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MECHANISM OF ALUMINUM TOXICITY AVOIDANCE IN TROPICAL RICE (*Oryza sativa*), MAIZE (*Zea mays*), AND SOYBEAN (*Glycine max*)

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ABSTRACT

Planting Al tolerant crops is an economically justifiable approach in crop production on acid soils. Experiments were conducted to study the mechanisms of Al tolerance among species and varieties of tropical rice, maize, and soybean with previously known levels of Al tolerance. These varieties were hydroponically cultured in 0, 5, 10, and 30 mg l^{-1} Al with complete nutrient solution at pH 4. The results show that root/shoot ratio of dry weight at 10 mg l^{-1} Al treatment was an important parameter to indicate differential Al tolerance in maize. Oxalic acid exudation from roots cannot always explain the Al tolerance. Total organic acid concentration in roots at 10 mg l^{-1} Al treatment indicated a difference of Al tolerance in soybean and lowland rice. Aluminum translocation from roots to shoots was lower in tolerant varieties than in sensitive varieties of soybean. Increased Al concentration in shoots with increased Al level in the solution was larger in soybean and maize than in lowland or upland rice. Among varieties of soybean, the Al concentration in shoots increased drastically in Wilis (Al-sensitive variety) with increase Al level, while in Kitamusume (Al-tolerant variety) it did not.

[*Keywords*: *Oryza sativa*, *Zea mays*, *Glycine max,* aluminum, soil toxicity]

INTRODUCTION

Acid soils cover approximately 30% of the total ice-free land area or about 3950 million ha of the earth's surface (Wright, 1989). Of the total acid soil area, 41% exists in America, 26% in Asia, 17% in Africa, 10% in Europe, and 6% in Australia and New Zealand. Indonesia has about 60 million ha of acid soils (Ultisols and Oxisols) which cover about 32% of the total land area of Indonesia (Subagyo *et al*., 2000). Sixty seven percent of the acid soil area in the world is under forest, 18% savannas and prairie vegetation, 4.5% arable crops, and <1% perennial tropical crops. Acid soils of the tropics represent the largest pool of potential land for future agricultural development.

The major constraints to plant growth in acid mineral soils are: (1) high hydrogen, aluminum, and manganese concentrations inducing toxicity; (2) low calcium, magnesium, potassium, phosphorus, and molybdenum concentrations inducing deficiency; and (3) inhibition of root growth and water uptake inducing nutrient deficiency, and drought stress (Marschner, 1997). From all constraints, Al toxicity is the most limiting factor to crop growth on acidic soils (Foy *et al*., 1978). The relative importance of these constraints, especially Al toxicity, depends on plant species and genotype, soil characteristics, and climate.

Several approaches have been suggested on how to increase crop production in acid soils. Acid soil improvement principally deals with reduction of acidity of the hydrogen ions by replacement with basic cations and reducing plant available Al in soil solution. This is commonly done by adding oxides, hydroxides, or carbonates of calcium and magnesium. However, these solutions are temporary and too expensive for the poor farmers of developing countries and is not always economically feasible, especially in strongly acid subsoils (Foy *et al*., 1978). Acid-tolerant crops offer an option that is environmentally friendly and relatively inexpensive for poor farmers to adopt. Selection or screening of plants, which are resistant to soluble Al in the root environment, is considered as an indiceous alternative approach.

The toxic actions of Al are primarily to the root system (Taylor, 1988). The root system becomes stubby as a result of inhibition of elongation of the main axis and lateral roots (Klotz and Horst, 1988). The severity of inhibition of root growth is a suitable indicator of genotypical differences in Al toxicity. The root apex (root cap, meristem, and elongation zone) accumulates more Al and tends to have a greater physical damage than the mature root tissue.

Indeed, only the apical 2-3 mm of maize roots (root cap and meristem) are usually affected by Al and this leads to growth inhibition (Ryan *et al*., 1993).

Physiological mechanisms of plant Al tolerance are grouped into avoidance and internal detoxification mechanisms. The avoidance mechanism includes exclusion of Al from sensitive sites such as Al exclusion from root and organic compound exudation for forming Al complex. An example of internal detoxification is the formation of Al organic acid complexes and Al protein complexes in the cells. Several studies provide strong evidence that Al-tolerant genotypes of wheat exclude Al from their root apices. Delhaize *et al*. (1993) showed that after exposure to Al, an Al-sensitive genotype accumulated many times more Al in the root apex (terminal 2 mm of root) than an Al-tolerant genotype, whereas no differences occurred in more mature root tissue.

One of the avoidance mechanisms of plant Al tolerance is exudation of organic acids from the root. The exudation of organic acids such as malate, citrate, succinate, and oxalate from roots has been suggested to play a role in Al exclusion. Aluminum-tolerant wheat varieties were able to excrete malic acid 3 to 5 fold higher than the Al-sensitive ones after exposure to Al. Beside malic acid, succinic acid was also excreted by wheat seedlings exposed to Al. Aluminum-tolerant genotypes excreted about 10 fold higher malic acid and about 3-5 fold higher succinic acid than Al-sensitive seedlings over 24-hour exposure to 50 μM Al (Salazar *et al*., 1997).

In response to Al stress, citric acid was also released by roots of an Al-resistant snapbean and this constituted a mechanism of Al tolerance (Miyasaka *et al*., 1991). Maize cultivars also excrete citric and malic acids in the presence of the Al. Pellet *et al*. (1995) found that exposure to Al trigered a dramatic stimulation in the rate of citrate release (3.5-7-fold increase) by roots of Al-tolerant SA 3, while in Al sensitive Tuxpeno, there was no significant stimulation of citrate release after exposure to Al. Secretion of oxalate was found in roots of taro cultivars (Bun-long and Lehua maoli) as a result of Al exposure. Addition of 900 μM Al in nutrient solution significantly stimulated oxalate excretion from roots of both taro cultivars, although no significant difference in oxalate exudation between the cultivars (Zhong Ma and Miyasaka, 1995). Earlier, Hue *et al*. (1986) found that several organic acids are effective in reducing Al toxicity and this implies that addition of such acids to soils, for instance through organic matter application, gives more opportunity to grow Al-sensitive crops on acid soils.

Based on the above findings, the objectives of this study were to study the mechanisms of Al tolerance in relation to Al concentration in plant tissues, organic acid concentrations in roots, and organic acid exudation from roots among species and varieties of upland rice, lowland rice, maize, and soybean.

MATERIALS AND METHODS

Plant Materials and Solution Culture

Experiments were conducted in a greenhouse of the Laboratory of Plant Nutrition, Faculty of Agriculture, Hokkaido University from May to October 1999. Seeds of Al-sensitive and Al-tolerant varieties of tropical upland rice (Dodokan, Cirata, Danau Tempe, Laut Tawar, IAC 165, and Oryzica sabana 6); lowland rice (Kapuas, Cisadane, IR66, KDML 105, RD 13, and RD 23); maize (Arjuna, Kalingga, Antasena, SA 3, SA 4, SA 5, P 3540, and PM 95A); and soybean (Wilis, Galunggung, Kerinci, INPS, and Kitamusume) taken from Indonesia, Thailand, South America, and Japan were used in this experiment. One variety of barley (Ryofu) was used as a comparison. Nursyamsi *et al*. (2000) reported that based on the sum of shoot and root Al_{RG50} values (Al concentration in solution when relative growth decreased to 50%), Al tolerance of crops was in the order of barley < maize < soybean < lowland rice < upland rice. By the same criteria, Al tolerance of maize varieties was in the order of Arjuna < Kalingga < P 3540 < SA 5 < SA 4 < PM 95A < SA 3 < Antasena. Al tolerance of soybean varieties was in the order of Wilis < INPS < Galunggung < Kerinci < Kitamusume. For lowland rice, the order of Al tolerance was RD 23 < Kapuas < Cisadane < KDML 105 < IR66 < RD 13, and for upland rice was in the order of Dodokan < IAC 165 < Cirata < Oryzica sabana 6 < Danau Tempe < Laut Tawar.

