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Differences Among Rice Cultivars in their Adaptation to Low Ionic Strength Solution with Toxic Level of Aluminum that Mimics Tropical Acid Soil Conditions

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ABSTRACT

Nutrient deficiencies are often an additional growth-limiting factor in tropical acid soils. Considering the potential interactions between Al stress and low-nutrient stress, we investigated differences among rice cultivars for Al tolerance, low-nutrient tolerance, and combined stress tolerance. The main objective of this study was to identify the predominant growth-limiting factor in tropical acid soils. Tolerance to low nutrient stress and combined stress did not show any relationship with Al tolerance indicating that these stress factors act independently. Al-tolerant cv. Rikuu-132 was tolerant to combined stress. Conversely, highly Al-sensitive cv. BR34 was most tolerant to combined and low nutrient stress. Combined stress tolerance of shoot was positively correlated with Ca content of shoot. The results indicate that Al tolerance alone is not adequate for superior performance on most acid soils. Tolerance to combined stress factors would be needed to improve productivity of rice on low fertility acid soils.

Keywords: acid soil, aluminum, low ionic strength solution, low nutrients, rice

Abbreviations: AN – Adequate nutrients, LN – Low nutrients, AN+Al – Adequate nutrients with aluminum, LN+Al – low nutrients with Al

INTRODUCTION

There are many causes for the poor growth of plants in acid soils. The common and primary stress factors are: 1) H^+ toxicity/low pH, 2) Al and Mn toxicities, 3) deficiencies of essential nutrients (N, P, K, Ca, Mg, Mo and B) (Rao, 2001; Rao et al., 1993). The major problems of these soils are the low content of cations, the toxicity of exchangeable Al and/or soluble Al, and low level of phosphorus and also low silicon availability due to long weathering (Okada and Fischer, 2001; Rao, 2001; Rao et al., 1993). Severals studies were conducted to identify major factors that influence plant growth in solution culture with or without Al in solution and on its related mechanisms (Ofei-Manu et al., 2001; Pavan et al., 1982; Pintro and Taylor, 2004). However, the number of studies that have simultaneously considered the two major factors (high Al and low nutrients) in tropical acid soils is limited (Wenzl et al., 2003). In high nutrient solutions, Al toxicity is alleviated by occurring physicochemical interaction between Al and other ions, the formation of non-toxic complexes with OH^-, SO_4^{2-} and precipitation of Al with high ionic strength solution (Blamey et al., 1983, 1991; Wheeler and Edmeads, 1995). The ionic strength of soil solutions of Savanna varied 1.3–1.7 mM in unfertilized conditions, which further increased to 5.4–13.4 mM after fertilization (Wenzl et al., 2003). Wenzl et al. (2001) found that root growth of *Brachiaria ruziziensis*, relatively Al-sensitive grass, was reduced in a solution containing toxic concentrations of Al and low nutrients than *B. decumbens*. An inadequate supply of nutrients may be one of the main factors that contribute to poor persistence of *B. ruziziensis* in infertile acid soils (Rao et al., 1998). Al activities in a solution of tropical acid soil ranged from 2.26 to 196.5 μmol L^{-1} (Pintro et al., 1999). Blamey et al. (1991) reported that realistic root growth inhibition of Lotus could be obtained from a low ionic strength of solution and a high Al concentration at similar levels to those found in acid soils. Watanabe and Okada (2005) investigated the difference in Al tolerance between Indica and Japonica cultivars under low ionic strength condition. To the best of our knowledge, no comprehensive studies on the identification of the primary inhibitory factors for plant growth have been studied except for the studies reported by Akhter et al. (2009a) and Khan et al. (2011) for sorghum and maize. The main objective of the present study was to determine differences among rice cultivars in their tolerance to Al, low nutrient stress and combined stress factors of Al and low nutriens by using low ionic strength nutrient solution that mimicks low fertility acid soils of the tropics. Rice is a major food crop in Bangladesh and identification of rice cultivars that are tolerant to low fertility acid soils in Bangladesh will contribute to improved food security and poverty alleviation.

