Screening of pigeonpea genotypes for nutrient uptake efficiency under aluminium toxicity

Arbind Kumar Choudhary · Dharmendra Singh

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Abstract For increasing pigeonpea production in India, it is necessary to expand its area in non-traditional areas such as north-eastern states, which have considerable area under acidic soils. In such soils, aluminium toxicity, which is a major yield limiting factor, interferes with nutrient uptake efficiency of crop plants. 32 genotypes of pigeonpea [*Cajanus cajan* (L.) Millsp.] were screened for tolerance to aluminium toxicity at four different aluminium concentrations (41, 82, 123 and 205 μM Al) by hematoxylin staining and root re-growth methods. The results of the two screening methods were consistent, suggesting that either of the two methods could be used for screening purpose. The most tolerant (IPA 7–10, T 7, 67 B and GT 101E) and sensitive (Bahar, Pusa 9 and Pusa 2002–2) genotypes were assessed for root and shoot aluminium contents in hydroponic assay at 0, 41, 82, 123 and 205 μM aluminium concentrations. Root and shoot aluminium contents were significantly lower in the tolerant than sensitive genotypes, indicating that aluminium tolerance mechanism involved aluminium exclusion and perhaps internal detoxification. Tolerant and sensitive genotypes were further assessed for phosphorus, potassium, calcium and magnesium contents in their root and shoot. Tolerant genotypes (IPA 7–10, T 7, 67 B and GT 101E) accumulated significantly high amounts of these nutrients (>1.5 times) compared to the sensitive ones. Better performance of tolerant genotypes could be ascribed

to better nutrient uptake efficiency and distribution within the plants.

Keywords Aluminium exclusion · Aluminium tolerance · Aluminium toxicity · Nutrient uptake · Pigeonpea

Introduction

Pigeonpea [*Cajanus cajan* (L.) Millspaugh] is a hardy, widely adapted and 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 (Saxena 2008). It is grown in Asia, Eastern and Southern Africa, Latin America and Caribbean countries. In India, it is cultivated on more than 4.0 million hectare area in almost all states. To meet the growing demand of pigeonpea, it is imperative to expand its cultivation on wider scale in non-traditional areas such as hilly tracts of north eastern states. There are other states like Bihar, Jharkhand and Chhattishgarh where increasing trend in pigeonpea cultivation has been observed in recent years. However, these states have considerable acreage under acidic soils with the serious problem of aluminium (Al) toxicity.

Aluminium toxicity (Al^{3+}) is a serious problem limiting crop productivity in acid soil which comprises large areas of the world (Kochian 1995) particularly in the tropics and subtropics (Foy et al. 1978). The problem is particularly serious in strongly acid subsurface soil horizons ($\text{pH} < 5.5$) that are difficult to lime. It can negatively influence growth of root and shoot, but many researchers have used root growth as the parameter to assess Al toxicity in crop plants (Kinraide et al. 1985). The major symptoms of Al toxicity is rapid inhibition of root elongation by destroying the root

A. K. Choudhary (✉)

Crop Improvement Division, Indian Institute of Pulses Research,
Kanpur 208 024 U.P., India
e-mail: akiipr23@yahoo.com

D. Singh

Department of Plant breeding and Genetics, College of
Horticulture and Forestry, Central Agricultural University,
Pasighat 791 102 Arunachal Pradesh, India

apex (Ryan et al. 1993) resulting in inefficient uptake of water and nutrients (Fageria 1985).

Al toxicity can be mitigated by liming and by using tolerant cultivars. Detoxification of aluminium by liming is possible in surface soil to a pH 5.5 or above in the field. However, liming does not remedy sub-soil acidity and it may not always be practical or cost effective (Tesfaye et al. 2001). Therefore, the use of tolerant cultivars may be a satisfactory solution to this problem. However, breeding for aluminium tolerance requires a rapid and effective technique to discriminate between tolerant and sensitive genotypes. The two most commonly used techniques are hematoxylin staining and root re-growth assay (Singh and Choudhary 2010). These two techniques have unconditional advantages over field screening because reliable ranking of tolerance in the field screening is difficult due to the large temporal and spatial variation in acidic soils. Moreover, screening at field level is very expensive and time consuming when a large number of genotypes are under evaluation (Garcia et al. 1979). Besides, the results obtained with solution culture screening method correlate positively with those obtained using field screening (Urrea-Gomez et al. 1996), indicating reliability and dependability of laboratory screening methods.

