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Deciphering Salinity Tolerance in Mungbean (*Vigna radiata* L.) through Root System Architecture, Multivariate Divergence Analysis and Multi-trait Ideotype Selection

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ABSTRACT

Background: Soil salinity is a major abiotic constraint on mung bean (*Vigna radiata* L.) productivity. Breeding resilient varieties requires an understanding of genetic diversity and the identification of salt-tolerant genotypes, with root system architecture being a key determinant of adaptation to saline conditions.

Methods: The experiment was conducted at Lovely Professional University, Punjab, using a factorial randomized block design (FRBD) with three replications. Root architecture was examined *via* scanning, while genetic divergence was assessed using Mahalanobis D^2 cluster analysis and principal component analysis. Genotype screening employed nine stress tolerance indices along with the multi-trait genotype Ideotype distance index (MGIDI).

Result: Root structure progressively deteriorated with increasing salinity, with KDMB-5023 and KDMB-5025 showing relatively greater structural resilience. Mahalanobis D^2 analysis grouped genotypes into four clusters, with the maximum inter-cluster distance observed between Cluster I and Cluster IV (D^2 = 8.498). Principal component analysis retained three components explaining 92.40% of total variation, with plant height, shoot length, vigour index and leaf area collectively contributing 82.47% of genetic divergence. Multi-index stress tolerance analysis identified KDMB-5020, KDMB-5030, KDMB-5014, KDMB-5025 and KDMB-5001 as the most tolerant genotypes, while MGIDI ranked KDMB-5001 (3.210), KDMB-5018 (3.459) and KDMB-5011 (3.500) as genotypes closest to the ideal type, indicating substantial potential gains through selection.

Key words: Ideotype selection, Mahalanobis D^2, MGIDI, PCA, Root system architecture, Salinity stress, Stress tolerance indices, *Vigna radiata*.

INTRODUCTION

Soil salinity is one of the most devastating abiotic stresses limiting global agricultural productivity, affecting over 833 million hectares (~8.7% of Earth's surface) with annual economic losses of US$ 27.3 billion (FAO-GSP, 2021; Shokri *et al*., 2024). It threatens 20% of cultivated and 33% of irrigated land globally, with escalation projected by 2050 due to climate change and poor irrigation practices (Negacz *et al*., 2022). In South Asia, expanding salt-affected soils threaten pulse crop sustainability critical to regional food security (Kumar and Sharma, 2020). Mungbean [*Vigna radiata* (L.) Wilczek], a short-duration legume cultivated on ~7.3 million hectares globally (India contributing ~41% of output), is rich in digestible protein (22-27%), fiber and micronutrients, making it vital for addressing malnutrition (Gayacharan *et al*., 2026). As a salt-sensitive glycophyte, mungbean is highly susceptible to salinity stress, which adversely affects seed germination, root growth, nodulation and photosynthetic activity, ultimately leading to significant reductions in yield (Chaurasia *et al*., 2024). Tolerance is linked to restricted Na^+/Cl^- transport and maintained K^+ levels in leaf mesophyll cells (Iqbal *et al*., 2024). Root system architecture (RSA) is a key adaptive trait for ion exclusion and water absorption, with root length, surface area and volume closely tied to salt tolerance (Farooq *et al*., 2024). However, RSA characterization integrated with genetic diversity and ideotype-based selection remains underexplored in mungbean.

