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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 9 (5): 954-960, 2006
ISSN 1028-8880
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Evaluation of Differences in Tolerance to Aluminium Toxicity among Some Tropical Cowpea (*Vigna unguiculata*) Genotypes

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Abstract: Aluminium (Al) toxicity is widespread in tropical and temperate acid soils. Eight cowpea (*Vigna unguiculata*) genotypes, G (Ife brown, IT87D-941-1, IT99K-1060, IT84S-2246-4, IT96D-610, IT93K-452-1, IT86D-719 and IT98D-810) were grown for 5 and 17 weeks (1st and 2nd experiments) and evaluated for their differential tolerance to 0, 20 and 50 μM $AlCl_3$ levels applied prior sowing in an Alfisol (Typic Paleudalf). Plant height at weekly intervals (from 2 weeks after planting, WAP), yield and post-cropping soil chemical parameters (pH, extractable-Al, extractable-Mn and available P) were estimated. Except at 2WAP, Al effect was insignificant ($p<0.05$) on plant height, though extractable-Al differed greatly ($p<0.01$) among soils sampled after cropping, suggesting need to test higher rates and/or continuous application through irrigation water. On the contrary, G and G×Al interaction significantly affected plant height, yield, soil pH, P-availability and Al tolerance potential. Plants of IT93K-452-1 variety were taller (71.6±3.38 cm) than individual plants of the other varieties at all Al application levels. Aluminium extracted from treated and untreated soils correlated linearly with Al addition levels, but not with the plant performance or other soil chemical parameters. Complexity in the soil environment increased with Al addition to the extent that crop performance became unpredictable and increasingly variable among the genotypes as tolerance to Al became more crucial. Biomass production (followed by pod weight) was the most sensitive parameter to Al addition while extractable Al changed maximally among the soil chemical parameters. The genotypes were categorized into efficient or non-efficient and tolerant or non-tolerant/susceptible types.

Key words: Genotypic differences, aluminium tolerance potential, *Vigna unguiculata*, $AlCl_3$ application levels

INTRODUCTION

Cowpea (*Vigna unguiculata*) is an important tropical and subtropical grain legume providing protein, vitamins and minerals for overwhelming majority of people in Latin America and Africa. It originated from West Africa with centre of domestication (Nigeria, Burkina Faso, Niger and Ghana) showing great diversity in their germplasm (Fawole *et al*., 2001). The crop's value also lies in its ability to tolerate drought and fix atmospheric nitrogen (N), which allows it to grow on and improve poor soils. However, agricultural and dietary survey reports on developing countries indicate that production has not kept pace with population growth and must increase by 72% (World Bank, 1989).

A major constraint to cowpea production is aluminium (Al), particularly on highly weathered and leached soils in humid tropical regions (Minella and Sorellis, 1992) where acidity causes infertility and generally limits crop production (Von Uexkull and Mutert, 1995). Deficiencies of phosphorous (P), calcium (Ca) and magnesium (Mg) coupled with the presence of phyto-toxic substances are responsible for the fertility limitation of acid solids as aggravated by industrial pollution and nitrification. Poor growth in acid soils could be related directly to Al saturation (Akinrinde *et al*., 2004). A common symptom of Al toxicity is P deficiency and the practical result is P deficiency symptom (Haynes, 1984; Huang *et al*., 1992).

Estimates of soil limitation to plant growth in developing countries show that an average of 23 % of soils used is constrained to Al toxicity (Anitzen and Ritter, 1984) that has limited the expansion of cowpea to important agricultural areas of the world (Alam, 1981). Genetic tolerance to toxic level of Al is of great importance for crop production on acid soils since increasing soil pH by liming is costly and limited to the surface layer (Alam, 1981; Foy, 1992). Restriction of crop growth by excess Al could be due to direct inhibition of nutrient uptake or disturbance of root cell functions (Kochian, 1995).