MATERIALS AND METHODS

Screening for short-term (24 h) aluminum tolerance

Indica type (Bangladeshi) rice seeds were collected from Bangladesh Rice Research Institute, Gazipur, Bangladesh. Seeds of Japonica cultivars were collected from the Faculty of Agriculture, Yamagata University, Japan; Shonai Branch Station, Yamagata Prefectural Agriculture Experiment Station, Yamagata, Japan or National Institute of Agro-Environmental Science, Tsukuba, Japan. All the chemicals were purchased from Wako Pure

Chemical Industries Ltd., Japan unless otherwise stated. Seeds were soaked in tap water under aeration for 24 h at 27^{o}C in a growth room, germinated under fluorescent white light (with a photosynthetic photon flux density of 81 μmol m^{-2} s^{-1}) and spread on nylon screen placed on a container filled with 9 L of tap water. Tap water contained 8.0, 2.92 and 1.95 mg L^{-1} of Ca, Mg and K, respectively. Temperature, light intensity and aeration were maintained as same throughout the experiment.

Seedlings with roots of 3-4 cm in length were selected and treated with 0.2 mM $CaCl_2$ for 6 h (pH 4.9). After measuring root length of the longest root by a ruler, roots of the seedlings were transferred to 0.2 mM $CaCl_2$ (control treatment), and 20 μM $AlCl_3$ in 0.2 mM $CaCl_2$ (Al treatment), and treated for 24 h at pH 4.9 for short-term treatment. At least 10 seedlings in each of control or Al treatments were used for short-term screening experiment. Just after 24 h, root length of the longest root for each seedling was measured again.

Plant culture and treatments in long-term (35 d) experiment

Elemental composition and pH in long-term culturing medium is shown in Table 1. All chemicals were purchased from Kanto Chemical Co., Inc., Japan. All seeds were soaked and spread on nylon screen for germination same as in short-term experiment. Just after sprouting, seedlings were transferred to the glasshouse for preculturing in tap water for 5 days. Seedlings with same size were selected and transplanted into the 40 L of low nutrient (LN) solution (one-fifth strength of adequate nutrients [AN]) for 2 d. Thereafter, all seedlings were treated with daily pH maintenance for 29 d as follows: 1) Control (AN, pH 5.2), 2) AN in Al conditions (42.6 μM soluble Al, pH 4.3), 3) LN (pH 5.2), 4) LN in Al conditions (pH 4.3). Ionic strength for AN was calculated as 22.6 mM whereas that of in LN was 4.7 mM. Mean concentrations of 42.6 μM soluble Al was obtained by mixing 370 μM Al and 230 μM P at pH 4.3 with frequent pH adjustment. Ionic activities of Al were calculated by a computer program developed by Wada and Seki (1994) (Fig. 1). Culture solutions just after daily pH adjustment were collected, filtered through membrane filter (0.2 μm in pore size), and P was measured. When necessary, P was added to maintain the recommended concentration in Table 1. P was measured colorimetrically by molybdenum blue method using spectrophotometer (U-2900, Hitachi, Japan) at 660 nm. Concentrations of each element were measured for confirmation by inductively coupled plasma atomic absorption spectrophotometer, ICP-AES (Liberty 220, Varian Australia Ptv. Ltd., Australia). The culture solutions were renewed weekly in first three weeks of culturing and every 5 d during the remaining days to maintain nutritional demand of the growing seedlings. Seedling replication was done 3 times whereas treatment replication was done twice. At harvest, 3 seedlings with similar sizes were selected, separated into shoots and roots, thoroughly washed, dried for 3 d at 70^{o}C in draft oven, and weighed.

Calculation of tolerances

The stress tolerances of the respective crops were calculated as % relative growth with respect to the plant dry weight, i.e.

$$\text{Al tolerance in AN (\%)} = \frac{\text{Dry weight in AN} + \text{Al}}{\text{Dry weight in AN}} \times 100$$

$$\text{Al tolerance in LN (\%)} = \frac{\text{Dry weight in LN} + \text{Al}}{\text{Dry weight in LN}} \times 100$$

$$\text{Low nutrient tolerance (\%)} = \frac{\text{Dry weightin LN}}{\text{Dry weightin AN}} \times 100$$

$$\text{Combined stress tolerance (\%)} = \frac{\text{Dry weight in LN} + \text{Al}}{\text{Dry weight in AN}} \times 100$$

Analysis of minerals in the plant samples

Dry root and shoot samples were homogenized and 0.1 g of each sample was taken for analysis. Wet ashing of samples was done by adding 4 ml of acid mixture (HNO_3:60%$HClO_4$ = 5:3, v/v) to the sample and heating. The ash was resolubilized with 1 M HCl followed by deionized water with repetitions and filtered. Measurement of P, K, Ca, Mg, Fe, Mn and Al concentration in the sample was carried out by ICP-AES (Liberty 220, Varian Australia Pvt. Ltd., Australia).

