

Principal Component and Cluster Analysis for Salinity Tolerance in Pigeon pea Genotypes (*Cajanus cajan* L.)

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ABSTRACT: Pigeon pea (*Cajanus cajan* L.) is a vital legume crop cultivated in tropical and subtropical regions, is impacted by salinity stress. It is a major abiotic stress caused by excessive salt accumulation in soil, severely affects crop growth and physiological processes. This study evaluated 99 pigeon pea genotypes sourced from ICRISAT for their salinity tolerance at the germination and seedling stages under six treatment conditions: control (0 mM NaCl), hydropriming, and four salinity levels (50, 100, 150, and 200 mM NaCl). The growth parameters including germination percentage, root length, shoot length, fresh weight, dry weight, and vigour index were recorded to assess stress responses. The experiment was conducted under a completely randomized design with four replications and data analysis employed Principal Component Analysis (PCA) and dendrogram clustering. PCA revealed that salinity stress negatively impacted most growth parameters, with genotype that express variation at higher NaCl concentrations. The genotypes G₅(ICP2947), G₃ (ICP 7380), G₂₀ (ICP 14961), G₂ (ICP 7024), and G₁₀ (ICP 16184) consistently performed well, showing higher germination, vigour index, and seedling lengths, indicating strong tolerance. Dendrogram analysis clustered genotypes into distinct tolerance groups. Germination percentage and vigour index values are subjected to dendrogram analysis, because these parameters are the key components of assessing the salt tolerance in seedling stage. Two clusters were formed for the germination percentage parameter and four distinct clusters were formed for the vigour index of the seedlings. Overall, the study identified key pigeon pea genotypes with significant salinity tolerance, which are promising genotypes for inclusion in breeding programs targeting salt-affected regions.

Keywords: Pigeon pea, germination percentage, vigour index, principal component analysis, dendrogram clustering.

INTRODUCTION

Pigeon pea (*Cajanus cajan* L.) is an important food legume crop mostly cultivated in tropical and subtropical regions of the world (Joshi *et al.*, 2022). Leguminous crops are suitable to increase the bio-productivity and reclamation of marginal soils (FAO, 2023). Salinity in soil is an abiotic stress caused by excessive salt accumulation in the soil surface. Around 10% of both irrigated and rainfed crop land is affected by salinity stress (Sahu *et al.*, 2023). The uptake of potassium ions from the soil water is reduced and the uptake of Na⁺ and Cl⁻ ions get increased and this affects the ionic balance and can potentially reduce the production of many high-value crops.

Salinity stress has a negative impact on pigeon pea (*Cajanus cajan* L.), a significant legume that is grown to meet the protein needs of the human population (USDA, 2023). Unlike cereals, pigeon peas are naturally susceptible to salt and waterlogging stress. Due to ion toxicity, nutritional imbalance, and disrupted hormone interactions, salinity produces osmotic imbalance to tissues and it negatively impacts overall growth and development of plants. The effect of salinity on the pigeon pea causes leaf necrosis and chlorosis, which results in lower photosynthetic rate and leaf ageing (Ahmed *et al.*, 2020). The initial stress imposition is done by soaking the seeds in distilled water and the salt solution with various concentration of sodium chloride. Initial stress imposition, like applying

a controlled salinity level, allow researchers to study salinity tolerance by directly observing and quantifying plant responses to the stress, revealing mechanisms of tolerance and sensitivity.

The salt tolerance capability of a plant differs among species and development stages, determining its capacity to withstand stress and finish its life cycle (Bhatt *et al.*, 2024). By utilization of the genetic heterogeneity found in the germplasm, salt-tolerant lines can be found (Ahmed *et al.*, 2020). The production of salinity tolerant varieties will be a suitable strategy for reducing yield losses under salinity stress conditions. Increase in NaCl have been shown to decrease dry weight, shoot and root length, and germination percentage (Gore *et al.*, 2024). Excessive salt concentration is due to the accumulation of Na⁺ and Cl⁻ ions in plant tissues is the major impact caused by salinity (Tharageshwari and Hemavathy 2020).

