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Multivariate Assessment of Genetic Variation and Trait Associations in Moth bean (*Vigna aconitifolia* Jacq.)

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Abstract: Genetic variability, trait associations, and genetic divergence was studied among 49 moth bean genotypes. Analysis of variance showed significant genetic variation for most of the growth and yield-related traits. Correlation analysis revealed strong positive associations of total pods, clusters, and reproductive traits on branches with seed yield, while test weight was largely independent, allowing simultaneous improvement of yield and seed size. Principal component analysis explained 67.4% of total variability with the first two components; PC1 highlighted reproductive traits driving yield variation, and PC2 emphasized seed size and pod distribution. Leveraging these results, three PCA-based multi-trait selection indices—Yield Index, Seed Size Index, and Architecture Index—were constructed using respective trait loadings to streamline simultaneous selection of complex traits aligned with breeding objectives. Cluster analysis using Mahalanobis D^2 distances and Ward's minimum variance method grouped genotypes into five distinct clusters revealing substantial genetic divergence. Unique genotypes in single-genotype clusters offer valuable genetic resources for broadening crop diversity. This integrative approach combining phenotypic variance, multivariate analysis, and genetic clustering provides a robust framework for effective parent selection and accelerated genetic gain in moth bean.

Key words: Genetic variability, moth bean, multi - trait selection indices, principal component analysis, trait associations.

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Moth bean (*Vigna aconitifolia* Jacq.), an underutilized yet highly resilient legume, is mainly cultivated in the arid and semi-arid zones of South Asia, with Rajasthan, India, as its principal growing region (Choudhary *et al*., 2024). The crop is uniquely adapted to withstand drought, extreme heat, and nutrient-poor soils, thereby serving as a vital source of food and fodder in marginal environments where few alternatives thrive (Sharma *et al*., 2024; Choudhary *et al*., 2024). However, despite its remarkable adaptability, moth bean remains underexploited, and its productivity is constrained by a narrow genetic base and limited systematic improvement efforts. Enhancing genetic gains in this crop requires a better understanding of the existing variability and the identification of reliable morphometric and

yield-related traits that can be effectively used in breeding programs.

In modern plant breeding, multivariate statistical tools have gained prominence for their ability to capture the complex interrelationships among traits and to classify genotypes based on genetic divergence. Correlation analysis offers insight into trait associations, while principal component analysis (PCA) helps in reducing dimensionality and identifying the traits contributing most to overall variability (Jolliffe and Cadima, 2016). Such approaches have proven valuable for orphan legumes like moth bean, where recent studies emphasize their role in identifying key traits, understanding trait interdependence, and selecting genetically diverse parents for crop improvement (Punia *et al*., 2023; Kohakade *et al*., 2017). Against this backdrop, the present study was conducted to evaluate genetic variability, trait associations, and genetic divergence in moth bean genotypes using ANOVA, correlation, PCA, and Mahalanobis D^2 clustering. Particular focus was given for identifying yield-contributing morphometric traits and grouping genotypes into divergent clusters for potential use in breeding.

Material and Methods

The present study was conducted during the Kharif-2022 season using 46 stable moth bean genotypes along with three checks (RMO-257, CZM-2, and RMO-2251) arranged in a randomized block design (RBD) with three replications. Data were recorded for 15 morphometric and yield-related traits encompassing both vegetative and reproductive characteristics including Plant height (PH), number of branches (BR), branch length (BL), clusters on the main stem (CLM), pods on the main stem (POM), clusters on branches (CLB), pods on branches (POB), total clusters (TCL), and total pods per plant (TP), peduncle length (PEL), seeds per pod (SPP), pod yield per plant (PY), test weight (TW) and seed yield per plant (SYPP), while the seed-to-pod ratio (SPR) was calculated to assess seed distribution efficiency.