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Mahalanobis D^2 and cluster analysis are widely used to assess genetic divergence and assist in selecting suitable parents for hybridization (Bužinskienė *et al*., 2023) while PCA identifies discriminating trait. Stress tolerance indices classify genotypes based on their yield performance under stress and non-stress conditions (Fischer and Maurer, 1978; Fernandez, 1992; Kaur *et al*., 2022; Patil *et al*., 2025; Askari *et al*., 2017), while MGIDI facilitates simultaneous selection for multiple traits (Olivoto and Nardino, 2021) and has been applied in the evaluation of diverse crop species (Pallavi *et al*., 2024; Suwardi *et al*., 2026). Despite these tools, an integrated framework combining RSA, D^2 clustering, PCA, stress indices and MGIDI for salt-tolerant mungbean screening is lacking. This study therefore aimed to characterize root architecture under salinity, evaluate genetic variability in RSA and agro-morphological traits, assess divergence via D^2 clustering and PCA, identify key discriminating traits, classify genotypes using stress indices and apply MGIDI for multi-trait ideotype-based selection of salt-tolerant genotypes.

MATERIALS AND METHODS

Experimental site and plant material

The experiment was conducted at the Agriculture Research Farm, Department of Genetics and Plant Breeding, Lovely Professional University (LPU), Phagwara, Punjab, India, using twenty diverse mungbean (*Vigna radiata* L.) genotypes evaluated for salinity tolerance at the seedling stage during the two-year Master's research programme.

Experimental design and salinity treatments

A factorial randomized block design (FRBD) with three replications was used, with each genotype evaluated under three NaCl levels: T1 (50 mM, low), T2 (100 mM, medium) and T3 (150 mM, high).

Seed germination and transplanting

Uniform seeds were germinated in a growth chamber on 1st November 2025, with germination monitored over four days. Seedlings were transplanted on 5th November 2025 into coco peat-filled plastic cups under controlled conditions.

Nutrient management and salinity imposition

Half-strength Hoagland solution was applied on 5th, 7th, 8th and 11th November to aid establishment. Salinity stress was then imposed from 13th November using full-strength Hoagland solution supplemented with NaCl at the designated concentrations, applied on 13th, 15th, 17th, 19th and 21st November.

Trait recording

Twelve traits were recorded post-stress: root length, shoot length, plant height, leaf area, Primary branches, shoot/root/total fresh weight, shoot/root/total dry weight and vigour Index I.

Statistical analysis

Genetic divergence was assessed *via* Mahalanobis' D^2 statistics (Rao, 1952) with Tocher's clustering method and PCA was performed on standardized trait data (Pearson, 1901) to identify key discriminating traits. Nine stress tolerance indices were calculated using vigour index values under non-stress (T1) and high-stress (T3) conditions. MGIDI was applied for simultaneous multi-trait selection (Olivoto and Nardino, 2021), with lower values indicating closer proximity to the ideotype. All analyses were performed in R using the metan package (Olivoto and Lúcio, 2020).

RESULTS AND DISCUSSION

Root system architecture under salt stress

Root scanning revealed clear genotypic differences in root morphology across salinity levels (Fig 1). Under low salinity (50 mM), KDMB-5004, KDMB-5005, KDMB-5011, KDMB-5023 and KDMB-5025 showed well-developed roots with uniform thickness and visible lateral emergence, indicating mild stress impact. Under high salinity (150 mM), KDMB-5010, KDMB-5011, KDMB-5014, KDMB-5023 and KDMB-5025 exhibited pronounced alterations root twisting, surface cracking and cortical fragmentation though KDMB-5023 and KDMB-5025 retained relatively better structural organization, indicating superior resilience. This aligns with known glycophyte responses to NaCl-induced osmotic and ionic stress, consistent with Chaurasia *et al.* (2024), who reported progressive declines in root volume, surface area and tip number with increasing NaCl, alongside tolerance indices ranging from 0.38 to 0.91 across accessions. The epidermal and cortical damage reflects reduced cell turgor and impaired expansion, restricting absorptive capacity (Farooq *et al*., 2024). Iqbal *et al.* (2024) linked mungbean salt tolerance to restricted root Na^+ transport and sustained K^+ retention and the resilience of KDMB-5023 and KDMB-5025 likely reflects similar mechanisms, supporting vascular continuity and water/assimilate transport under salinity (Munns and Tester, 2008).