Most of the grain legumes including chickpea (Singh and Chaturvedi 2007), pigeonpea (Singh and Choudhary 2009), pea (Singh and Choudhary 2010) and alfalfa (Campbell et al. 1988) are sensitive to aluminium. Considerable variation for tolerance to aluminium toxicity in plant species and genotypes within species has been reported (Kinraide et al. 1985; Singh and Choudhary 2009). The work on screening for tolerance to Al toxicity in pigeonpea is only a few and also not well-documented. It is also not known whether aluminium tolerance of pigeonpea genotypes stems from their better nutrient uptake ability. This paper reports the results of screening and selection for tolerance to Al toxicity in pigeonpea and describes the effects of high aluminium concentrations on nutrient uptake of selected genotypes.

Materials and methods

Plant materials

All the 32 pigeonpea genotypes (Table 1), which were used in the present study, are available and maintained at the Indian Institute of Pulses Research Research, Kanpur (Uttar Pradesh), India. These genotypes belong to three distinct maturity groups (early, medium and late) and have originated at different places in India. Some of these genotypes, viz., Bahar, Pusa 9, T 7 and Narendra Arhar 1 (late group), Asha, BSMR 736, BDN 2 and ICP 8863

(medium group), GT 100, GT 101E, UPAS 120 and Pusa 992 (early group) are released cultivars and cultivated widely in the area of their adoption.

Hematoxylin assay (Experiment I)

The staining protocol was assayed as per Polle et al. (1978) with minor modifications for visual detection of aluminium in the roots. Seeds were disinfected with 1 % sodium hypochlorite and then germinated in filter paper. After 8 days, seedlings were transferred to plastic containers in nutrient solution (4.0 mM CaCl_2 , 6.5 mM KNO_3 , 2.5 mM MgCl_2 , 0.1 mM $(\text{NH}_4)_2\text{SO}_4$, 0.4 mM NH_4NO_3) that was adjusted to pH 4.5 with 1 M HCl solution. Seedlings were kept in above nutrient solution for 2 days under continuous light and aeration. The seedlings were then grown for 24 h on the fresh nutrient solution containing 41, 82, 123 and 205 μM aluminium concentrations. The roots of seedlings were then placed in aerated distilled water for 60 min to remove aluminium on the root surface. The stain solution consisted of 2 g/l hematoxylin and 0.2 g/l KIO_3 which was prepared in distilled water. The roots of seedlings were immersed in the hematoxylin stain for 30 min after which the seedlings were placed in flowing distilled water for 30 min thrice. Each seedling was visually scored for stain intensity of the root tips. Seedlings were tested in completely randomized design with two replications. Six seedlings per genotypes per replication were visually scored as none (0) or partial (≤ 1), moderate (≤ 2) and completely (≤ 3) stained. The zero and partially stained (≤ 1.0) seedlings were classified as *tolerant*, seedlings taking moderate stain as moderately tolerant and those deeply stained as *sensitive*.

Root re-growth assay (Experiment II)

The protocol of this procedure was given by Nava et al. (2006). Seeds were disinfected and germinated in the same manner as used for the hematoxylin assay in experiment I. The seedlings were transferred to a plastic container in nutrient solution without aluminium for 48 h. The seedlings were then transferred to a solution with 41, 82, 123 and 205 μM aluminium concentrations for another 48 h. Finally, seedlings were transferred to the solution free of aluminium for 72 h further. Root growth was reinitiated after removal from the aluminium solution and root re-growth of the primary root of each seedling was measured starting from the point of root thickening (callosity). The response of each genotype was determined as re-growth of the primary root after exposition to Al^{3+} . The seedlings were evaluated in a complete randomized design with two replications. Data from the two replicates were combined to generate mean primary root re-growth for each genotype. Each replicate consisted of a sample of 6 seedlings and the

