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# A comparative study of screening methods for tolerance to aluminum toxicity in pigeonpea [*Cajanus cajan* (L.) Millspaugh]

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**Abstract**

Aluminum toxicity is a major factor limiting pigeonpea productivity in acid soils of North-eastern states of India. However, information regarding screening of genotypes for aluminum tolerance in pigeonpea is meagre. The effects of five levels of aluminum concentrations (0, 10, 20, 30 and 50 µg $ml^{-1}$ Al) on 32 pigeonpea genotypes were studied by four different methods: hydroponic and sand assays (growth response methods), root re-growth and hematoxylin root staining. Significant variability was noted for tolerance to aluminum toxicity among the pigeonpea genotypes. The results of all the four screening methods were consistent, suggesting that any one of the four methods could be used for screening purpose. However, due to operative simplicity, reliable and better precision and short test period, the hematoxylin staining at 30 µg $ml^{-1}$ aluminum concentration was suggested as the best method to discriminate pigeonpea genotypes for aluminum tolerance. Based on the results, most tolerant (IPA 7-10, T 7and 67 B) and most sensitive (Bahar, Pusa 9 and Pusa 2002-2) genotypes were identified for future use in breeding for aluminum tolerance in pigeonpea.

**Keywords:** *Cajanus cajan,* aluminum tolerance, aluminum toxicity, pigeonpea, hematoxylin staining, screening methods.
**Abbreviations:** Al- Aluminum; IIPR- Indian Institute of Pulses Research; LSD- Least significant differences.

## Introduction

Aluminum (Al) toxicity is a well known limitation to crop production in 30% of arable lands (Campbell et al., 1988). The problem is particularly serious in strongly acid subsurface soil horizons (pH<5.5) that are difficult to lime. Aluminum toxicity (Clarkson, 1967; Delhaize and Ryan, 1995) negatively affects growth of both root and shoot. However, many researchers have considered root as the primary growth parameter to assess aluminum toxicity in crop plants (Foy et al., 1978; Kinraide et al., 1985). The root growth reduction may stem from restricted absorption of water and nutrients and ultimately causes yield reduction in such problem soils. There are two most common ways to mitigate Al toxicity: liming and use of tolerant cultivars. Detoxification of Al by liming is possible in surface soil in the field 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. Use of tolerant genotypes and breeding of crops for aluminum tolerance is a reliable approach to enhance production on acidic soils. This requires a rapid and effective technique to discriminate between tolerant and sensitive genotypes. There are several screening methods for aluminum tolerance such as solution, sand and soil cultures, root re-growth and hematoxylin staining techniques, and field screening. However, reliable ranking of tolerance in the field screening is difficult because of the 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). Selection of seedlings in hydroponic assay has been used as a rapid screening method to screen for aluminum tolerance in several crops (Fageria and Carvalho, 1982; Fageria, 1985). Hematoxylin staining and root re-growth techniques are also frequently used techniques because they have produce consistent results (Reid et al., 1971; Luo and Dvorak, 1996). In addition, the results obtained with solution culture screening method correlate positively with those obtained using field screening (Urrea-Gomez et al., 1996), showing that this method could be representative of what happens in the field. Hydroponic assay including hematoxylin staining has been recommended to identify aluminum tolerant genotypes in several crops (Singh et al., 2009; Singh and Choudhary, 2010; Choudhary and Singh, 2011). The reaction of hematoxylin with aluminum–stressed roots has been used by several researchers in different crop species such as wheat (Polle et al., 1978; Carver et al., 1988; Rincon and Gonzales, 1992; Tice et al., 1992; Carver and Ownby, 1995), soybean (Sartain and Kamprath, 1978), maize (Cancado, 1999; Lidon et al., 2007), pea (Singh et al., 2009; Singh and Choudhary, 2010), chickpea (Singh and Chaturvedi, 2007), and the like.

Pigeonpea [*Cajanus cajan* (L.) Millspaugh] is a short-lived perennial shrub that is cultivated in a wide range of environments and cropping systems (Saxena, 2008). Globally, pigeonpea is cultivated on 4.92 million hectares (Mha) with an annual production of 3.65 million tons and productivity of 898 kg $ha^{-1}$ (http://faostat.fao.org/). India accounts for about 75% of the world acreage under

pigeonpea. It is grown in almost all the states including north-eastern states, Jharkhand, Chhatishgarh and M.P., which have large acreage under acid soils with the problem of Al toxicity. There are no specific varieties for cultivation in these problem areas. Available literature on Al toxicity is only a few and also not well documented. The present paper provides a comparative analysis of four different methods vis-à-vis screening of pigeonpea lines for Al tolerance.

**Results and discussion**

No distinct and visible symptoms of aluminum toxicity were observed in the shoot of pigeonpea genotypes. However, the symptoms were evident in the root. The primary effect of aluminum toxicity was the restriction of root growth. Shorter roots with absence of normal branching pattern were observed at higher levels of aluminum (30 and 50 µg $ml^{-1}$ Al) compared to the control treatment (0 µg $ml^{-1}$ Al).