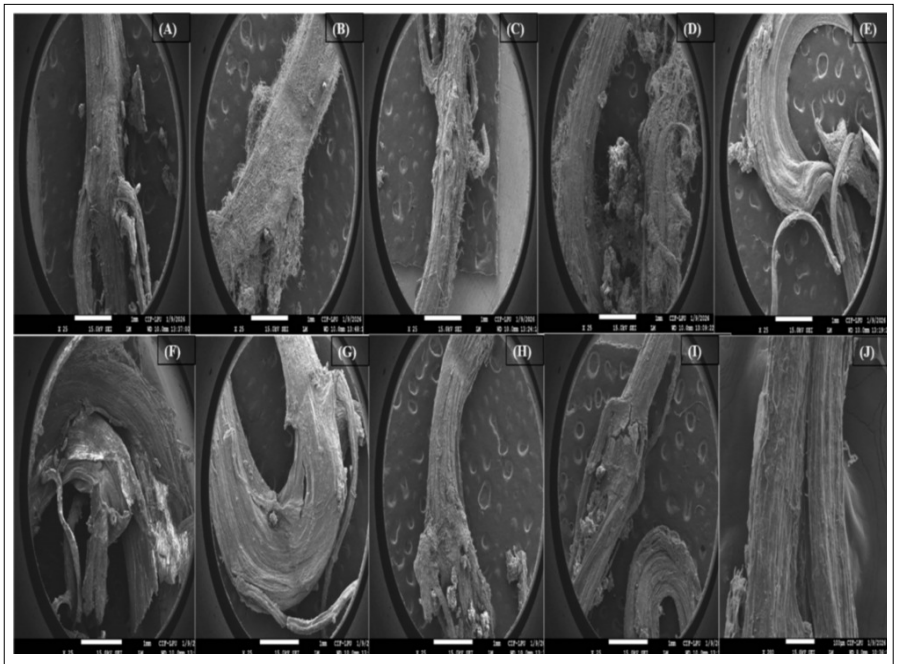
Cluster analysis (Mahalanobis D^2)

Grouping of genotypes into clusters

Mahalanobis D^2 cluster analysis grouped the 20 mungbean genotypes into four distinct clusters, reflecting considerable genetic diversity among the evaluated material (Table 1; Fig 2). Cluster II was the largest, comprising 11 genotypes (KDMB-5009, KDMB-5010, KDMB-5012, KDMB-5013, KDMB-5014, KDMB-5017, KDMB-5023, KDMB-5025, KDMB-5030, KDMB-5003 and KDMB-5006), indicating a high degree of similarity among these genotypes for the studied traits. Cluster I contained six genotypes (KDMB-5004, KDMB-5007, KDMB-5015, KDMB-5020, KDMB-5028 and KDMB-5005), while Cluster IV comprised two genotypes (KDMB-5011 and KDMB-5018). Cluster III was a monogenotypic cluster containing only KDMB-5001, indicating its high genetic divergence from all other genotypes.

Cluster mean values

Cluster mean analysis revealed substantial variation across clusters (Table 2). Cluster IV showed the highest



Root architecture under low salinity (50 mM NaCl) for (A) KDMB-5004, (B) KDMB-5005, (C) KDMB-5011, (D) KDMB-5023 and (E) KDMB-5025 and root architecture under high salinity (150 mM NaCl) for (F) KDMB-5010, (G) KDMB-5011, (H) KDMB-5014, (I) KDMB-5023 and (J) KDMB-5025.

Fig 1: Field emission scanning electron microscopy (FESEM) images of roots from selected mungbean genotypes (*Vigna radiata* L.) genotypes under contrasting salinity levels.