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Combination of sound management practices with tolerance to Al is capable of ameliorating the negative impact of acid stress on cowpea performance (Akinrinde *et al.*, 2004). This is influenced by genetic background (Bona *et al.*, 1994). Several studies (Oikeh *et al.*, 2003; Hogh-Jensen and Pedersen, 2003; Kadiata and Lumpungu, 2003) have also highlighted the fact that plant species and even varieties within species vary in their capacity for biomass and/or grain production. This characteristic is yet to be fully explored, especially for cowpea. Identification of cowpea varieties that can tolerate excess Al in acid soils would assist in improving the yield of the crop. Thus, a major step in breeding Al tolerant cultivars is to identify Al-tolerant cowpea genotypes. The work being reported was principally conducted to evaluate differences in Al tolerance among eight cowpea genotypes grown on a Nigerian alfisol.

MATERIALS AND METHODS

The investigation was conducted between October 2004 and February 2005 in a greenhouse at the Agronomy Department, University of Ibadan (UI) Nigeria. The experimental soil was loamy sand (0-15 cm) Alfisol (Typic Paleudalf) collected from the Teaching and Research Farm (Parry road) at UI. The major soil chemical properties included $pH_{(H2O)}$: 6.6; 19 g C kg^{-1} soil organic C; 4.1g N kg^{-1} soil; 7.01 mg kg^{-1} extractable P (Bray 1); 1.15 cmol kg^{-1} K; 1.45 cmol kg^{-1} Ca and 4.07 cmol kg^{-1} effective CEC. Soil pH (in H_2O) was determined using a 1:2.5 (l. c) soil: water ratio. Sand, clay and silt contents were determined by the hydrometer method (Bouyoucos, 1951). Organic matter was determined by dichromate oxidation (Walkley and Black, 1934). The other soil analysis methods used are described in International Institute for Tropical Agriculture (IITA) soil analysis manual (Juo, 1981).

The soil was air-dried, thoroughly mixed up and passed through 2 mm sieve prior to filling in plastic pots up to 2 kg each. There were two experiments; both being of factorial arrangement involving treatments replicated three times in Completely Randomised Design (CRD):

- In the first experiment, treatments consisted of three Al levels (0, 20 and 50 μM $AlCl_3$) and eight cowpea genotypes (Ife brown, IT87D-941-1, IT99K-1060, IT84S-2246-4, IT96D-610, IT93K-452-1, IT86D-719 and IT98K-810), giving a total of 72 units that were used for the evaluation of growth responses of the test crop to the Al treatment.
- In the second experiment, there were only two levels of Al treatment (0 and 50 μM $AlCl_3$) applied on the eight-cowpea genotypes, resulting in a total of 2×8×3 (= 48) experimental units used for evaluating the yield and yield components of the treated and control plants.

In both cases, each kilogram soil received basal supply of 100 mg P as KH_2Po_4 in addition to Al treatment. There were two plants (established from pre-germinated seeds) in each pot and soil was maintained at 60% Field Capacity (FC). An insecticide (Karate-2.5 Kimbola Cythalothin) was sprayed (once in the first experiment but twice in the second experiment) at 1 mL per 160 mL water.

Plant height measurement (commenced after 2 weeks of establishment) was done at weekly intervals in the first experiment whereas harvesting was done after 5 weeks of growth. Fresh shoot were recorded immediately after harvesting the tops while dry shoot were recorded after oven drying at about 100°C. Roots were separated from the soil by washing several times with water to make nodule count possible. Soils sampled after cropping were also analysed for pH (H_2O), available P as well as 0.01N HCl-extractable Al and Mn. Pods were harvested in the second experiment after 17 weeks of growth.

All data were analysed by analysis of variance (ANOVA), using Sigmastat software and F-test was employed to evaluate the significance of the treatments. The student-Newman-Keul's test was used to compare means at both 1 and 5% probability levels.

RESULTS AND DISCUSSION

The effect of Al levels on plant height was insignificant ($p<0.05$) except at the second week of growth, indicating the ineffectiveness of rates as well as insufficiency of the single dose applied (Fig. 1) whereas there were significant differences among the cowpea genotypes in this trait. Pinheiro de Carvalho *et al.* (2003) noted that survival could occur in several wheat germplasm (screened at 100 and 200 μM Al) after withdrawal of Al stress.