***Screening of Genotypes in Sand and Hydroponic assays***

Growths of pigeonpea genotypes as expressed by root and shoot lengths and dry weights of root and shoot were significantly reduced with increasing level of Al concentrations in both sand and hydroponic assays. There was a sharp and progressive decline in growth parameters with increasing levels of aluminum toxicity from 0 to 50 µg $ml^{-1}$ in sand assay but the degree of reduction in growth parameters varied among the genotypes. The effect was more prominent on root growth than the shoot growth. There was a highly significant interaction between genotype and aluminum concentration ($P = 0.001$) in sand assay. In the control treatment (0 µg $ml^{-1}$ Al concentration), Pusa 9 and Bahar had significantly shorter tap roots than those of T 7 and IPA 7-10 (Table 2). Among the thirty-two pigeonpea genotypes screened for Al tolerance, IPA 7-10 and T 7 showed only 32.40% and 36.25% decrease in their root lengths, respectively compared to Bahar (63.04%) and Pusa 9 (66.66%) from 0 to 50 µg $ml^{-1}$ Al concentrations. The percentage root length reduction in the Pusa 9 was significantly greater than all other genotypes except Bahar and Pusa 2002-2. The correlation between hydroponic and sand assays was determined for all the four parameters (Table 3). The reduction in root and shoot lengths and root and shoot dry matters in hydroponic assay was correlated significantly ($P < 0.01$) with those in sand assay, indicating that both assays gave similar responses (Fig. 1) and allowed selection of genotypes differing markedly in Al tolerance for more detailed study. The same degree of association was also observed among the four parameters themselves within each assay, indicating that any one of the four could be used as a selection criterion in sand or hydroponic assay. In the sand assay, there was a significant interaction between genotype and sand Al for root dry matter ($P < 0.01$) and total (root + shoot) dry matter ($P < 0.03$). Following exposure to 50 µg $ml^{-1}$ Al concentration, root dry matter reduction was more than the total dry matter. The total dry matter reduced by only 28 – 35% in IPA 7-10 and T 7, but by 56 – 64% in Bahar and Pusa 9 (Table 4).

***Hematoxylin Staining and Root Re-growth Studies***

Root staining techniques have shown that aluminum accumulates principally in the root tips of the main root and lateral root tissue. Hematoxylin staining and root re-growth analyses were conducted on the pigeonpea genotypes grown at each of the four Al concentrations (10, 20, 30 and 50 µg $ml^{-1}$ aluminum). The root tips exhibited the greatest degree of staining. The root tips of control plants showed no stain (data not shown). The stain score ranged from 0.0 (none) to 3.0 (complete stain). Variation in the mean hematoxylin score (over four concentrations of aluminum) from partial ($\leq 1.0$) to complete stain (3.0) was observed. Based on mean stain score (or stain score at 30 µg $ml^{-1}$ Al), IPA 7-10, T 7, 67 B and GT 101 and Bahar, Pusa 2002-2 and Pusa 9 were classified as tolerant and sensitive, respectively to aluminum toxicity. Root re-growth of all genotypes decreased significantly with an increase in aluminum concentration in nutrient solution (data not presented). Root re-growth virtually ceased in Bahar, Pusa 2002-2 and Pusa 9 at higher Al concentrations (30 or 50 µg $ml^{-1}$ Al) due to irreversible damage caused to the root tips. Four genotypes namely, IPA 7-10, T 7, GT 101 and 67 B had larger mean root re-growth ($> 1.5$ cm) and thus were classified as tolerant to aluminum toxicity. Tolerant genotypes had partial hematoxylin stain scores ($\leq 1.0$) and large root re-growth ($> 1.5$ cm) at 30 µg $ml^{-1}$ aluminum concentration (Fig. 2) and this level of Al concentration (30 µg $ml^{-1}$) was sufficient to discriminate between tolerant and sensitive genotypes.