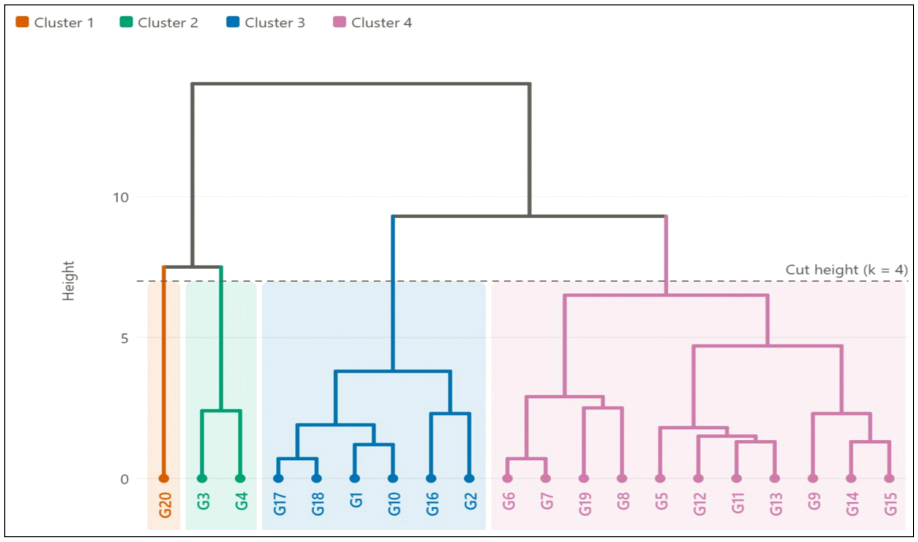


Fig 2: Hierarchical clustering of mungbean genotypes.

Table 1: Distribution of 20 mungbean genotypes into clusters based on Mahalanobis D^2 analysis.

Cluster	Genotypes
I	KDMB-5004, KDMB-5007, KDMB-5015, KDMB-5020, KDMB-5028, KDMB-5005
II	KDMB-5009, KDMB-5010, KDMB-5012, KDMB-5013, KDMB-5014, KDMB-5017, KDMB-5023, KDMB-5025, KDMB-5030, KDMB-5003, KDMB-5006
III	KDMB-5001
IV	KDMB-5011, KDMB-5018

means for root and shoot growth traits root length (6.21 cm), shoot length (18.13 cm), plant height (24.34 cm), root fresh weight (0.72 g), shoot dry weight (0.64 g), total dry weight (0.80 g) and vigour index (16.33) indicating superior vigour under salinity. Cluster III led in leaf area, primary branches, fresh weight and root dry weight, reflecting stronger biomass accumulation. Cluster II showed intermediate performance, while Cluster I recorded the lowest values overall, including vigour index (8.79), indicating poor salinity performance.

Inter- and intra-cluster distances

Intra-cluster distances ranged from 1.61 (Cluster III) to 2.89 (Cluster II), reflecting greater within-cluster variability in larger clusters. Inter-cluster distances ranged from 3.33 (Clusters I-II) to 8.49 (Clusters I-IV), indicating maximum divergence between Clusters I and IV, followed by Clusters I and III (8.34) (Table 3). The markedly larger inter- versus intra-cluster distances confirm substantial genetic diversity among the evaluated genotypes.

Clustering of 20 mungbean genotypes into four groups confirmed substantial genetic diversity, consistent with Meena *et al.* (2025), whose D^2-based clustering and PCA (first three components >62% variation) showed similar results. KDMB-5001's monogenotypic cluster reflects high divergence, aligning with Rahangdale *et al.* (2023). The maximum inter-cluster distance (Clusters I-IV, $D^2 = 8.49$) suggests these crosses would maximize recombination and yield superior segregants, supporting Bužinskienė *et al.* (2023), who linked distant-cluster crosses to high heterosis. Superior growth/vigour traits in Cluster IV and biomass traits in Cluster III mark them as promising parents, consistent with Iqbal *et al.* (2024).

