Greater contribution of low-nutrient tolerance to the growths of sorghum and maize under combined stress conditions with high-aluminum and low-nutrients in solution culture simulating the nutrient status of tropical acid soils

Afrin Akhter[1], Md. Shahadat Hossain Khan[1], Egashira Hiroaki[1], Keitarou Tawaraya[1], Idupulapati M. Rao[2], Peter Wenzl[3], Satoru Ishikawa[4], Tadao Wagatsuma[1*]

[1] Faculty of Agriculture, Yamagata University, Tsuruoka, Yamagata 997-8555, Japan
[2] International Center for Tropical Agriculture, Cali, Colombia

[3] Diversity Arrays P/L, PO Box. 7141, Yarralumla, Canberra ACT 2600, Australia

[4] National Institutes for Agro-Environmental Sciences 3-1-3, Kannondai, Tsukuba, Ibaraki 305-8604, Japan

* Correspondence: Tadao Wagatsuma
Faculty of Agriculture, Yamagata University
Tsuruoka, Yamagata 997-8555
Tel: 0235-28-2864
Fax: 0235-28-1812
E-mail: wagatuma@tds1.tr.yamagata-u.ac.jp

Running title: Low-nutrient tolerance in acid soils

Abstract

Aluminum (Al) tolerance is usually regarded as the determining factor for plant growth in acid soils and nutrient deficiencies are often additional growth-limiting factors in tropical acid soils. Considering potential interactions between Al toxicity and nutrient deficiencies, we investigated sorghum (*Sorghum bicolor* Moench [L.]) and maize (*Zea mays* L.) cultivar differences for (a) Al tolerance (relative growth in a one-fifth strength nutrient solution [low-nutrient medium, ionic strength: 4.5 mM] with Al and without Al), (b) low-nutrient tolerance (relative growth in the low-nutrient medium compared with growth in a full-strength nutrient solution) and (c) combined tolerance (relative growth in the low-nutrient medium containing Al compared with the full-strength medium lacking Al). The goal of this study was to identify the predominant growth-limiting factor using a solution culture medium that simulates the nutrient status of tropical acid soils. Differential Al tolerance among 15 cultivars of sorghum and 10 cultivars of maize in short-term assays (2.5 or 20 μM $AlCl_3$ in 0.2 mM $CaCl_2$ at pH 5.0 or 4.9, respectively, for 24 h) was positively correlated with Al tolerance in long-term cultures (11.1 or 42.6 μM soluble Al in the low-nutrient medium at pH 4.5 or 4.3, respectively, for 29 days). However, the level of Al tolerance in short-term assays did not correlate with the combined tolerance as defined above suggesting that the short-term screening technique may not be practically useful for estimating cultivar adaptation to the combination of stress factors in tropical acid soils. In sorghum, a less Al-tolerant plant species, higher Al tolerance was associated with less Al absorption by roots and a greater K translocation into shoots. In maize, a more Al-tolerant plant species, there was no correlation between the accumulation or transport of elements and Al tolerance. Standardized partial regression coefficients suggested that low-nutrient tolerance contribute more to combined tolerance than Al tolerance under most conditions (except for Al-sensitive sorghum at 42.6 μM $AlCl_3$). A greater combined tolerance was associated with a higher K shoot concentration in sorghum and a higher Ca shoot level in maize. We propose that plant nutritional characteristics linked to low-nutrient tolerance should be evaluated as an important strategy for plant production in tropical acid soils, both for Al-tolerant plant species and for Al-sensitive plant species under low-Al conditions.