***Aluminum Contents in Tolerant and Sensitive Genotypes***

Based on the four different methods of screening, four tolerant (IPA 7-10, T 7, GT 101 and 67 B) and three sensitive (Bahar, Pusa 2002-2 and Pusa 9) genotypes were identified. Root aluminum contents (mg $g^{-1}$) of five such genotypes were estimated at four aluminum levels (0, 10, 30 and 50 µg $ml^{-1}$) in hydroponic assay. There was a highly significant interaction ($P < 0.01$) between genotype and root aluminum concentration in hydroponic assay. Root aluminum content (mg $g^{-1}$) was greater than that of shoot (Table 5). Root aluminum contents of tolerant genotypes (IPA 7-10, T 7 and 67 B) were significantly lower than those of sensitive genotypes (Bahar and Pusa 9) at 10, 30 and 50 µg $ml^{-1}$ aluminum concentrations (Fig. 3). The increase in shoot aluminum contents in IPA 7-10, 67 B and T 7 at both levels (30 and 50 µg $ml^{-1}$) compared to the control (0 µg $ml^{-1}$) was non-significant. Aluminum concentration in the roots of both tolerant and sensitive genotypes was greater than that for the shoots. Root aluminum contents were significantly lower for the tolerant genotypes (IPA 7-10, T 7 and 67 B) than for the sensitive genotypes (Bahar and Pusa 9) at both 30 and 50 µg $ml^{-1}$ Al concentrations in hydroponic assay. It is, therefore, reasonable to assume that the aluminum tolerance in these accessions of pigeonpea involved aluminum exclusion (Delhaize and Ryan, 1995; Kochian, 1995) from the root. Although shoot aluminum content was also considerably lower for the tolerant genotypes (IPA 7-10, T 7 and 67 B) than for the sensitive genotypes (Bahar and Pusa 9), no indication of internal detoxification (Ma et al., 2001) was observed.

***Relationship among Variability Parameters in Four Screening Methods***

The occurrence of significant differences among pigeonpea genotypes for tolerance to aluminum toxicity indicated the scope of genetic improvement for Al tolerance in pigeonpea. The variation in response was most likely due to difference in genetic potential of pigeonpea 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 root length reduction in hydroponic assay was a good predictor of

**Table 1.** Description of pigeonpea genotypes

| Genotype | Pedigree |
|---|---|
| IPA 7-10 | Selection from a local land race belonging to Varanasi district in U.P. |
| T 7 | Selection from a land race belonging to the Lucknow district in U.P. |
| 67 B | Unknown |
| GT 101E | ICPL 269 × Pusa Sweta |
| MAL 13 | (MA 2 × MA 160) × Bahar |
| UPAS 120 | Selection from P 4768 |
| Asha | C 11 × ICPL 6 |
| Amar | Selection from Bahar |
| Ranchi Local | A land race of Ranchi (Jharkhand) |
| IPA 92 | Selection from a local collection, 98-3 |
| Azad | Bahar × KPBR 80-1 |
| BDN 2 | Selection from local Bori II-132-A-1 |
| PI 397430 | A selection from primary gene pool |
| IPA 204 | Bahar × Ac 314-314 |
| Narendra Arhar 1 | Selection from Faizabad land race |
| IPA 234 | T 7 × WRP 1 |
| PAU 881 | H 89-22 × ICPL 85024 |
| AL 15 | Selection from P 8-9 |
| Pusa 992 | Selection from 90306 |
| IPA 6-1 | Selection from a land race of Etwah district of U.P. |
| BDN 1 | Selection from local land race 'Bori' |
| MA 6 | MA 2 × Bahar |
| Kudrat 3 | Selection from a land race of Mirzapur district of U.P. |
| AL 201 | AL 16 × LP 200 |
| Dholi Dwarf | Selection from a land race of Darbhanga district of Bihar |
| MA 3 | Selection from land race no. MA 2 |
| GT 100 | T 15-15 × S 5 |
| BSMR 736 | CIP 7217 × No 148 |
| Sharad | (Bahar × NPWR 15) × PS 16 |
| Pusa 2002-2 | Sel 90310 × H 88-45 |
| Bahar | Selection from a land race of *Motihari* district of Bihar |
| Pusa 9 | UPAS 120 × 3673 |

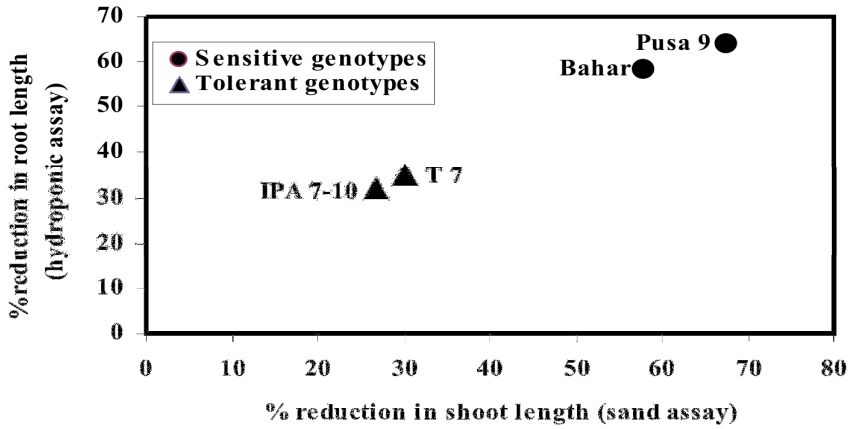


**Fig 1.** Relationship between root length reduction in the hydroponic assay (0 compared to 50 µg ml-1 Al) and shoot length reduction in the sand assay (0 compared to 50 µg ml-1 Al) for four pigeonpea genotypes