The recorded data were subjected to comprehensive statistical analyses to evaluate genetic variability, trait associations, and genetic divergence. Analysis of variance (ANOVA) was performed to determine the significance of differences among genotypes. Pearson correlation coefficients were calculated to examine relationships among growth and yield traits. Multivariate analyses, including Principal Component Analysis (PCA) (Jolliffe and Cadima, 2016) and cluster analysis based on Mahalanobis D^2 distances (Mahalanobis, 1936), were employed to explore patterns of genetic divergence among genotypes. PCA was used to reduce dimensionality and identify key traits contributing to total variation, while Mahalanobis D^2 analysis coupled with Ward's minimum variance method (Ward, 1963) classified genotypes into distinct clusters. To address singularity in the covariance matrix, the Moore–Penrose generalized inverse (Moore, 1920) was applied for stable computation of inter-genotypic distances. The clustering results facilitated the identification of genetically diverse parental combinations for hybridization, and PC-based scores were further used to construct selection indices (Debnath *et al*., 2022). All statistical analyses were performed using R version 4.5.1 (R Core Team, 2025; r-project.org).

Results and Discussion

Analysis of variance

The analysis of variance in the study revealed significant genetic variability among 49 moth bean genotypes (Table 1) for

Table 1. Mean Sum of squares of different morphometric traits in moth bean genotypes

Source	df	PH	BR	BL	CLM	POM	CLB	POB	TCL
Genotype	48	214.7**	1.30**	773.9**	12.57**	245.71**	180.85**	1359**	236.11**
Replication	2	151.55	0.03	259.3	0.28	11.07	73.89	201.2	74.4
Error	96	58.01	0.61	98.4	5.91	97.06	71.05	695.6	98.29
Source	df	TP	PEL	SPP	PY	TW	SYPP	SPR	
Genotype	48	1628.9*	28.18**	0.12**	43.34*	2.01**	26.46*	6.42	
Replication	2	282.8	0.71	0.03	16.27	2.14 *	10.31	7.11	
Error	96	994	2.413	0.06	28.9	0.54	16.54	5.16	