The cowpea genotypes were significantly different in the entire yield and yield component parameters, except number of pods, whereas Al treatments could not influence any of them. The genotypes also had significant effects on soil pH and available P contents while Al treatment was only able to increase the amount of Al extractable from the soil and extractable Mn did not vary among the plants. Interaction between Al and genotype was significant for plant height (at all the successive growth periods), nodulation, weight of pods and biomass production. It also had effect on soil P availability. As such, level of performance of a variety may be specific for particular Al level and evaluation under different Al

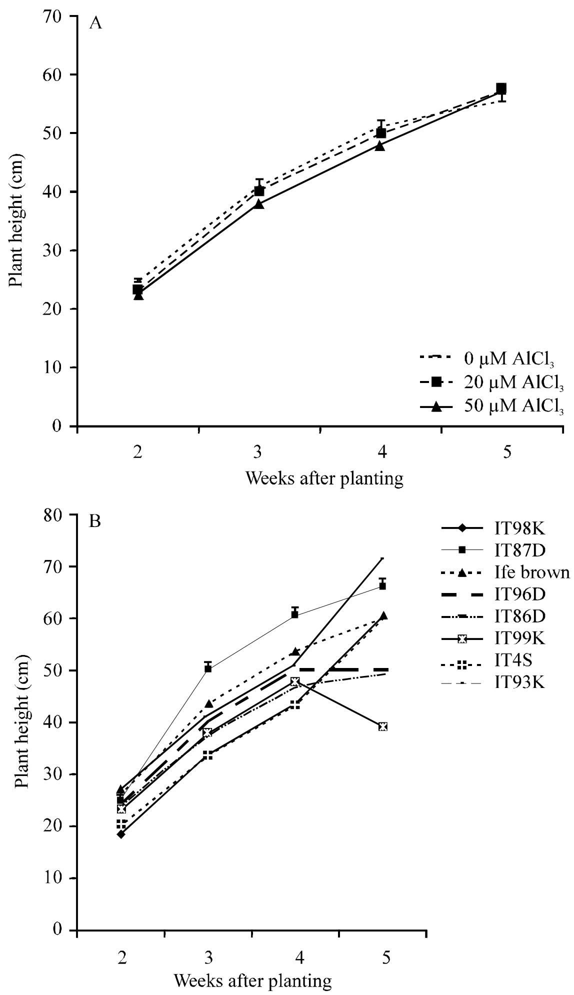

Fig. 1: Effects of [A] $AlCl_3$ application levels and [B] genotypic differences on height of cowpea (*Vigna unguiculata*)

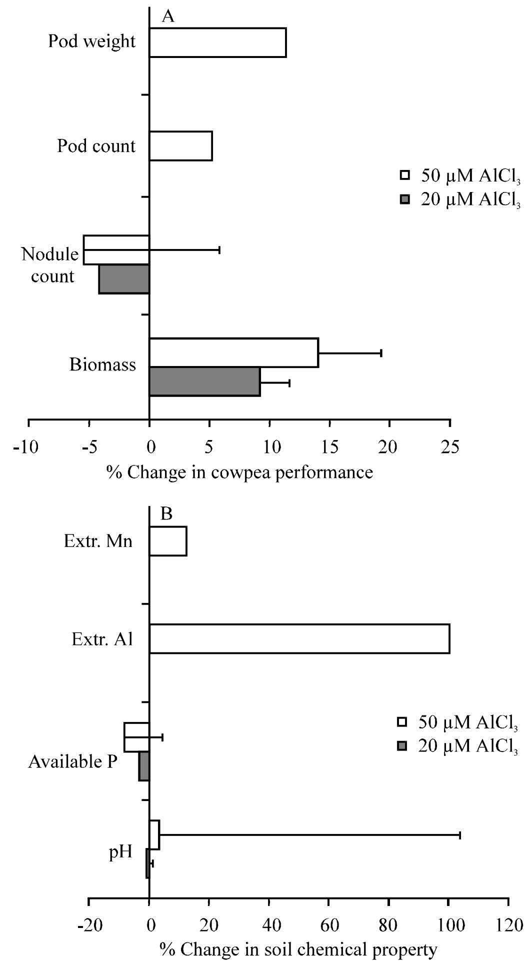

Fig. 2: Percentage change (increase or decrease) in: [A] cowpea performance parameters and [B] soil chemical property parameters at the two aluminium addition levels (20 and 50 µM $AlCl_3$). [% change = (cowpea performance parameter or soil property parameter value at specific Al addition level-cowpea performance parameter or soil property parameter value at 0 µM $AlCl_3$/ cowpea performance parameter or soil property parameter value at 0 µM $AlCl_3$)×100]