Table 1 Hematoxylin stain score and root re-growth of pigeonpea genotypes

Genotype ^a	Hematoxylin stain score		Genotype ^a	Root re-growth (cm)	
	123 μ M Al concentration	Mean of all Al concentration		123 μ M Al concentration	Mean of all Al concentration
IPA 7-10	1.00	0.68	IPA 7-10	2.05	2.17
T 7	1.00	0.69	67 B	1.65	1.78
67 B	1.00	0.84	GT 101E	1.59	1.74
GT 101E	1.00	0.88	T 7	1.53	1.71
Ranchi Local	1.55	1.59	AL 15	0.78	0.88
BDN 2	1.60	1.68	PAU 881	0.68	0.82
Pusa 992	1.65	1.69	Kudrat 3	0.67	0.80
PAU 881	1.75	1.75	PI 397430	0.60	0.76
AL 201	1.75	1.75	Pusa 992	0.59	0.73
Sharad	1.85	1.85	AL 201	0.55	0.70
Dholi Dwarf	1.85	1.88	BDN 1	0.51	0.66
GT 100	1.85	1.88	BDN 2	0.59	0.65
IPA 234	1.85	1.89	Ranchi Local	0.58	0.63
Asha	1.90	1.90	UPAS 120	0.51	0.63
MA 3	1.90	1.93	IPA 204	0.43	0.62
AL 15	1.90	1.94	IPA 234	0.48	0.60
MAL 13	1.90	1.94	Dholi Dwarf	0.43	0.59
Narendra Arhar 1	1.90	1.94	Narendra Arhar 1	0.38	0.57
Azad	1.95	1.94	BSMR 736	0.35	0.57
Amar	1.95	1.94	IPA 06-1	0.41	0.56
BSMR 736	1.95	1.95	MA 6	0.40	0.56
IPA 06-1	1.95	1.95	IPA 92	0.42	0.55
IPA 204	2.00	1.96	GT100	0.40	0.55
Kudrat 3	2.05	1.96	Asha	0.45	0.54
IPA 92	2.05	1.98	MAL 13	0.38	0.54
BDN 1	2.10	1.98	MA 3	0.40	0.53
UPAS 120	2.10	1.99	Sharad	0.38	0.53
MA 6	2.15	1.99	Azad	0.36	0.52
PI 397430	2.20	1.99	Amar	0.38	0.52
Pusa 9	2.85	2.55	Pusa 9	0.25	0.40
Pusa 2002-2	3.00	2.67	Pusa 2002-2	0.18	0.39
Bahar	3.00	2.69	Bahar	0.13	0.34

^a Genotypes are arranged in ascending and descending order of mean hematoxylin score and root re-growth, respectively; CD ($P=0.05$) values for Al concentration $\times$ genotype interaction are 0.07 and 0.06 in hematoxylin and root re-growth assay, respectively

average root re-growth of each sample was used as the replicate value.

Hydroponic assay (Experiment III)

Seeds were disinfected and germinated in the same manner as used for the hematoxylin assay in experiment I. After 8 days, the seedlings were transferred to dilute nutrient solution: KNO_3 (0.5 mM), $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (0.5 mM), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.2 mM), KH_2PO_4 (0.1 mM), KCl (50 μM), H_3BO_3 (46 μM), Fe-EDTA (20 μM), $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (2 μM),

$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (1 μM), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.3 μM) and $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$ (0.5 μM) (Simon et al. 1994) having 0 (control), 41, 82, 123 and 205 μM aluminium concentrations. The aluminium treatments were supplied as $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$. 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 uniform (8 days old) plants

of each genotype were grown in duplicate trays (2 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 for 20 s in distilled water to remove surface contamination followed by blotting to eliminate the entrained moisture. The 30 days 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 two growth parameters namely, root dry wt (RW) and shoot dry wt (SW). Data on dry weights of root (g) and shoot (g) were collected from each treatment in each replication.