Seeds of each plant were sterilized with 1% sodium hypochlorite for 10 minutes, washed with deionized water, and germinated on moist perlite and vermiculite applied with standard nutrient composition. Three weeks (for rice) and one week (for maize, soybean, and barley) after germination seedlings were pre-cultivated in the complete nutrient solution consisted of 30 mg N l^{-1} (NH_4NO_3), 1 mg P l^{-1} ($NaH_2PO_42H_2O$), 30 mg K l^{-1} (K_2SO_4:KCl=1:1), 50 mg Ca l^{-1} ($CaCl_22H_2O$), 20 mg Mg l^{-1} ($MgSO_47H_2O$), 2 mg Fe l^{-1} ($FeSO_47H_2O$), 0.5 mg Mn l^{-1} ($MnSO_44H_2O$), 0.5 mg B l^{-1} (H_3BO_3), 0.2 mg Zn l^{-1} ($ZnSO_47H_2O$), 0.01 mg Cu l^{-1} ($CuSO_45H_2O$), and 0.005 mg Mo l^{-1} ($(NH_4)_6Mo_7O_{24}4H_2O$) (Osaki *et al*., 1997). The pH of the solution was adjusted using pH meter to 5.0 + 0.1 for rice and to 4.7 + 0.1 for soybean and maize.

After pre-culture, the seedlings (two plants per hill) were transferred to the hydroponic container (360 l) having identical nutrient solution and treated with 0, 5, 10, and 30 mg l^{-1} Al using $Al_2(SO_4)_3$. The Al and P concentrations in the solution were adjusted by the addition of adequate amounts of Al and P until Al-P equilibrium state was reached at pH 4.0 + 0.1. Each treatment was replicated four times. During the experiment, culture solution was constantly aerated, solution pH was controlled at 4.0 + 0.1 and the nutrient concentrations in solution were adjusted to the initial concentrations every 10 days.

Growth Measurements

Plants were harvested 14 days for rice and barley, 7 days for maize, and 12 days for soybean after transferring from pre-culture to treatment media. After washing with deionized water, samples were separated into roots and shoots. Then, dry weight of each organ was measured after drying in the oven at 80°C for 2 days. Relative growth (RG) of each organ at each Al treatment was calculated using the formula:

$$RG = (DW\ Al_X - DW\ O)/(DW\ Al_0 - DW\ O)$$

where DW Al_X is plant dry weight (g $hill^{-1}$) treated with "X" Al concentration; DW Al_0 is plant dry weight (g $hill^{-1}$) without Al; and DW O is plant dry weight (g $hill^{-1}$) at 0 day (after pre-culture). Order of relative Al tolerance was determined according to Al_{RG50} value. Al_{RG50} value was calculated as Al concentration in the nutrient solution at which RG decreased 50% compared to that of 0 Al culture solution. The higher the value of Al_{RG50} the more tolerant the varieties are and vice versa. Nursyamsi *et al*. (2000) presented data on relative growth and Al_{RG50} of tested varieties.

Aluminum Analysis

Concentrations of Al in shoots and roots were analyzed after 0.1 g (shoots) and 0.06 g (roots) of ground samples were digested with H_2SO_4-H_2O_2 and the volume adjusted to 25 ml. Samples were filtered, then Al was determined by atomic absorption spectrophotometry.

Analysis of Organic Acid Exudation from Roots

One day before harvest or 13 days (for rice), 6 days (for maize), and 11 days (for soybean) after treatment, plants were rinsed with deionized water, and root exudate was collected in a 500 ml flask containing 200 mM $CaCl_2$ ($CaCl_2 2H_2O$) and aerated for 24 hours under normal light. Root exudates were filtered with filter paper (type 5C, Adventic Toyo) and concentrated by a rotary evaporator at 40°C until nearly dry and diluted to 1 ml. The solution was then stored at -80°C before analysis of organic acids.

Before analysis of organic acids, about 0.3 ml of root exudate solution was filtered with a disposable syringe filter (cellulose acetate, 0.45 µm). Organic acids were analyzed with a Capillary Ion Analyzer (Waters type). The buffer electrolyte solution was made by adding 2.5 ml CIA-PAC™ OFM Anion-BT into 97.5 ml 120 mM $Na_2B_4O_7$ (as $Na_2B_4O_7 10H_2O$). Waters capillary fused silica 50 µM x 60 cm with detection 185 nm was used in this analysis.

Analysis of Organic Acid Concentrations in Roots

After collection of organic acid exudation from roots, samples were separated into roots and shoots and plant fresh weight was measured. Subsamples of roots were frozen at -80°C, then lyophilized and stored again at -80°C until analysis of organic acid concentrations in tissue. About 0.1 g lyophilized sample of roots was grounded in 10 ml Tris (hydroxymethyl) aminomethane buffer solution, pH 7.4 at 25°C. The buffer solution was made from 50 ml 0.1 M Tris (12.114 g $C_4H_{11}NO_3$ l^{-1}) and 42 ml of 0.1 M HCl, diluted to 100 ml with deionized water. After grinding, suspensions were centrifuged with Avanti™ 30 Centrifuge at 10,000 rpm for 20 minutes at 0°C. About 0.3 ml solution of root extract was filtered with a disposable syringe filter unit (cellulose acetate 0.45 µm). Concentrations of organic acids in roots were measured using the previously explained method.

RESULTS AND DISCUSSION

Plant Growth

The relationship between relative growth rate (RGR) at 0 mg l^{-1} Al level in solution and Al_{RG50} for each crop is shown in Fig. 1. The correlation between RGR and Al_{RG50} is not significant at the 5% level in upland rice (r = 0.13), lowland rice (r = 0.25), maize (r = 0.31), and soybean (r = 0.67). It is assumed that plants with low RGR have a high Al tolerance because slow growth confers a benefit to Al detoxification and Al exclusion. However as RGR at 0 mg l^{-1} Al has no