The primary goal for crop establishment in pigeon pea is to identify the salt tolerant genotypes, at early growth stages (seedling stages). In order to explore the potential for salt tolerance, the current study was conducted by evaluating the genotypes of pigeon pea in terms of morphological parameters at various salinity levels of 0 mM NaCl, hydropriming, 50 mM NaCl, 100 mM NaCl, 150 mM NaCl and 200 mM NaCl concentration at the germination. The variability in salt tolerance of the genotypes can be identified using the cluster analysis and principal component analysis (Hari Prasanth *et al.*, 2023).

MATERIALS AND METHODS

A. Site of experiment and design

The experiment was performed in the laboratory of Genetics and Plant Breeding, in association with Seed Science and Technology, SRM College of Agricultural Sciences, SRM Institute of Science and Technology at Chennai, Tamil Nadu. Ninety-nine Pigeon pea genotypes were sourced from International Crop Research Institute for Semi-Arid Tropics (ICRISAT, Hyderabad) and the genotypes are evaluated for salinity stress tolerance.

B. Treatment details

The treatments include a control group with 0 mM NaCl as T₀ and hydropriming was done using distilled water for T₁. At various concentrations of NaCl, the treatments T₂, T₃, T₄, T₅ denote 50 mM NaCl, 100 mM NaCl, 150 mM NaCl and 200 mM of NaCl respectively. The sterilized sand is filled in plastic trays was arranged according to the Factorial Completely Randomized Design (FCRD) with four replicates.

The genotypes are exposed to initial stress by soaking the seeds for three hours in distilled water and NaCl solutions according to the treatment. For each genotype, 25 seeds are soaked for every treatment. During the experiment, the germination data was recorded, which helped in evaluating the seed characters and their response to salt stress at the seedling stages. The parameters observed for studying the salt stress tolerance in this research include root length, shoot length, germination percentage, fresh weight, dry weight and vigour index.

C. Observations recorded

According to the International Seed Testing Association guidelines (ISTA, 2012), the experiment was carried out with notable results. The germination (%) was calculated on the 10th day of emergence, when the final counts were taken. On the 10th day of emergence, ten seedlings in healthy condition were taken out from each replication, for measuring the root length. It is denoted in 'cm'. On the 10th day of emergence, ten seedlings in healthy condition were taken out from each replication, for measuring the shoot length. It is denoted in 'cm'. For computing the fresh weight, on the 10th day of emergence, ten healthy seedlings were taken out from each replication. It is denoted in 'mg 10 seedlings⁻¹'. The ten healthy and uprooted seedlings were shade dried for 24 hours to compute the final root and shoot biomass. The dried seedlings were kept in hot air oven for 20 hours, at 70°C and then weighed and it is denoted in 'mg 10 seedlings⁻¹'. The total seedling length is calculated by adding the values of root length and shoot length. It is expressed in 'cm'. The vigor index values were determined using the given formula, and the average values were represented as whole numbers (Abdul-Baki and Anderson 1973).

D. Statistical Analysis

The pigeon pea genotypes and different salinity levels were treated as two independent factors with Principal Component Analysis (PCA) and dendrogram. The statistical analysis was done using SPSS version 16.0 (SPSS Inc., Chicago, USA). This is done by converting the original variables into a new set of uncorrelated variables referred to as principal components, where each component records the direction of maximum variance (Jolliffe and Cadima 2016). In this study, biplots are obtained based on the described methodology (Gabriel, 1971) were produced separately for each level of salinity and highlighted the major factors that were responsible for the large majority of the variability. Dendrogram provides a pictorial representation of clusters, where the genotypes which contribute to the variability are grouped according to the extent of its contribution (Hari Prasanth *et al.*, 2023).

RESULTS AND DISCUSSION

A. Principal Component Analysis (PCA)

The Principal Component Analysis (PCA) is used to find the divergence between various treatments, across all the genotypes. PCA measures divergence using the concept of spatial distance, through pictorial representation (Kanwar *et al.*, 2020). The PCA was conducted to assess the contribution of growth parameters of pigeon pea, under six different levels of salinity: Control (T₀), hydropriming (T₁), 50 mM NaCl (T₂), 100 mM NaCl (T₃), 150 mM NaCl (T₄), 200 mM NaCl (T₅).

