*Indian Journal of Agricultural Sciences* **93** (2): 221–224, February 2023/Short Communication
https://doi.org/10.56093/ijas.v93i2.130327

# Rapid screening of pea (*Pisum sativum*) genotypes against aluminium toxicity

MOHD TALHA ANSARI[1], P SARMA[1], SANDEEP KUMAR[2] and KRIPA SHANKAR[2]*

*College of Horticulture and Forestry, Central Agricultural University, Pasighat, Arunachal Pradesh 791 102, India*

Received: 16 November 2022; Accepted: 18 January 2023

**Keywords**: Aluminium toxicity, Haematoxylin staining, Root tolerance index

Soil acidity is a detrimental factor for the successful production of legume due to nutrient deficiency and aluminium (Al) toxicity. Pea is important legume crop in which Al toxicity damages root growth, callose and lignin deposition and elevated reactive oxygen species (Shetty *et al.* 2020). Thus, the present study was carried out to screen 25 pea (*Pisum sativum* L.) genotypes for Al tolerance at 12 ppm and 24 ppm in the laboratory of Basic Sciences and Humanities, College of Horticulture and Forestry, Central Agricultural University (CAU), Pasighat, Arunachal Pradesh. Garden pea (*Pisum sativum* var. hortense) genotypes were collected from different institutes, industries and locations. Pea genotypes were grown in trays containing sterilized sand supplemented with Hoagland solution under a controlled environment. Screening of pea genotypes was performed using growth characters, root tolerance index and haematoxylin staining. Scoring for haematoxylin staining was done by the amount of stain taken up by the roots (Singh *et al.* 2009). K-mean clustering was carried out to categorize genotypes into tolerant, intermediate and susceptible. The genotypes Kashi Samridhi, CHFGP-1 and CHFGP-14 exhibited an increase in root and shoot biomass. Nearly all the genotypes exhibited reduction in root and shoot length. Based on the K-clustering score sum P.S.M.3, Kashi Samrath, Kashi Samridhi, CHFGP-1, CHFGP-7 and CHFGP-55 were observed as tolerant at 24 ppm Al level. Matar Ageta-7, Pb-89, AP-3, Pusa Pragati, Arka Priya, Kashi Shakti, CHFGP-6, VRPE-29, NO-17 and CHFGP-15 were categorized as susceptible genotypes. These genotypes can also be used to examine the breeding and inheritance of Al tolerance.

The major mechanism operating in pea resistant to Al exhibits higher *p*H in root zone, ability to resist oxidative stress and reduced lignification's (Matsumoto and Motoda 2012, Ansari 2019). Therefore, this experiment was conducted at College of Horticulture and Forestry, CAU, Pasighat, Arunachal Pradesh in 2019 to screen 25 pea genotypes for Al tolerance at 12 ppm and 24 ppm. The 25 genotypes of garden peas were raised in the growth chamber in January month with a day length of 14 hours and mean temperature of 18±3°C and relative humidity of 80%. The seedlings were established initially for 6 days and supplemented with Hoagland Solution (Hoagland and Arnon 1950). Further, the seedlings were treated with two-level (12 and 24 ppm) along with control. Nine plants (3 from each replication) were collected for haematoxylin staining. Root length and biomass were measured by photosynthetic pigment Lichtenthaler's method (Lichtenthaler 1987) and the root tolerance index (Taylor and Foy 1985). Three seedlings per genotype per replication were visually scored: 1=no or partial staining, 2=moderate staining and 3=deep staining. The 'no staining' and 'partial staining' seedlings were classified as tolerant, the 'moderate staining', seedlings as moderately tolerant and those deeply stained as sensitive as per Haematoxylin staining (Singh *et al.* 2009). Gomez and Gomez (1984) recommended procedure was used to calculate the analysis of variance. Using SPSS (Version 21), K-Mean clustering was carried out. LSD was used to compare means at 5% probability value.

*Root biomass:* The root biomass of pea genotypes at seedling stage was reduced or unaffected in all genotypes except for Kashi Samridhi, VRP-5, CHFGP-1 and CHFGP-14, where a significant increase was observed at 12 ppm Al level. At 24 ppm Al concentration, P.S.M.3 showed a significant increase in root biomass, and Matar Ageta-7, Pb-89 and Pusa Pragati showed a significant reduction in root biomass with respect to control. In the genotypes where root biomass was increased after treatment with Al, have been associated with the lessening of $H^+$ toxicity. Kichigina *et al.* (2017) observed a similar outcome in which root biomass of some genotypes was unaffected or increased.