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). Calcium, magnesium and aluminium contents of respective plant parts were estimated by Perkin-Elmer atomic absorption spectrophotometer (Model 5000, Perkin-Elmer, Shelton, CT-USA). Phosphorus and potassium contents were determined by spectrophotometer and flame photometer, respectively. Phosphorus, potassium, calcium, magnesium and aluminium contents were expressed in percentage of total dry matter (%) and mg/g respectively.

These three 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.

Statistical analysis

Data were subjected to two-way analyses of variance to determine the significance of individual effects and genotype $\times$ Al treatment interactions. Least significant differences (LSD) were calculated for significant interactions ($P=0.05$). In the hydroponic assay, where multiple comparisons were made (32 genotypes at 5 aluminium concentrations), analysis was performed using SPSS software and LSD was calculated for significant interactions.

Results

Screening and selection of genotypes

Root staining techniques have shown that aluminium accumulates principally in the root tips of the main root and lateral root tissue. Hematoxylin staining analysis was conducted on the 32 pigeonpea genotypes grown at each of the four Al concentrations (41, 82, 123 and 205 μM aluminium). The root tips exhibited the greatest degree of staining. The root tips of control plants showed no stain (data not shown). Significant genotype $\times$ Al concentration interaction ($p<0.05$) was observed for stain intensity of

genotypes. The stain score ranged from 0.0 (none) to 3.0 (complete stain). Significant differences among genotypes were observed for stain scores. The variation in the mean hematoxylin score (over 4 levels of aluminium) from partial (0.68) to complete stain (2.69) was observed (Table 1). Based on mean stain score at 123 μM Al, IPA 7–10, T 7, 67 B and GT 101E and Bahar, Pusa 2002–2 and Pusa 9 were classified as tolerant and sensitive, respectively to Al toxicity.

To confirm the screening results, root re-growth assay was also conducted on all the 32 genotypes. There was significant genotype $\times$ Al concentration interaction ($p<0.05$) for root re-growth. Root re-growth of all genotypes decreased significantly with an increase in aluminium concentration in nutrient solution (data not presented). Four genotypes namely, IPA 7–10, T 7, 67 B and GT 101E had larger mean root re-growth (>1.5 cm) and thus were classified as tolerant to Al toxicity (Table 1). Tolerant genotypes had partial hematoxylin stain scores (≤ 1.0) and large root re-growth (>1.5 cm) at 123 μM aluminium concentration and this level of Al concentration (123 μM) was sufficient to discriminate between tolerant and sensitive genotypes. All the 32 genotypes could obviously be classified into three discrete groups viz., tolerant, sensitive and intermediate (Table 2) although variation within the group was also present.

Hydroponic assay: symptoms of Al toxicity

In the present experiment, no distinct visible symptoms were observed in the shoot of pigeonpea genotypes but symptoms of Al toxicity were evident in the root. The root was affected more than the shoot. The primary effect of Al toxicity was the restriction of root growth. Injured roots did not branch normally and were shorter at higher levels of aluminium (123 or 205 μM Al) than the roots grown in nutrient solution without aluminium (control).

Hydroponic assay: aluminium contents in extreme genotypes

Based on the two methods of screening, four tolerant (IPA 7–10, T 7, 67 B and GT 101E) and three sensitive (Bahar, Pusa 2002–2 and Pusa 9) genotypes were identified. Root aluminium contents (mg/g) of these genotypes were estimated at four aluminium levels (0, 41, 123 and 205 μM) in hydroponic assay. There was a highly significant interaction ($P<0.01$) between genotype and root aluminium concentration. Root aluminium content (mg/g) was greater than that of shoot (Table 3). Root aluminium contents of tolerant genotypes (IPA 7–10, T 7, 67 B and GT 101E) were significantly lower than those of sensitive genotypes (Bahar, Pusa 2002–2 and Pusa 9) at 41, 123