shoot growth reduction in sand assay. Genotypes that had the largest dry matter reduction in the sand assay also had the largest root length reduction so that the in-sand responses of pigeonpea genotypes could reasonably be predicted from the hydroponic root length assay. Although tap root length was also reduced in IPA 7-10 and T 7, but the reduction was most acute and highly significant in Bahar and Pusa 9 at 50 µg $ml^{-1}$ in both hydroponic and sand assays. The reduction in shoot length was comparatively less in hydroponic assay, indicating lesser severity of aluminum on shoot growth. Between the tolerant and sensitive genotypes, a number of genotypes showed intermediate response and skewed towards either side. Similar trend of response for root length reduction was observed at 30 µg $ml^{-1}$ Al concentration. Even this concentration of aluminum was sufficient to discriminate between tolerant and sensitive genotypes as has been used in pea and other crops (Singh and Choudhary, 2010; Choudhary et al., 2011). The correlation among four growth parameters

**Table 2.** Effect of five aluminum concentrations on tap root length (cm) of thirty-two pigeonpea genotypes in the sand assay

| Genotype | Al concentration | | | | | % reduction* |
|---|---|---|---|---|---|---|
| | 0 µg ml$^{-1}$ | 10 µg ml$^{-1}$ | 20 µg ml$^{-1}$ | 30 µg ml$^{-1}$ | 50 µg ml$^{-1}$ | |
| IPA 07-10 | 24.41 | 22.56 | 18.67 | 17.10 | 16.50 | 32.40 |
| T 7 | 21.57 | 18.77 | 17.79 | 14.69 | 13.75 | 36.25 |
| 67B | 22.19 | 20.31 | 18.16 | 15.70 | 13.78 | 37.89 |
| GT 101E | 20.72 | 18.90 | 17.69 | 15.41 | 12.79 | 38.27 |
| MAL-13 | 19.55 | 15.97 | 15.4 | 12.68 | 11.99 | 38.67 |
| PAU 881 | 19.88 | 14.41 | 13.80 | 12.67 | 11.91 | 40.09 |
| Kudrat 3 | 17.15 | 14.38 | 12.25 | 11.43 | 10.23 | 40.38 |
| Asha | 17.86 | 13.85 | 12.84 | 11.35 | 09.99 | 44.05 |
| Al 15 | 20.54 | 15.93 | 14.17 | 13.08 | 11.03 | 46.29 |
| Sharad | 19.71 | 16.45 | 12.72 | 11.17 | 10.32 | 47.64 |
| IPA 204 | 19.45 | 15.41 | 12.93 | 11.61 | 10.03 | 48.43 |
| Dholi dwarf | 21.46 | 16.91 | 13.59 | 11.67 | 10.91 | 49.15 |
| Al 201 | 18.23 | 14.03 | 13.15 | 11.67 | 09.13 | 49.93 |
| IPA 92 | 19.45 | 16.59 | 13.03 | 10.20 | 09.74 | 49.95 |
| BDN 1 | 18.71 | 15.26 | 13.41 | 10.40 | 09.20 | 50.83 |
| PI 397430 | 20.55 | 16.37 | 11.73 | 11.05 | 10.10 | 50.88 |
| IPA 234 | 18.68 | 15.08 | 13.37 | 12.21 | 09.12 | 51.17 |
| Azad | 18.55 | 15.30 | 13.23 | 10.93 | 09.01 | 51.42 |
| BSMR 736 | 20.25 | 16.44 | 13.73 | 11.01 | 09.45 | 53.33 |
| MA 6 | 20.41 | 16.72 | 13.73 | 10.94 | 09.45 | 53.70 |
| UPAS 120 | 19.57 | 15.98 | 13.37 | 11.41 | 09.05 | 53.76 |
| Pusa 992 | 21.53 | 17.25 | 16.16 | 12.40 | 09.81 | 54.43 |
| GT 100 | 22.46 | 19.02 | 14.64 | 11.45 | 10.18 | 54.67 |
| Amar | 19.25 | 16.55 | 12.31 | 11.14 | 08.63 | 55.17 |
| MA 3 | 21.45 | 17.43 | 14.17 | 10.70 | 09.41 | 56.13 |
| Narendra Arhar 1 | 22.56 | 18.80 | 14.85 | 13.32 | 09.88 | 56.20 |
| Ranchi local | 19.75 | 15.38 | 12.73 | 11.67 | 08.50 | 56.96 |
| BDN 2 | 21.46 | 16.08 | 12.78 | 10.63 | 09.09 | 57.67 |
| IPA 6-1 | 20.97 | 17.69 | 12.68 | 12.08 | 08.80 | 58.03 |
| Pusa 2002-2 | 19.57 | 14.66 | 11.20 | 11.00 | 08.15 | 58.34 |
| Bahar | 18.40 | 12.00 | 09.00 | 08.01 | 06.80 | 63.04 |
| Pusa 9 | 21.00 | 15.56 | 10.70 | 08.10 | 7.00 | 66.66 |