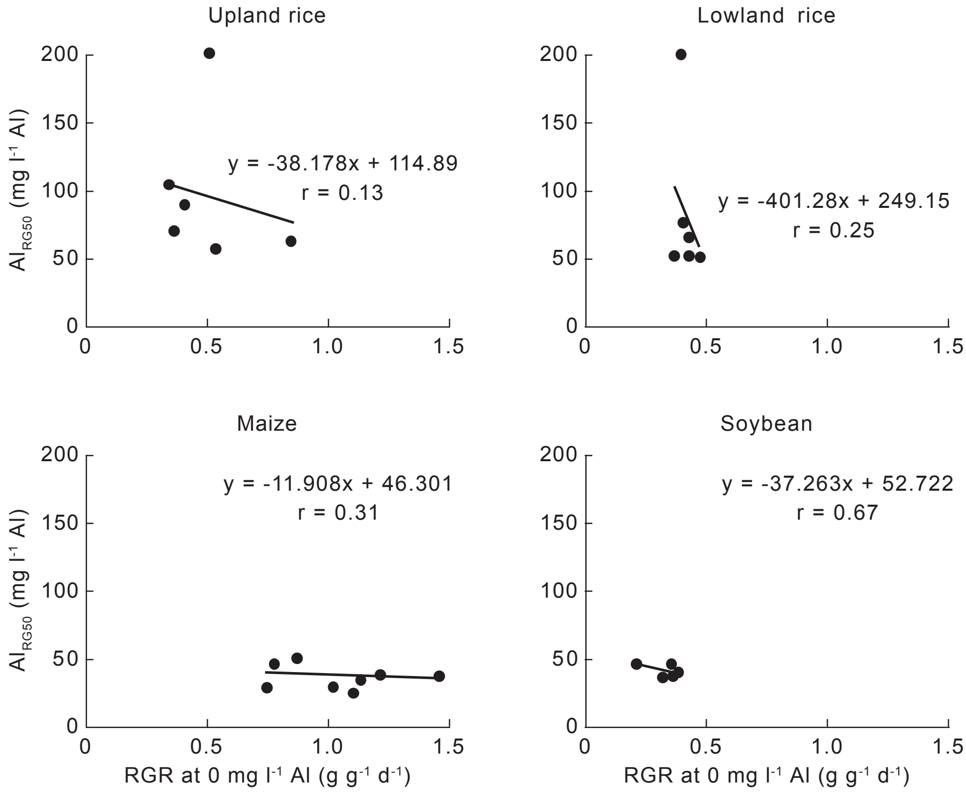

Fig. 1. Relationship between relative growth rate (RGR) at 0 mg l^{-1} Al and Al_{RG50}.

relationship with Al_{RG50}, RGR is not proven to be a factor of Al tolerance. Correlation between growth parameters (shoot and root dry weight, Al and organic acid concentrations) at 5 and 30 mg l^{-1} Al levels in nutrient solution and Al_{RG50} were not significant (data not shown).

The relationship between root/shoot dry weight ratio (root dry weight/shoot dry weight) and Al_{RG50} was calculated in each crop at 10 mg l^{-1} Al level in nutrient solution. Significant positive correlation between root/shoot dry weight ratio at 10 mg l^{-1} Al level and Al_{RG50} was found in maize ($r = 0.78$, $P < 0.05$). However, in upland rice, lowland rice, and soybean, the correlation between root/shoot dry weight ratio at 10 mg l^{-1} Al level and Al_{RG50} was not significant at 5% level ($r = 0.03$, 0.66 and 0.56, respectively) (Fig. 2). Maize was more sensitive to Al than upland rice, lowland rice, and soybean. Root inhibition in maize varieties was stronger than that in other species. Thus, this ratio is an important parameter to indicate differential Al tolerance in maize.

Organic Acids

As organic acids can make chelates and detoxify Al in the rhizosphere, it is suggested that organic acid exudation from roots is an important Al tolerance mechanism of some species (Miyasaka *et al*., 1991). In the current experiment, oxalic acid exudation from roots was found only in some varieties of maize (PM 95A and SA 3) and in soybean (Wilis, INPS, Galunggung, and Kitamusume) as a result of Al treatment (Tables 1-4), while in upland and lowland rice, oxalic acid exudation was not found. The relationship between oxalic acid exudation from roots and Al_{RG50} was not found in upland rice, lowland rice, and maize because nearly no oxalic acid was exuded from roots. Also in soybean, the correlation between oxalic acid exudation from roots and Al_{RG50} was not significant (data not shown) because the oxalic acid exudation was not clearly different between Wilis (Al-sensitive variety) and Kitamusume (Al-tolerant variety). Thus, organic acid exudation is not always a factor indicating Al tolerance.

In soybean, total organic acid concentration in roots increased with increasing Al level, and was higher in Kitamusume (Al-tolerant variety) than in Wilis (Al-sensitive variety). However, in other crops, the total organic acid concentration in roots was not clearly different between tolerant and sensitive varieties (Tables 1-4). This response indicates that there is possible relationship between total organic acid

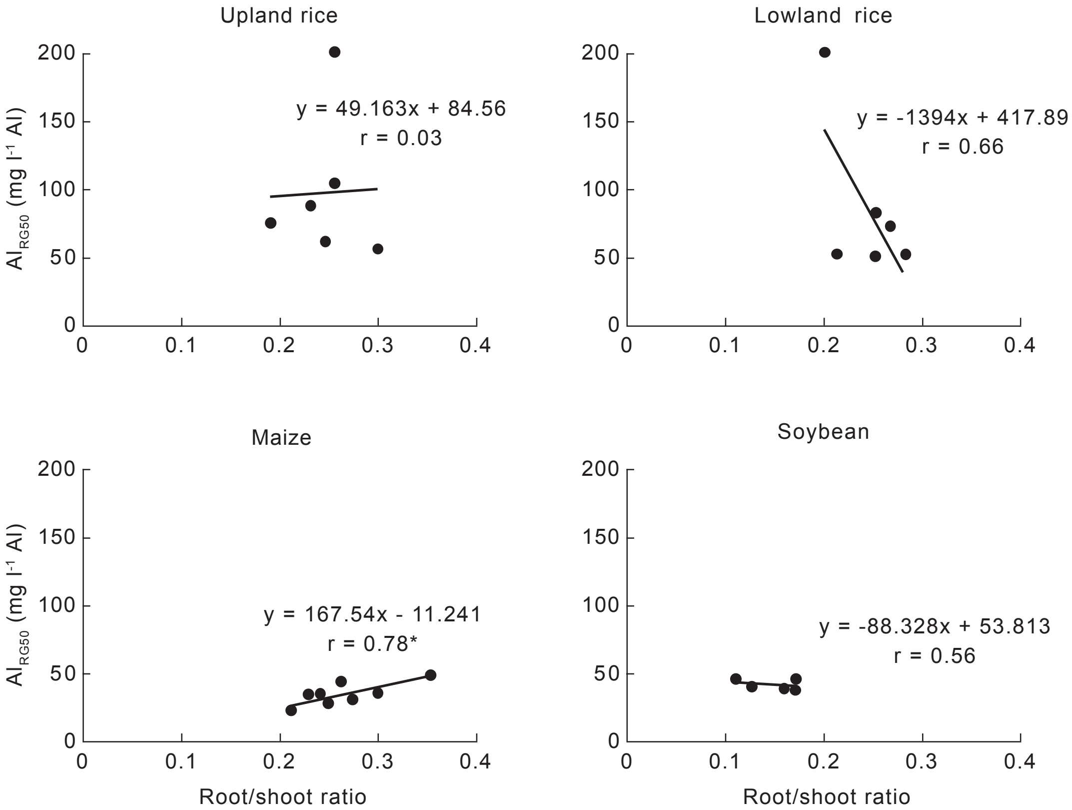

Fig. 2. Relationship between root/shoot ratio (calculated as root dry weight/shoot dry weight) at 10 mg l^{-1} Al and Al_{RG50}. * indicates significance at 5% level.

concentration in roots and the degree of Al tolerance in soybean. Besides protein, organic acids play an important role in reducing Al toxicity by forming Al-organic acid complexes in the cells.