Table 2 Grouping of pigeonpea genotypes based on hematoxylin stain intensity and root re-growth assay over all the four (41, 82, 123 and 205 μM) Al concentrations

Hematoxylin stain intensity	Root re-growth (cm)	Genotypes	Al toxicity reaction
Partial (≤ 1.0)	Large (>1.5)	IPA 7–10, T 7, 67 B and GT 101E	Tolerant
Moderate (1.0–2.0)	Medium (0.5–1.5)	MAL 13, UPAS 120, Asha, Amar, Ranchi Local, IPA 92, Azad, BDN 2, PI 397430, IPA 204, Narendra Arhar 1, IPA 234, PAU 881, AL 15, Pusa 992, IPA 06–1, BDN 1, MA 6, Kudrat 3, AL 201, Dholi Dwarf, MA 3, GT 100, BSMR 736 and Sharad	Intermediate reaction
Complete (>2.0)	Small (<0.5)	Bahar, Pusa 2002–2 and Pusa 9	Sensitive

and 205 μM aluminium concentrations. The increase in shoot aluminium contents in tolerant genotypes at both levels (123 and 205 μM) compared to the control (0 μM) was non-significant. However, it (shoot Al content) increased more than 9 and 15 times at 123 and 205 μM aluminium concentrations, respectively for sensitive genotypes (Bahar, Pusa 2002–2 and Pusa 9). Both root and shoot aluminium uptake was significantly lower in tolerant genotypes (IPA 7–10, T 7, 67 B and GT 101E) than the sensitive ones (Bahar, Pusa 2002–2 and Pusa 9) at both 123 and 205 μM Al concentrations. In fact, percentage reduction (Table 4) in the root dry matter and total dry matter (root + shoot) of tolerant genotypes at 205 μM (over 0 μM) Al concentration was comparatively less (38–44 and 28–35 %, respectively) than the sensitive ones (67–72 and 56–64 %, respectively).

Hydroponic assay: nutrient uptake behaviour

There was a highly significant interaction ($P<0.01$) between genotype and Al concentration for nutrient uptake behaviour (P, K, Ca and Mg) in root and shoot. Highly significant correlation ($r=0.93^{**}$ – 0.97^{**} , $p<0.001$) was noticed between root and shoot for percentage reduction in the uptake of all the four nutrients (P, K, Ca and Mg) at 205 μM Al concentration over control (0 μM Al concentration). However, the degree of association for % reduction

in the uptake of these nutrients in the root was slightly low, although significant ($r=0.78^{*}$ – 0.96^{**}). Similar association trend was also observed in the shoot ($r=0.73^{*}$ – 0.96^{**}). In general, there was a gradual reduction in the uptake of these four nutrients with the increase in Al concentration from 0 μM to 205 μM in all the genotypes (Tables 5 and 6). However, conspicuous difference was observed for percentage reduction between tolerant and sensitive genotypes. The reduction in phosphorus uptake was higher in shoots than roots at 205 μM Al level. The percentage reduction varied from 32.93 % (IPA 7–10) to 62.35 % (Pusa 2002–2) and from 41.13 % (67 B) to 63.89 % (Pusa 2002–2) in the root and shoot. Similar trend was observed for potassium uptake excepting highest reduction (55.59 %) recorded in the shoot of Pusa 9. Tolerant genotypes (IPA 7–10, T 7, 67 B and GT 101E) had higher concentrations (>1.75 times) of P and K in their roots than the sensitive genotypes (Bahar, Pusa 2002–2 and Pusa 9) at 205 μM Al concentration. The percentage reduction in uptake of both the nutrients at 205 μM compared to the control was also high (>1.6 times) in Bahar, Pusa 2002–2 and Pusa 9. Similar trends were observed for Ca and Mg concentrations in the shoot of tolerant and sensitive genotypes (Table 6). However, the magnitude of percentage reduction at 205 μM in both tolerant and sensitive genotypes was comparatively low for Ca uptake. Pusa 9 was the worst affected sensitive genotype for Mg uptake in both root and shoot (Table 6).