*Root length:* Among the pea genotypes grown in sand culture, Kashi Shakti, Matar Ageta-7, Lincoln and Kashi Ageti was having long roots. However, a higher level of Al treatment resulted in a significant reduction of root length.

[1]College of Horticulture and Forestry, Central Agricultural University, Pasighat, Arunachal Pradesh; [2]ICAR-Indian Agricultural Research Institute, New Delhi. *Corresponding author email: shankar_11783@iari.res.in

With the Al treatment root length of pea genotypes decreased or remained unaffected in the genotypes except for Arka Apoorva, Priya and KK-10 which showed a significant increase in root length compared to no Al. Similarly, Blair *et al.* (2009) also reported varying responses of common bean genotypes towards aluminium toxicity where they observed that the Andean gene pool was Al resistant compared to Mesoamerican genotypes in respect of root growth in the presence of toxic Al in the solution culture.

*Root tolerance index (RTI):* At 12 ppm Al level, RTI was highest in Kashi Shakti followed by AP-3, Kashi Samrath, CHFGP-1 and the lowest RTI was observed in CHFGP-14 and Arkel. At 24 ppm Al level, RTI was found highest in Arka Apoorva followed by KK-10, Priya and the lowest RTI was observed in CHFGP-14. These genotypes can also be used to examine the breeding and inheritance of Al tolerance. Similarly, Belachew and Stoddard (2017) also reported varying responses of common bean genotypes towards aluminium toxicity.

*Photosynthetic pigments:* At 12 ppm Al level, photosynthetic pigments were highest in Kashi Shakti followed by AP-3, Kashi Samrath, CHFGP-1 and the lowest RTI was observed in CHFGP-14 and Arkel. Under various stresses, chlorophyll in the chloroplast was broken down resulting in the loss of thylakoid structure (Rout *et al.* 2001). At 24 ppm Al level, Arka Priya and Kashi Ageti showed a significant reduction as compared to control. The result is supported by the findings of Pandey *et al.* (2014) in rice, where chlorophyll content of sensitive cultivar reduced significantly, whereas in the tolerant cultivar either low or non-significant decrease in chlorophyll content was observed due to the Al stress.

*Total carotenoids:* Carotenoids are non-enzymatic antioxidant that play an important part in scavenging ROS generated because of stress. Averaged across genotypes, there was a significant increase of nearly 5% in carotenoid content at 24 ppm Al level. Significant variation was observed for mean carotenoids content across Al concentrations in 25 pea genotypes after 14 days of Al treatment. EC-9485 and VRPE-29 had significantly higher carotenoids content in leaves at 12 ppm Al level with respect to control. The carotenoid content decreased in some genotypes, viz. Arka, Priya, Kashi Ageti and CHFGP-14, whereas some genotypes, viz. CHFGP-6, VRPE-29, CHFGP-15 and KK-10 showed a significant increase in carotenoids content at 24 ppm Al concentration compared to control. The increase in carotenoids content provided direct evidence of the effects of heavy metal stress on plant (Murtaza *et al.* 2019). The results are in accordance with the findings of Lazarevic *et al.* (2016) that Al treatment caused an increase of carotenoids in potato cultivars.

*Haematoxylin staining:* Scoring for haematoxylin staining was done by observing the amount of stain taken up by the roots and were scored accordingly. Based on staining, at 24 ppm Al level, root of Arkel, Kashi Samrath, Kashi Samridhi, NO-17 and CHFGP-55 had less/no staining