The principal components PC₁ & PC₂ showed the cumulative variation of 67.01% in the control (T₀), with 47.11% and 19.90% of the variance respectively in control (T₀). The early-stage growth parameters like germination percentage and vigour index show greater values of differentiation along PC₁, across the

genotypes G₅, G₂₀, G₃, G₂ and G₁₀. Due to non-stress conditions, there is a lack of differential performance and uniform expression of traits. The shift in genotype positioning along PC₁ and P₂ in hydropriming treatment, indicates moderate trait divergence. The PC₁-60.23% and PC₂- 22.27% captured 82.51% of cumulative variation. The negative trait loading values across PC₁ and PC₂ indicate that there is a metabolism difference (Afzal *et al.*, 2023). The hydropriming facilitates enzyme activation, improved germination and membrane repair due to increased rate of imbibition of water (Nedunchezhiyan *et al.*, 2020).

At 50 mM NaCl (T₂), Germination percentage, vigour index, total seedlings length and shoot length contributed to PC₁. The genotype G₅, G₃, G₂₀, G₂ and G₁₀ along PC₁ showed tolerance to salinity. The component PC₁ explained 69.42% and PC₂ explained 10.14%. The dry weight of seedlings had high loading value in PC₂. The biomass accumulation in terms of dry weight, under mild stress is an important factor for the notable performance (Shashibhushan *et al.*, 2022). The principal component PC₁ & PC₂ explained 79.17% and 8.13% respectively at 100 mM NaCl (T₃). It contributes for 87.30% of variation. The traits including germination percentage, vigour index, shoot length and total seedling length maintained positive contributions to PC₁. The osmotic stress produced due to increased uptake Na⁺ and Cl⁻ might pose negative effects on the metabolic and structural stability (Ahmed *et al.*, 2020). Under 150 mM NaCl (T₄), principal component analysis revealed PC₁ and PC₂ explained 76.68% and 10.74% of variability. Dry weight exhibited strong negative loading in PC₂. The positive influence of vigour index and total seedling length suggests that seedling vigour was due to altered dilution mechanisms in the membranes and cellular components (Ouma *et al.*, 2024). The physiological processes that aid in the growth and development of the seedlings were affected in the higher levels of salinity (Dar *et al.*, 2024).

At the highest concentration of 200mM NaCl (T₅), the principal components PC₁ and PC₂ accounted for 80.46% cumulatively. The growth parameters like germination percentage, shoot length and total seedling length contributed positively to PC₁. The root length emerged as the influential trait in PC₂. The stress tolerance mechanisms were reflected in G₅, G₂₀, G₃, G₂ & G₁₀. The tolerant genotypes exhibited an avoidance mechanism to the salt accumulation and water access was maintained. The PCA biplot at this level showed the most dispersed genotype distribution, signifying maximal divergence in seedling responses (Mukhopadhyay *et al.*, 2021). Majority of the genotypes did not to maintain balance among the growth parameters, but the genotypes with higher germination percentage, total seedling length and root length stood apart as potentially highly salt-tolerant. The highly salt tolerant genotypes were identified and can be further recommended for breeding programs (Mukhopadhyay *et al.*, 2021).

B. Dendrogram

Clustering dendrogram is a diagrammatic representation of hierarchical clustering. It is used to

group the parameters based on similarities in their traits. The dendrogram provide classification of genotypes into tolerant, moderately tolerant and susceptible groups (Kalyan *et al.*, n.d.). The higher divergence in the dendrogram indicates the dissimilarity and moderate divergence indicates that there is absence of extreme differences among the pigeon pea genotypes across various treatments. The germination percentage and vigour index were subjected to dendrogram clustering, because they revealed the establishment of seedlings, upon exposure to NaCl at different compositions (Mishra *et al.*, 2024).

The cluster I (red colored) comprise a comparatively smaller group of genotypes, that exhibited similarity. The genotypes G₅, G₃, G₂₀, G₂, G₁₀, G₄₈, G₈, G₅₀, G₄ & G₁₉ showed highest germination percentage amongst other genotypes. All the other genotypes were grouped in cluster II (bluish green color). This cluster represented the larger and heterogenous group of genotypes with more internal variations. The cluster II constitutes moderately tolerant to sensitive genotypes. At increasing levels of salinity, the germination percentage reduced due to osmotic stress & ionic stress. This has in turn increased the time taken for the seed to germinate (Alagupalamuthirsolai and Vijayabharathi 2020).