Table 3 Root and shoot Al uptake (mg/g) of five pigeonpea genotypes under four different aluminium concentrations in hydroponic assay

Genotype	Al uptake (root)				Al uptake (shoot)			
	Al concentration				Al concentration			
	0 μM	41 μM	123 μM	205 μM	0 μM	41 μM	123 μM	205 μM
IPA 7-10	0.095	1.926	3.142	3.650	0.040	0.116	0.170	0.189
T 7	0.090	2.049	3.431	3.918	0.041	0.117	0.170	0.189
67 B	0.091	2.080	3.457	3.906	0.041	0.118	0.173	0.191
Bahar	0.058	3.002	5.030	5.640	0.023	0.145	0.249	0.357
Pusa 9	0.060	2.981	4.964	5.316	0.023	0.146	0.248	0.358

CD ($P=0.05$)=0.36 and 0.10 for Al concentration $\times$ genotype interaction for root and shoot Al content, respectively

Table 4 Effects of three aluminum concentrations on total dry matter (g plant⁻¹) of five pigeonpea genotypes in hydroponic assay

Genotype	Root dry matter				Total dry matter (shoot + root)			
	Al concentration				Al concentration			
	0 μ M	123 μ M	205 μ M	% reduction ^a	0 μ M	123 μ M	205 μ M	% reduction ^a
IPA 7-10	0.114	0.099	0.070	38.59	0.422	0.344	0.303	28.19
T 7	0.111	0.083	0.060	44.44	0.404	0.311	0.266	35.18
67 B	0.113	0.089	0.068	39.82	0.412	0.322	0.286	30.58
Bahar	0.099	0.041	0.033	67.44	0.311	0.181	0.143	56.27
Pusa 9	0.081	0.044	0.022	72.83	0.314	0.166	0.112	64.33

CD ($P=0.05$)=0.01 g and 0.04 g for Al concentration $\times$ genotype interaction for root and total dry matter, respectively

^a represents the reduction in total dry matter from 0 to 205 μ M Al concentration

Discussion

The ranking of pigeonpea genotypes on the basis of mean hematoxylin stain score over all the 4 concentrations of and at 123 μ M of Al was almost similar. This implied that 123 μ M of Al concentration could be used to discriminate tolerant (IPA 7–10, T 7, 67 B and GT 101E) and sensitive (Bahar, Pusa 2002–2 and Pusa 9) genotypes of pigeonpea by hematoxylin stain technique. Many researchers have recommended 123 μ M Al concentration as the optimum level to distinguish between tolerant and sensitive genotypes (Singh and Chaturvedi 2007; Singh et al. 2009). Similar results were noticed when genotypes were rated through root re-growth assay. Root re-growth virtually ceased in Bahar, Pusa 2002–2 and Pusa 9 at higher Al concentrations (123 or 205 μ M Al) due to irreversible damage caused to the root tips. For efficient discrimination among genotypes against Al toxicity through root re-

growth assay, 123 μ M Al level was found optimum as has also been observed in other crops (Echart et al. 2002; Nava et al. 2006; Singh and Choudhary 2010).

The variable response of pigeonpea genotypes to Al toxicity was most likely due to difference in genetic potential of pigeonpea genotypes. Hematoxylin staining and root re-growth assays consistently discriminated between tolerant (IPA 7–10, T 7, 67 B and GT 101E) and sensitive (Bahar, Pusa 2002–2 and Pusa 9) genotypes of pigeonpea. The response of these four tolerant genotypes for hematoxylin stain score was a good predictor of root re-growth under Al toxicity. Genotypes that had the partial hematoxylin stain score had the largest root re-growth when relieved from stress of Al toxicity and vice versa so that the *in-hematoxylin* responses of pigeonpea genotypes could reasonably be predicted from the root re-growth assay.