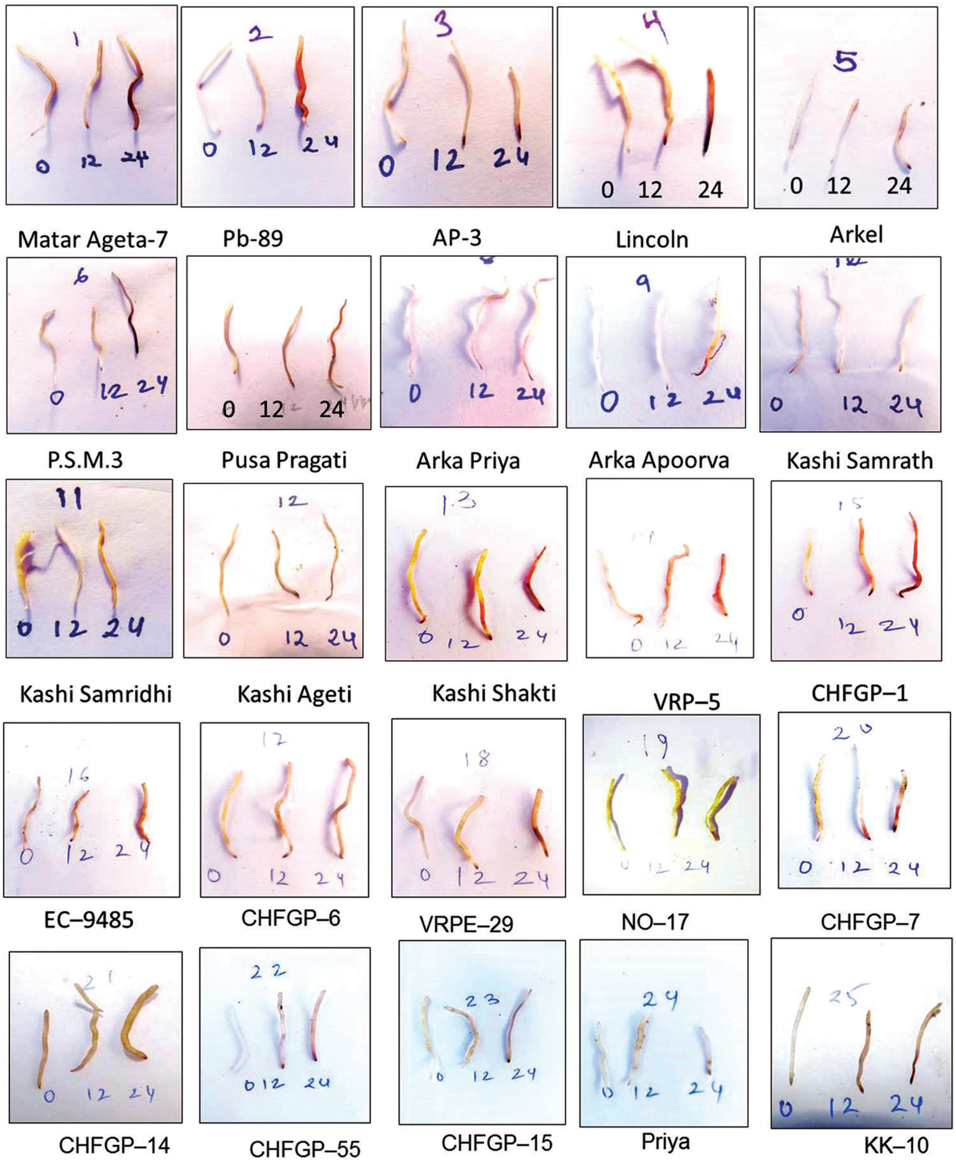


Fig 1 Haematoxylin staining of 25 genotypes at different Al levels (Control, 12, 24 ppm Al).

Table 1 Rankings of genotypes for their relative tolerance in terms of seedling and yield character using K–mean cluster analysis at 24 PPM Al level