RESULTS

Mean value of Al tolerance under short-term treatment of simple nutrient solution for eight cultivars of rice was 43.3% (Fig. 2). Al tolerance was in the order of Rikuu-132, Kamenoo>Sasanishiki, BR41>>>Aikokuu, Rikuu-20, Domannaka, BR34. Under long-term conditions the whole plant average Al tolerance was 83% and 72% in AN and LN conditions, respectively (Fig. 3 A, B). Al stress in both nutritional conditions decreased the plant growth, but tolerant and sensitive cultivars maintained identical tendency i.e., whole plant Al tolerance in AN was in the order of BR41, Sasanishiki>Rikuu-132, Kamenoo>>> Domannaka> Aikoku>Rikuu-20, BR34 and whole plant Al tolerance in LN was in the order of BR41 > Rikuu-132, Sasanishiki> Domannaka > Kamenoo >>> BR34, Rikuu-20>Aikoku (Fig. 3 A, B).

Tolerance to low nutrient conditions was almost in reverse order of Al tolerances except for Rikuu-132 (Fig 4 C) and Al sensitive cultivars showed rather higher tolerance to low nutrients. Under combined stress conditions, i.e., combined tolerance did not follow any trend and are in random order when compared with Al tolerance and low nutrient tolerance (Fig. 4 D). Further, the combined tolerance did not show any correlation with Al tolerance or low nutrient tolerance (Table 2). Short-term Al tolerance showed significant positive relationship with Al tolerance in both nutrition (AN and LN) conditions (Table 2). As expected, significant negative correlation (R^2 = -0.884**) was observed between root Al concentration and Al tolerance in LN condition (Fig. 4 A). On the other hand, root Al concentration did not show any relationship with tolerance to combined stress conditions (Fig. 4 B).

Ca concentration in the shoot showed significant positive correlation with combined tolerance of shoot (R^2 = 0.507*) indicating the Ca in the shoot playing the most important role to ameliorate or minimize the effects of combined stress conditions (Fig. 5). However, in the absence of Al, shoot Ca level did not increase under LN conditions (Fig. 5).

DISCUSSION

We initially screened 23 Bangladeshi (Indica type) and 6 Japanese (Japonica type) rice cultivars for Al tolerance and further screened 18 pedigree cultivars of one Japanese tolerant cultivar. Among these 47 rice cultivars, we selected 8 cultivars (Fig. 2) and investigated long-term Al tolerance in the presence of differential nutrient concentrations, i.e., adequate nutrient (AN) and low nutrient (LN, 1/5th of AN). Among the selected cultivars, the relationship between short-term and long-term Al tolerances was 0.781** (r = 0.884**) and 0.653* (r = 0.808*) for AN and LN conditions, respectively (Table 2). These results suggest that the short-term (24h) screening technique for Al tolerance may be useful for estimating Al tolerance in long-term culture conditions with nutrients. Akhter et al. (2009a) also found similar results for sorghum and maize.

No correlations were found between short-term Al tolerance and the combined tolerance (R^2 = 0.09, r = 0.3) (Table 2). Although investigations based on similar short-term screening techniques have been reported (Akhter et al., 2009b; Khan et al., 2009a; 2009b; Kobayashi et al., 2004), our results suggest that a short-term screening technique may not be practically useful for estimating cultivar adaptation to the combination of stress factors found in tropical acid soils.

A significant negative correlation was observed with root Al concentrations and Al tolerance (y = -0.06x + 7.78, R^2 = 0.884**) (Fig. 4 A) suggesting greater uptake of Al ions with increasing sensitivity of the cultivars. On the other hand, root Al concentrations did not show any relationship with combined stress tolerance suggesting independence of combined stress tolerance from Al absorption ability. Similar results were also observed by Akhter et al. (2009a) and Khan et al. (2011) for sorghum and maize.