The relationship between total organic acid concentration in roots and Al_{RG50} was shown in each crop at 10 mg l^{-1} Al level in solution (Fig. 3). Significant positive correlation between total organic acid concentration in roots at 10 mg l^{-1} Al treatment and Al_{RG50} was found in soybean ($r = 0.98$, $P < 0.01$) and lowland rice ($r = 0.83$, $P < 0.05$), but not found in upland rice ($r = 0.17$) and maize ($r = 0.70$). This correlation indicates that in the case of soybean and lowland rice, total organic acid concentration in roots increased with the increase of Al tolerance. Based on these data, total organic acid concentrations in roots can be used as an indicator for differential Al tolerance in soybean and lowland rice, but not for upland rice and maize.

Aluminum Accumulation and Concentration

Aluminum tolerance may be achieved by deposition in surface cell wall of Al or exclusion of Al (excluder). In excluder types, Al tolerance may be achieved by exclusion from sensitive sites at least from the shoots, or from uptake in general by root-induced changes in the rhizosphere (Marschner, 1997). Figures 4-7 showed that Al concentration was higher in roots than in shoots of all crops. This response indicates that the crops may achieve Al tolerance by exclusion from the shoots (excluder types).

The relationship between shoot/root ratio of amount of Al and Al_{RG50} in each species at 10 mg l^{-1} Al level in nutrient solution is shown in Fig. 8. Significant negative correlation between the shoot/root ratio of amount of Al and Al_{RG50} at 10 mg l^{-1} Al level was found in soybean ($r = 0.99$, $P < 0.01$), but not found in upland rice ($r = 0.06$), lowland rice ($r = 0.18$), and maize ($r = 0.66$). In soybean, shoot/root ratio of amount of Al decreased with increased Al tolerance. This response indicates that Al translocation from roots to shoots was lower in tolerant varieties than in sensitive varieties, because soybean roots have high ability to deposit Al in root surface probably by complexing with organic acids. In other words, there was inhibition in translocation of Al from roots to shoots in tolerant varieties of soybean. Thus, the inhibition in translocation of Al

Table 1. Effect of Al treatment on organic acid concentration in roots and organic acid exudation rate from roots of upland rice.

Variety	ppm Al	Organic acid concentration (nmol g^{-1} root DW)						Exudation rate (nmol g^{-1} root DW 24 hr^{-1})
		Oxalic acid	Fumaric acid	Succinic acid	Malic acid	Citric acid	Total	
Dodokan	0	334 + 6	Trace	Trace	2268 + 595	Trace	2602 + 601	Trace
	5	465 + 54	Trace	Trace	2777 + 108	Trace	3242 + 162	Trace
	10	541 + 70	Trace	Trace	3449 + 495	Trace	3990 + 565	Trace
	30	113 + 367	Trace	Trace	2492 + 472	Trace	3624 + 839	Trace
IAC 165	0	318 + 31	Trace	Trace	1481 + 88	Trace	1799 + 119	Trace
	5	612 + 43	Trace	Trace	1967 + 100	Trace	2479 + 143	Trace
	10	693 + 116	Trace	Trace	2486 + 122	Trace	3179 + 238	Trace
	30	1262 + 184	Trace	Trace	3057 + 780	Trace	4319 + 964	Trace
Cirata	0	526 + 177	405 + 11	556 + 15	5584 + 48	398 + 17	7469 + 267	Trace
	5	698 + 13	644 + 152	759 +159	8019 +1248	1451 + 96	11571 +1667	Trace
	10	751 + 69	598 + 87	740 +126	6340 + 586	2317 + 494	10746 +1362	Trace
	30	675 + 65	517 + 85	782 +145	6045 + 444	1879 + 427	9898 +1165	Trace
Oryzica sabana 6	0	253 + 19	Trace	Trace	3566 + 189	Trace	3819 + 208	Trace
	5	264 + 15	Trace	Trace	3600 + 831	Trace	3864 + 846	Trace
	10	277 + 12	Trace	Trace	3633 + 831	Trace	3910 + 843	Trace
	30	413 + 28	Trace	Trace	2874 + 643	Trace	3287 + 671	Trace
Danau Tempe	0	243 + 0	Trace	Trace	3617 + 700	Trace	3860 + 700	Trace
	5	265 + 2	Trace	Trace	2430 + 429	Trace	2695 + 430	Trace
	10	391 + 46	Trace	Trace	2092 + 556	Trace	2483 + 602	Trace
	30	638 + 152	Trace	Trace	3323 + 708	Trace	3961 + 860	Trace
Laut Tawar	0	417 + 20	116 + 0	Trace	2581 + 283	Trace	3113 + 303	Trace
	5	419 + 51	215 + 0	Trace	3067 + 98	Trace	3701 + 149	Trace
	10	693 + 146	497 + 17	Trace	3686 + 142	Trace	4876 + 305	Trace
	30	1062 + 284	371 + 5	Trace	2757 + 880	Trace	4190+1 168	Trace

Table 2. Effect of Al treatment on organic acid concentration in roots and organic acid exudation rate from roots of lowland rice.

Variety	ppm Al	Organic acid concentration (nmol g^{-1} root DW)						Exudation rate (nmol g^{-1} root DW 24 hr^{-1})
		Oxalic acid	Fumaric acid	Succinic acid	Malic acid	Citric acid	Total	
RD 23	0	496 + 54	301 + 60	Trace	2061 + 179	Trace	2857+2 93	Trace
	5	566 + 106	475 + 24	788 + 0	3009 + 346	Trace	4837 + 475	Trace
	10	478 + 82	577 + 161	627 + 0	3779 + 576	Trace	5461 + 819	Trace
	30	584 + 120	487 + 154	574 + 0	3430 + 142	Trace	5076 + 416	Trace
Kapuas	0	823 + 24	Trace	Trace	2526 + 892	Trace	3349 + 915	Trace
	5	592 + 51	Trace	Trace	3039 + 350	Trace	3631 + 400	Trace
	10	646 + 107	Trace	Trace	3626 + 194	Trace	4272 + 301	Trace
	30	606 + 174	Trace	Trace	2747 + 728	Trace	3353 + 901	Trace
Cisadane	0	497 + 36	Trace	Trace	1793 + 153	326 + 58	2615 + 247	Trace
	5	482 + 107	Trace	Trace	2036 + 518	548 + 0	3066 + 624	Trace
	10	524 + 21	Trace	Trace	1829 + 298	503 + 89	2856 + 408	Trace
	30	835 + 275	Trace	Trace	1603 + 476	327 + 0	2765 + 750	Trace
KDML 105	0	378 + 51	Trace	Trace	1049 + 287	526 + 0	1953 + 337	Trace
	5	602 + 49	Trace	Trace	2339 + 466	466 + 0	3407 + 515	Trace
	10	591 + 88	Trace	Trace	2596 + 437	669 + 150	3856 + 675	Trace
	30	465 + 42	Trace	Trace	1546 + 132	Trace	2011 + 174	Trace
IR 66	0	572 + 128	Trace	535 + 0	2532 + 167	Trace	3639 + 294	Trace
	5	550 + 121	Trace	642 + 0	2616 + 330	Trace	3808 + 451	Trace
	10	599 + 76	Trace	679 +169	2626 + 305	Trace	3903 + 550	Trace
	30	484 + 42	Trace	527 + 93	2729 + 81	Trace	3740 + 215	Trace
RD 13	0	776 + 51	284 + 6	460 + 30	3144 + 398	1322 + 0	5986 + 485	Trace
	5	1193 + 121	465 + 43	631+114	5264 + 746	1802 + 0	9355 +1025	Trace
	10	1092 + 122	508 + 48	555+104	4442 + 609	1226 + 0	7823 + 883	Trace
	30	856 [redacted] 174	615 + 23	803+152	3695 [redacted] 380	2207 + 175	8176 [redacted] 904	Trace

Table 3. Effect of Al treatment on organic acid concentration in roots and organic acid exudation rate from roots in maize.