application levels may be needed when any of these parameters are used as basis for their selection. Except for Al extractable, all the measured chemical parameters from post-cropped soil samples had insignificant linear relationship with Al application levels, implying that only the former can be said to increase as the soil Al level was increased. In addition, the relationship between Al levels and plant height (at all growth periods) was not significant. There seemed to be a tendency for increased complexity in soil reaction and other chemical properties with Al addition while growth and yield became unpredictable whereas high Al tolerance potential of the cultivar is more crucial. This is an indication of the diversity in cowpea germplasm (Fawole *et al*., 2001). The cultivars used varied in their capacity for growth and/or biomass production.

To evaluate the sensitivity of the parameters to Al toxicity, changes in weight and number of pods, biomass production, nodulation, as well as pH, available P and extractable Al and Mn at each of the Al addition levels as compared with 0 µM Al level were estimated and presented in Fig. 2. Among the yield components, biomass production exhibited the maximum change with Al addition. It was followed by pod weight. Among the soil chemical properties measured, extractable Al (followed by extractable Mn and available P) changed

Table 1: Significance of F values and orthogonal contrasts derived from analysis of variance (ANOVA) for performance parameters (measured on eight cowpea genotypes) and some soil chemical properties (determined after cropping) at three Aluminium (Al) levels

Parameter	Cowpea Genotype (CG)	Al-application level (Al-AL)	CG×Al-AL interaction	Linear Al-AL/ Parameter Regression	CV (%)
Performance Parameters					
Plant height (cm) at successive growth stages/weeks					
2	**	*	**	NS	18.3
3	**	NS	**	NS	20.2
4	**	NS	**	NS	16.1
5	**	NS	**	NS	20.8
Pod count	NS	NS	NS	NS	21.1
Nodule count	**	NS	**	NS	19.2
Yield components (g pot^{-1})					
Pod weight	**	NS	**	NS	47.0
Biomass	**	NS	**	NS	21.1
Post-cropping soil chemical properties					
pH (H_2O)	**	NS	NS	NS	4.9
Available P	**	NS	**	NS	39.6
Extractable-Al	NS	**	NS	**	35.7
Extractable-Mn	NS	NS	NS	NS	131.4
Al-tolerance potential (g pod g^{-1} $AlCl_3$ applied)	**	ND	ND	-	43.3

*, ** Indicates significance at 0.05 and 0.01 probability levels, respectively, NS = Not Significance, ND = Not Determined

Table 2: Influence of $AlCl_3$ application levels on performance parameters across eight genotypes of cowpea and on some soil chemical properties

Parameter	$AlCl_3$-application level (μM) 0	20	50	Average	±SE
Plant height (cm) at successive growth periods (weeks)	24.7	23.1	22.7	23.47	0.53
Pod count	3.3	ND	3.5	3.3	0.50
Nodule count	16.0	15.4	15.2	15.53	0.59
Pod weight (g pot^{-1})	4.79	ND	5.33	5.06	0.25
Biomass production (g pot^{-1})	19.23	ND	20.70	19.97	0.66
Post-cropping soil chemical properties					
pH (H_2O)	6.3	6.3	6.4	6.33	0.10
Available P (mg kg^{-1})	46.58	45.05	42.86	44.83	2.18
Extractable-Al (mg kg^{-1})	0.002	ND	0.004	0.003	0.0001
Extractable Mn (mg kg^{-1})	0.005	ND	0.006	0.0055	0.002
Al-tolerance potential (g g^{-1})					
Biomass	-	6795.63	2366.04	4580.84	597.29
Pod weight	-	ND	887.29	887.29	123.09

Means for each of the parameters (under different P application levels) that are followed by the same letter are not significantly different at the 0.05 level by the Student-Newman-Keul's method, ND = Not Determined