Although we measured nutrient elements in the plant samples and studied correlations with combined stress tolerance, no correlation of combined stress tolerance with any of the nutrient elements was observed except for shoot Ca and shoot combined stress tolerance (Fig. 5) that constituted almost three-quarters of the whole plant. Akhter et al. (2009a) reported significant correlation with maize between shoot Ca and combined stress tolerance, for an Al-tolerant crop species whereas Al-sensitive sorghum did not show such relationship. `

Some of the nutritional characteristics of plants grown in the presence of Al have already been reported (Foy and Brown, 1964; Hãussler *et al*., 2006; Mariano and Keltjens, 2005). We propose that the plant nutritional characteristics linked to low-nutrient tolerance observed in the present investigation should be further evaluated as an important strategy for plant adaptation in tropical acid soils for both Al-tolerant plant species and Al-sensitive plant species under low-Al conditions. Aluminum tolerance and low-nutrient tolerance for plant production in these soils may vary depending on the plant nutritional characteristics that are related to Al tolerance and low-nutrient tolerance. The soluble Al concentration and nutrient status of these soils are also important factors to consider for evaluation. A short-term screening technique that can be applied to these soils should, alternatively, be established in the future. So far, investigations were carried out using only monocotyledonous species. The recommended plant nutritional characteristics required to cope with low-nutrients containing Al may be different between plant species, for example, for dicotyledonous plants. Further research is needed using other crops, such as grain legumes, root and tuber crops and other high value crops grown in tropical areas.

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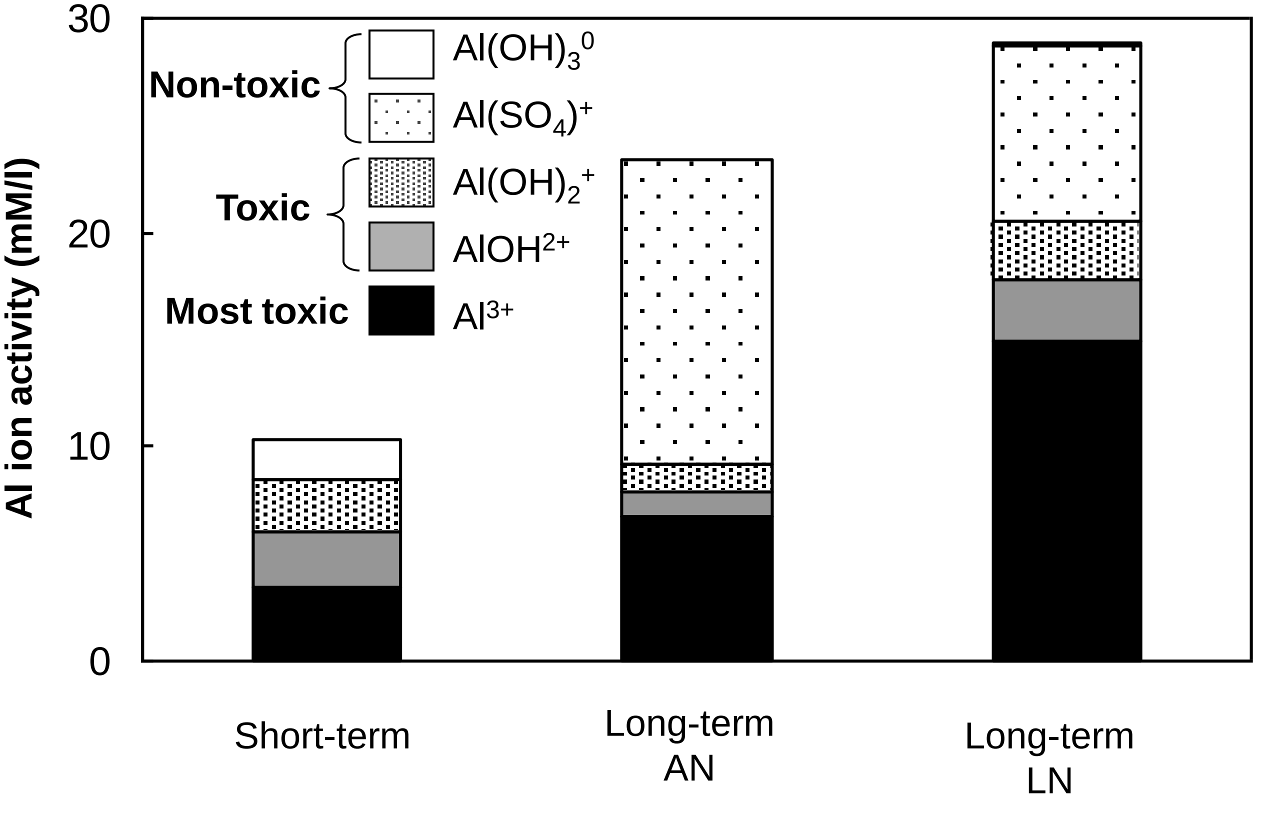