Principal component analysis

PCA retained three components (Eigenvalue >1.0) explaining 92.40% of total variation (Table 4; Fig 3A). PC1 (62.58%) showed strong positive loadings for shoot length, plant height, root dry weight, total dry weight and vigour index, representing overall seedling growth under stress. PC2 (15.85%) was linked to primary branches and fresh weight traits, with root length contributing negatively. PC3 (13.95%) reflected dry matter accumulation (shoot/total dry weight, root fresh weight), with negative loadings for leaf area and primary branches. The biplot (Fig 3B, C) identified KDMB-5010 (G20) as a distinct outlier with exceptional biomass, while KDMB-5018 and KDMB-5011 showed superior shoot growth and vigour. KDMB-5005, KDMB-5020, KDMB-5028, KDMB-5007 and KDMB-5009 clustered in the negative quadrant, indicating poor performance. This high variance capture confirms strong trait interdependency with PC1's dominance aligning with Azam *et al.* (2023) and PC2/PC3 patterns mirroring osmotic-ionic stress partitioning (Iqbal *et al.*, 2024). The biplot separation corroborates cluster findings, consistent with Sivakumar *et al.* (2020). Plant height contributed most to genetic divergence (32.50%), followed by shoot length (18.87%), vigour index (15.90%) and leaf area (15.21%) (Fig 3D), with total fresh weight, shoot fresh weight and root length contributing moderately (3.10-7.42%); remaining traits were negligible (≤0.08%). Together, plant height, shoot length, vigour index and leaf area accounted for 82.47% of divergence, aligning with Chaurasia *et al.* (2024). The negligible dry-weight contribution suggests shoot

Table 2: Cluster mean values for twelve traits in mungbean genotypes under salinity stress.

Cluster	I	II	III	IV
RL	3.85	5.71	4.73	6.21
SL	8.70	13.01	17.03	18.13
PH	12.42	18.73	21.77	24.34
LA	4.01	6.73	11.53	9.21
PB	1.07	1.02	1.33	1.00
SFW	0.57	1.21	8.71	3.96
RFW	0.17	0.32	0.26	0.72
TFW	0.74	1.52	8.97	4.68
SDW	0.08	0.18	0.20	0.64
RDW	0.03	0.06	0.16	0.16
TDW	0.10	0.24	0.36	0.80
VI	8.79	13.22	13.99	16.33

Table 3: Intra-(diagonal) and inter-cluster (off-diagonal) D^2 distances among four clusters of mungbean genotypes.

Cluster	I	II	III	IV
I	2.08	3.33	8.34	8.49
II	3.33	2.89	6.86	5.72
III	8.34	6.86	1.61	6.37
IV	8.49	5.72	6.37	2.23

Diagonal values = intra-cluster distances; off-diagonal values = inter-cluster distances.

Table 4: Eigenvalues, percentage variance, cumulative variance and trait loadings for the first three principal components.

PC's	PC1	PC2	PC3
Eigenvalue	7.51	1.90	1.67
Variance %	62.58	15.85	13.9
Cumulative.variance. %	62.58	78.43	92.39
Loadings	PC1	PC2	PC3
RL	0.21	-0.44	-0.33
SL	0.35	-0.06	-0.11
PH	0.33	-0.19	-0.18
LA	0.25	-0.06	-0.45
PB	0.03	0.51	-0.43
SFW	0.27	0.45	-0.04
RFW	0.30	-0.14	0.32
TFW	0.29	0.41	-0.01
SDW	0.30	-0.02	0.39
RDW	0.32	0.18	0.15
TDW	0.31	0.01	0.35
VI	0.31	-0.25	-0.19