Variety	ppm Al	Organic acid concentration (nmol g^{-1} root DW)										Exudation rate (nmol g^{-1} root DW 24 hr^{-1})
		Oxalic acid	Malonic acid	Fumaric acid	Maleic acid	Succinic acid	Malic acid	Citric acid	Tartaric acid	Pyruvic acid	Total	
Arjuna	0	726 + 20	281 + 0	1087 + 327	290 + 84	293+ 10	2034 + 134	766 + 20	154+ 0	Trace	5631 + 595	Trace
	5	812 + 72	384 + 0	1169 + 356	383 + 16	613+ 118	2278 + 291	925 + 109	1229 + 587	Trace	7793 + 1549	Trace
	10	1130 + 92	321 + 26	2162 + 397	815 + 244	613+ 215	2976 + 1	1227 + 89	1108+509	Trace	10350 + 1571	Trace
	30	1350 +202	341 + 12	2625 + 329	636 + 75	810+ 80	5936 + 404	1776 + 395	Trace	Trace	13474 + 1497	Trace
Kalingga	0	534 + 35	Trace	1026 + 19	Trace	Trace	998 + 220	517 + 126	Trace	Trace	3076 + 400	Trace
	5	809 + 91	Trace	1202 + 87	Trace	Trace	1328 + 244	726 + 0	Trace	Trace	4065 + 422	Trace
	10	968 +145	Trace	1083 + 67	Trace	Trace	1727 + 296	1074 + 216	Trace	Trace	4852 + 724	Trace
	30	1337 +164	Trace	1742 + 578	Trace	Trace	2078 + 472	1203 + 0	Trace	Trace	6360 + 1214	Trace
P 3540	0	617 + 95	Trace	1644 + 211	362 + 81	Trace	1917 + 530	Trace	1192 + 11	Trace	5732 + 928	Trace
	5	519 + 40	Trace	1877 + 112	499 + 183	570 + 0	2667 + 457	1187 + 238	1757 + 142	Trace	9076 + 1171	Trace
	10	814 + 65	Trace	2478 + 673	641 + 226	604 +129	2462 + 288	1668 + 148	1668 + 8	Trace	10335 + 1536	Trace
	30	958 + 70	Trace	3118 + 679	505 + 50	524 + 42	3338 + 78	1980 + 242	Trace	Trace	10423 + 1160	Trace
SA 5	0	711 + 61	311 + 22	1200 + 241	Trace	254 + 0	2943 + 988	Trace	Trace	Trace	5419 + 1292	Trace
	5	784 +189	555 + 88	1135 + 346	Trace	398 +166	2930 + 963	Trace	Trace	Trace	5802 + 1751	Trace
	10	1246 +165	577 + 0	1878 + 388	Trace	521 +234	4372 + 581	Trace	Trace	Trace	8593 + 1367	Trace
	30	1293 +182	515 +217	3316 + 36	Trace	528 + 68	6200 + 319	Trace	Trace	Trace	11851 + 822	Trace
SA 4	0	1027 + 7	Trace	1259 + 200	372 + 60	968 +924	2005 + 785	676 + 18	Trace	Trace	6307 + 1162	Trace
	5	1094 +329	Trace	1679 + 266	514 + 137	841 +252	1958 + 772	1325 + 297	Trace	442 + 0	7853 + 2053	Trace
	10	1013 + 44	Trace	2384 + 87	771 + 222	439 + 0	2498 + 503	961 + 18	Trace	703 + 190	8768 + 1063	Trace
	30	1026 + 22	Trace	1966 + 133	Trace	342 + 0	2826 + 122	1587 + 467	Trace	2727 + 293	10474 + 1037	Trace
PM 95 A	0	494 + 99	256 + 0	756 + 104	Trace	747 +240	2628 + 892	Trace	Trace	Trace	4881 + 1334	137 + 8
	5	648 +145	360 + 10	1670 + 400	Trace	854 +203	2989 + 917	Trace	Trace	Trace	6521 + 1675	222 + 48
	10	761 + 45	256 + 0	2195 + 195	Trace	734 +149	3543 + 52	Trace	Trace	Trace	7489 + 441	312 + 55
	30	1072 + 74	155 + 0	2064 + 292	Trace	734 +234	3967+3 86	Trace	Trace	Trace	7992 + 986	338 + 4
SA 3	0	537 + 5	Trace	857 + 244	183 + 0	479 +186	2468 + 824	Trace	Trace	Trace	4524 + 1258	173 + 24
	5	540 + 66	Trace	820 + 276	239 + 0	510 + 0	2621 + 420	Trace	Trace	Trace	4729 + 762	197 + 58
	10	699 + 90	Trace	893 + 240	Trace	Trace	2602 + 17	Trace	Trace	Trace	4194 + 347	215 + 28
	30	929 +209	Trace	1966 + 133	321 + 41	293 + 47	4828 + 191	Trace	Trace	Trace	8337 + 621	316 + 27
Antasena	0	768 + 61	Trace	513 + 123	Trace	410 + 0	1849 + 463	Trace	Trace	Trace	3540 + 647	Trace
	5	805 + 18	Trace	737 + 207	423 + 0	796 + 0	1971 + 158	660 + 0	Trace	Trace	5392 + 383	Trace
	10	971 +163	Trace	606 + 10	329 + 0	555 +149	1676 + 105	733 + 0	Trace	Trace	4870 + 427	Trace
	30	1049 +233	Trace	2141 + 227	529 + 39	706 + 94	3016 + 902	1460 + 537	Trace	Trace	8901 + 2032	Trace

Table 4. Effect of Al treatment on organic acid concentration in roots and organic acid exudation rate from roots in soybean.

Variety	ppm Al	Organic acid concentration (nmol g^{-1} root DW)							Exudation rate (nmol g^{-1} root DW 24 hr^{-1})
		Oxalic acid	Malonic acid	Fumaric acid	Succinic acid	Malic acid	Citric acid	Total	
Wilis	0	1491 + 236	915 + 128	Trace	Trace	Trace	Trace	2406 + 364	444 + 68
	5	2378 + 67	1147 + 32	Trace	Trace	Trace	Trace	3523 + 99	576 + 70
	10	2770 + 57	1180 + 339	Trace	260 + 91	Trace	Trace	4209 + 476	973 + 232
	30	2566 + 50	1603 + 14	Trace	149 + 3	Trace	Trace	4318 + 525	2278 + 74
INPS	0	2325 + 563	1008 + 237	Trace	Trace	142 + 0	Trace	3475 + 800	Trace
	5	3147 + 334	1048 + 38	Trace	554 + 55	104 + 11	Trace	4853 + 488	216 + 9
	10	2861 + 84	1627 + 750	Trace	187 + 0	150 + 44	Trace	4823 + 877	881 + 21
	30	3187 + 36	2830 + 408	Trace	519 + 11	201 + 45	Trace	6737 + 500	998 + 233
Galunggung	0	2314 + 381	888 + 11	Trace	252 + 61	180 + 6	Trace	3633 + 459	908 + 203
	5	2698 + 133	1719 + 368	Trace	246 + 24	144 + 3	Trace	4806 + 528	641 + 101
	10	2989 + 282	1751 + 822	Trace	464 + 0	224 + 42	Trace	5428 +1146	1594 + 339
	30	2568 + 175	1880 + 44	Trace	461 + 0	799 + 20	Trace	5707 + 317	2122 + 335
Kerinci	0	2847 + 319	1367 + 68	203 + 7	370 + 47	Trace	594 + 145	5381 + 578	Trace
	5	3011 + 211	1745 + 341	341 + 40	302 + 40	Trace	845 + 6	6244 + 598	Trace
	10	3221 + 273	1432 + 410	892 + 7	493 + 74	Trace	2105 + 145	8143 + 901	Trace
	30	3345 + 407	1646 + 450	Trace	440 + 71	Trace	2719 + 254	8150 +1182	Trace
Kitamusume	0	1547 + 136	1436 + 67	954 + 11	Trace	Trace	Trace	3937 + 317	116 + 4
	5	2232 + 229	1642 + 570	905 + 15	671 + 13	392 + 65	725 + 41	6567 +1063	487 + 105
	10	3922 + 760	2137 + 422	1821 + 46	1127 + 222	739 + 256	1340 + 328	11085 +2451	526 + 114
	30	3088 + 113	1621 + 260	202 + 0	1041 + 155	932 + 296	1820 + 753	8710 + 903	2233 + 358