Fig. 1 Al ion activity in the culture solution. In short-term (24 h) added Al concentration was 20µM. In long-term experiments added Al and P concentration was 370µM and 230µM respectively.

Fig. 1, Khan et al. 2012

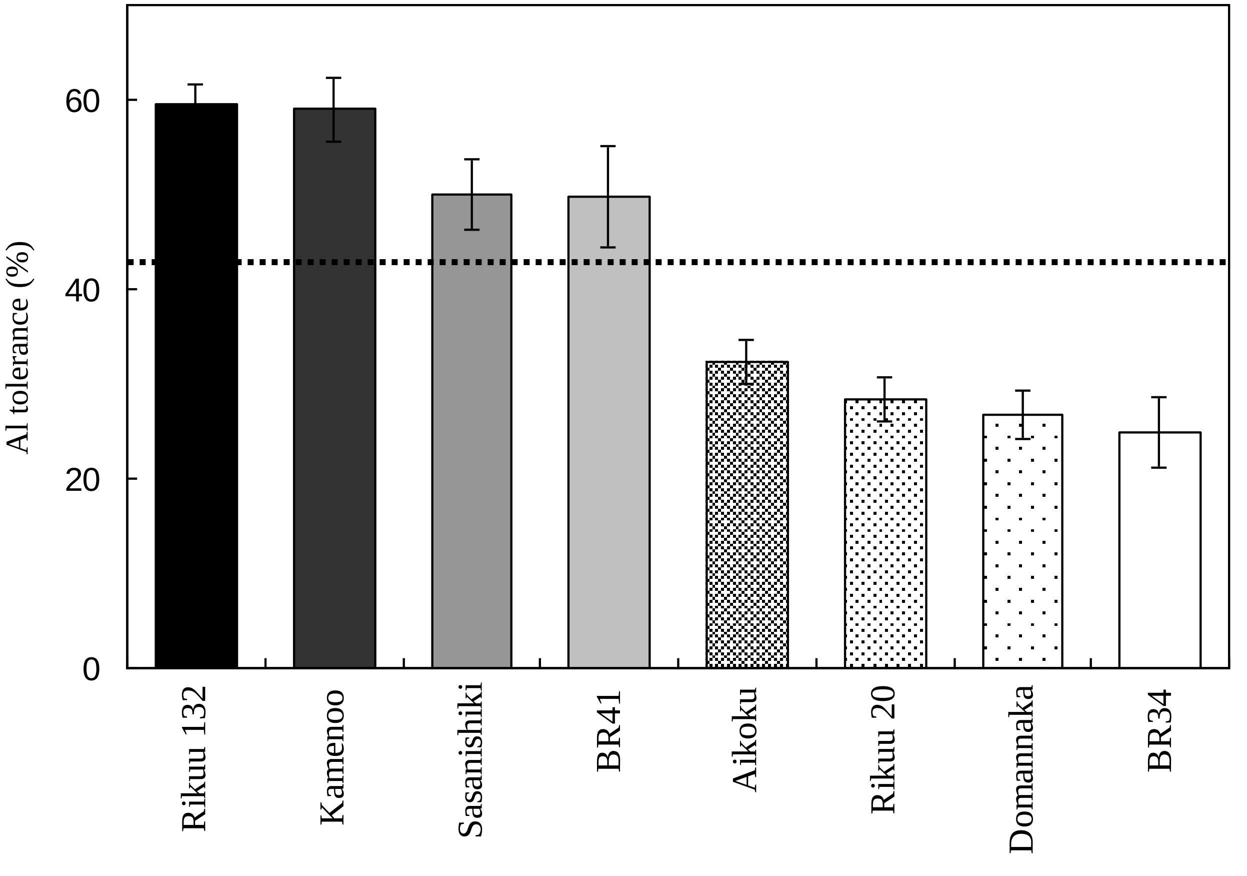

Fig. 2. Short-term aluminum (Al) tolerance for selected rice cultivars. 20 μM AlCl3 in 0.2 mM CaCl2 for 24 h (pH 4.9). Al tolerance is expressed as net root elongation of the longest root in treatment/net root elongation of control. Dotted line