

MORPHO-PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF PIGEONPEA (*Cajanus cajan* L.) GENOTYPES IN ALUMINIUM TOXIC SOIL OF THE NORTH EASTERN HILL (NEH) REGION

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ABSTRACT

In the North Eastern Hill (NEH) region of India, pigeonpea (*Cajanus cajan* L.) is a promising crop due to its adaptability to various environmental conditions. However, the region's acidic soils exacerbated by aluminium (Al) toxicity, present significant cultivation challenges. An experiment was carried out in the Department of Genetics and Plant Breeding, School of Agricultural Sciences (SAS), Medziphema Campus; Nagaland University (NU) during the period of July to December, 2022 to examine the morpho-physiological and biochemical responses of seven pigeonpea genotypes to Al stress, aiming to identify those with enhanced tolerance. For this experiment, seven genotypes (PA-3, PA-291, PA-406, PA-414, PA-421, GT-101, and PA-640) were assessed in field pots utilizing three treatments, such as control, 100 μ M and 225 μ M of Al concentration, using a Factorial Completely Randomized Design (FCRD) with three replications.

Significant genetic variability was found among the genotypes. The interaction of genotypes and treatments effects was found significant for the characters except for number of branches plant⁻¹ and test weight. Aluminium stress (100 μ M Al and 225 μ M Al) resulted in reduction of plant height, number of branches plant⁻¹, root volume, flower number plant⁻¹, plant dry weight, shoot dry weight, root dry weight, nitrate reductase activity, pod number plant⁻¹ seed yield, and test weight, while increasing leaf proline, chaffy pods percentage and root aluminium content. Notably, genotypes GT-101, PA-291 and PA-640 had lower root aluminium accumulation with superior performance making them strong candidates for breeding in aluminium-toxic soils. The correlation analysis of yield and biochemical parameters revealed that seed yield plant⁻¹ had positive significant association with all the parameters except pod number plant⁻¹, chaffy pods percentage, root aluminium content and test weight. These findings highlight the genetic diversity and potential for selecting aluminium-tolerant pigeonpea genotypes to enhance cultivation in the NEH region.

(Key words: Aluminium toxicity, pigeonpea genotypes, morpho-physiological responses, biochemical analysis, genetic variability, tolerant genotypes)

INTRODUCTION

Pigeonpea (*Cajanus cajan* L.) is a key legume crop widely cultivated in tropical and subtropical regions. It is valued for its drought resilience and ability to thrive in poor soil conditions, making it crucial for sustainable agriculture and food security, especially in developing countries. Globally, pigeonpea covers around 4.92 million hectares, producing approximately 4.8 million tons annually, with major cultivation areas in India, East Africa, and the Caribbean (Anonymous, 2020). Its capability to fix atmospheric nitrogen, enhance soil fertility, and generate substantial biomass makes pigeonpea an essential part of cropping systems, especially in areas prone to soil degradation and low fertility. India leads in both the production and consumption of pigeonpea, accounting for

about 70% of global output, primarily in Maharashtra, Karnataka, Madhya Pradesh, Uttar Pradesh, and Andhra Pradesh, spanning roughly 3.9 million hectares and yielding 3.3 million tons annually (Anonymous, 2020 a). Pigeonpea is a vital protein source, significantly contributing to dietary protein intake in vegetarian diets. Its deep-rooting system and drought tolerance make it a preferred choice in rainfed agriculture, supporting the stability and sustainability of Indian farming practices.

In the North Eastern Hill (NEH) region of India, pigeonpea is emerging as a promising crop. The NEH region's unique topography, diverse agro-climatic conditions, and acidic soils offer both opportunities and challenges for pigeonpea cultivation. Despite favourable climatic conditions for its growth, the region's acidic soils and high rainfall lead to aluminium (Al) toxicity, posing

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significant constraints. However, pigeonpea's adaptability to diverse environmental conditions makes it a promising crop for enhancing food security and livelihoods in the NEH region.

Aluminium toxicity is a major issue in acidic soils (pH below 5.5) prevalent in many parts of the world, including the NEH region (Debnath *et al.*, 2021; Sharma and Sarangthem, 2021). Acidic soils solubilize aluminium into toxic forms, mainly Al^{3+} , which adversely affects plant growth by impairing root elongation, disrupting nutrient and water uptake, and inducing oxidative stress, resulting in reduced crop yields (Kochian *et al.*, 2004; Yamamoto *et al.*, 2002). The global prevalence of acidic soils and the resulting aluminium toxicity underscore the need to develop crop varieties with enhanced tolerance to Al toxicity to ensure sustainable agricultural production.