Key words: aluminum tolerance, combined tolerance, low-nutrient tolerance, gramineous plants, tropical acid soils

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 and 3) a deficiency of essential nutrients (N, P, K, Ca, Mg, Mo and B) (Rao et al. 1993; Rao 2001). Major problems of acid soils of South American Savannas are the low content of cations, the toxicity of exchangeable Al and/or soluble Al and low levels of phosphorus as well as low silicon availability due to long weathering (Okada and Fischer 2001, Rao et al. 1993; Rao 2001). Many studies have focused on the identification of major factors that cause a decrease of plant production in solution culturing with or without Al and its related mechanisms (Pavan et al. 1982; Wright et al. 1989; Ofei-Manu et al. 2001; Pintro and Taylor 2004). However, the number of research in simultaneous consideration of the major two factors (high Al and low nutrients) in tropical acid soils is limited (Wenzl et al. 2003). In high nutrient solution, Al toxicity is alleviated by physicochemical interaction between Al and other ions, formation of nontoxic complexes with OH^- and SO_4^{2-}, and the precipitation of aluminum phosphates from high ionic strength solutions (Blamey et al. 1983, 1991; Wheeler and Edmeads 1995). The ionic strength of Savanna soil solutions vary from 1.3–1.7 mM for unfertilized samples and increased to 5.4–13.4 mM after fertilization (Wenzl et al. 2003). Wenzl et al. (2001) found that root growth of *Brachiaria* *ruziziensis*, a relatively Al-sensitive grass, was reduced in a solution containing toxic concentrations of Al and a low amount of nutrients than *B. decumbens*. An inadequate supply of nutrients may be one of the main factors that contribute to the poor persistence of *B. ruziziensis* in infertile acid soils. The range of Al activities in the solution of tropical acid soil was found to be 2.26 to 196.5 μM (Pintro et al. 1999). Blamey et al. (1991) reported that realistic root growth inhibition of *Lotus* could be obtained from a low ionic strength solution and a high Al concentration at similar levels to those in acid soils. Watanabe and Okada (2005) investigated the difference in Al tolerance between Indica and Japonica cultivars under low ionic strength conditions. As far as we know, no comprehensive studies on the identification of the primary inhibitory factor for plant production have been reported. Specifically, the clarification of plant nutritional characteristics for better plant production using two plant species each composed of a wide variation of cultivars has not been reported. Our interest would be different Al tolerances and plant nutritional characteristics under solution culture that simulates the nutrient status and the concentration of soluble Al in tropical acid soils. The proposal of a method for the isolation of the primary factor from complicated factors in tropical acid soils is urgent. Here we report a greater contribution of low-nutrient tolerance and related plant nutritional characteristics for the improved production of two gramineous plant species (sorghum and maize) under the above-mentioned experimental conditions.

MATERIALS AND METHODS

Source of seeds and germination

For sorghum, the following cultivars were used: Lucky-1, King, Takii, Toumitsu-1, Lucky-2 and Meter (Takii Co., Ltd., Japan); Sudax-1, Super sugar, Hybrid-1, Little, Sudax-306 and Kaneko (Kaneko Seeds Co., Ltd., Japan) and Koutoubun, Hazuki and Green (Snow Brand Seed Co., Ltd., Japan). For maize, the following cultivars were used: Royaldent TX115, Royaldent-110, Royaldent TH472 (Takii Co., Ltd., Japan); Golddent KD500, Golddent KD850, Golddent KD777, Golddent KD459, Golddent KD670, Golddent KD620 and Golddent KD520 (Kaneko Seed Co., Ltd., Japan). Seeds were soaked in tap water under aeration for 24 h at 27 °C in a growth room and germinated under fluorescent white light (80.7 μmol m^{-2} s^{-1}), spread on a nylon screen placed on a container that was filled with 9 L of tap water. The 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 unchanged throughout the experiment.

Screening for short-term Al tolerance

Seedlings with roots 3-4 cm in length were selected and treated with 0.2 mM $CaCl_2$ for 6 h (pH 5.0 or 4.9 for low or high Al, respectively). After measuring the root length of the longest root with a ruler, roots of the seedlings were transferred to a 0.2 mM $CaCl_2$ solution (control treatment, pH 5.0 or 4.9 for low or high Al, respectively) and either a 2.5 µM (low Al) or 20 µM (high Al) $AlCl_3$ solution containing 0.2 mM $CaCl_2$ (Al treatment). pH of the solutions was adjusted at 5.0 or 4.9 for low Al or high Al, respectively. At least 10 seedlings in each of control and Al treatments were used for the short-term screening experiment. After 24 h the root length of the longest root for each seedling was measured again.