indicates average Al tolerance in all cultivars. Data are mean ±SE (n ≥ 10).

Fig. 2, Khan et al. 2012

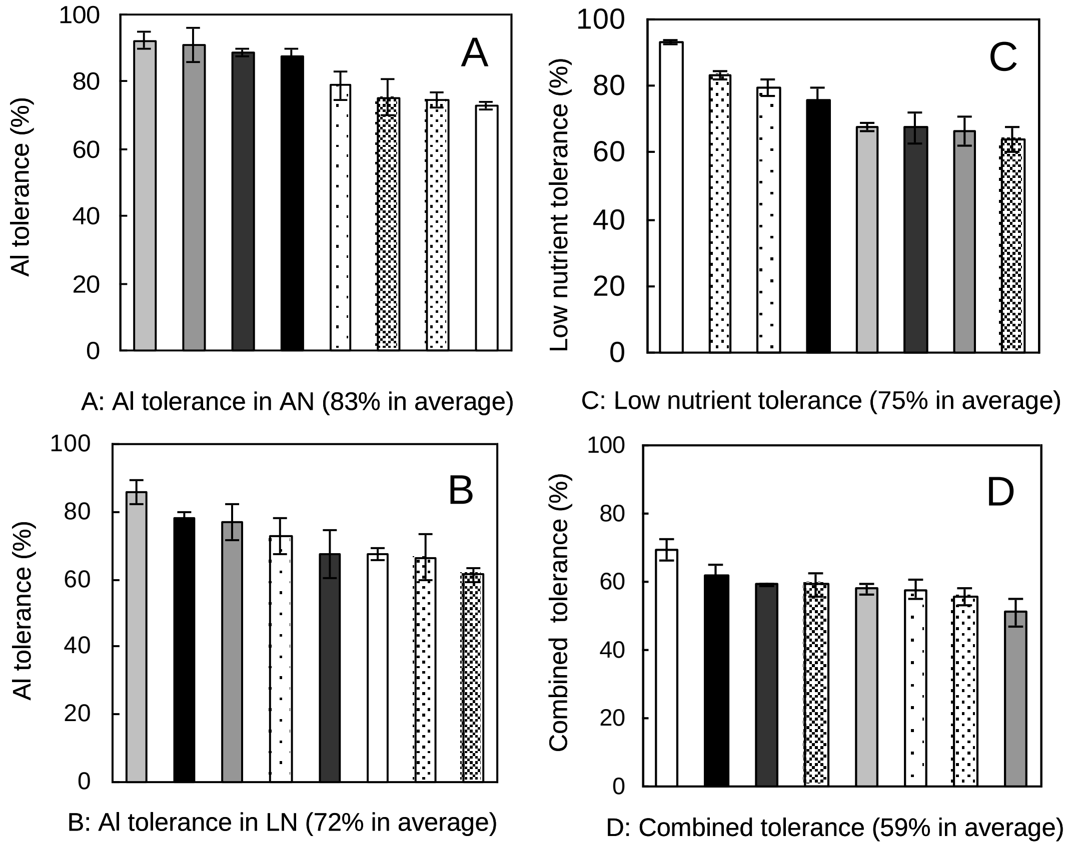

Fig. 3 Relative tolerances in long-term (35 d) hydroponics experiment. A. Al tolerance in AN conditions, B. Al tolerance in LN conditions, C. Low nutrient tolerance and D. Combined stress tolerance. Definitions of the tolerances were described in Materials and Methods. Color of each bar represents the same cultivar as shown in Fig. 2. Bar indicate ±SE, n=4