LSD (P = 0.05) = 1.93 cm for Al concentration x genotype interaction. LSD (P = 0.05) for % reduction in tap root length was 8.73
*represents the reduction in tap root length from 0 to 50 µg ml$^{-1}$ Al concentration

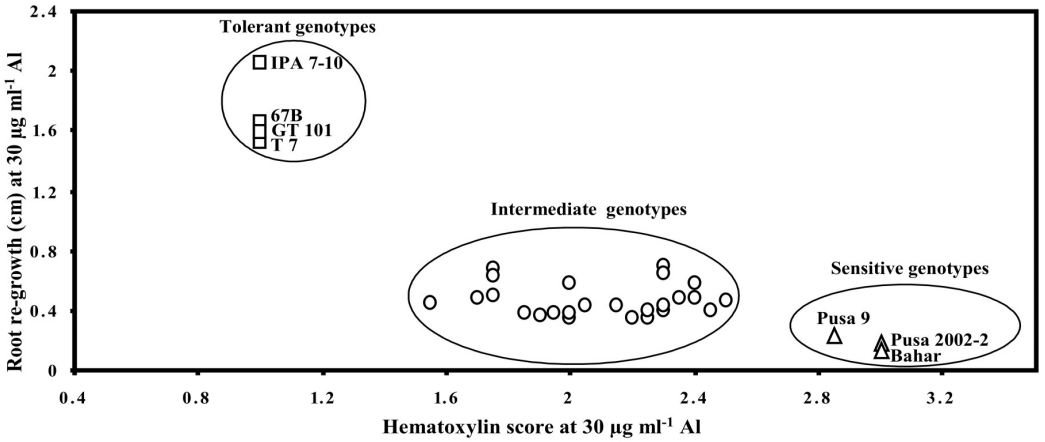


**Fig 2.** Relationship between root re-growth and hematoxylin scores of 32 genotypes of pigeonpea at 30 µg ml$^{-1}$ Al concentration.

in both assays indicated that any one of these could be used to screen for Al tolerance. Foy et al. (1993) also observed that genotypic correlation between shoot and root growth was good and both parameters could be used to evaluate for aluminum tolerance. The reduction in root dry matter, that was probably provoked by phytotoxic effect of $Al^{3+}$, was greater than total dry matter reduction in both tolerant and sensitive genotypes, indicating root as the primary site of aluminum toxicity (Ryan et al., 1993). Relative root length has been proposed as a measure of tolerance to excess aluminum (Reid et al., 1971; Foy, 1974; Kochian, 1995; Singh and Chaturvedi, 2007). Comparative assessment of reduction in root dry matter and root length indicated clearly that aluminum had significant effect on root growth. The decreased root growth might be the main cause for reduction in stem growth. Restriction in root growth brings about physiological stresses such as water deficit and nutrient deficiency (Sarkunan and Bidappa, 1982). Aluminum has also been reported to cause a decrease in the relative root growth rate (Oleksyn et al., 1996; Neogy et al., 1999).

**Table 3.** Correlation coefficients for different pairs of characters in hydroponic and sand assay over five levels of aluminum concentrations

| Trait | $RL_{hydro}$ | $SL_{hydro}$ | $RW_{hydro}$ | $SW_{hydro}$ | $RL_{sand}$ | $SL_{sand}$ | $RW_{sand}$ | $SW_{sand}$ |
|---|---|---|---|---|---|---|---|---|
| $RL_{hydro}$ | 1.00 | 0.68** | 0.77** | 0.62** | 0.79** | 0.73** | 0.83** | 0.64** |
| $SL_{hydro}$ | 0.68** | 1.00 | 0.80** | 0.82** | 0.84** | 0.80** | 0.746** | 0.85** |
| $RW_{hydro}$ | 0.77** | 0.80** | 1.00 | 0.81** | 0.86** | 0.74** | 0.829** | 0.81** |
| $SW_{hydro}$ | 0.62** | 0.82** | 0.81** | 1.00 | 0.75** | 0.65** | 0.641** | 0.83** |
| $RL_{sand}$ | 0.79** | 0.84** | 0.86** | 0.75** | 1.00 | 0.86** | 0.851** | 0.84** |
| $SL_{sand}$ | 0.73** | 0.80** | 0.74** | 0.65** | 0.86** | 1.00 | 0.818** | 0.78** |
| $RW_{sand}$ | 0.83** | 0.75** | 0.83** | 0.64** | 0.85** | 0.82** | 1.000 | 0.71** |
| $SW_{sand}$ | 0.64** | 0.85** | 0.81** | 0.83** | 0.84** | 0.78** | 0.711** | 1.00 |

**significant at P = 0.01

#RL, SL, RW and SW represent root length, shoot length, root weight and shoot weight, respectively