Culturing and treatments in a long-term experiment

The elemental composition and pH of the long-term culturing medium is shown in Table 1. All seeds were soaked and spread on a nylon screen for germination in the same way as the short-term experiment. Just after germination, seedlings were transferred to a glasshouse for preculturing in tap water for 5 days. Seedlings of equal size were selected and transplanted into 40 L of low nutrient (LN) solution (one-fifth strength of the full nutrients [FN] solution) for 2 d. Thereafter, all seedlings were cultured with daily pH maintenance for 29 d as follows: 1) Control (FN, pH 5.2), 2) FN under low Al conditions (11.1 μM soluble Al, pH 4.5), 3) FN under high Al conditions (42.6 μM soluble Al, pH 4.3), 4) LN (pH 5.2), 5) LN under low Al conditions (pH 4.5), 6) LN under high Al conditions (pH 4.3). Ionic strength for the FN solution was calculated as 22.6 mM based on the method of Wada and Seki (1994). Because P and Al co-precipitate, their soluble concentration in the solution was measured as follows. After mixing 35 μM $AlCl_3$ with culture solution containing 55 μM NaH_2PO_4 at pH 4.5 or mixing 370 μM $AlCl_3$ with that containing 230 μM NaH_2PO_4 at pH 4.3 respectively with frequent pH adjustment for 1d, culture solutions were collected just after the pH adjustment and filtered through a membrane filter (0.2 μM in pore size), after which soluble concentrations of Al and P were measured. Finally, mean concentrations of 11.1 or 42.6 μM soluble Al and 5 μM soluble P were obtained. P was measured colorimetrically by the molybdenum blue method (Jackson 1958) using a spectrophotometer (U-2900, Hitachi, Japan) at 660 nm and Al was measured by ICP-AES, Varian-2000, Australia). While long-term culturing, pH was adjusted daily and thereafter culture solution was collected just after pH adjustment and soluble concentration of P was measured. When solution P concentration was found below the required level (5 μM), requisite amount of P was added to maintain the recommended concentration as shown in Table 1 and this daily adjustment of soluble concentration of P was continued throughout the treatment period. In another experiment, elements in the culture solutions were measured after 4^{th} week of treatments by ICP-AES, where elemental decrease was negligible (decrease was within range of 3-11% for all major nutrients [K, Ca, Mg, Fe and Mn] in all treatments). Culture solutions were renewed weekly in the first three weeks of culturing and then every 5 d after that to maintain the nutritional demand of the growing seedlings. Replication was done on 6 seedlings for both crops with three container repetition. At harvest 3 seedlings of similar size were selected, separated into shoots and roots, thoroughly washed and dried for 3 d at 70 °C in a draft oven and weighed.

Calculation of tolerances

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

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

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

$$\text{Low nutrient tolerance(\%)} = \frac{\text{Dry weight in LN}}{\text{Dry weight in FN}} \times 100$$

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

Analysis of elements in the plant samples

Homogenized dry samples for shoots and roots were digested by the electric digestion apparatus (Fujiwara Company Co., Ltd., Japan) at 200W with an acid mixture (HNO_3:60% $HClO_4$ = 5:3 v/v). Concentrations of each element in plant samples were measured by flame (K, Ca, Mg, Fe, Mn) or flameless (Al) atomic absorption spectrophotometry (Zeeman 5000, Hitachi, Japan) in the existence of 1000 ppm La.

Calculation of ionic activities

Ionic activities of Al were calculated using a computer program developed by Wada and Seki (1994).

Statistical analysis

Significance of difference among the cultivars in short-term experiment was tested by Fisher's LSD (Fisher 1958). Significance of correlations was tested by simple linear correlation coefficient. To compare the influence of explanatory variable on dependent variable, we calculated standardized partial regression coefficient using STB option for REG procedure of SAS® (SAS Institute 1988).