Ryan et al. (1993), based on their observation on root and total dry matter, proposed that root is the primary site

Table 5 Phosphorus and potassium concentrations (% dry matter) in the root of seven pigeonpea genotypes under four different aluminium concentrations in hydroponic assay

Genotype	P uptake					K uptake				
	Al concentration					Al concentration				
	0 μ M	41 μ M	123 μ M	205 μ M	% reduction ^a	0 μ M	41 μ M	123 μ M	205 μ M	% reduction ^a
IPA 7-10	4.19	3.82	3.42	2.81	32.93	3.08	2.86	2.10	2.05	33.50
T 7	3.50	3.12	2.29	2.18	37.71	3.11	2.82	2.11	1.91	38.58
67 B	3.65	3.15	2.38	2.25	38.36	3.27	3.08	2.15	1.93	40.98
GT 101E	3.52	3.14	2.70	2.15	38.92	3.06	2.74	1.99	1.87	39.54
Bahar	2.71	1.92	1.33	1.23	54.61	2.76	2.14	1.45	1.17	57.79
Pusa 9	2.56	2.23	1.71	1.16	54.68	2.60	2.19	1.38	1.10	57.69
Pusa 2002-2	3.40	2.96	1.60	1.28	62.35	2.68	2.22	1.59	1.15	57.08

^a represents the reduction in total dry matter from 0 to 205 μ M Al concentration; CD ($P=0.05$)=0.18 and 0.13 for Al concentration $\times$ genotype interaction for P and K uptake, respectively

Table 6 Calcium and magnesium concentrations (% dry matter) in the shoot of seven pigeonpea genotypes under four different aluminium concentrations in hydroponic assay

Genotype	Ca uptake					Mg uptake				
	Al concentration					Al concentration				
	0 μ M	41 μ M	123 μ M	205 μ M	% reduction ^a	0 μ M	41 μ M	123 μ M	205 μ M	% reduction ^a
IPA 7-10	2.81	3.05	2.30	2.03	27.75	3.36	3.08	2.38	2.27	32.44
T 7	2.99	2.90	2.55	2.18	27.09	3.20	2.94	2.33	2.20	31.25
67 B	2.86	2.73	2.30	1.93	32.51	3.20	2.77	2.19	2.08	35.00
GT 101E	3.08	2.73	2.37	1.99	35.38	3.08	2.88	2.63	1.92	37.66
Bahar	2.46	2.38	1.85	1.40	43.08	2.83	2.56	1.99	1.40	50.53
Pusa 9	2.28	2.26	1.85	1.35	40.78	3.74	2.53	2.02	1.52	59.35
Pusa 2002-2	2.53	2.28	1.87	1.51	40.31	2.86	2.49	1.70	1.39	51.39

^a represents the reduction in total dry matter from 0 to 205 μ M Al concentration; CD ($P=0.05$)=0.12 for Al concentration $\times$ genotype interaction for both Ca and Mg uptake

of aluminium toxicity. In the present experiment, the reduction in root dry matter was greater than total dry matter reduction in both tolerant and sensitive pigeonpea genotypes, also indicating root as the primary site of aluminum toxicity. It is interesting to note that out of the four tolerant genotypes, three (IPA 7–10, T 7 and GT 101E) have indeterminate growth habit and two (67 B and GT 101E) are early maturing. Besides IPA 7–10 and T 7, 67 B and GT 101E belong to Northeast plain zone (NEPZ), South zone (SZ) and Central zone (CZ), respectively. This indicates that tolerance to Al toxicity in pigeonpea has no correlation with growth habit, maturity period and geographical origin.