Table 3: Plant height and production of biomass and pod of eight cowpea genotypes treated with different $AlCl_3$ application levels

$AlCl_3$ level (μM)	Cowpea genotype IT98K-810	IT87D-941-1	Ife brown	IT96D-610	IT86D-719	IT99K-1060	IT84S-2246-4	IT93K-452-1	F-test	CV(%)
Plant height (cm) at 5 weeks of growth										
0	60.0	69.0	59.0	52.0	46.7	43.0	46.3	70.0	**	20.4
20	55.7	69.0	51.3	52.3	52.0	36.7	68.0	74.0	**	21.9
50	64.0	60.3	70.0	45.7	48.7	37.3	63.0	70.7	**	20.6
Mean	59.9	66.1	60.1	50.0	49.1	39.0	59.1	71.6	**	20.8
±SE	±1.42	±1.19	±4.15	±2.81	±0.95	±0.99	±1.19	±3.38		
Biomass production (g pot^{-1})										
0	9.93	24.67	21.57	16.73	21.87	16.30	20.17	22.63	**	26.3
50	22.27	20.50	22.93	22.03	19.57	16.47	19.33	22.50	NS	16.7
Mean	16.10	22.58	22.25	19.38	20.72	16.38	19.75	22.57	**	21.1
±SE	±2.95	±1.31	±1.06	±1.68	±0.91	±0.86	±0.64	±1.43		
Number of pods										
0	2.7	3.3	3.3	3.3	3.7	3.0	3.3	3.7	NS	51.4
50	4.0	3.0	3.3	4.0	3.7	3.0	3.0	3.7	NS	15.1
Mean	3.33	3.17	3.33	3.67	3.67	3.00	3.17	3.67	NS	16.9
±SE	±0.42	±0.17	±0.21	±0.21	±0.21	±0.00	±0.17	±0.21		
Weight of pods (g pot^{-1})										
0	1.67	9.13	7.40	3.20	4.63	3.17	4.33	4.77	**	17.0
50	8.83	6.53	7.33	5.27	3.63	2.73	3.70	4.57	**	43.3
Mean	5.25	7.83	7.37	4.23	4.13	2.95	4.02	4.67	**	46.9
±SE	±1.75	±0.81	±0.38	±0.71	±0.45	±0.22	±0.27	±0.29		

**Significant at 0.01 probability level, NS = Not Significant, Means for each of the parameters (under different rice varieties) that are followed by the same letter are not significantly different at the 0.05 level by the Student-Newman-Keul's method