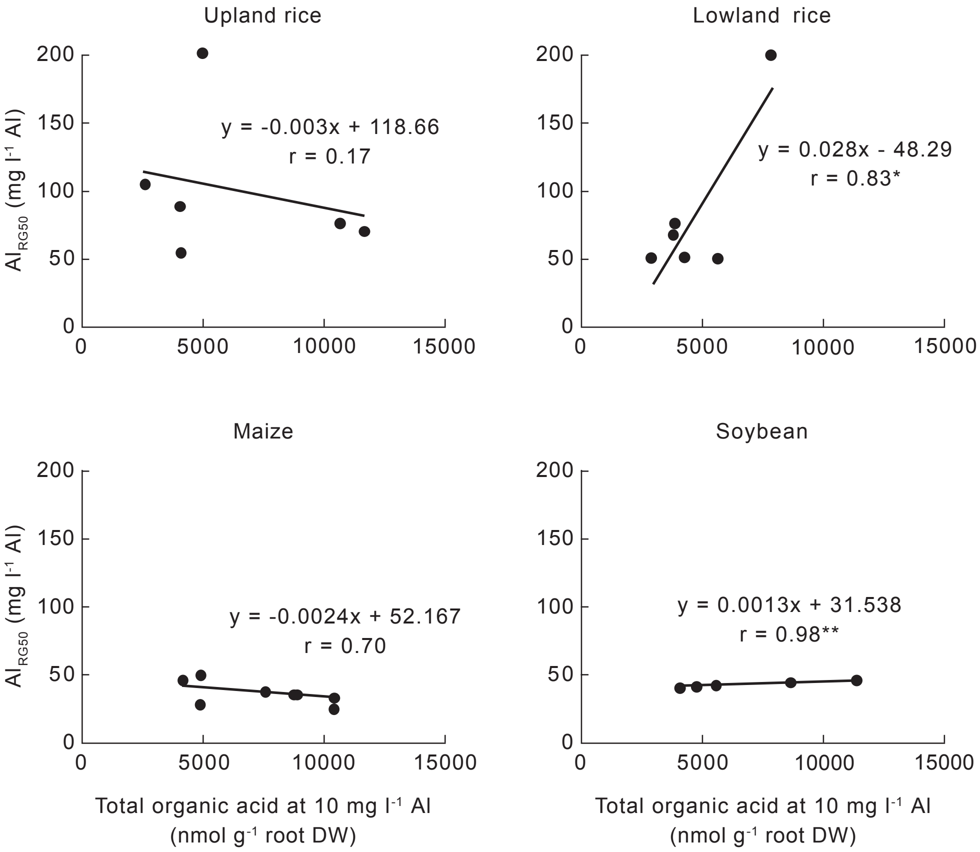

Fig. 3. Relationship between total organic acid concentration in roots at 10 mg l^{-1} Al and Al_{RG50}. * and ** indicate significance at 5% and 1% levels, respectively.

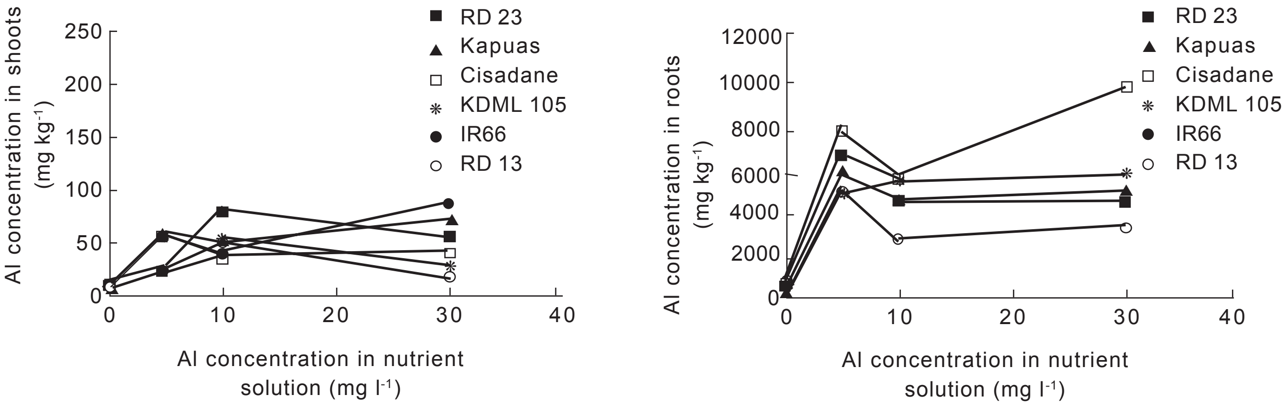

Fig. 4. Aluminium concentration in shoots and roots of lowland rice as affected by Al concentration in the nutrient solution.

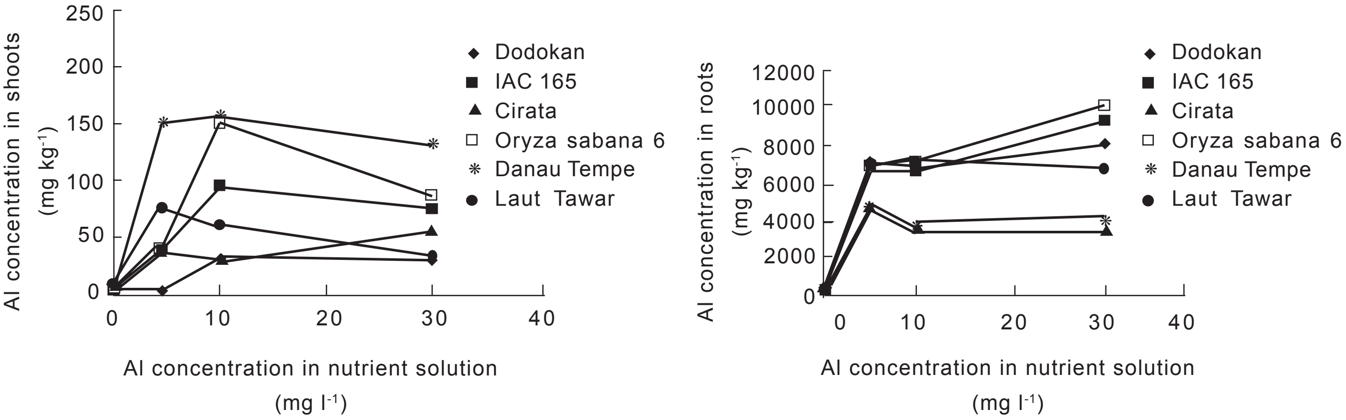

Fig. 5. Aluminum concentration in shoots and roots of upland rice as affected by Al concentration in the nutrient solution.