| | Shoot length | Root length | Shoot biomass | Root biomass | Shoot dry matter | Root dry matter | Chl. a | Chl. b | Carot-enoids | Stain-ing* | Sum | Ranking |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Matar Ageta–7 | 2 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 17 | Susceptible |
| Pb–89 | 2 | 1 | 1 | 2 | 2 | 2 | 3 | 3 | 2 | 1 | 19 | susceptible |
| AP–3 | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 1 | 18 | Susceptible |
| Lincoln | 3 | 3 | 2 | 2 | 2 | 2 | 2 | 3 | 2 | 1 | 22 | Intermediate |
| Arkel | 3 | 2 | 1 | 2 | 2 | 2 | 2 | 3 | 2 | 3 | 22 | Intermediate |
| P.S.M. 3 | 2 | 3 | 2 | 3 | 3 | 2 | 3 | 3 | 2 | 1 | 24 | Tolerant |
| Pusa Pragati | 2 | 1 | 2 | 2 | 2 | 1 | 2 | 3 | 2 | 2 | 19 | Susceptible |
| Arka Priya | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 1 | 1 | 17 | Susceptible |
| Arka Apoorva | 2 | 3 | 2 | 2 | 2 | 2 | 3 | 3 | 2 | 1 | 22 | Intermediate |
| Kashi Samrath | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 2 | 3 | 23 | Tolerant |
| Kashi Samridhi | 3 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 2 | 3 | 24 | Tolerant |
| Kashi Ageti | 3 | 2 | 3 | 3 | 3 | 2 | 2 | 3 | 2 | 2 | 25 | Tolerant |
| Kashi Shakti | 3 | 1 | 1 | 2 | 2 | 1 | 2 | 2 | 2 | 1 | 17 | Susceptible |
| VRP–5 | 2 | 2 | 2 | 2 | 2 | 3 | 2 | 3 | 2 | 2 | 22 | Intermediate |
| CHFGP–1 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 1 | 25 | Tolerant |
| EC–9485 | 2 | 1 | 2 | 2 | 2 | 2 | 3 | 3 | 2 | 2 | 21 | Intermediate |
| CHFGP–6 | 1 | 1 | 2 | 1 | 1 | 1 | 3 | 2 | 3 | 2 | 17 | Susceptible |
| VRPE–29 | 2 | 1 | 2 | 2 | 2 | 2 | 2 | 1 | 3 | 1 | 18 | Susceptible |
| NO–17 | 2 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 19 | Susceptible |
| CHFGP–7 | 3 | 1 | 1 | 3 | 3 | 3 | 3 | 3 | 2 | 1 | 23 | Tolerant |
| CHFGP–14 | 3 | 1 | 3 | 2 | 2 | 1 | 2 | 3 | 2 | 3 | 22 | Intermediate |
| CHFGP–55 | 3 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 2 | 3 | 24 | Tolerant |
| CHFGP–15 | 3 | 1 | 1 | 2 | 1 | 1 | 2 | 3 | 3 | 2 | 19 | Susceptible |
| Priya | 2 | 2 | 2 | 3 | 3 | 2 | 2 | 3 | 2 | 1 | 22 | Intermediate |
| KK–10 | 2 | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 1 | 21 | Intermediate |

*For staining score were given based on staining magnitude.

and was given 3 scores. At the lower level of Al, roots of Matar Ageta-7, Arkel, P.S.M.3, Arka Priya, Kashi Samrath, Kashi Samridhi, EC-9485, NO-17, CHFGP-14, CHFGP-15 and CHFGP-55 had low staining. However, at 24 ppm roots of Arkel, Kashi Samrath, Kashi Samridhi, NO-17 and CHFGP-55 had less/no staining which shows less injury and better Al tolerance (Fig 1).

*Grouping of genotypes into clusters (tolerant, intermediate and susceptible)*: K-mean clustering was done using the difference value between control and Al stress for each character except haematoxylin staining. Scoring for haematoxylin staining was done by observing the amount of stain taken up by the root and scored accordingly. Clusters were formed at 24 ppm Al level. The cluster group rankings were obtained from the cluster centers in the order from the lowest to highest. Based on the K-clustering score, amongst all the studied characters P.S.M.3, Kashi Samrath, Kashi Samridhi, CHFGP-1, CHFGP-7 and CHFGP-55 were classified as tolerant at 24 ppm Al level (Table 1). Matar Ageta-7, Pb-89, AP-3, Pusa Pragati, Arka Priya, Kashi Shakti, CHFGP-6, VRPE-29, NO-17 and CHFGP-15 were classified as susceptible genotypes and the remaining were intermediate performing. Similarly, Kichigina *et al.* (2017) classified pea genotypes into tolerant and susceptible clustering using the Ward method based on phenotypic and economic traits. Belachew and Stoddard (2017) also used to rank for the screening of faba bean accession to screen acidity and Al stresses.