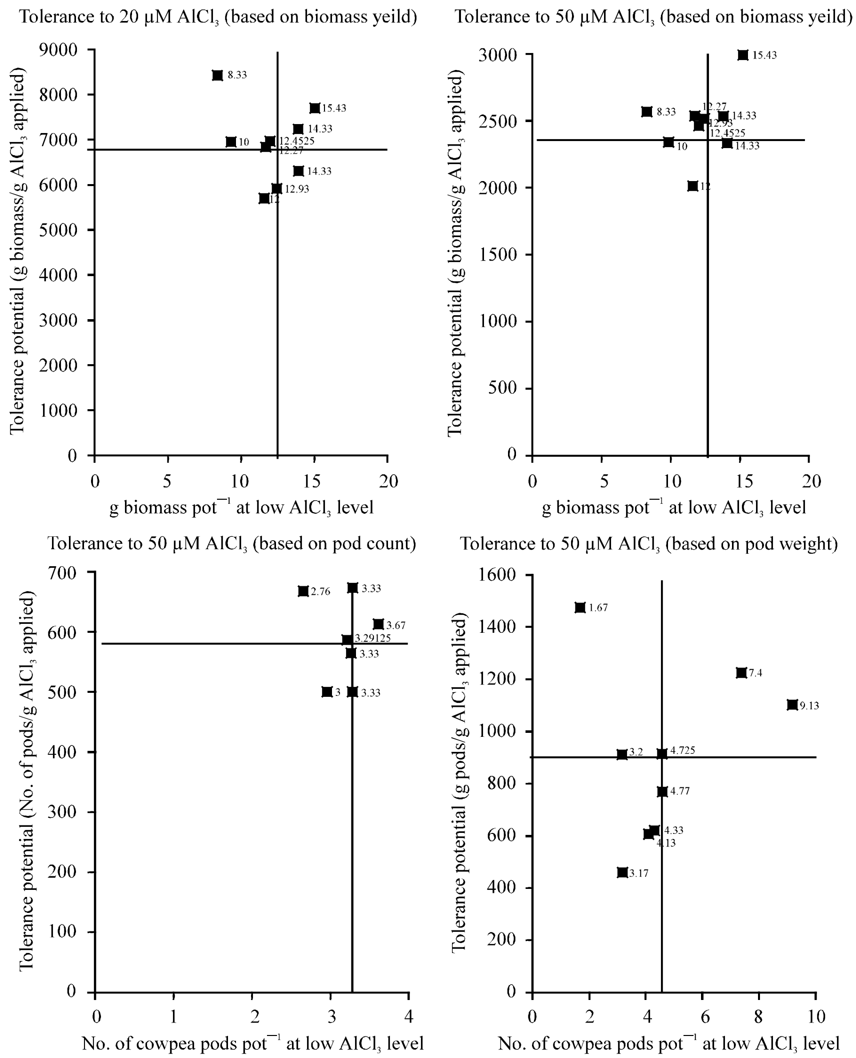

Fig. 3: Categorization of the eight cowpea genotypes on the basis of aluminium tolerance potential [The indicated values represent the actual tolerance potential by each cultivar as plotted against the respective total biomass (dry root+dry shoot) produced or number or weight of pods. The value in circle is the overall average Al-tolerance potential for all the cultivars plotted against the average total biomass/pod number/pod weight used in demarcating the categories]

maximally. Thus, biomass production, extractable Al as well as extractable Mn and available P were the most sensitive response parameters to Al toxicity. Since biomass production is the most easily determined among these parameters, it can be used for screening cowpea genotypic responses to Al.

Genotype×aluminium interactions for plant height (between 2-5 weeks of growth), number of nodules, pod weight, biomass production and soil available P were significant (Tables 1 and 2), indicating that the three Al levels caused different responses in the growth and yield in different genotypes beside influencing P availability in soil. Except for the 50 µM Al level, there were significant ($p<0.01$) differences among genotypes for each Al level with respect to biomass production, plant height measured at 5 WAP and pod weight (Table 3). Number of pods, however, was not significant both across and within the Al levels. Individual plants of the "IT93K-452-1 " variety, on the average, were significantly taller than the individual plants of the other varieties at all the Al application levels. Genotype "Ife brown" could be said to have medium height at 0 µM Al, but interestingly had the highest pod yield.

Aluminium tolerance potential also differed significantly among genotypes across Al levels (Table 4). On biomass production basis, genotype IT99K-1060 had the highest Al tolerance potential at both 20 and 50 µM Al levels whereas genotype IT86D-719 had the lowest.

Table 4: Aluminium tolerance potential of eight cowpea genotypes (estimated on biomass, number of pods and pod weight basis) as influenced by different $AlCl_3$ application levels

Cowpea genotype	Biomass yield at 0 μM $AlCl_3$	Al tolerance potential ($g\ g^{-1}$) 20 μM Al	Al tolerance potential ($g\ g^{-1}$) 50 μM Al	Number of pods at 0 μM Al	Al tolerance potential ($g\ g^{-1}$)	Pod weight at 0 μM Al	Al tolerance potential ($g\ g^{-1}$)
IT98K-810	14.33	7250	2195	2.67	666.67	1.67	1471.67
IT87D-941-1	15.43	7700	2850	3.33	500.00	9.13	1088.33
Ife brown	12.93	5750	2405	3.33	555.00	7.40	1221.67
IT96D-610	14.33	6165	2445	3.33	666.67	3.20	878.33
IT86D-719	12.00	5585	1916.67	3.67	611.67	4.13	605.00
IT99K-1060	8.33	8415	2466.67	3.00	500.00	3.17	455.00
IT84S-2246-4	10.00	6835	2205	3.33	500.00	4.33	616.67
IT93K-452-1	12.27	6665	2445	3.67	611.67	4.77	761.67
Average	12.45	6795.63	2366.04	3.29	576.46	4.73	887.30
±SE	0.84	343.68	95.89	0.12	25.60	0.86	123.09