RESULTS

Differential Al tolerances among sorghum and maize cultivars under short-term screening conditions

The average Al tolerance for sorghum in the short-term under low Al conditions among all cultivars was higher (43%) than that under high Al conditions (21%) (Fig. 1A). Cultivars Super sugar and King were most tolerant while cultivars Kaneko and Takii were most sensitive to Al in the medium in the case of low Al conditions (57.8%, 60.3% and 29.2%, 29.2%, respectively). For maize the average Al tolerance in the short-term under low Al conditions was 71%, and that under high Al conditions was 55% (Fig. 1B). Among maize cultivars, Golddent KD520 was most tolerant and cultivar Golddent KD500 was most sensitive to Al (83.1% and 39.4% in high Al conditions, respectively).

Al tolerance under low Al conditions was positively correlated with that under high Al conditions for both plant species, i.e., differential Al tolerance among cultivars was the same irrespective of Al concentration in the media for both plant species (R^2 = 0.561** for sorghum and R^2 = 0.914** for maize). Larger variations in Al tolerance were observed under low Al conditions for sorghum (29.2–60.3%) and under high Al conditions for maize (34.9–83.1%).

Al tolerance, low-nutrient tolerance and combined tolerance among sorghum and maize cultivars under long-term culturing conditions

In low Al conditions the combined tolerance for sorghum shoots was in the range of 54.0–88.8% and the average value was 72.8% and that in high Al conditions (sorghum whole plant) ranged from 14.2–40.7% and the average value was 21% (Fig. 2A, B). For maize (whole plant) under low Al conditions the combined tolerance ranged from 60.8–91.7% and the average value was 76.8% and that under high Al conditions ranged from 36.5–59.1% and the average value was 46.9% (Fig. 2C, D).

Relationships for each tolerance with combined tolerance for sorghum were indicated in Fig. 3. For sorghum whole plant in low Al conditions, Al tolerance in the LN ranged from 66.5–87.6% and the average value was 76.9%. Al tolerance in the FN condition ranged from 71.2–93.2% and the average value was 85.5%. For the whole plant, LN tolerance ranged from 66.2–88.8% and the average value was 75.9%. The combined tolerance was positively correlated with the Al tolerance in the FN condition (R^2 = 0.278*) and with LN tolerance (R^2 = 0.408*). In high Al conditions, Al tolerance in LN ranged from 26.3–52.6% and the average value was 37.9%. Al tolerance in the FN condition ranged from 37.2–94.6% and the average value was 53.0%. The combined tolerance was positively correlated with Al tolerance in the FN condition (R^2 = 0.803**) and with LN tolerance (R^2 = 0.321*). However, Al tolerance in LN conditions (in both low and high Al) did not show any relationship with combined tolerance. (Fig. 3).

Relationships for each tolerance with combined tolerance for maize were indicated in Fig. 4. In maize low Al conditions, Al tolerance under LN ranged from 63.6–92.2% and the average value was 79.1%. Al tolerance in the FN condition ranged from 76.9–90.6% and the average value was 85.7%. LN tolerance ranged from 61.7–87.2% and the average value was 71.7%. The combined tolerance was positively correlated with Al tolerance in the LN condition (R^2 = 0.556*), and with LN tolerance (R^2 = 0.697**). In high Al conditions, Al tolerance in LN ranged from 57.0–76.0% and the average value was

65.4%. Al tolerance in the FN condition ranged from 67.9–88.6% and the average value was 77.5%. The combined tolerance was positively correlated with Al tolerance in the LN condition ($R^2 = 0.522$*) and with LN tolerance ($R^2 = 0.595$**) (Fig. 4).

Changes in growth and nutritional status after long-term culturing with a low nutrients solution or Al

Focusing on the characteristic nutritional aspects after lowering nutrient concentration of medium and Al addition to low nutrient for consideration of actual tropical acid soils, the remarkable results in Fig. 5 are as follows: LN decreased considerably P and Mg in shoot for both plant species, and for maize LN considerably decreased also K in both plant parts. In root, LN decreased considerably P, K and Fe for both plant species. In both plant parts for both plant species, considerable decrease of P in FN with both concentration of Al was considered to be ascribed to the lower concentration of soluble P in media.