Fig. 3, Khan et al. 2012

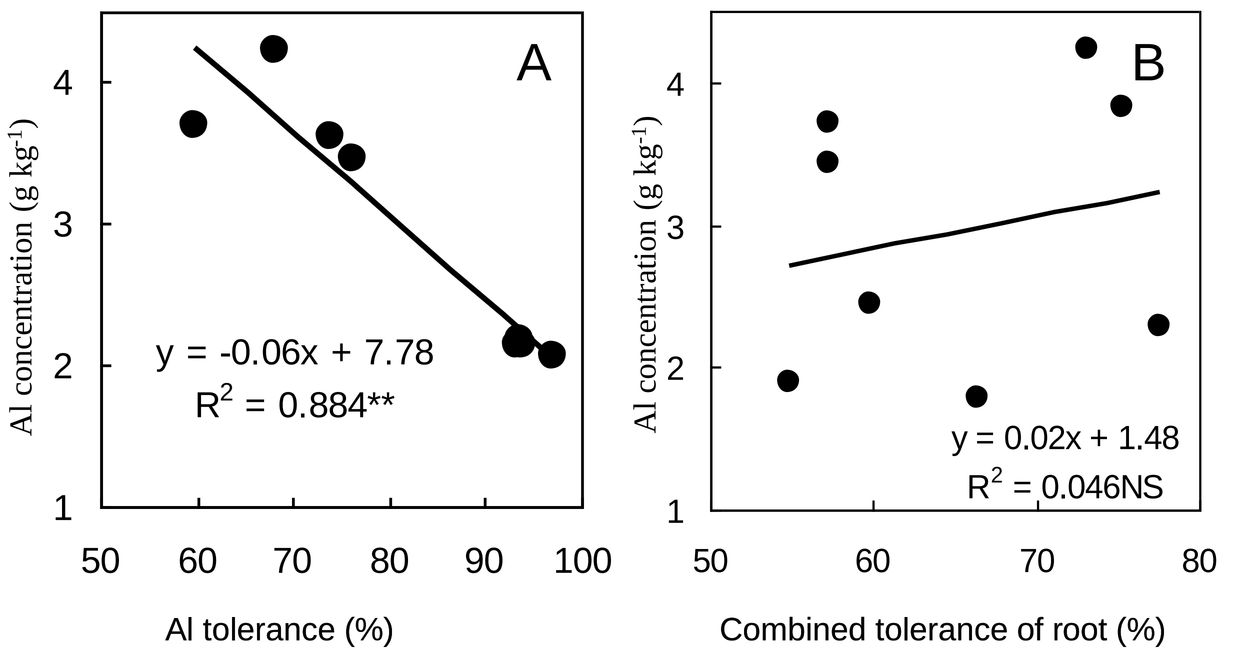

Fig. 4 Relationship of root Al concentration with Al tolerance (A) and combined stress tolerance (B)