Table 5: Rating of aluminium tolerance potentials for eight cowpea genotypes estimated on biomass production and pod weight basis

Al tolerance estimation parameter	$AlCl_3$ addition	Yield at 0 μM Al	Al tolerance ($g\ g^{-1}$)	Efficient and tolerant	Non-efficient but tolerant	Efficient but Non-tolerant	Non-efficient and Non-tolerant
Biomass production ($g\ pot^{-1}$)	20 μM	15.43	7700	IT87-941-1			
		14.33	7250	IT98K-810			
		10.00	6835		IT4S-2246-4		
		8.33	8415		IT99K-1060		
		12.00	5585			IT86-719	
		12.93	5750				Ife brown
		14.33	6165				IT96D-610
		12.27	6665				IT93K-452-1
Biomass production ($g\ pot^{-1}$)	50 μM	15.43	2850	IT87-941-1			
		14.33	2195	IT98K-810			
		12.27	2445	IT93K-452-1			
		8.33	2467		IT99K-1060		
		12.00	1917			IT86-719	
		10.00	2205			IT4S-2246-4	
		12.93	2405				Ife brown
		14.33	2445				IT96D-610
Pod weight ($g\ pot^{-1}$)	50 μM	7.40	1222	Ife brown			
		9.13	1088	IT87D-941-1			
		4.77	762	IT93K-452-1			
		1.67	1472		IT98K-810		
		3.20	878		IT96D-610		
		3.17	455			IT99K-1060	
		4.33	617			IT4S-2246-4	
		4.13	602				IT86D-719

Interestingly, the three most tolerant of Al with regard to pod yield were in the decreasing order: IT98K-810>Ife brown>IT87D-941-1. It is note worthy that Al tolerance potential declines with increase in the Al level, being much lower at 50 μM Al than at 20 μM Al.

By plotting the values of the respective yield components when no Al was applied against the respective genotype Al tolerance potential values, the eight genotypes were fitted into four categories. As evident from Fig. 3 and Table 5, the categories (on the basis of biomass production at 20 μM $AlCl_3$ addition) are:

- Efficient and Al-tolerant genotypes with performance higher than the average of all the genotypes considered when no Al was applied. To fit this class, they were also observed to tolerate Al addition such that their average Al tolerance potential was higher than the average for the eight genotypes. Only two of the 8 genotypes (IT87-941-1 and IT98K-810) fall into this category.
- Non-efficient but Al-tolerant genotypes. Varieties IT4S-2246-4 and IT99K-1060 fall under this category as they produced less than average biomass yield. Their Al tolerant potentials were, however, higher than the average for the 8 varieties tested.
- The efficient but non-tolerant genotypes produced higher than average biomass yield and had average Al-tolerant potential lower than the average obtained for all genotypes tested. One of the genotypes (IT86-719) used in this study fall into this group.
- Non-efficient and non tolerant. Three genotypes (Ife brown, IT96D-610 and IT93K-452-1) were neither tolerant nor efficient as they produced lower than average yield and also had lower than average Al-tolerance potential.

The ratings for the genotypes with respect to biomass or pod yield at 50-μM $AlCl_3$ additions are further given in Table 5. The groups of genotypes that are efficient in yield performance and also tolerant to Al are the best as they can produce well under high Al levels of acid soils.

CONCLUSION

The increasing costs of lime and chemical P fertilizers usually disable small and medium scale farmers in developing nations of the world to have ready access to these important sustainable farming inputs. Those in advanced nations are also not excluded from possible environmental pollution that could result from continuous use of the chemicals in efforts directed to alleviate the deleterious effects of acid soil infertility. Given the differential responses of cowpea genotypes to Al-toxicity stress, evaluation of the Al tolerant potentials (yield per unit of Al concentration) can be used in Al-tolerance screening studies of the crop under greenhouse conditions.

ACKNOWLEDGMENT

The John D. and Catherine T. MacArthur Foundation Grant facilitated by the University of Ibadan funded the research.

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