As compared with the results after Al addition to FN, Al addition to LN decreased more considerably DW and K in both plant parts and Ca in root for sorghum; for maize it decreased more considerably Fe and Ca in shoot and K in root.

The relationship between Al tolerance and elemental characteristics

For sorghum, the Al in roots was negatively correlated with the Al tolerance in the LN treatment under high Al conditions ($R^2 = 0.278$*) (Fig. 7A). In maize, however, no such correlation was observed (data not shown). For sorghum, shoot K was positively correlated with the Al tolerance in the LN treatment under high Al conditions ($R^2 = 0.284$*) (Fig. 7B). No other significant correlations were observed between nutrient status and Al tolerance for sorghum (data not shown). For maize, no correlations were observed between nutrient status and Al tolerance. Additionally, no correlations were observed between nutrient status in LN conditions and low nutrient tolerance for both plant species (data not shown).

DISCUSSION

Maize (*Zea mays* L.) is the third most important cereal crop grown in the world (Baligar et al. 1997). In South and Central America, maize is mostly grown in acidic soils where yields are limited by deficient levels of available P, Ca, and Mg and toxic levels of Al and Mn (Baligar et al. 1997). On the other hand, Sorghum (*Sorghum bicolor* [L.] Moench) is the fifth most important cereal crop in the world (Sere and Estrada 1987). In South America it is grown mainly in acidic soils (4.6 million ha). Sorghum production in South America is limited by deficient levels of available P, Ca, Mg and micronutrients as well as toxic levels of Al and Mn (Sere and Estrada 1987).

We initially screened for short-term Al tolerance using 15 cultivars of sorghum and 10 cultivars of maize. A wide range of Al tolerance was observed among cultivars of sorghum and maize (Fig. 1). We also investigated long-term Al tolerance in the presence of all nutrients using the same cultivars for both plant species. In sorghum, the relationship between long-term and short-term Al tolerance was $R^2 = 0.267^*$ for whole plant under low nutrients and low Al conditions. For maize, relationship between long-term and short-term Al tolerance was $R^2 = 0.462^*$ for whole plant under low nutrients and high Al conditions (Fig. 8). This suggests that the short-term (24 h) screening technique for Al tolerance may be useful for the estimation of Al tolerance in long-term culturing with nutrients.

No correlations were found between short-term Al tolerance and the combined tolerance: R^2 = 0.106 and 0.002 for high Al and low Al conditions, respectively, for sorghum. For the whole maize plant in high and low Al conditions we found R^2 = 0.172 and 0.035, respectively (data not shown). Although investigations based on similar short-term screening techniques have been reported (Ishikawa et al. 2001; Ma et al. 2002; Kobayashi et al. 2004; You et al. 2005; Wagatsuma et al. 2005; Khan et al. 2008), 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.

Al in nutrient solutions decreased DW and also decreased the concentrations of all the nutrients measured (P, K, Ca, Mg, Fe and Mn) for sorghum more than for maize (Fig. 5). A less inhibitory effect of Al on DW in maize indicates greater Al tolerance for maize as compared with sorghum. The activity of all Al ion species ($Al[OH]_3^0$ + $AlSO_4^+$+ $Al[OH]_2^+$ + $AlOH^{2+}$ + Al^{3+}) in the low-nutrient medium was higher than that in full-nutrient medium as determined by the calculation of Wada and Seki (1994). The equation for the regression line of the average Al concentration in the roots of all cultivars (μg g^{-1} [y]) and the activity of the Al ion in the medium (x) for sorghum under 4 different medium conditions, i.e., FN + low Al, FN + high Al, LN + low Al and LN + high Al, was y = 159x – 303 (R^2 = 0.982**) (Al concentrations was within range between 402-4359 μg g^{-1}). The equation for the regression line between above two factors for maize was y = 66.7x – 40 (R^2 = 0.965*) (Al concentrations was within range between 202-1905 μg g^{-1})