The dendrogram of vigour index showed that the genotypes can form 4 clusters based on their salt tolerance ability (Fig. 3). The cluster I (red color) included only three genotypes G₅, G₃ and G₂₀ showed grater values of vigour index across all levels of induce salinity stress. The cluster II (green color) comprises of G₂, G₁₀, G₄₈, G₄, G₅₀, G₈ & G₁₉ which are also considered to thrive well even under induced saline conditions. The above-mentioned clusters exhibit highest vigour index values under salinity due to mechanisms like ionic homeostasis, anti-oxidant defense mechanisms. The proper functioning of enzymes was maintained by appropriate ratio of potassium and sodium uptake (Ditta and Ullah 2023).

The cluster III (bluish green) represented the moderately salt tolerant genotypes, which were slightly sensitive to the salinity levels. The cluster IV (purple) consisted of genotypes that were sensitive to higher salinity levels and this cluster showed maximum divergence with significantly lower vigour index. The salt sensitivity inhibits the germination, due to imbalances of ions and the reduced water uptake (Kimaro *et al.*, 2021).

The shift from a two-cluster (Fig. 2) to a four-cluster (Fig. 3) classification provides increased resolution in genotype discrimination. The genotypes in Clusters I and II (Fig. 3) are prime candidates for advancement in breeding programs and field trials. The moderate performers are cluster III genotypes can be further evaluated under variable salinity levels to confirm stability. The cluster IV genotypes may be excluded from breeding pools but can serve as checks or controls in stress studies. When mapped against individual growth parameter, these clusters can be quantitatively validated to select the top performers for salt-affected regions (Tanwar *et al.*, 2023).