Pigeonpea, with its diverse genetic base, is a valuable resource for developing Al-tolerant genotypes. Previous studies have shown significant variability in Al tolerance among pigeonpea genotypes (Sharma *et al.*, 2012). Understanding pigeonpea's morpho-physiological and biochemical responses to Al toxicity is crucial for breeding programs aiming to improve Al tolerance. Key traits associated with Al tolerance in pigeonpea include root growth parameters, organic acid exudation, antioxidant enzyme activities, and the expression of Al-tolerance genes (Sharma *et al.*, 2012). Selecting or developing better-adapted genotypes based on these traits is essential. Our study aims to identify genotypes with superior performance in acidic soils, contributing to the development of Al-tolerant pigeonpea varieties for sustainable agriculture in the NEH region.

MATERIALS AND METHODS

The present investigation was carried out at the Department of Genetics and Plant Breeding, School of Agricultural Sciences (SAS), Medziphema Campus; Nagaland University (NU) during the period of July to December, 2022 with a total of seven pigeonpea genotypes (PA-3, PA-291, PA-406, PA-414, PA-421, GT-101, and PA-640) collected from AICRP-Pigeonpea, NU, SAS, Medziphema.

All the genotypes were screened for aluminium toxicity with three treatments used, *viz.*, control (0 μ M), 100 (μ M)Al, and 225 (μ M) Al. Each treatment replicated three times in a factorial CRD design. The seeds of the each genotype were grown in three series of pots (10 cm \times 15 cm). One series contained field soil (control), while the other two contained aluminium treatment with moderate (100 mg/1000 g⁻¹ soil) and higher (225 mg/1000 g⁻¹ soil) concentrations. During growing period all agronomic management practices were implemented equally as per the recommendations.

All morpho-physiological and biochemical observations were recorded during the flower initiation stage, while yield and yield-related parameters were noted

after harvest. The observations included plant height, number of branches plant⁻¹, root volume, number of flowers plant⁻¹, plant dry weight, dry weight of shoot, dry weight of root, nitrate reductase activity (Hageman and Hucklesby, 1971), proline content (Bates *et al.*, 1973), number of pods plant⁻¹, seed yield plant⁻¹, test weight, chaffy pods percentage and aluminium content in root biomass.

To analyze the data, standard statistical procedures were utilized (Panse and Sukhathme, 1967). Specifically, analysis of variance (ANOVA) was conducted using the R software 4.2 version to identify significant differences among the treatments.

RESULTS AND DISCUSSION

Seven pigeonpea genotypes of different genetic backgrounds were used for screening under various levels of aluminium by evaluating the morphological characters. The leaves of different genotypes exhibited necrosis symptoms when plants were treated with higher concentrations of aluminium, specifically 250 μ M and 350 μ M of Al. In similar studies majority of the immature leaves curled, impeding the growth and development of the petiole (Rout *et al.*, 2001, Debnath *et al.*, 2017).

The results of the analysis of variance showed a high degree of variability with significant differences among the seven (7) genotypes and the treatments (Al) combination for all the characters studied, except the number of branches and test weight. Mean performance genotypes and the treatments are represented in Table 1. The morphological parameters *viz.*, plant height, root volume, number of flowers plant⁻¹, plant dry weight, dry weight of shoot and dry weight of root showed significant variation among the genotypes and treatments under study and reduced with the increasing of aluminium concentration. The results revealed that the genotypes PA-291 was found superior for most of the characters studied whereas the moderate performance was recorded in genotypes PA-640, GT-101, PA-3 and PA-421 (Table 1). In contrast, the lowest performing genotype was PA-406 for most of the traits. Interaction were found significant. It was maximum in all morphological traits except number of branches plant⁻¹ and highest interaction was found in genotype PA-291 with control and next to this was P-291 with 100 μ M concentration.

The plant height was significantly increased in the control condition compared with Al treatments. It has been revealed that highest plant height was observed in PA-291 in control condition (154.70 cm) but it was at par with the 100 μ M of Al treatment (150.40). Reduced effect of plant height by 225 μ M of Al treatment was found among all the genotypes. Legesse *et al.* (2017) also reported a significant decrease in plant height with aluminium treatment at higher concentrations in common bean. Root growth is inhibited by short and long-term exposure to hazardous levels of aluminium (Ryan and Kochian, 1993; Kochian, 1995). Among the genotypes, PA-406 showed

high per cent of reduction under treatment 100 μM Al and 225 μM Al when compared with control condition in terms of root volume and root dry weight. The apexes of the primary and lateral roots thicken and turn brownish-gray, indicating the quick inhibition of root growth (Rout *et al.*, 2001). A reduction in cell division occurs together with radical inhibition (Frantzios *et al.*, 2003) and elongation of

the root cells, which causes considerable lignification of the cell wall through pectin-crossing (Rout *et al.*, 2001; Jones *et al.*, 2006). This modification inhibits water absorption, which is necessary for the transportation of nutrients through the apoplast, eventually leading to a loss in production and grain quality (Zheng and Yang, 2005; Raman *et al.*, 2002).

