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Short Communication

Selection of pigeonpea genotypes for tolerance to aluminium toxicity

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With 2 tables

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Abstract

Aluminium toxicity is a major growth limiting factor for pigeonpea [*Cajanus cajan* (L.) Millspaugh] production in acid soils. Thus, screening and selection of pigeonpea genotypes for aluminium tolerance is important. The effects of five aluminium concentrations (0, 10, 20, 30 and 50 ppm Al) on 32 genotypes of pigeonpea were studied in hydroponic and sand assays (growth response methods). Ratings of genotypes were similar for the two screening methods, suggesting that either of the two could be used for evaluation of genotypes for aluminium tolerance. Root and shoot aluminium contents were significantly lower in the tolerant ('IPA 7-10′ and 'T 7') than sensitive genotypes ('Bahar' and 'Pusa 9'), indicating that aluminium tolerance mechanism *per se* in the tolerant genotypes involved aluminium exclusion. Genotypes 'IPA 7-10' and 'T 7' will be useful in breeding programmes to improve aluminium tolerance in pigeonpea.

Key words: aluminium toxicity — *Cajanus cajan* (L.) Millspaugh — aluminium tolerance — aluminium exclusion

Aluminium toxicity (Al^{3+}) is a serious problem limiting crop productivity in acid soils which comprises large areas of the world (Kochian 1995) particularly in the tropics and subtropics (Foy et al. 1978). The major symptoms of aluminium (Al) toxicity is rapid inhibition of root elongation by destroying the root apex (Ryan et al. 1993) resulting in inefficient uptake of water and nutrients (Fageria and Carvalho 1982, Fageria 1985).

The two most common ways to alleviate aluminium toxicity are by liming and by using tolerant cultivars. It is possible to detoxify aluminium in surface soil in the field by liming to a pH 5.5 or above. However, liming does not remedy sub-soil acidity and it may not always be practical or cost effective (Tesfaye et al. 2001). Under such situations, use of tolerant cultivars may be a satisfactory solution to this problem. Breeding of crops for aluminium tolerance requires a rapid and effective technique to discriminate between tolerant and sensitive genotypes. Field screening for aluminium tolerance is difficult because of the temporal and spatial variation in acidic soils. Moreover, screening in the field is expensive and time consuming when a large number of genotypes are under evaluation (Garcia et al. 1979). Therefore, selection for tolerance in hydroponic or sand assay based on growth response traits of seedlings has been used as a rapid method to screen for aluminium tolerance in several crops (Singh and Chaturvedi 2007, Singh et al. 2009). In addition, the results obtained with solution culture screening method correlate positively with those obtained using field screening (Urrea-Gomej et al. 1996).

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is a hardy, widely adapted, drought-tolerant crop with a large temporal variation (90–300 days) for maturity. These traits allow its cultivation in a range of environments and cropping systems. It is grown in Asia, Eastern and Southern Africa, Latin America and Caribbean countries (Saxena 2008). In India, it is cultivated on more than 3.40 million hectares which include the North-eastern states, Bihar, Jharkhand and Chhatishgarh that have considerable acidic soils. In these states, pigeonpea cultivation has been increasing in recent years. Therefore, it is imperative to have suitable varieties for acidic soils of these areas. Little work on screening for tolerance to aluminium toxicity in pigeonpea has been reported. This paper reports the results of selection for tolerance to aluminium toxicity in pigeonpea.

Materials and Methods

Thirty-two (32) pigeonpea genotypes (Table 1) were chosen for study and acquired from the Indian Institute of Pulses Research, Kanpur (Uttar Pradesh), India. These genotypes belong to three distinct maturity groups (early, medium and late) and have originated from different places in India. Some of these genotypes ('Bahar', 'Pusa 9', 'UPAS 120', 'Narendra Arhar 1', 'BSMR 736', 'Asha', etc.) are released cultivars and cultivated widely in the area of their adaptation.