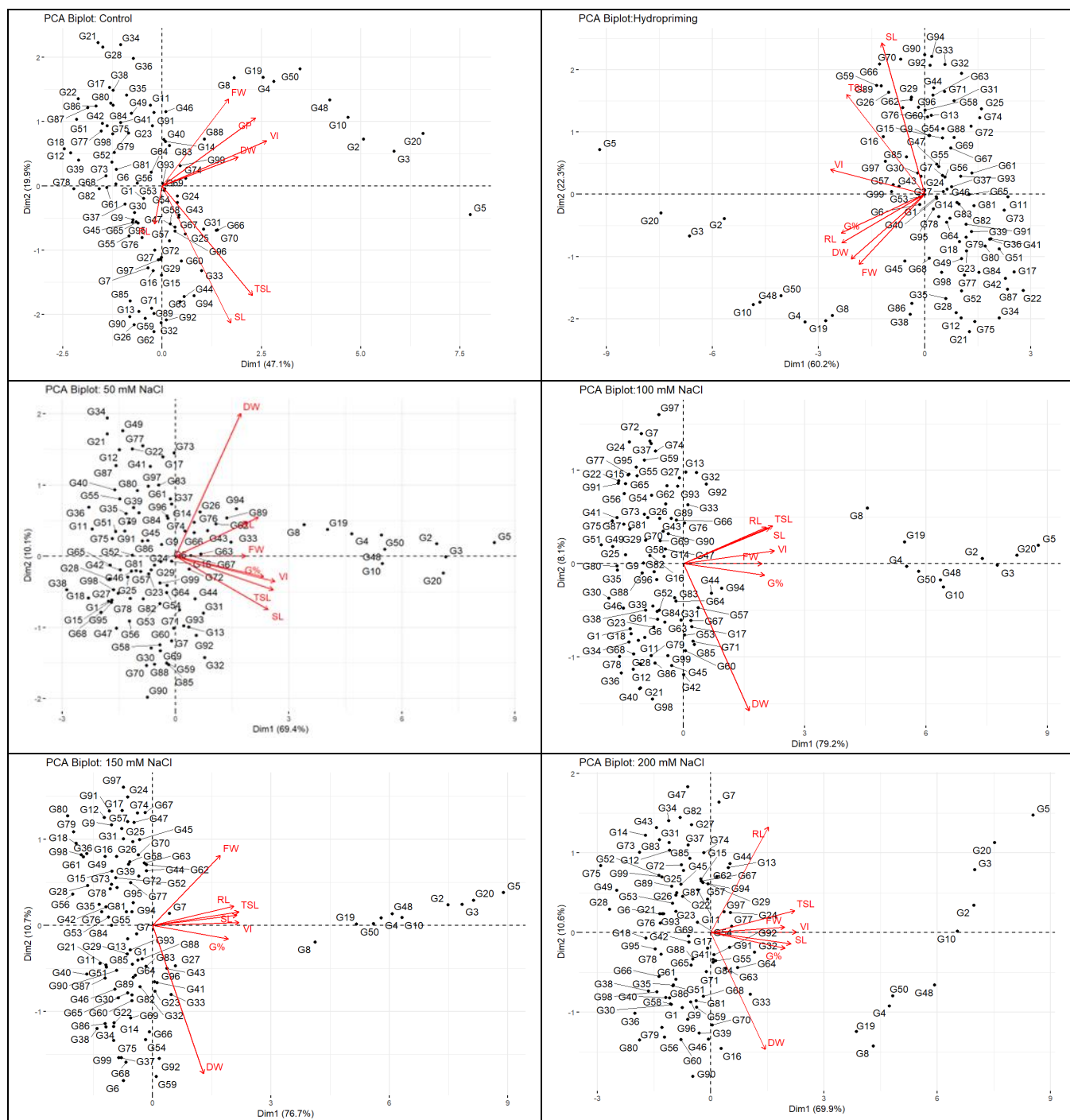


Fig. 1. Principal component biplot graphs of Pigeon pea genotypes under various levels of salinity treatments - Control (T0), Hydropriming (T1), and 50 mM NaCl (T2), 100 mM NaCl (T3), 150mM NaCl (T4) and 200mM NaCl (T5). The observed growth parameters include germination percentage (G%), shoot length (SL), root length (RL), total seedling length (TSL), fresh weight (FW), and dry weight (DW).