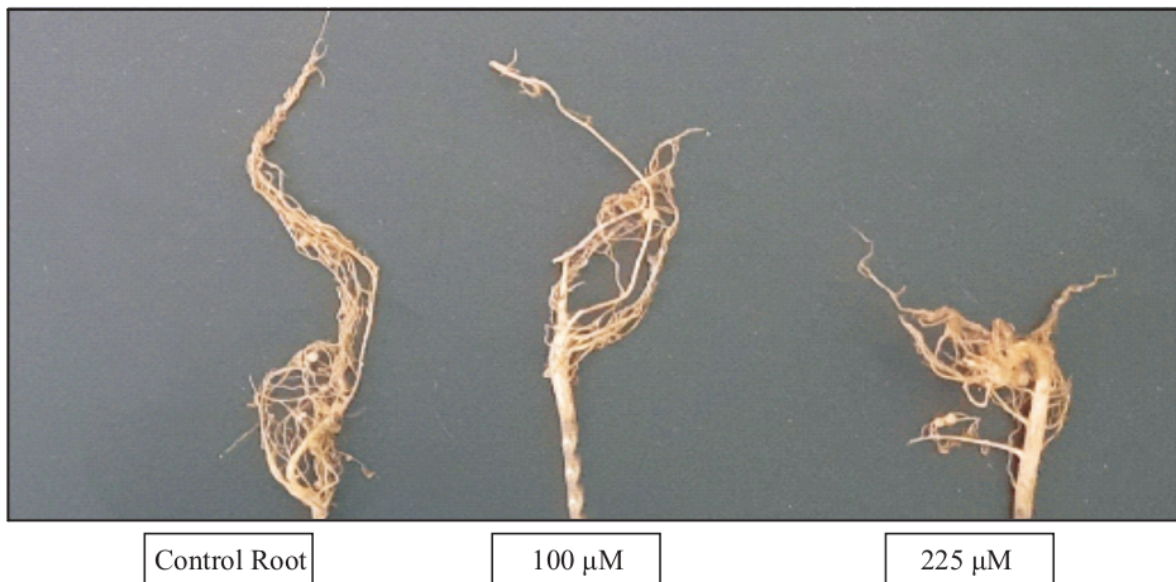


Figure 1. Representative picture showing reduction of root growth under different aluminium treatment

For the trait Aluminium content in root biomass, the superior performer was GT-101 genotype followed by PA-291 and PA-640 which performed better with lower aluminium content in root and performed superior for other agronomic traits. The findings are in line with those made by Tan and Keltjens (1990a, b) and Kolawole *et al.* (2000), who noted a comparable decrease in nutrient uptake by Al treatment in genotypes of sorghum and cowpea. Rapid root development suppression is the main sign of aluminium toxicity (Kochian *et al.*, 2005). The reduction percentage was higher in 225 μM Al than 100 μM Al when compared with the control condition as shown in Figure 1. Similar finding was reported by Choudhury and Sharma (2014) that Al³⁺ treatment is a dose dependent resulted in the inhibition of root growth. Minimum reduction in shoot, root volume and root dry weight was recorded in genotypes PA-291 and GT-101. According to Panda *et al.* (2009) Al exposure inhibits cell growth and division, which causes root stunting and decreased water and nutrient intake.

A significant decrease in nitrate reductase (NR) was observed in the higher dose of aluminium concentration, genotypes PA-291 and PA-640 showed less reduction in treatment 100 (μM) Al and 225 (μM) Al when compared with the control condition. According to the studies of Sharma and Dubey (2005) Al toxicity and water stress decreased total amount of functional NR. The level of proline increased in higher aluminium concentration, genotypes PA-291 and PA-640 showed less reduction in treatment 100 μM Al and 225 μM Al when compared with the control condition. Bhamburdekar and Chavan (2011)

observed similar results, noting that plants accumulate proline under various stress conditions to mitigate damage. Their study on pigeonpea germinating seeds revealed that proline levels increased in response to NaCl, Boron, and Aluminium treatments. Specifically, proline content was significantly elevated under salinity stress, and all tested aluminium concentrations induced proline accumulation, particularly at 48, 72, and 120 hours of seed germination. Proline accumulation is one of the adaptive responses in different species of plants to stress from heavy metals (Ziaei *et al.*, 2014). At higher concentration there was decrease in proline content which may be due to degradation of protein involved in proline biosynthesis.