df, degrees of freedom; * significant at p=0.05; ** significant at p=0.01

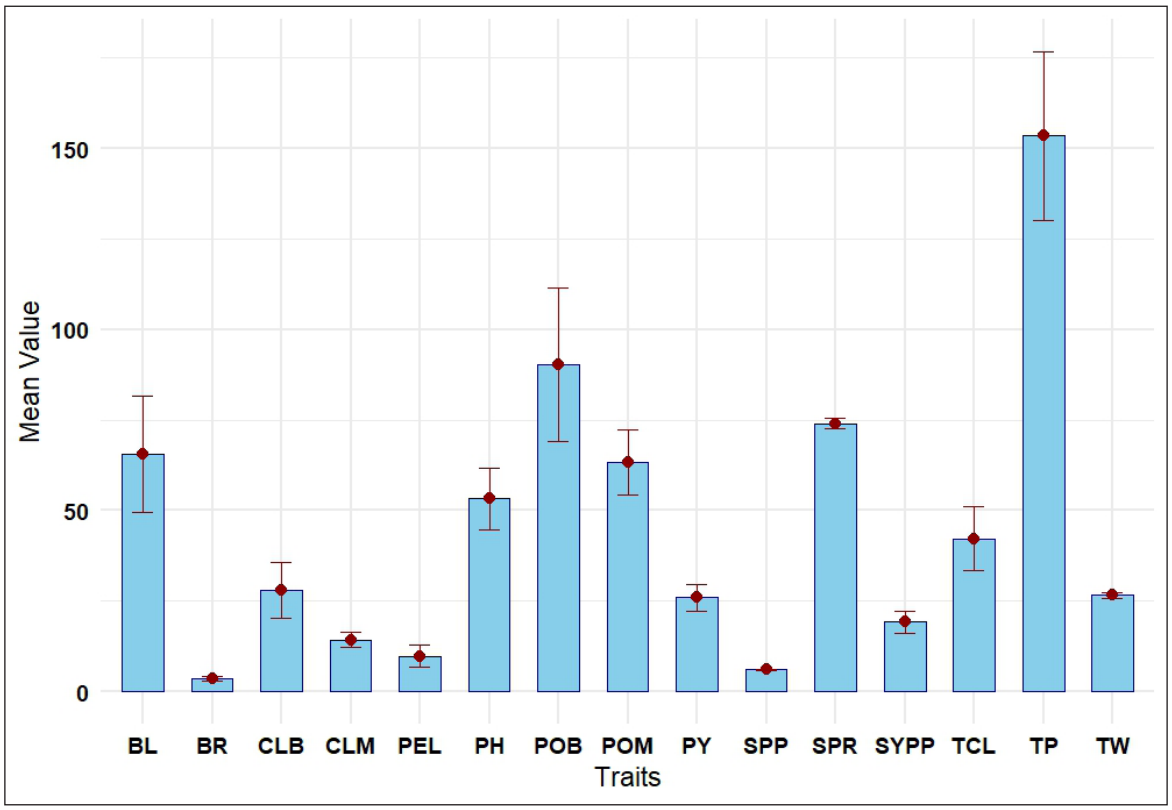


Fig. 1. Variability in different morphometric traits.

most morphometric and yield-related traits, reflecting the strong influence of genotype on growth, reproductive, and yield components (Fig. 1). Significant differences were observed in architectural traits such as peduncle length (Fig. 2) plant height, number of branches, branch length, clusters, and pods on both main stem and branches, indicating these traits are genetically controlled and can serve as effective selection criteria for breeding programs aimed at improving plant architecture and yield potential. Similarly, yield and seed-related traits including total pods per plant, peduncle length, seeds per pod, pod yield, test weight and seed yield per plant (Fig. 2) also showed substantial variation, further supporting the existence of useful genetic diversity.

PH-Plant Height (cm); BR-Number of Branches; BL-Branch length(cm); CLM-Clusters on main stem; POM-Number of Pods on main stem; CLB- Number of clusters on branches; POB-Pods on branches; TCL-Total clusters per plant; TP-Total pods per plant; PEL- Peduncle length(cm); SPP- Number of seeds per pod; PY-Pod yield per plant (g); TW- test weight(g); SYPP- Seed yield per plant(g); SPR-Seed to pod ratio.

However, seed-to-pod ratio did not differ significantly among genotypes, suggesting that the variation may be more of environmental influence rather than genetically determined, and could require indirect improvement through correlated traits or agronomic practices.

These findings are consistent with previous research demonstrating high genetic variability and heritability for branching, pods per plant, plant height, and seed yield components in moth bean, which are predominantly governed by additive gene action and thus amenable to selection (Kohakade *et al*., 2017; Chaudhary *et al*., 2021; Soni and Modi, 2023). The observed significant genotypic variation for yield-contributing traits supports their use as reliable targets for breeding and genetic enhancement, while the limited variability in seed-to-pod ratio aligns with reports suggesting its environmental sensitivity (Kohakade *et al*., 2017).

Trait association between different morphometric traits

The correlation analysis revealed significant associations that provide valuable insights into the yield-determining components. Seed yield

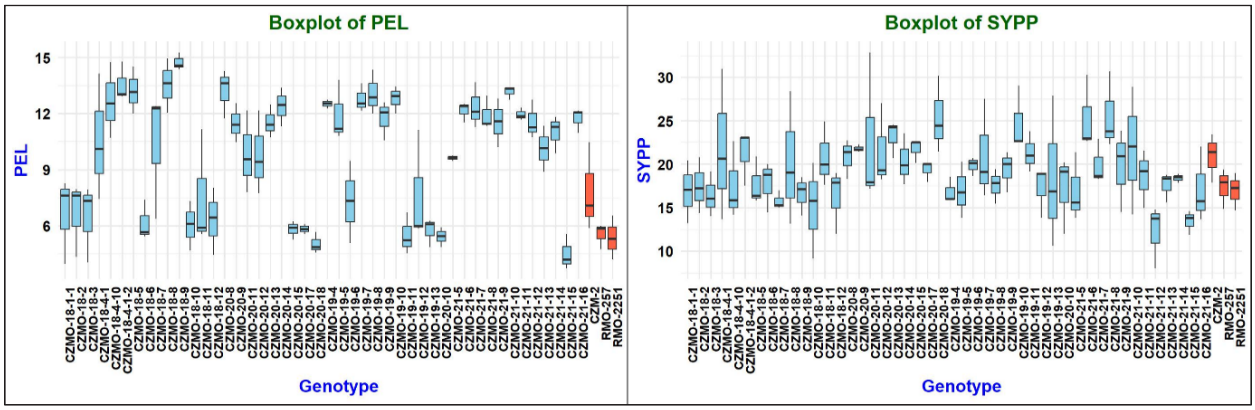


Fig. 2. Variability in moth bean genotypes for PEL (peduncle length) and SYPP (seed yield per plant).

per plant exhibited a highly significant and positive correlation with pod yield per plant (r = 0.993**), total pods per plant (r = 0.935**), pods on branches (r = 0.824**), total clusters per plant (r = 0.690**), and clusters on branches (r = 0.677**). These strong associations indicate that improvement in seed yield can be effectively achieved through indirect selection for these yield-contributing traits.