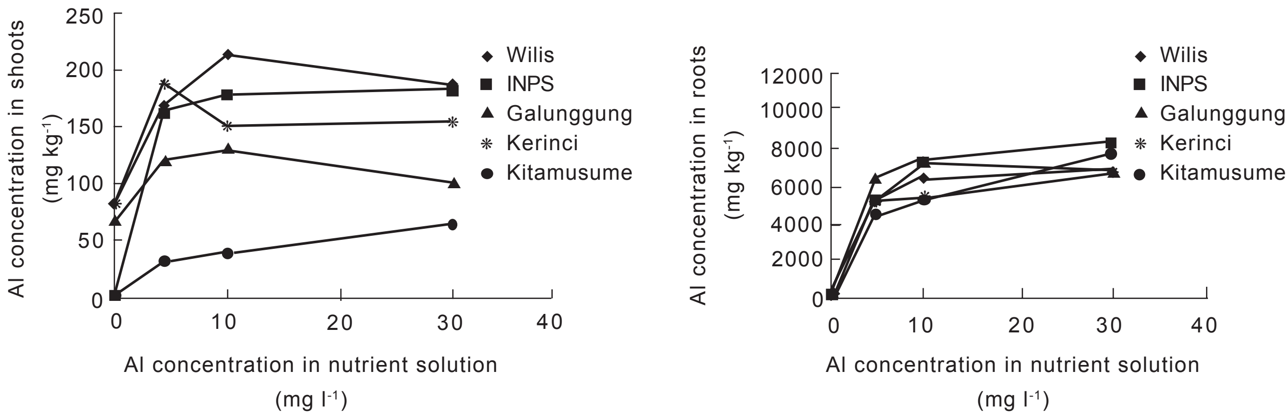

Fig. 6. Aluminum concentration in shoots and roots of soybean as affected by Al concentration in the nutrient solution.

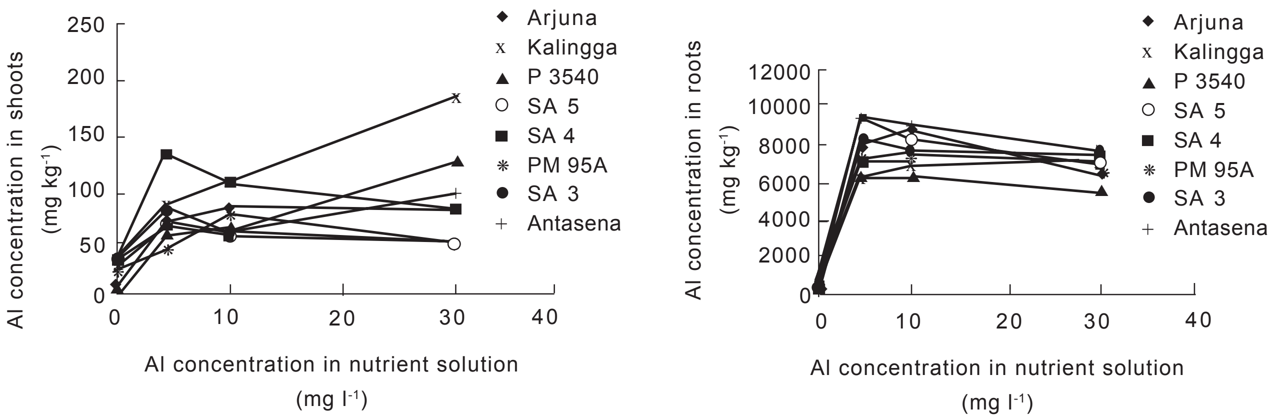

Fig. 7. Aluminum concentration in shoots and roots of maize as affected by Al concentration in the nutrient solution.

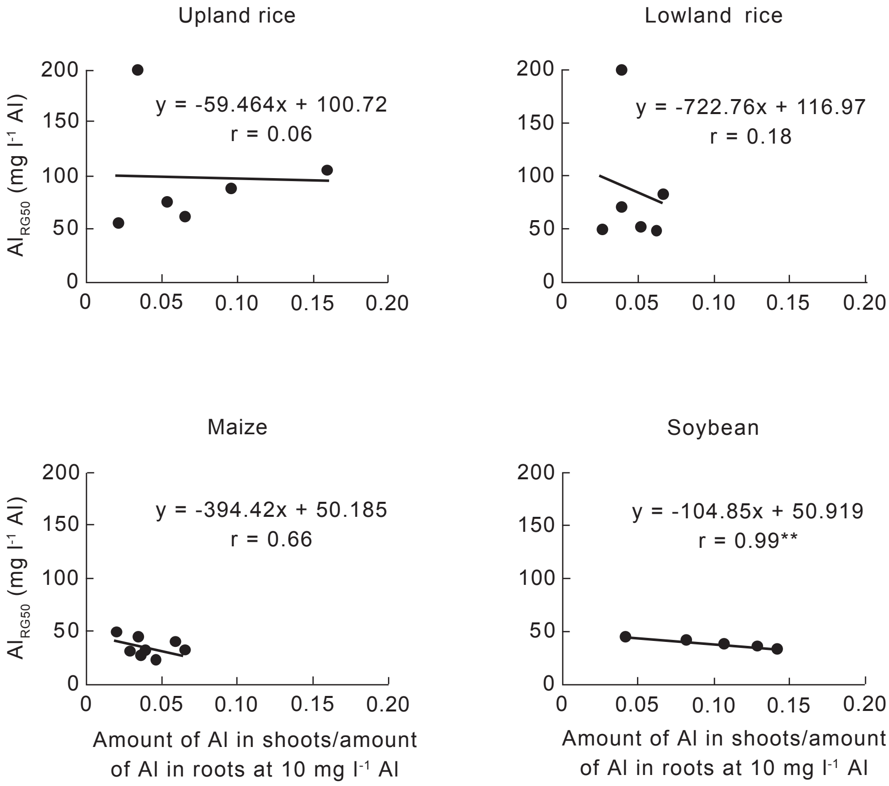

Fig. 8. Relationship between amount of Al in shoots/amount of Al in roots at 10 mg l^{-1} Al and Al_{RG50}. ** indicates significance at 1% level.

from roots to shoots can be suggested as a mechanism to achieve Al tolerance in soybean.

Aluminum concentration in shoots increased with increased Al level and this trend was more clear in soybean and maize than in lowland or upland rice (Figs. 4-7). In lowland rice, Al concentration in shoots was the lowest (Fig. 5). Among varieties of soybean, the Al concentration in shoots increased drastically in Wilis (Al-sensitive variety), while in Kitamusume (Al-tolerant variety) it did not. Rice was more tolerant to Al than maize and soybean. Also in soybean, Kitamusume was more tolerant to Al than Wilis. The tolerant varieties may exclude Al from shoots more strongly than sensitive ones.