In case of yield related traits *viz.*, number of pods plant⁻¹, seed yield plant⁻¹, test weight were higher under control condition than those grown in aluminium treated soil. In contrast chaffy pods percentage was higher in treatment conditions as compared to control. For the trait number of pods plant⁻¹, genotype PA-291 and PA-640 performed better, while the lowest performance was noticed in genotype GT-101, whereas the moderate performance was recorded in genotypes PA-3, PA-421, PA-414 and PA-406. In seed yield plant⁻¹, the highest performer was genotype PA-291, moderate performance was noticed in genotype PA-640 and PA-3 while the lowest performance was recorded in genotype PA-406 and PA-414. The effect Al concentration with 100 μM was found better response in genotypes PA-291 and GT-101. The moderate performance was noted in genotypes

PA-640, PA-414 and PA-421. Genotype PA-291 and PA-640 showed highest performance for test weight, moderate in PA-3, PA-406 and PA-421 while the lowest in genotype GT-101. For the trait chaffy pods percentage, the lower value showed by genotype PA-291 followed by PA-640 whereas higher value was measured in genotype PA-3. In agreement with the results of this study, Kushwaha *et al.* (2017) reported a decrease in pod weight and pod yield in response to aluminium treated soils. Interactions were found to be significant, showing maximum effects in all yield-related traits except for test weight. The highest interaction was observed in genotype PA-291 with the control followed by PA-291 with the 100 μM concentration.

The character association study of morpho-physiological biochemical, and yield component traits among the different genotypes are presented in Table 2. It was found that seed yield plant^{-1} had positive and significantly association with plant height plant^{-1} , number of branches plant^{-1} , root volume plant^{-1} , flower number plant^{-1} , plant dry weight plant^{-1} , shoot dry weight plant^{-1} , root dry weight plant^{-1} , nitrate reductase activity, and leaf proline content. Whereas, it was significantly and negatively associated with pod number, test weight, chaffy pods percentage and root aluminium content.

The study on the morpho-physiological and

biochemical responses of pigeonpea in aluminium toxic soil of the North Eastern Hill (NEH) region demonstrated that high aluminium levels (100 μM Al and 225 μM Al) substantially impacted various plant characteristics. Under these stress conditions, significant reduction was observed in plant height plant^{-1} , number of branches plant^{-1} , root volume plant^{-1} , flower number plant^{-1} , plant dry weight plant^{-1} , shoot and root dry weights, nitrate reductase activity, pod number plant^{-1} , seed yield plant^{-1} , and test weight. In contrast, there was an increase in leaf proline content, chaffy pods percentage and aluminium concentration in root biomass compared to the control. These findings indicate that aluminium stress negatively affected the growth, physiological processes, and yield of pigeonpea. Interestingly, the genotypes PA-291, PA-640 and GT-101 showed lower aluminium accumulation in root biomass and performed better in other traits, indicating some tolerance to aluminium toxicity. This suggests potential for genetic enhancement through breeding programs aimed at improving aluminium tolerance. Therefore, genotypes PA-291, PA-640 and GT-101 can be further validated and potentially used in breeding programs to develop aluminium-tolerant pigeonpea varieties for cultivation in acidic soils, especially in North East India.