Aluminium concentration in the roots of both tolerant and sensitive genotypes was greater than that for the shoots. Root aluminium contents were significantly lower for the tolerant genotypes (IPA 7–10, T 7, 67 B and GT 101E) than for the sensitive genotypes (Bahar, Pusa 2002–2 and Pusa 9) at both 123 and 205 μ M Al concentrations in hydroponic assay. It is, therefore, reasonable to assume that the aluminium tolerance in the tolerant accessions of pigeonpea stemmed partly from aluminium exclusion from the root (Choudhary et al. 2011). No evidence of other mechanisms operating at the root level in pigeonpea was observed. Shoot aluminium content was also considerably lower for the tolerant genotypes (IPA 7–10, T 7, 67 B and GT 101E) than for the sensitive genotypes (Bahar, Pusa 2002–2 and Pusa 9). However, no indication of internal detoxification (Ma et al. 2001) was observed. Therefore, aluminium tolerance per se in tolerant genotypes of pigeonpea involved aluminium exclusion (Delhaize and Ryan 1995; Kochian 1995).

Aluminium is known to negatively affect nutrient uptake by the crop plants. Tolerant (IPA 7–10, T 7, 67 B and GT 101E) and sensitive genotypes (Bahar, Pusa 2002–2 and

Pusa 9) gave differential root and shoot responses to phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) uptake at four aluminium concentrations. Significant reductions in the uptake of P and K (root) and of Ca and Mg (shoot) were noticed for both tolerant and sensitive genotypes. However, the extent of reduction was significantly low for tolerant genotypes compared to sensitive genotypes. Tolerant genotypes significantly contained comparatively high percentages of phosphorus, potassium, calcium and magnesium at both 123 and 205 μ M Al concentrations. Better performance of tolerant genotypes could be ascribed to better regulation of ionic uptake and their distribution within the plants under different aluminium concentrations. Such nutrients have been reported key nutrients involved in aluminium toxicity and genotypes with the ability to maintain a less inhibited uptake of these four nutrients have been found tolerant. Tolerant genotypes (IPA 7–10, T 7, 67 B and GT 101E) well accumulated high amounts of these nutrients in the given period compared to sensitive ones (Bahar, Pusa 2002–2 and Pusa 9). Decrease of plant uptake of phosphorus in different plant species due to excess aluminium in nutrient solution has been reported by Pavan and Bingham (1982) and Lidon et al. (2007). The decrease in K uptake due to the presence of excess Al in the medium was noticed in rice by Sarkunan and Bidappa (1982). However, aluminium has been reported to increase the uptake of K in tea plants (Konishi et al. 1985). The inhibitory influence in Ca uptake was also found in soybean (Sartain and Kamprath 1978). Concentration of calcium and magnesium in shoot were found to be higher than that of root thereby indicating the higher translocation to shoot and less retention in roots as compared to either element Al, P or K. Pintro et al. (1996) also recorded similar observations. The observed reduction in Ca and Mg of both root and shoot with respect to increased Al concentration of nutrient solution could be attributed to the competition of

aluminium with these elements for common binding sites at or near the root surface and subsequent reduction in uptake of these elements. Genotypes tolerant to aluminium toxicity indeed absorbed relatively high amounts of four nutrients compared to the sensitive genotypes and this might have contributed to better growth of plants with consequent high biomass production. Baligar et al. (1995), while evaluating toxic effect of aluminium on the mineral nutrition of plant species, found that genotypes showing resistance to aluminium also had shown a less impaired nutrient uptake than sensitive genotypes in sorghum.

In conclusion, both hematoxylin staining and root re-growth assays consistently discriminated between tolerant and sensitive genotypes of pigeonpea. Both root and shoot aluminium uptake was much lower for tolerant than sensitive genotypes, indicating that both aluminium exclusion and perhaps internal detoxification impart tolerance to aluminium toxicity in pigeonpea. Tolerant genotypes such as IPA 7–10, T 7, 67 B and GT 101E will be used in future breeding programme to develop aluminium tolerant pigeonpea cultivars. However, further study involving land races and wild accessions (especially from *Cajanus scarabaeoides* and *C. platycarpus*) of pigeonpea for tolerance to aluminium toxicity under field condition (natural acid soil) is still required. This may generate comprehensive data for even higher degree of Al tolerance vis-à-vis reproductive parameters such as yield. This will also corroborate whether tolerance to Al toxicity in pigeonpea imparts only survival advantage or also confers increased reproductive fitness on the tolerant genotypes.

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