Hydroponic assay (Experiment 1): Seeds were disinfected with 1% sodium hypochlorite and then germinated in filter paper. After 8 days, the seedlings were transferred to dilute nutrient solution: KNO_3 (0.5 mM), $Ca(NO_3)_2.4H_20$ (0.5 mM), $MgSO_4.7H_2O$ (0.2 mM), KH_2PO_4 (0.1 mM), KCl (50 μM), H_3BO_3 (46 μM), FE-EDTA (20 μM), $MnCl_2.4H_2O$ (2 μM), $ZnSO_4.7H_2O$ (1 μM), $CuSO_4.5H_2O$ (0.3 μM) and $NaMoO_4.2H_2O$ (0.5 μM) (Simon et al. 1994) having 0 (control), 10, 20, 30 and 50 ppm aluminium concentrations. The aluminium treatments were supplied as $AlCl_3.6H_20$. The pH of nutrient solution was maintained at 4.5 for all the treatments using 1 M HCl. The pH of the aluminium-treated nutrient solution was measured daily. The solution was regularly aerated by bubbling air into the nutrient solution with an aquarium air pump and replaced after every 4 days to maintain the proper nutrient and aluminium concentration. Four (8 days old) plants of each genotype selected for uniformity were grown in duplicate trays (two replications) for each of the five aluminium concentrations. After 22 days of growth, the root and shoot were harvested separately and the roots were rinsed 20 s in distilled water to remove surface contamination followed by blotting

Table 1: Effects of five aluminium concentrations on tap root length (RL) of 32 pigeonpea genotypes in the hydroponic assay

Genotype	Tap RL (cm) for different Al concentration					% reduction*
	0 ppm	10 ppm	20 ppm	30 ppm	50 ppm	
IPA 7-10	20.1	17.0	16.1	15.0	13.7	31.8
T 7	24.2	23.0	18.6	16.9	15.8	34.8
67 B	25.5	23.2	20.8	17.6	16.1	36.9
MAL 13	18.0	16.1	13.1	12.2	11.4	37.1
GT 101E	20.4	19.9	17.1	13.9	12.6	38.0
UPAS 120	18.4	15.1	13.8	12.3	11.4	38.3
Asha	23.5	17.9	16.7	15.8	14.2	39.6
Amar	24.2	18.2	16.2	15.3	14.1	41.7
Ranchi Local	24.5	18.7	17.2	15.8	14.3	41.8
IPA 92	23.9	17.4	15.2	14.8	13.8	42.1
Azad	22.6	17.6	15.6	14.7	13.0	42.1
BDN 2	23.7	17.6	16.2	15.3	13.7	42.2
PI 397430	24.6	17.3	16.2	15.8	14.1	42.5
IPA 204	23.5	16.9	15.7	14.7	13.5	42.5
Narendra Arhar 1	20.3	16.3	14.5	12.7	11.7	42.6
IPA 234	24.7	18.7	16.2	15.8	14.0	43.2
PAU 881	19.6	14.5	13.2	12.1	11.1	43.3
AL 15	18.9	14.5	13.2	11.1	10.6	43.8
Pusa 992	20.5	17.8	15.8	13.5	11.5	43.9
IPA 6-1	24.6	17.7	15.6	14.8	13.7	44.1
BDN 1	24.5	16.6	15.7	14.7	13.6	44.5
MA 6	24.6	17.6	16.1	15.1	13.6	44.6
Kudrat 3	24.8	18.1	16.7	15.3	13.6	45.1
AL 201	18.5	17.4	13.8	11.1	10.1	45.6
Dholi Dwarf	18.7	12.6	11.4	10.8	9.8	47.3
MA 3	21.4	18.3	16.2	12.6	11.0	48.6
GT 100	24.5	17.9	15.3	13.7	12.4	49.2
Pusa 2002-2	16.6	11.5	09.4	08.4	08.0	51.7
BSMR 736	24.7	21.9	16.5	12.8	11.2	54.5
Sharad	25.5	22.7	16.9	14.1	11.5	54.9
Bahar	17.1	11.9	08.8	08.0	07.2	57.9
Pusa 9	16.6	09.6	08.7	05.7	05.4	67.5