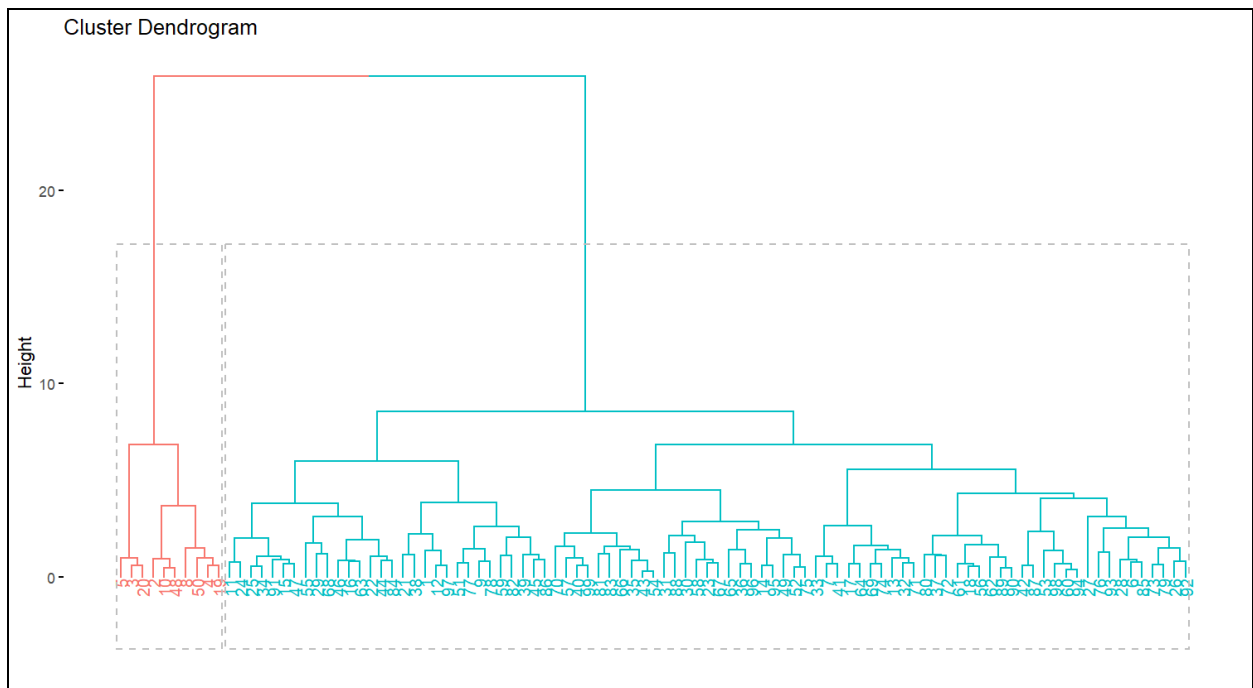


Fig. 2. Cluster dendrogram of germination percentage of pigeon pea genotypes under various levels of salinity: Control (T_0), Hydropriming (T_1), and 50 mM NaCl (T_2), 100 mM NaCl (T_3), 150 mM NaCl (T_4) and 200 mM NaCl (T_5).

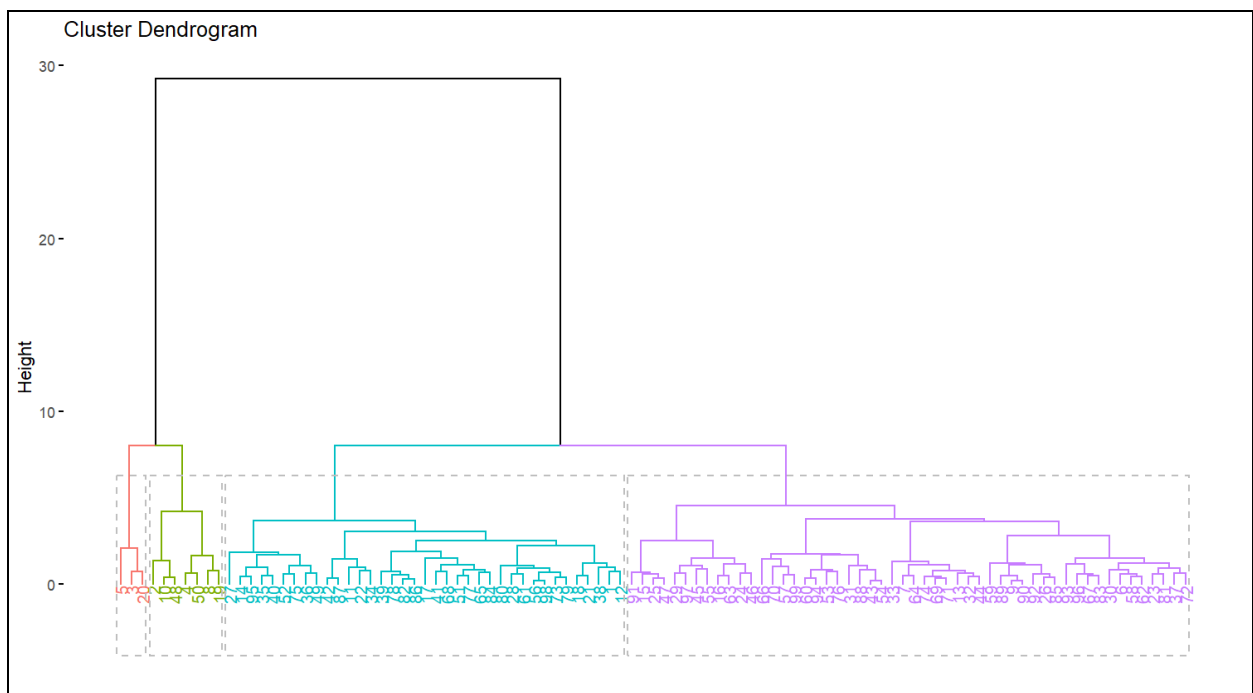


Fig. 3. Cluster dendrogram of vigour index of pigeon pea genotypes under various levels of salinity: Control (T_0), Hydropriming (T_1), and 50 mM NaCl (T_2), 100 mM NaCl (T_3), 150mM NaCl (T_4) and 200mM NaCl (T_5).

Table 1: Principal factors of principal component analysis and their eigenvalues, variability and cumulative variability for control, hydropriming and four different salinity treatments.