Pod yield per plant also displayed significant positive correlations with total pods per plant (r = 0.942**), pods on branches (r = 0.827**), clusters on branches (r = 0.680**), and total clusters (r = 0.692**), suggesting that these parameters function collectively in determining the productivity potential of individual genotypes. The number of pods on branches was strongly correlated with total pods (r = 0.918**) and total clusters (r = 0.699**), highlighting the crucial contribution of branch fertility toward yield enhancement.

Plant height exhibited significant and positive correlations with number of branches (r = 0.686**), branch length (r = 0.749**), clusters on branches (r = 0.712**), and total clusters per plant (r = 0.709**), suggesting that taller and well-branched plants tend to bear more clusters and pods. Similarly, the number of branches and branch length were strongly intercorrelated (r = 0.862**) and showed significant positive associations with clusters on branches (r = 0.829** and r = 0.795**, respectively). These findings indicate that vigorous vegetative growth promotes reproductive sink development, thereby improving yield potential (Fig. 3).

Peduncle length demonstrated a strong positive association with branch length (r = 0.907**) and plant height (r = 0.625**), implying that robust plant architecture contributes to longer peduncles, which may enhance pod exposure and filling. The number of clusters on the main stem showed a significant positive correlation with pods on the main stem (r = 0.485**) and total clusters per plant (r = 0.623**), signifying the contribution of main stem productivity to the overall reproductive output.

In contrast, test weight exhibited non-significant correlations with most other yield components, suggesting that improvement in seed size can be pursued independently without negatively influencing yield-related attributes. The seed-to-pod ratio showed a weak but positive association with seed yield per plant (r = 0.436**), indicating that efficient seed set contributes marginally to total yield.

Similar trends have been reported earlier. Yadav *et al*. (2023) observed that seed yield per plant was positively correlated with number of pods per plant, plant height, and number of branches. Sahoo *et al*., 2018 also identified number of pods per plant, branches per plant, and seeds per pod as highly variable and yield-contributing traits. Chaudhari *et al*. (2021) further confirmed significant positive correlations of seed yield with peduncle length, clusters per branch, and branches per plant, underscoring the importance of branching traits in yield improvement. Moreover, Bhavsar and Birari (1989), who noted that plant height, peduncle length, and branching traits were positively correlated with seed yield in moth bean. More recently, Punia *et al*. (2023) demonstrated that number of branches, number of pods per plant, and cluster number per plant exhibited high variability and were reliable indicators of yield even under stress conditions. Similarly, Soni and Modi (2023) confirmed significant positive