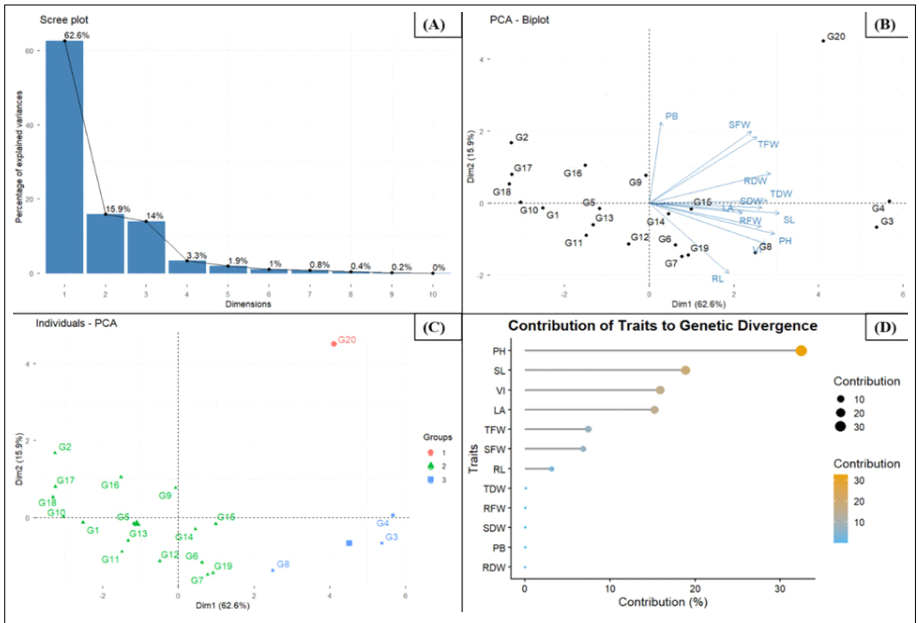
morphology drives inter-genotypic discrimination, while dry matter differences manifest within salinity levels, consistent with Talakayala *et al.* (2025). These findings support plant height, shoot length, vigour index and leaf area as efficient, non-destructive selection criteria for early-generation salinity tolerance breeding in mungbean.

Stress tolerance indices

Considerable genotypic variation was observed across all nine stress indices. KDMB-5018, KDMB-5025, KDMB-5030 and KDMB-5013 consistently showed high STI, GMP, YSI, YI and HM values, indicating stable performance under salinity, while KDMB-5006 recorded the lowest SSI (-0.83) and SSPI (-9.43), confirming its tolerance. Conversely, KDMB-5017, KDMB-5005 and KDMB-5028 exhibited high SSI, TOL and SSPI, indicating greater susceptibility. Based on mean ranks across all indices, KDMB-5020 emerged as the most tolerant genotype (mean rank: 6.33), followed by KDMB-5030 (7.00), KDMB-5014 (7.11), KDMB-5025 (7.22) and KDMB-5001 (7.89) (Fig 4). The most susceptible were KDMB-5010 (17.56) and KDMB-5007 (17.44). This multi-index approach confirmed that no single index fully captures salinity tolerance, reinforcing the value of integrating complementary indices consistent with Sarkar *et al.* (2025), who showed that combining multiple indices with PCA and cluster analysis improves screening reliability in lentil under abiotic stress. The superior STI, GMP and HM values of KDMB-5018 and KDMB-5025 indicate genotypes sustaining productivity under both stress and non-stress conditions, a key criterion for variety development (Fernandez, 1992). This aligns with Pour *et al.* (2019), who established that high STI, MP, GMP and HM identify genuinely stress-tolerant, high-yielding genotypes, while SSI > 1 denotes above-average susceptibility correctly classifying KDMB-5017, KDMB-5005 and KDMB-5028 as susceptible here. The negative SSI and SSPI of KDMB-5006 suggest possible stress-induced compensatory responses, warranting further investigation.