## SUMMARY

Twenty-five pea genotypes were grown in trays under a controlled environment in the laboratory of Basic Sciences and Humanities, College of Horticulture and Forestry, CAU, Pasighat, Arunachal Pradesh (2018–2021) and evaluated at 3 levels of Al (0 ppm, 12 ppm and 24 ppm). Al treatment was given through Hoagland solution. Screening of pea genotypes was performed using growth characters, root tolerance index and haematoxylin staining. The genotypes Kashi Samridhi, CHFGP-1 and CHFGP-14 exhibited an increase in root and shoot biomass. Nearly all the genotypes

exhibited reduction in root and shoot length. Based on the K-clustering score P.S.M.3, Kashi Samrath, Kashi Samridhi, CHFGP-1, CHFGP-7 and CHFGP-55 were observed as tolerant at 24 ppm Al level. Matar Ageta-7, Pb-89, AP-3, Pusa Pragati, Arka Priya, Kashi Shakti, CHFGP-6, VRPE-29, NO-17 and CHFGP-15 were categorized as susceptible genotypes.

## REFERENCES

Ansari M T, Pandey A K, Mailappa A S and Singh S. 2019. Screening of Dolichos bean [*Lablab purpureus* (L.) Sweet] genotypes for aluminium tolerance. *Legume Research-An International Journal* **42**(4): 495–99.

Belachew K Y and Stoddard F L. 2017. Screening of faba bean (*Vicia faba* L.) accessions to acidity and aluminium stresses. *PeerJ* **5**: e2963.

Blair M W, Lopez-Marin H D and Rao I M. 2009. Identification of aluminum resistant Andean common bean (*Phaseolus vulgaris* L.) genotypes. *Brazilian Journal of Plant Physiology* **21**: 291–300.

Gomez K A and Gomez A A. 1984. *Statistical procedures for agricultural research*. John wiley and sons (Eds), U.S.

Hoagland D R and Arnon D I. 1950. *The water-culture method for growing plants without soil*, Vol. 347, pp. 1–32. California agricultural experiment station, U.S.

Kichigina N E, Puhalsky J V, Shaposhnikov A I, Azarova T S, Makarova N M, Loskutov S I, Safronova V I, Tikhonovich I A, Vishnyakova M A, Semenova E V and Kosareva I A. 2017. Aluminum exclusion from the root zone and maintenance of nutrient uptake are principal mechanisms of Al tolerance in (*Pisum sativum* L.). *Physiology and molecular biology of plants* **23**(4): 851–63.

Lazarevic B, Jurkic V, Mussic M and Poljak M. 2016. Effect of aluminium toxicity on concentration of photosynthetic pigments in two potato cultivars with different aluminium sensitivity. *Acta horticulture* **1142**(10): 61–66.

Lichtenthaler H K. 1987. Chlorophylls and carotenoids: Pigments of Photosynthetic Biomembranes. *Methods in Enzymology,* Vol. 148, pp. 350–382. Academic Press, Cambridge.

Matsumoto H and Motoda H. 2012. Review: aluminium toxicity recovery processes in root apexes. Possible association with oxidative stress. *Plant Science* **185**: 1–8.

Murtaza B, Naeem F, Shahid M, Abbas G, Shah N S, Amjad M, Bakhat H F, Imran M, Niazi N K and Murtaza G. 2019. A multivariate analysis of physiological and antioxidant responses and health hazards of wheat under cadmium and lead stress. *Environmental Science and Pollution Research* **26**: 362–70.

Pandey P, Srivastava R K and Dubey R S. 2014. Water deficit and aluminum tolerance are associated with a high antioxidative enzyme capacity in Indica rice seedlings. *Protoplasma* **251**: 147–60.

Rout G, Samantaray S and Das P. 2001. Aluminium toxicity in plants: a review. *Agronomie* **21**: 3–21.

Shetty R, Vidya C S N, Prakash N B, Lux A and Vaculík M. 2020. Aluminum toxicity in plants and its possible mitigation in acid soils by biochar: A review. *Science of the Total Environment* **765**: 142744.

Singh D, Rai A K and Panyang O. 2009. Hematoxylin staining as a potential screening technique for aluminium tolerance in pea (*Pisum sativum* L.). *Current Science* **96**(8): 1029–30.

Taylor G J and Foy C D. 1985. Mechanism of aluminum tolerance in *Triticum aestivum* L. (wheat). II. Differential *p*H induced by spring cultivars in nutrient solution. *Australian Journal of Botany* **72**: 702–06.