (n = 3).

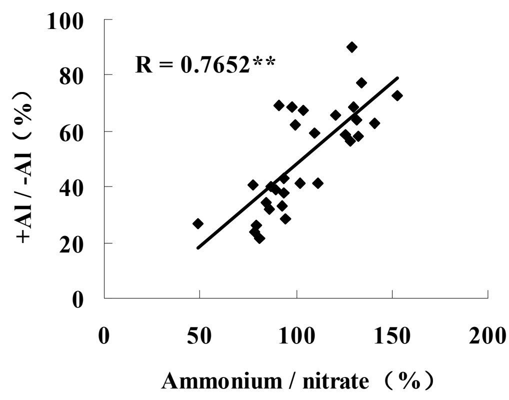

Figure 4. The correlation analyse between Al resistance and N utilization in 30 rice cultivars. +Al / -Al (%) expresses relative root elongation. Ammonium / nitrate (%) expresses relative root weight.

Conclusion

It can be concluded that: (1) compared with nitrate, ammonium can alleviate Al toxicity or enhance Al resistance of rice; (2) Al does not inhibit ammonium uptake of Al-resistant rice cultivar but does that of Al-sensitive cultivar, and Al-resistant rice cultivar obtains higher ammonium uptake rate under Al stress compared with Al-sensitive cultivar; (3) Al resistance is positively correlated with ammonium utilization. According to these conclusions, we point out that it is possible to enhance Al resistance and N utilization of rice at the same time in acidic soils through the selection of rice cultivars, soil fertilizer management and molecular genetic modification. More experimental evidences of field experiments and molecular biology are needed in future research.

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