Table 1. Influence of Aluminium concentration on pigeonpea genotypes

Genotypes	Plant height plant (cm)	Number of branches plant	Root volume plant (cm)	Number of flowers plant	Plant dry weight plant(g)	Dry weight of shoot(g)	Dry weight of root(g)	Nitrate reductase activity (μM)	Proline content (nmol mg^{-1})	Number of pods plant	Seed yield plant (g)	Test weight (g)	Chaffy pods percentage in root biomass (μM)
PA-3	133.59	70.89	9.84	123.22	17.84	12.05	5.59	2.56	138.63	105.33	22.33	71.11	6.00
PA-291	151.35	73.22	12.68	131.22	23.96	15.88	7.07	2.90	169.55	120.78	29.24	71.52	3.13
PA-406	80.42	69.22	5.38	118.00	9.83	8.87	3.21	2.40	135.62	102.00	20.96	70.92	3.74
PA-414	99.03	69.67	6.86	122.89	12.57	9.69	3.81	2.72	158.92	104.89	20.78	70.12	4.45
PA-421	110.83	70.44	8.52	122.78	15.58	11.06	4.43	2.72	150.21	105.22	21.58	70.34	3.75
GT-101	94.33	70.33	7.25	108.56	13.10	9.40	3.90	2.72	146.06	91.56	21.24	69.24	5.19
PA-640	144.16	71.22	11.79	140.67	20.43	14.11	6.30	2.87	162.24	110.44	25.86	70.11	3.34
SE(m) \pm	1.86	0.27	0.15	1.20	0.23	0.27	0.10	0.01	0.45	1.01	0.33	0.16	0.18
CD at 5%	5.50	0.80	0.45	3.58	0.68	0.80	0.29	0.03	1.18	3.00	0.98	0.47	0.54
Concentrations													
Control(0 μM)	121.03	71.71	9.42	130.86	17.43	12.52	5.23	2.76	148.33	111.29	24.19	72.42	3.52
Al(100 μM)	115.36	70.62	8.96	123.24	16.23	11.65	4.91	2.70	151.29	105.19	23.17	71.85	4.04
Al(225 μM)	112.34	69.81	8.33	117.62	14.90	10.56	4.57	2.64	155.20	100.76	22.07	71.45	5.13
SE(m) \pm	1.156	0.181	0.099	0.791	0.156	0.182	0.065	0.003	0.296	0.661	0.154	0.110	0.120
CD at 5%	3.40	0.543	0.296	2.400	0.460	0.520	0.190	0.009	0.888	1.960	0.450	0.330	0.350
Interactions													
SE(m) \pm	1.49	0.48	0.26	2.09	0.41	0.48	0.17	0.01	0.78	1.75	0.40	0.29	0.31
CD at 5%	4.45	-	0.77	6.00	1.22	1.43	0.50	0.03	2.33	5.23	1.19	-	0.93

Table 2. Character association analysis of genotypes and Al treatments

Genotypes	Plant height plant ⁻¹ (cm)	Number of branches plant ⁻¹	Root volume plant ⁻¹ (cm ³)	Number of flowers plant ⁻¹	Plant dry weight of shoot (g) plant ⁻¹	Dry weight of root (g)	Nitrate reductase activity (μ M)	Proline content (nmol mg ⁻¹)	Numbers of pods plant ⁻¹	Chaffy pods (%)	Aluminium content in root biomass (μ M)	Test weight (g)	Seed yield plant (g)
Plant height (cm)	1.000	0.673**	0.980**	0.616**	0.940**	0.854**	0.972**	0.558**	0.578**	0.352 ^{NS}	-0.129 ^{NS}	-0.324 ^{NS}	0.798**
Number of branches plant ⁻¹		1.000	0.702**	0.420 ^{NS}	0.740**	0.735**	0.468*	0.116 ^{NS}	0.285 ^{NS}	-0.101 ^{NS}	-0.266 ^{NS}	0.128 ^{NS}	0.701**
Root volume (cm ³)			1.000	0.609**	0.954**	0.901**	0.719**	0.568**	0.519*	0.292 ^{NS}	-0.119 ^{NS}	-0.281 ^{NS}	0.848**
Number of flowers plant ⁻¹				1.000	0.565**	0.699**	0.613**	0.378 ^{NS}	0.860**	0.040 ^{NS}	0.105 ^{NS}	-0.471*	0.664**
Plant dry weight (g)					1.000	0.922**	0.759**	0.602**	0.427 ^{NS}	0.186 ^{NS}	-0.265 ^{NS}	-0.179 ^{NS}	0.894**
Dry weight of shoot (g)						1.000	0.687**	0.484*	0.526*	0.097 ^{NS}	-0.105 ^{NS}	-0.227 ^{NS}	0.914**
Dry weight of root (g)							0.750**	0.553**	0.473*	0.250 ^{NS}	-0.204 ^{NS}	-0.176 ^{NS}	0.865**
Nitrate reductase activity (μ M)								1.000	0.347 ^{NS}	-0.075 ^{NS}	-0.555**	0.010 ^{NS}	0.786**
Proline content (nmol.mg ⁻¹)									0.087 ^{NS}	0.038 ^{NS}	-0.365 ^{NS}	-0.279 ^{NS}	0.668**
Number of pods plant ⁻¹										1.000	0.321 ^{NS}	-0.543*	0.370 ^{NS}
Chaffy pods percentage											0.323 ^{NS}	-0.362 ^{NS}	-0.063 ^{NS}
Aluminium content in root biomass (μ M)												1.000	-0.514*
Test weight (g)													1.000
													-0.131 ^{NS}

*and** indicate significance at 5% and 1% levels, respectively

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