LSD = 2.4 cm (P = 0.05) for Al concentration × genotype interaction. LSD (P = 0.05) for % reduction in tap RL was 10.1.
*The reduction in tap RL from 0 to 50 ppm Al concentration.

to eliminate the entrained moisture. The 30-day-old plants were dried at 80°C for 72 h to determine dry weight of roots and shoots. The experiment was conducted in a completely randomized design with factorial combinations and two replications. Data were recorded on four growth parameters namely root length (RL), shoot length (SL), root dry weight (RW) and shoot dry wt (SW). RL was measured from the base of the cotyledon to the tip of the roots for each seedling in each treatment. Data on RL (cm), shoot length (cm), and dry weights of root (g) and shoot (g) were collected from each replication and each treatment. Percentage response to aluminium treatments for these parameters was calculated according to the following equations (Gudu et al. 2001):

% response = [RL (Al treated plants) − RL (control plants)]/[RL (control plants)] × 100

Dry samples of root and shoot were ground and dissolved in a di-acid mixture (nitric acid and perchloric acid) in a 3 : 1 ratio (v/v). Aluminium contents (mg/g) of respective plant parts were estimated by Perkin-Elmer atomic absorption spectrophotometer (Model 5000; Perkin-Elmer, Shelton, CT, USA).

Sand assay (Experiment 2): Seeds were disinfected and germinated in the same manner as used for the hydroponic assay (experiment 1) and then seedlings were transferred to plastic pots (15 cm diameter) containing quartz acid washed sand (Mugai and Agony 1997). The aluminium (Al) treatment solutions were prepared as described in experiment 1. The irrigation solution was maintained at a pH 4.5 using 1 M HCl. The various aluminium treatment solutions (0, 10, 20, 30 and 50 ppm) were supplied to plants daily. The sand was washed with distilled water once each week during the entire experimental period. The experiment was laid out in a completely randomized design with factorial set up and two replications. Plants were harvested after 22 days of growth, and the sand was washed off the roots under tap water. The shoots were excised from the roots and both were rinsed in distilled water. The plant tops and roots were dried separately in a hot air oven at 80°C for 72 h and the dry matter yields were determined. The same growth parameters were taken as described in experiment 1.

These two experiments were conducted during the year 2008 in the Department of Plant Breeding and Genetics, College of Horticulture and Forestry, Central Agricultural University, Pasighat, India. The two experiments were repeated to show whether the two methods correlate well and are comparable over time and space.

Statistical analysis: Data were subjected to two-way analyses of variance to determine the significance of individual effects and genotype × Al treatment interactions. Least significant differences (LSD) were calculated at P = 0.05 for significant interactions. In the hydroponic assay, where multiple comparisons were made (32 genotypes at five aluminium concentrations), analysis was performed using SPSS 13.0 statistical software (SPSS Inc., Chicago, IL, USA) and LSD was calculated for significant interactions.

Results and Discussion

In the present investigation, no distinct visible symptoms were observed in the shoot of pigeonpea, but root growth was highly restricted. Injured roots did not branch normally and were shorter at higher levels of aluminium than the control plants in all genotypes.

Table 2: Root and shoot Al contents (mg/g) of four pigeonpea genotypes under three different aluminium concentrations in hydroponic assay

	Al content (root)			Al content (shoot)		
	Al concentration			Al concentration		
Genotype	0 ppm	20 ppm	50 ppm	0 ppm	20 ppm	50 ppm
IPA 7-10	0.10	2.84	3.65	0.040	0.142	0.189
T 7	0.09	3.10	3.92	0.041	0.145	0.189
Bahar	0.06	4.23	5.64	0.023	0.203	0.357
Pusa 9	0.06	4.20	5.32	0.023	0.202	0.358

LSD = 0.36 and 0.15 (P = 0.05) for Al concentration × genotype interaction for root and shoot Al content, respectively.

Genotype selection (hydroponic and sand assays)

There was a highly significant interaction between genotype and aluminium concentration (P = 0.001) in hydroponic assay (Table 1). Among the 32 pigeonpea genotypes screened for Al tolerance, 'IPA 7-10' and 'T 7' showed only 31.8% and 34.8% decrease in their RLs, respectively, compared to 'Bahar' (57.9%) and 'Pusa 9' (67.5%) from 0 to 50 ppm Al concentrations.