(Fig. 6). Comparing each slope of regression line for sorghum and maize, i.e., 159 and 66.7, the absorbability of Al ions in sorghum roots was estimated to be more than twice that of maize roots. Together with the negative correlation between Al tolerance and Al in the roots ($R^2 = 0.278^*$, Fig. 7A), the lower level of Al tolerance for sorghum is due to the greater absorbability of Al ions by roots. This negative correlation between Al tolerance and Al concentrations agrees with previous reports (Wagatsuma et al. 1995; Ofei-Manu et al. 2001; Piñeros et al. 2005).

A positive correlation was also observed between Al tolerance and K in sorghum shoots (Fig. 7 B). No correlations were, however, observed between Al tolerance and all other nutrients for sorghum and between Al tolerance and all elements, including Al, for maize (data not shown). These results suggest the possible significance of higher K absorption / translocation for better growth of sorghum in the medium with Al.

In all these experimental conditions, the combined tolerance was significantly correlated both with Al tolerance and low-nutrient tolerance for both plant species (Figs. 2, 3 and 4, Table 2). To evaluate the relative importance of these two factors on the combined tolerance we performed multiple regression analysis and further calculated a standardized partial regression equation (to treat both factors equally) among the combined tolerance, Al tolerance and low-nutrient tolerance (Table 2). Although we did not find correlation of combined tolerance for sorghum whole plant in low Al condition, shoot part which constituted almost $3/4^{th}$ of whole plant and is the harvested plant part showed correlation with other tolerances. A greater contribution of low-nutrient tolerance than Al tolerance under most conditions were evident (0.46 > 0.39 for sorghum under low Al conditions, 0.69 > 0.57 for maize under low Al conditions and 0.69 > 0.64 for maize under high Al conditions). The exception was for the Al-sensitive sorghum under high Al conditions (0.67 > 0.02). From these findings it could be suggested that Al tolerance for low Al is more important for sorghum and that for high Al is more important for maize, depending on the relative tolerance to Al for each plant species (Fig. 8, Table 2). Although similar results have already been reported (Okada and Fischer 2001; Wenzl et al. 2003), our finding may be considered to be definitive evidence based on a more comprehensive experiment. In sorghum, the combined tolerance was positively correlated with the tissue concentration of K in shoot under combined stress conditions (R^2 = 0.491*). In maize, the same correlation was recognized between the combined tolerance and that of Ca in shoot under combined stress conditions (R^2 = 0.477*) (Fig. 9). No correlations were observed between the combined tolerance and any other nutrients including Al (data not shown). A greater potential for K translocation in sorghum and Ca translocation in maize is suggested as a strategy for better plant production in tropical acid soils.

Although soluble P concentration was rather low at 5μM, no positive correlations were observed between shoot P and any kind of shoot tolerance (R^2 = 0.116, 0.017, 0.166, 0.191 and 0.256 for Al tolerance [low Al], Al tolerance [high Al], LN tolerance, combined tolerance [low Al] and combined tolerance [high Al] in sorghum, respectively; and R^2 = 0.095, 0.334, 0.061, 0.037 and 0.396 for Al tolerance [low Al], Al tolerance [high Al], LN tolerance and combined tolerance [low Al] as well as combined tolerance [high Al] in maize, respectively) (data not shown). The soluble concentration of P in the medium is, therefore, not considered to be a determining factor for plant growth in this study.

Some nutritional characteristics of plants that were grown in the presence of Al have already been reported (Foy and Brown 1964; Mariano and Keltjens 2005; Hãussler et al. 2006). We propose here that the plant nutritional characteristics linked to low-nutrient tolerance demonstrated in the present investigation should be evaluated as an important strategy for plant production in tropical acid soils. This should be for both Al-tolerant plant species and for Al-sensitive plant species under low-Al conditions. The Al tolerance and low-nutrient tolerance for plant production in these soils may fluctuate 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. A short-term screening technique that can be applied to these soils should, alternatively, be established in the future.