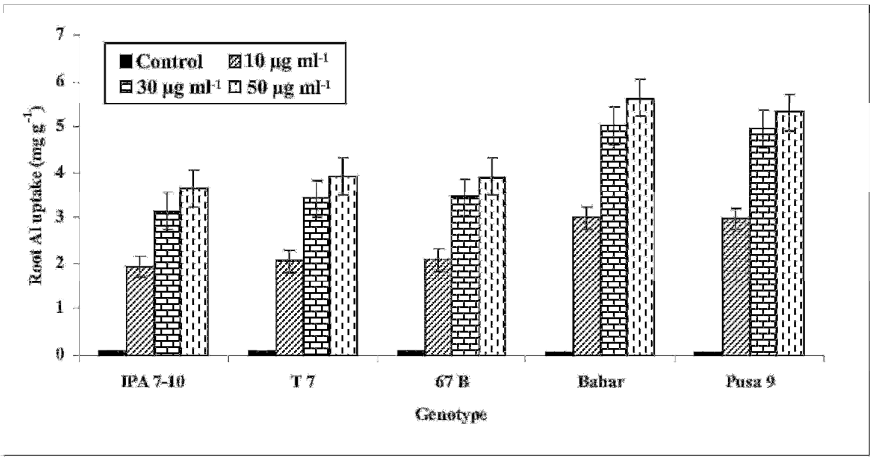


**Fig 3.** Root aluminum uptake of five genotypes of pigeonpea at four Al concentrations.

Root traits have been recommended for screening against aluminum stress in wheat (Delhaize and Ryan, 1995) and rye (Gallego and Benito, 1997). Therefore, traits related to roots should be preferred to shoot traits while exercising selection for Al tolerance in pigeonpea also. The two screening methods almost consistently discriminated between tolerant (IPA 7-10, T 7 and 67 B) and sensitive (Bahar and Pusa 9) genotypes of pigeonpea. Among the 32 genotypes tested, IPA 7-10, T 7 and 67 B had minimum reduction for most of the growth parameters at 50 µg $ml^{-1}$ aluminum concentration. However, the difference for the percentage reduction was also significant and similar even at 30 µg $ml^{-1}$ Al concentration. Moreover, the relative rating of the genotypes was also similar for both tolerant and sensitive genotypes. Therefore, 30 µg $ml^{-1}$ of aluminum could be considered as the effective and efficient level for discriminating tolerant and sensitive genotypes of pigeonpea for aluminum toxicity in both sand and hydroponic assays.

The intensity of hematoxylin stain in the seedlings' roots increased progressively as the aluminum concentration in nutrient solution was increased from 10 to 50 µg $ml^{-1}$. The occurrence of significant genotypic variation for the hematoxylin score (from no stain to complete stain) was similar to those observed in wheat and barley and other crops (Polle et al., 1978; Minella and Sorrells, 1992; Singh et al., 2009). Tolerant genotypes (IPA 7-10, T 7 and 67 B) had partial stain score (≤ 1.0) due to lower concentration of aluminum in the root tip meristem, presumably because of aluminum exclusion. However, none or partial hematoxylin stain in other crop plants have been reported due to the high pH of the cell wall (Polle et al., 1978). The high pH immobilizes aluminum and thus protects the plants from aluminum toxicity (Ownby, 1993). Sensitive genotypes (Bahar, Pusa 9 and Pusa 2002-2) do not have this mechanism to deal with aluminum toxicity and thus accumulated higher concentration of aluminum in their roots, giving complete stain score (3.0). Effective and efficient differentiation was also observed at 30 µg $ml^{-1}$ Al, where root tips of IPA 7-10, T 7, 67 B and GT 101 and of Pusa 9, Pusa 2002-2 and Bahar appeared partially and darkly stained, respectively. The other genotypes showed moderate stain in their root tips. The aluminum tolerance of genotypes based on hematoxylin staining was similar to that of growth response methods based on growth parameters. Aluminum tolerance of genotypes was also similar when assessed by root re-growth method. IPA 7-10, 67 B, T 7 and GT 101 had the highest mean value of root re-growth, whereas Pusa 2002, Bahar and Pusa 9 showed minimum mean value of root re-growth across the aluminum levels. Although the same pattern of root re-growth was observed at 50 µg $ml^{-1}$ Al level for the above-mentioned genotypes, 30 µg $ml^{-1}$ Al concentration was sufficient to discriminate between tolerant and sensitive genotypes. At this level of aluminum (30 µg $ml^{-1}$), tolerant genotypes (IPA 7-10, 67 B, T 7 and GT 101) showed larger root re-growth (>1.5 cm) while the sensitive genotypes (Pusa 2002-2, Bahar and Pusa 9) exhibited very small (≤ 0.25 cm) root re-growth. The same level of aluminum concentration (30 µg $ml^{-1}$) has been used to discriminate tolerant and