Multi-trait genotype ideotype distance index (MGIDI)

MGIDI analysis revealed substantial variation in multi-trait performance under salinity (Fig 5A). KDMB-5001 recorded the lowest MGIDI value (3.210), ranking first as closest to the ideotype, followed by KDMB-5018 (3.459) and KDMB-5011 (3.500). Other relatively low-scoring genotypes (4.640-4.780) included KDMB-5017, KDMB-5010, KDMB-5003, KDMB-5023, KDMB-5013 and KDMB-5025, while KDMB-5006, KDMB-5015, KDMB-5007, KDMB-5005 and KDMB-5020 (5.307-5.482) showed the greatest deviation from the ideotype. Selection differential analysis showed strong positive gains for biomass traits, particularly under medium/high salinity: TDW-High (188.64%), SDW-High (173.57%), SDW-Medium (163.96%), TDW-Medium (147.01%), SFW-Low (303.91%) and TFW-Low (278.16%) (Fig 5B). Factor contributions showed FA1 capturing medium/high-salinity biomass, FA2 low-salinity dry matter, FA3 fresh biomass and root dry weight, FA4 shoot/plant height under stress and FA5 seedling vigour at low salinity. A near-100% goal percentage across traits indicated minimal genetic trade-offs, confirming MGIDI's reliability



(A) scree plot showing eigenvalues and proportion of variance explained by each principal component; (B) biplot illustrating genotype distribution and trait loadings along PC1 and PC2; (C) scatter plot depicting grouping of genotypes into three distinct PCA-derived clusters and (D) lollipop plot representing the percentage contribution of individual traits to total genetic divergence.

Fig 3: Principal component analysis of mungbean genotypes under salinity stress.

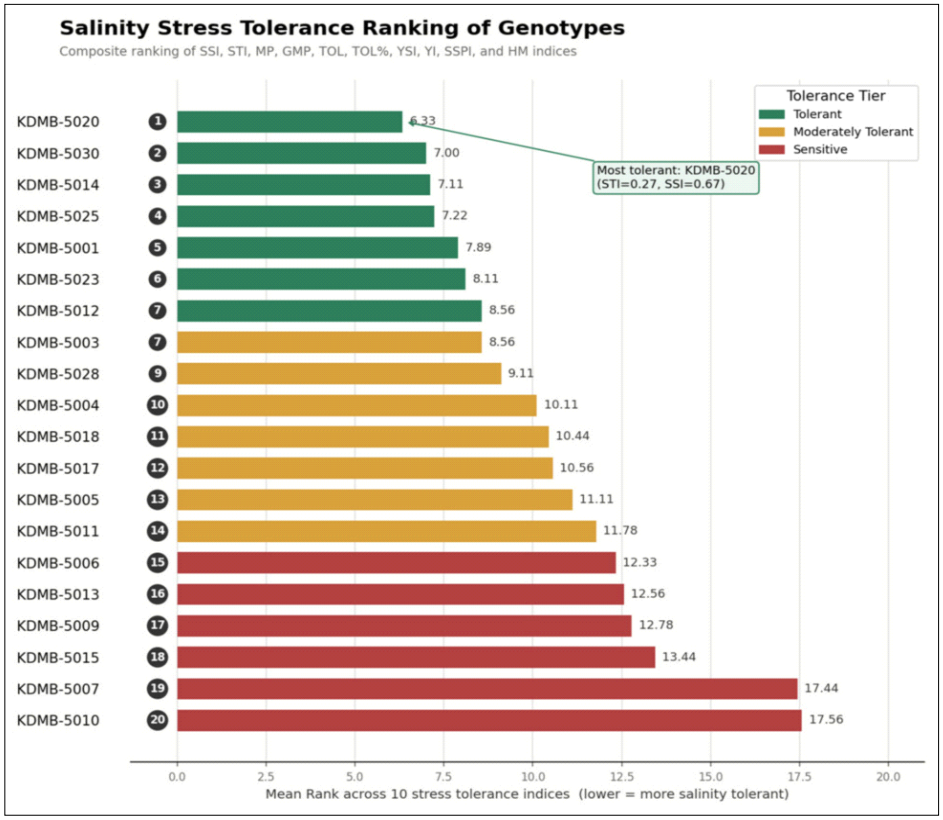
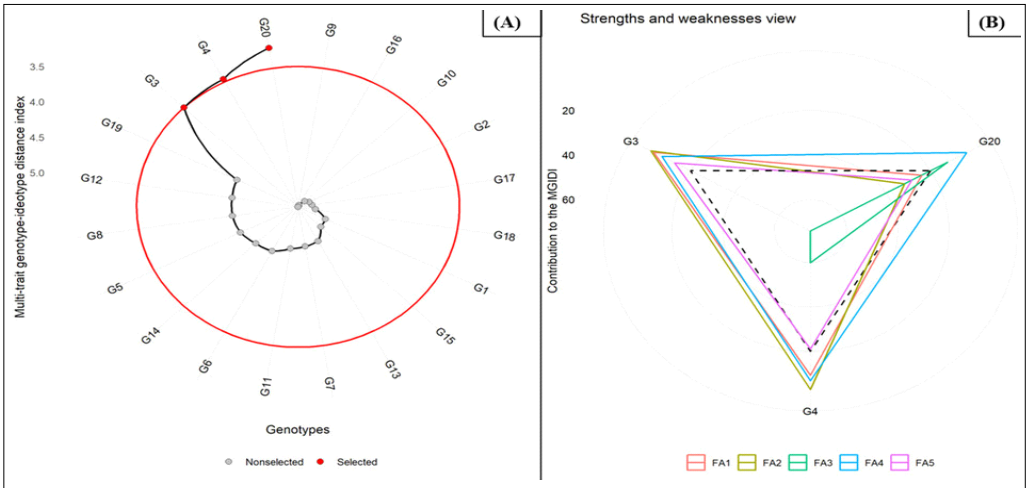