The reduction in root and shoot lengths and root and shoot dry matters in hydroponic assay was correlated (r = 0.64** – 0.86**) significantly (P < 0.01) with those in sand assay, indicating that both assays gave similar responses and allowed selection of genotypes differing markedly in Al tolerance for more detailed study. The degree of association was also highly significant and strong among the four parameters themselves within each assay ($r_{hydroponic}$ = 0.62** – 0.82**, r_{sand} = 0.71** – 0.86**), indicating that any one of the four could be used as a selection criterion in hydroponic or sand assay. Foy et al. (1993) also observed that genotypic correlation between shoot and root growth in soybean [*Glycine max* (L.) Merr] was good and both parameters could be used to assess aluminiumtolerance among genotypes.

Hydroponic and soil assays consistently discriminated between tolerant ('IPA 7-10' and 'T 7') and sensitive ('Bahar' and 'Pusa 9') genotypes of pigeonpea. The response of these four genotypes for RL reduction in hydroponic assay was a good predictor of shoot growth reduction in sand assay. Genotypes that had the largest dry matter reduction in the sand assay (data not presented) also had the largest RL reduction so that the in-sand responses of pigeonpea genotypes could reasonably be predicted from the hydroponic RL assay. A number of genotypes showed intermediate response for RL reduction (Table 1). Genotypes like '67 B', 'MAL 13' and 'GT 101E' and 'Sharad', 'BSMR 736' and 'Pusa 2002-2' skewed towards aluminium tolerance and sensitivity, respectively. Similar trend of response for RL reduction was observed at 30 ppm Al concentration. Even this concentration of aluminium was sufficient to discriminate between tolerant and sensitive pigeonpea genotypes as used in pea and other crops (Singh and Choudhary 2010).

Similar results and trends were obtained when the two experiments were repeated. Only slight variations were observed in the rating of genotypes within the tolerant ('IPA 7-10' and 'T 7') and sensitive ('Bahar' and 'Pusa 9') groups. The percentage reduction in the RL over 50 ppm Al concentration was comparable in both hydroponic (34.95–72.47%) and sand (33.54–64.21%) assays. The correlation among the four parameters for percentage reduction *within* hydroponic (0.47** – 0.98**) and sand (0.49** – 0.98**) assays was strong and significant. The degree of association *between* hydroponic and sand assays for percentage reduction in the four parameters was also highly significant (0.48** – 0.82**), indicating that both assays gave similar responses.

Aluminium concentration in the roots of both tolerant and sensitive genotypes was greater than that for the shoots (Table 2). Root aluminium contents were significantly lower for the tolerant genotypes ('IPA 7-10' and 'T 7') than for the sensitive genotypes ('Bahar' and 'Pusa 9') at both 20 and 50 ppm Al concentrations. This indicated that aluminium tolerance in these accessions of pigeonpea was a result of aluminium exclusion from the root. In addition, shoot aluminium content was also considerably lower for the tolerant genotypes than for the sensitive genotypes. This implied that aluminium tolerance *per se* in tolerant genotypes involved aluminium exclusion (Delhaize and Ryan 1995, Kochian 1995).

In conclusion, both hydroponic and sand assays consistently discriminated between tolerant and sensitive genotypes of pigeonpea. Any of the two methods could be used for screening pigeonpea genotypes as both methods provided similar results. The two methods correlated well and could be comparable over time and space. Hydroponic assay is the most commonly used screening technique for it provides precise measurement of tolerance. However, sand assay appears to be less expensive. Both root and shoot aluminium uptake was much lower for tolerant than sensitive genotypes, indicating that aluminium exclusion *per se* is controlling tolerance to aluminium toxicity in pigeonpea. Tolerant genotypes 'IPA 7-10' and 'T 7' will be used in future breeding programmes to develop aluminium-tolerant pigeonpea cultivars.

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