**Table 4.** Effects of three aluminum concentrations on total dry matter (g plant$^{-1}$) of four pigeonpea genotypes in sand assay

| Genotype | Root dry matter Al concentration | | | | Total dry matter (shoot + root) Al concentration | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 µg ml$^{-1}$ | 30 µg ml$^{-1}$ | 50 µg ml$^{-1}$ | % reduction* | 0 µg ml$^{-1}$ | 30 µg ml$^{-1}$ | 50 µg ml$^{-1}$ | % reduction* |
| IPA 7-10 | 0.11 | 0.09 | 0.07 | 38.59 | 0.42 | 0.34 | 0.30 | 28.19 |
| T 7 | 0.11 | 0.08 | 0.06 | 44.44 | 0.40 | 0.31 | 0.26 | 35.18 |
| Bahar | 0.09 | 0.04 | 0.03 | 67.44 | 0.31 | 0.18 | 0.14 | 56.27 |
| Pusa 9 | 0.08 | 0.04 | 0.02 | 72.83 | 0.31 | 0.16 | 0.11 | 64.33 |

LSD (P = 0.05) = 0.01g and 0.04g for Al concentration x genotype interaction for root and total dry matter, respectively.
*represents the reduction in total dry matter from 0 to 50 µg ml$^{-1}$ Al concentration

**Table 5.** Root and shoot Al uptake (mg g$^{-1}$) of five pigeonpea genotypes under four different aluminum concentrations in hydroponic assay

| Genotype | Al uptake (root) Al concentration | | | | Al uptake (shoot) Al concentration | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 µg ml$^{-1}$ | 10 µg ml$^{-1}$ | 30 µg ml$^{-1}$ | 50 µg ml$^{-1}$ | 0 µg ml$^{-1}$ | 10 µg ml$^{-1}$ | 30 µg ml$^{-1}$ | 50 µg ml$^{-1}$ |
| IPA 7-10 | 0.10 | 1.93 | 3.14 | 3.65 | 0.04 | 0.12 | 0.17 | 0.19 |
| T 7 | 0.09 | 2.05 | 3.43 | 3.92 | 0.04 | 0.12 | 0.17 | 0.19 |
| 67 B | 0.09 | 2.08 | 3.46 | 3.91 | 0.04 | 0.12 | 0.17 | 0.19 |
| Bahar | 0.06 | 3.00 | 5.03 | 5.64 | 0.02 | 0.15 | 0.25 | 0.36 |
| Pusa 9 | 0.06 | 2.98 | 4.96 | 5.32 | 0.02 | 0.15 | 0.25 | 0.36 |

LSD (P = 0.05) = 0.36 and 0.10 for Al concentration x genotype interaction for root and shoot Al content, respectively.

sensitive genotypes for aluminum toxicity in pea (Singh and Choudhary, 2010) and other crops using root re-growth method. All the four methods (hydroponic and sand assays, hematoxylin staining and root re-growth methods) almost consistently discriminated between tolerant (IPA 7-10, T 7 and 67 B) and sensitive (Pusa 9, Bahar and Pusa 2002-2) genotypes of pigeonpea at 30 µg ml$^{-1}$ Al concentration. Aluminum exclusion from the roots appeared as the primary mechanism for tolerance to aluminum toxicity in the tolerant genotypes. Tolerant genotypes IPA 7-10, T 7 and 67 B will be used in future breeding programme to develop aluminum tolerant pigeonpea cultivars. However, screening of pigeonpea genotypes including wild accessions (especially from *Cajanus scarabaeoides* and *C. platycarpus*) under field condition (natural acid soil) is still needed to amass comprehensive data for even higher degree of tolerance to aluminum toxicity and for reproductive parameters such as yield (Choudhary and Singh, 2011). This will confirm whether tolerance to aluminum toxicity in pigeonpea implies merely survival advantage or also results in increased biological fitness.

**Materials and methods**

The pigeonpea genotypes used in this study were obtained from the Indian Institute of Pulses Research (IIPR), Kanpur (Uttar pradesh), India. The genotypes were IPA 7-10, T 7, 67 B, MAL 13, GT 101E, 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 6-1, BDN 1, MA 6, Kudrat 3, AL 201, Dholi Dwarf, MA 3, GT 100, BSMR 736, Sharad, Pusa 2002-2, Bahar and Pusa 9. Some of these genotypes are released varieties and cultivated widely in the area of their adoption (Table 1). These are maintained at the IIPR, Kanpur and also at the places of their origin.