Fig 4: Overall salinity stress tolerance ranking of 20 mungbean (*Vigna radiata* L.) genotypes derived from mean rank scores across nine complementary stress tolerance indices.



(A) ranking of 20 genotypes by MGIDI scores across 11 traits under three salinity levels, with lower scores indicating closer proximity to the ideotype; (B) strengths-and-weaknesses radar diagram showing where selected genotypes meet or fall short of ideotype targets, aiding parental selection for salinity tolerance breeding.

Fig 5: MGIDI analysis for identifying salt-tolerant mungbean genotypes.

for simultaneous multi-trait selection. By integrating twelve traits across three environments, MGIDI identified KDMB-5001, KDMB-5018 and KDMB-5011 as most ideotype-proximal, consistent with Debnath *et al.* (2024). KDMB-5001's ranking is further validated by its monogenotypic cluster status and strong stress index performance. Similar utility was reported by Zahedi *et al.* (2026) in wheat and Chaity *et al.* (2026) in rice (genetic gains of 28.51% and 25.86%). The high biomass gains and near-universal goal achievement confirm simultaneous trait improvement with minimal trade-offs, an advantage over conventional mean-rank methods (Olivoto and Nardino, 2021), corroborated by Palaniyappan *et al.* (2025) in fodder maize.

CONCLUSION

Twenty mungbean genotypes showed wide genetic diversity under NaCl salinity, with root traits declining progressively as salinity rose; KDMB-5023 and KDMB-5025 were the most resilient. The largest inter-cluster distance (D^2 = 8.498, Clusters I and IV) suggests useful parental combinations and plant height, shoot length, vigour index and leaf area explained 82.47% of the divergence, making them early-generation selection criteria. KDMB-5020, KDMB-5030, KDMB-5014 and KDMB-5025 were the most salt-tolerant across stress indices, while KDMB-5001, KDMB-5018 and KDMB-5011 ranked closest to the ideotype by MGIDI. These genotypes are priority resources for breeding salt-tolerant mungbean.

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Disclaimers

This disclaimer clarifies that the views expressed herein belong exclusively to the authors and do not reflect the official positions of their affiliated institutions.

Informed consent

NA.

Conflict of interest

All authors declared that there is no conflict of interest.

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