Our investigation was carried out using gramineous plant species. The recommended plant nutritional characteristics required to cope with low-nutrients containing Al may be different between plant species such as for dicotyledonous plants. Further research is needed in future using other plant species such as other popular and important crop plant species grown in tropical areas.

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Figure captions:

Fig. 1. Short-term aluminum (Al) tolerance for cultivars of sorghum (A) and maize (B). Low Al, 2.5 μM $AlCl_3$ in 0.2 mM $CaCl_2$ for 24 h (pH 5.0); high Al, 20 μM $AlCl_3$ in 0.2 mM $CaCl_2$ for 24 h (pH 4.9). Al tolerance is expressed as the net root elongation of the longest root in Al treatment/net root elongation of the control. Data are mean ±SE ($n \geq 10$). Average values with the same letter(s) are not significantly different at the 5% significance level (Fisher's least significant difference). * $P < 0.05$; ** $P < 0.01$. The R^2 value is the determination coefficient between Al tolerance in low Al and that in high Al.

Fig. 2. Combined tolerances (%) of sorghum (A, B) and maize (C, D) in low Al (A, C) and high Al (B, D) conditions. Tolerances were calculated based on the dry weight after culturing in different treatment solutions as shown in Table 1 for 29 d with a daily pH adjustment. The combined tolerance was defined as relative dry weight in LN+Al to that in FN. All tolerance values were calculated for the whole plant; * the combined tolerance of sorghum under low Al conditions for the shoot part only.

Fig. 3. Relationship for Al tolerance in LN, Al tolerance in FN and low-nutrient tolerance with combined tolerance for sorghum in low Al and high Al conditions. Tolerances were calculated based on the dry weight after culturing in different treatment solutions as shown in Table 1 for 29 d with a daily pH adjustment. Combined tolerance has been defined in Fig. 2. Al tolerance in LN, relative dry weight in LN+Al to that in LN; Al tolerance in FN, relative dry weight in FN+Al to that in FN; LN tolerance, relative dry

weight in LN to that in FN. Dotted lines indicate non significant relations. ns, not significant; * $P < 0.05$; ** $P < 0.01$.

Fig. 4. Relationship for Al tolerance in LN, Al tolerance in FN and low-nutrient tolerance with combined tolerance for maize in low Al and high Al conditions. Treatments conditions, materials and definitions are same in Fig. 3. All tolerance values were calculated for the whole plant. Dotted line indicates non significant relation. ns, not significant; * $P < 0.05$; ** $P < 0.01$.

Fig 5. Relative values on average values of dry weight and mineral concentration for 15 cultivars of sorghum (A) and 10 cultivars of maize (B) grown in long-term culturing. ◐ LN or FN without Al, dry weight or mineral concentration in LN (% of FN), LN under low Al conditions (% of LN), LN under high Al conditions (% of LN), FN under low Al conditions (% of FN), FN under high Al conditions (% of FN).

Fig. 6. Relationship between activity of whole Al ion species in medium and average Al concentration in roots for all sorghum cultivars (open symbols) and all maize cultivars (closed symbols). ○, ●, FN + low Al; ◇, ◆, FN + high Al; Δ, ▲, LN+ low Al; □, ■, LN+ high Al. Values are means ± SD (n = 3).

Fig. 7. Relationship between Al tolerance in LN and Al concentration in roots (A) or between Al tolerance and K concentration in shoots (B) for sorghum in long-term culturing. Al tolerance in LN was calculated as the ratio of growth in LN under high Al conditions to that in LN. * $P < 0.05$.

Fig. 8. Relationship between short-term Al tolerance and long-term Al tolerance for sorghum (A) and maize (B). The Al tolerance for sorghum is under low Al conditions and that for maize under high Al conditions. * $P < 0.05$; ** $P < 0.01$.

Fig. 9. Relationship between combined tolerance and K concentration for sorghum shoots (A) and between the Ca concentrations for maize shoots (B) in LN under high Al conditions. * $P < 0.05$; ** $P < 0.01$.