The relationship between Al concentration and Al_{RG50} in each species at 10 mg l^{-1} Al level in nutrient solution is shown in Fig. 9 (shoots) and Fig. 10 (roots). A significant negative correlation between Al concentration in shoots and Al_{RG50} was found in soybean ($r = 0.89$, $P < 0.01$), but not found in other crops (r = 0.08, 0.52, and 0.52 for upland rice, lowland rice, and maize, respectively).

A significant negative correlation between Al concentration in roots and Al_{RG50} was also found only in soybean ($r = 0.80$, $P < 0.05$). However, in other crops the correlation was not significant at the 5% level (r = 0.25, 0.37, and 0.31 for upland rice, lowland rice, and maize, respectively). In soybean, Al concentration in shoots and roots increased with increase of Al level in nutrient solution. However, Al concentration in shoots and roots decreased with increasing Al tolerance at 10 mg l^{-1} Al level. This response indicates that Al-tolerant varieties can exclude Al from shoots and roots stronger than Al sensitive ones.

The use of crops tolerant to Al toxicity in acid soils was recommended to reduce the application of soil amendments. Based on root/shoot dry weight ratio and Al concentration in shoot parameters, rice is an adaptable crop to be planted in acid soils. In addition, according to total organic acid concentration in roots, Kitamusume (soybean) and RD 13 (lowland rice) are also suggested in the soils.

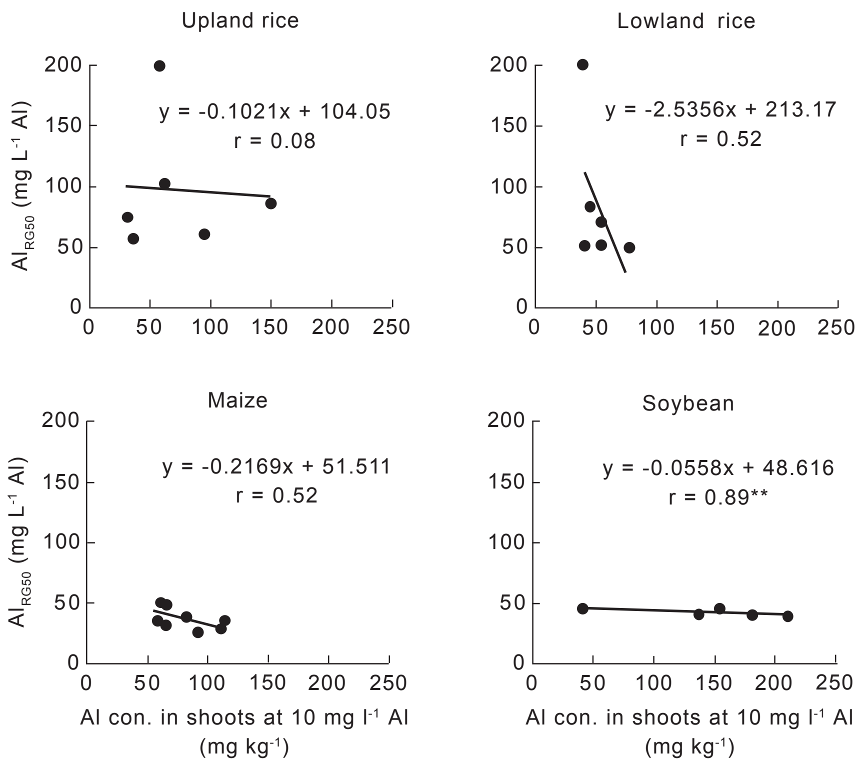

Fig. 9. Relationship between Al concentration in shoots at 10 mg l^{-1} Al and Al_{RG50}. ** indicates significance at 1% level.

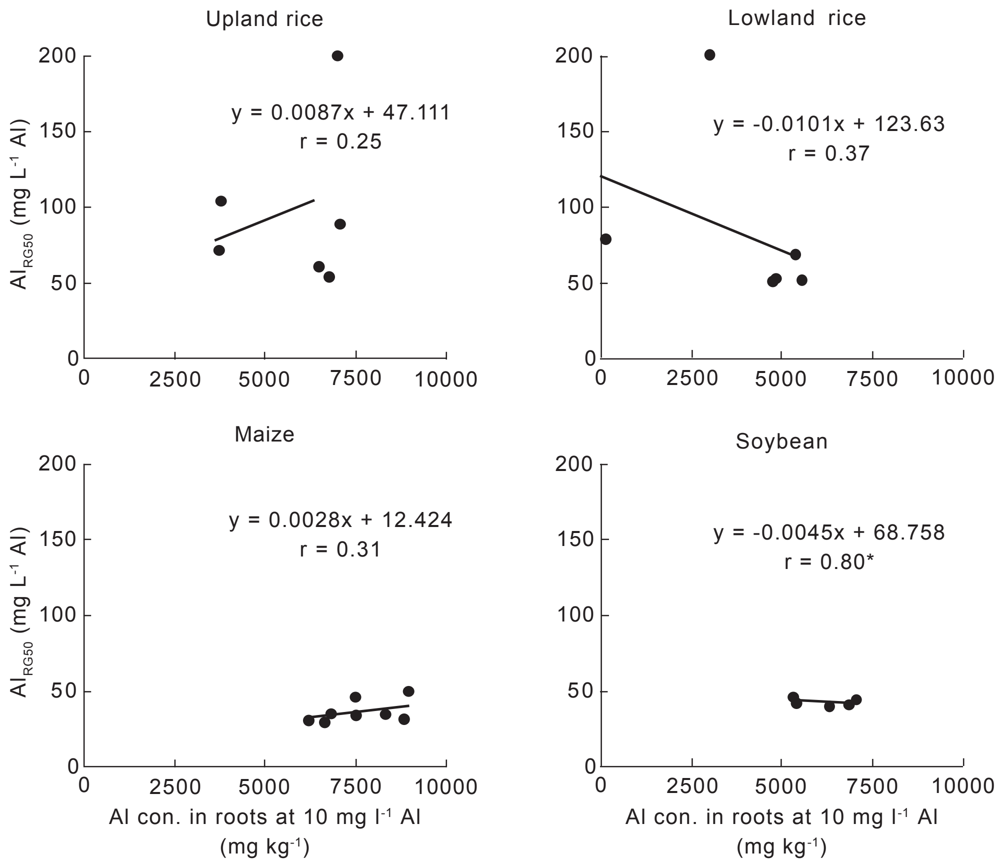

Fig. 10. Relationship between Al concentration in roots at 10 mg l^{-1} Al and Al_{RG50}. * indicates significance at 5% level.

CONCLUSION

The root/shoot dry weight ratio at 10 mg l^{-1} Al treatment is an important parameter to indicate differential Al tolerance in maize. Oxalic acid exudation from roots cannot always explain the Al tolerance. Total organic acid concentration in roots at 10 mg l^{-1} Al treatment indicated a difference of Al tolerance in soybean and lowland rice.

Aluminum translocation from roots to shoots was lower in tolerant varieties than in sensitive varieties of soybean. The increase in Al concentration in shoots with increasing Al level in the solution was larger in soybean and maize than in lowland or upland rice. Among varieties of soybean, the Al concentration in shoots increased drastically in Wilis (Al sensitive variety) with increasing Al level, while in Kitamusume (Al tolerant variety) it did not. Al concentration in shoots and roots at 10 mg l^{-1} Al level is an important parameter to indicate a difference of Al tolerance in soybean.

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