***Sand Assay (Experiment-1)***

Seeds were disinfected with 1% sodium hypochlorite (w/v) and then germinated in filter paper. Thereafter, seedlings were transferred to plastic pots (15 cm diameter) containing quartz acid washed sand (Mugai and Agony, 1997). The Al treatments were supplied as $AlCl_3.6H_20$. The irrigation solution was maintained at a pH 4.5 using 1M HCl. The treatment solutions were supplied daily to plants. The sand was washed with distilled water after every seven days during the entire experimental period. The experiment was laid out in a factorial randomized block design with two replications. Plants were harvested after 22 days of growth and the sand was washed off from 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 hours and the dry matter yields were determined. Data on root and shoot length, and dry weight of root and shoot were collected from each treatment in each replication. Percentage response to aluminum treatments for these parameters was calculated according to the following equation (Gudu et al., 2001):

% response = [(growth parameters in control – growth parameters in Al treatments)/ growth parameters in control] x 100

***Hydroponic Assay (Experiment-2)***

Seeds were disinfected and germinated in the same manner as used for the sand assay in experiment-1. After 8 days, the seedlings were transferred in dilute nutrient solution **:** $KNO_3$ (0.5mM), Ca $(NO_3)2.4H_20$ (0.5mM), $MgSO_4.7H_2O$ (0.2mM), $KH_2PO_4$ (0.1mM), KCl (50µM), $H_3BO_3$ (46µM), FE-EDTA (20µM), $MnCl_2.4H_2O$ (2µM), $ZnSO_4.7H_2O$ (1µM), $CuSO_4.5H_2O$ (0.3µM) and $NaMoO_4.2H_2O$ (0.5µM) (Simon et al., 1994) having 0, 10, 20, 30 and 50 µg ml$^{-1}$ Al concentrations. The aluminum (Al) treatment solutions were prepared as described in experiment-1. The pH of nutrient solution was maintained at 4.5 for all the treatments using 1 M HCl and was monitored daily. The solution was regularly aerated by bubbling air into the nutrient solution with aquarium air pump and replaced every 4 days to maintain nutrient and aluminum concentration. Four uniform plants of each genotype were grown in each of the duplicate trays for each aluminum concentration. After 22 days of growth, the root and shoot were harvested separately and the roots were given 20 second rinse in distilled water to remove surface contamination followed by blotting to eliminate the entrained

moisture. The age of the plant at harvest was 30 days and the duration of aluminum treatment was 22 days. Same procedures as used in experiment-1 were repeated for recording data on root and shoot length, dry weight of root and shoot and percent response. 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). Aluminum contents (mg $g^{-1}$) in the respective plant parts were estimated by Perkin-Elmer atomic absorption spectrophotometer (Model 5000, Perkin-Elmer, Shelton, CT-USA).

***Hematoxylin Assay (Experiment-3)***

The staining protocol (Polle et al., 1978) was partially modified for visual detection of aluminum in the roots. Seeds were disinfected and germinated in the same manner as used for the sand assay in experiment-1. After 8 days, seedlings were transferred to plastic containers in nutrient solution (4.0 mM $CaCl_2$, 6.5 mM $KNO_3$, 2.5mM $MgCl_2$, 0.1 mM $(NH4)_2$ $SO_4$, 0.4 mM $NH_4NO_3$) that was adjusted to pH 4.5 with 1M HCl solution. Seedlings were kept in the nutrient solution for 2 days under continuous light and aeration. The seedlings were then grown for 24 hours on the fresh nutrient solution containing 10, 20, 30 and 50 µg $ml^{-1}$ Al concentrations. The roots of seedlings were then placed in aerated distilled water for 60 minutes to remove aluminum on the root surface. The staining solution consisted of 2 g $l^{-1}$ hematoxylin and 0.2 g $l^{-1}$ $KIO_3$ prepared in distilled water. The roots of seedlings were immersed in the hematoxylin stain for 30 minutes after which the seedlings were placed three times in flowing distilled water for 30 minutes. Each seedling was visually scored for the staining pattern 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 complete (3) staining. Classification of genotypes into discrete categories such as tolerant (0-1), moderate (2) and sensitive (3) was done based on intensity of stain.

***Root Re-growth Assay (Experiment-4)***

The protocol of the procedure followed for the assay of root re-growth has been given by Nava et al. (2006). Seeds were disinfected and germinated in the same manner as used for the sand assay. The seedlings were transferred to plastic containers in nutrient solution without aluminum for 48 hours. The seedlings were then transferred to a solution with 10, 20, 30 and 50 µg $ml^{-1}$ Al concentrations for another 48 hours. Finally, seedlings were transferred further to the solution free of aluminum for 72 hours. Root growth was reinitiated after removal from the aluminum 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 completely 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 average root re-growth of each sample was used as the replicate value. These four 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 × Al treatment interactions. Least significant differences (LSD) were calculated at P = 0.05 for significant interactions. In the sand and hydroponic assays, where multiple comparisons were made (32 genotypes at 5 aluminum concentrations), analyses were performed using SPSS software and LSD was calculated for significant interactions. Pearson's correlation coefficient for different pairs of parameters (*within* and *between* hydroponic and sand assays) was also computed and subjected to test of significance.

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