Variable		PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	Control	1.81	1.18	1.03	0.81	0.72	0.20
	Hydropriming	2.05	1.24	0.74	0.60	0.55	0.02
	50mM	2.20	0.84	0.77	0.67	0.60	0.02
	100mM	2.35	0.75	0.62	0.52	0.47	0.02
	150mM	2.31	0.86	0.69	0.48	0.39	0.03
	200mM	2.21	0.86	0.76	0.69	0.54	0.03
Variability (%)	Control	47.11	19.90	15.16	9.53	7.54	2.08
	Hydropriming	60.23	22.27	7.87	5.25	4.36	0.00
	50mM	69.42	10.14	8.64	6.50	5.27	0.00
	100mM	79.17	8.13	5.54	3.92	3.20	0.00
	150mM	76.68	10.74	6.95	3.36	2.25	0.00
	200mM	69.85	10.61	8.39	6.89	5.44	3.34
Cumulative variability (%)	Control	47.11	67.01	82.16	91.69	99.24	99.86
	Hydropriming	60.23	82.51	90.37	95.63	99.99	100.00
	50mM	69.42	79.56	88.20	94.71	99.99	100.00
	100mM	79.17	87.30	92.85	96.78	99.98	100.00
	150mM	76.68	87.41	94.36	97.72	99.98	100.00
	200mM	69.85	80.46	88.85	95.74	99.98	100.00

Table 2: Contribution of morphological and physiological traits in the principal factors under different salinity treatments (principal component loading values).

Character	Control		Hydropriming		50mM		100mM		150mM		200mM	
	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2
G%	0.45	0.31	-0.41	-0.17	0.38	-0.12	0.37	-0.07	0.37	-0.08	0.38	-0.09
RL	-0.03	-0.17	-0.41	-0.22	0.36	0.23	0.37	0.22	0.39	0.11	0.29	0.66
SL	0.33	-0.63	-0.21	0.70	0.40	-0.32	0.39	0.22	0.41	0.06	0.41	-0.06
TSL	0.43	-0.50	-0.38	0.46	0.42	-0.20	0.40	0.23	0.42	0.08	0.43	0.13
VI	0.50	0.20	-0.46	0.11	0.43	-0.15	0.41	0.07	0.42	0.01	0.43	0.00
FW	0.32	0.39	-0.32	-0.32	0.30	0.00	0.36	-0.00	0.33	0.42	0.37	0.03
DW	0.36	0.13	-0.36	-0.29	0.28	0.86	0.30	-0.91	0.25	-0.89	0.27	-0.72

CONCLUSIONS

The present study evaluated the salinity tolerance of 99 pigeon pea (*Cajanus cajan* L.) genotypes under varying levels of NaCl stress using morphological parameters at the germination and seedling stages. The findings revealed significant genotypic variation in response to salinity, with increased salt concentrations leading to a general decline in germination percentage, shoot and root length, fresh and dry weight, and vigour index. Principal Component Analysis (PCA) effectively computed the variability and highlighted the key growth parameters contributing to salt tolerance across different salinity treatments. Genotypes such as G₅ (ICP 2947), G₃ (ICP 7380), G₂₀ (ICP 14961), G₂ (ICP 7024), and G₁₀ (ICP 16184) consistently demonstrated superior performance under saline conditions, indicating their potential for tolerance and adaptability. Dendrogram clustering further categorized the genotypes into tolerant, moderately tolerant, and sensitive groups, providing a clearer understanding of genotype performance and aiding in selection for breeding programs. The integration of PCA and dendrogram clustering approaches provided a robust framework for identifying salt-tolerant genotypes. These tolerant lines offer valuable genetic resources for the development of pigeon pea varieties suited to salt-

affected regions, contributing to improved crop productivity and resilience in marginal environments.

FUTURE SCOPE

This study lays the foundation for developing salt-tolerant pigeon pea varieties. Future research should focus on field trials to validate the performance of identified tolerant genotypes under natural saline conditions. Molecular and physiological studies are needed to uncover the genetic basis of salinity tolerance, enabling marker-assisted selection. Advanced phenotyping and biochemical assays can provide deeper insights into stress response mechanisms. The identified genotypes can be used in breeding programs to develop high-yielding, salt-resilient cultivars. These efforts will support sustainable agriculture and improve pigeon pea productivity in salt-affected areas, contributing to food security and climate-resilient farming systems.

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Conflicts of Interest. None.

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