Fig. 4, Khan et al. 2012

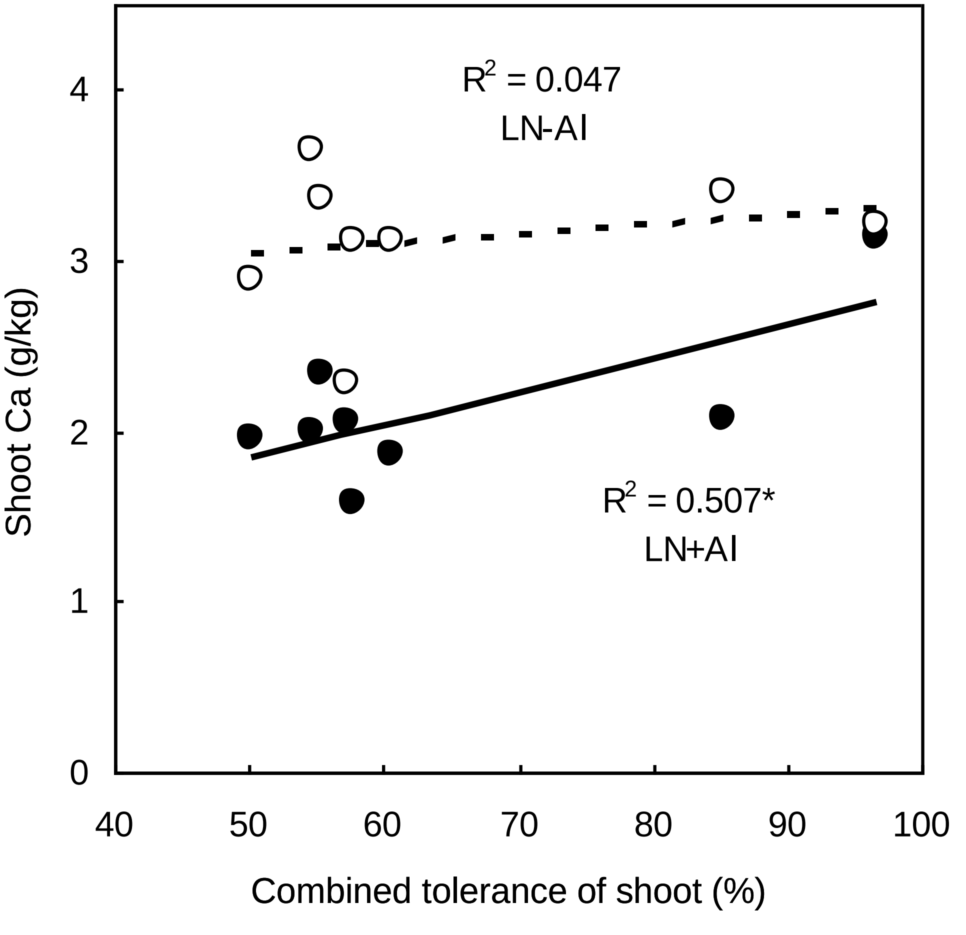

Fig. 5 Relationship of shoot Ca concentration with combined stress tolerance. Open circles are LN condition, closed circles LN+Al conditions

Fig. 5, Khan et al. 2012

Table 1: Elemental composition (mmol L-1 or µmol L-1) and pH of the long-term solution culture medium. Except for P and Al, all nutrients are the added concentrations. Phosphorus and Al are the measured concentrations.

Element		Treatments			
		Adequate nutrients (AN)		Low nutrients (LN)	
Salt	Element	AN-Al	AN+Al	LN-Al	LN+Al
NH_4NO_3	NO_3-N (mM)	2.86	2.86	0.57	0.57
NH_4NO_3	NH_4-N (mM)	1.43	1.43	0.29	0.29
NaH_2PO_4	P (mM)	0.26	0.005	0.05	0.005
K_2SO_4	K (mM)	1.53	1.53	0.31	0.31
$CaCl_2$	Ca (mM)	2.0	2.0	0.40	0.40
$MgSO_4$	Mg (mM)	1.65	1.65	0.33	0.33
$FeSO_4$	Fe (µM)	35.8	35.8	7.16	7.16
$MnSO_4$	Mn (µM)	18.2	18.2	3.64	3.64
$CuSO_4$	Cu (µM)	0.16	0.16	0.03	0.03
$(NH_4)_6Mo_7O_{24}$	Mo (µM)	0.05	0.05	0.01	0.01
H_3BO_3	B (µM)	37.0	37.0	7.4	7.4
$ZnCl_2$	Zn (µM)	3.06	3.06	0.61	0.61
$AlCl_3$	Al (µM)	—	42.6	—	42.6
pH		5.2	4.3	5.2	4.3

Table 2: Correlation coefficients (r value) among the tolerances (whole plant)

	Al tolerance in AN	Al tolerance in LN	Short-term Al tolerance	Low nutrient tolerance	Combined tolerance
Al tolerance in AN	1				
Al tolerance in LN	0.711*	1			
Short-term Al tolerance	0.884**	0.808*	1		
Low nutrient tolerance	-0.638	-0.389	-0.609	1	
Combined tolerance	-0.449	-0.187	-0.300	0.605	1