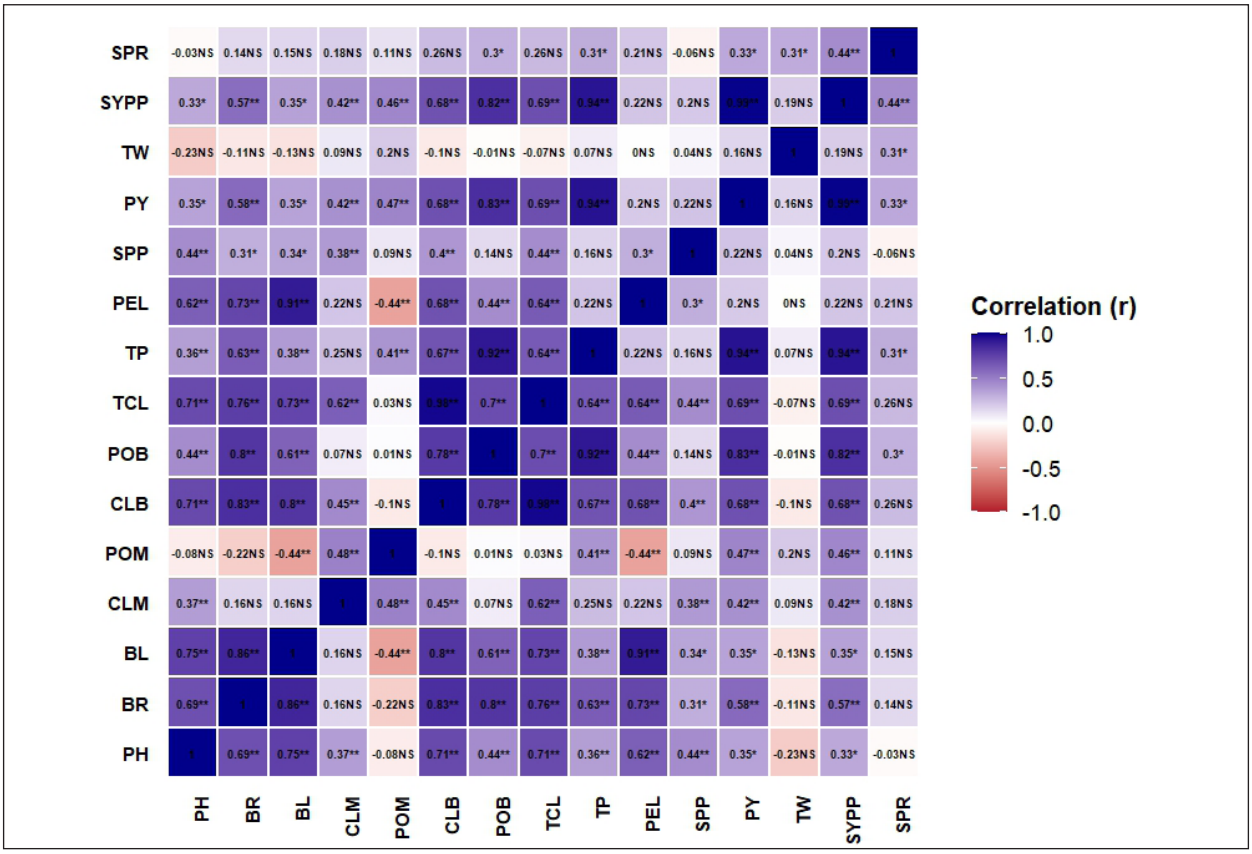


Fig. 3. Correlation heatmap between studied morphometric traits in moth bean genotypes.

correlations between grain yield and plant height, branches per plant, and pod length. Chaudhari *et al*. (2021) and Bhavsar and Birari (1989) noted that seed weight showed little or no association with yield, suggesting that yield improvement in moth bean is primarily determined by pod and branching traits, while seed size can be targeted separately.

Overall, the correlation pattern clearly identifies pod yield per plant, total pods, and branch-associated reproductive traits (pods and clusters on branches, total clusters) as the most influential factors determining seed yield in moth bean. Hence, these traits may serve as reliable selection criteria in breeding programs aimed at yield enhancement under arid conditions.

Principle component analysis

Principal component analysis provides a dimensional reduction that simplifies complex trait data, enabling breeders to design efficient selection indices that target multiple correlated traits simultaneously, thus accelerating genetic gain in moth bean breeding programs (Debnath *et al*., 2022; Shojaei *et al*., 2024). In the present study, it revealed significant genetic variation concentrated primarily over few key components. The first principal component (PC1), explaining 48.8% of total variability, was strongly influenced by reproductive traits such as clusters on branches, pods on branches, total clusters, total pods, pod yield, and seed yield per plant (Fig. 4).

This indicates that variation in reproductive output on secondary branches is the main driver of genetic diversity among genotypes. Moderate expression of plant height, number of branches, and branch length on PC1 further highlight the role of plant architecture in supporting pod formation and yield. The second component (PC2), accounting for 18.6% of variation, differentiated genotypes mainly through pods on the main stem, seed weight, and total weight, reflecting variation in pod distribution and seed size. Subsequent components (PC3–PC10) captured minor but relevant variations in traits such as peduncle length, seeds per pod, clusters on the main stem, and seed-to-pod ratio (Table 2).

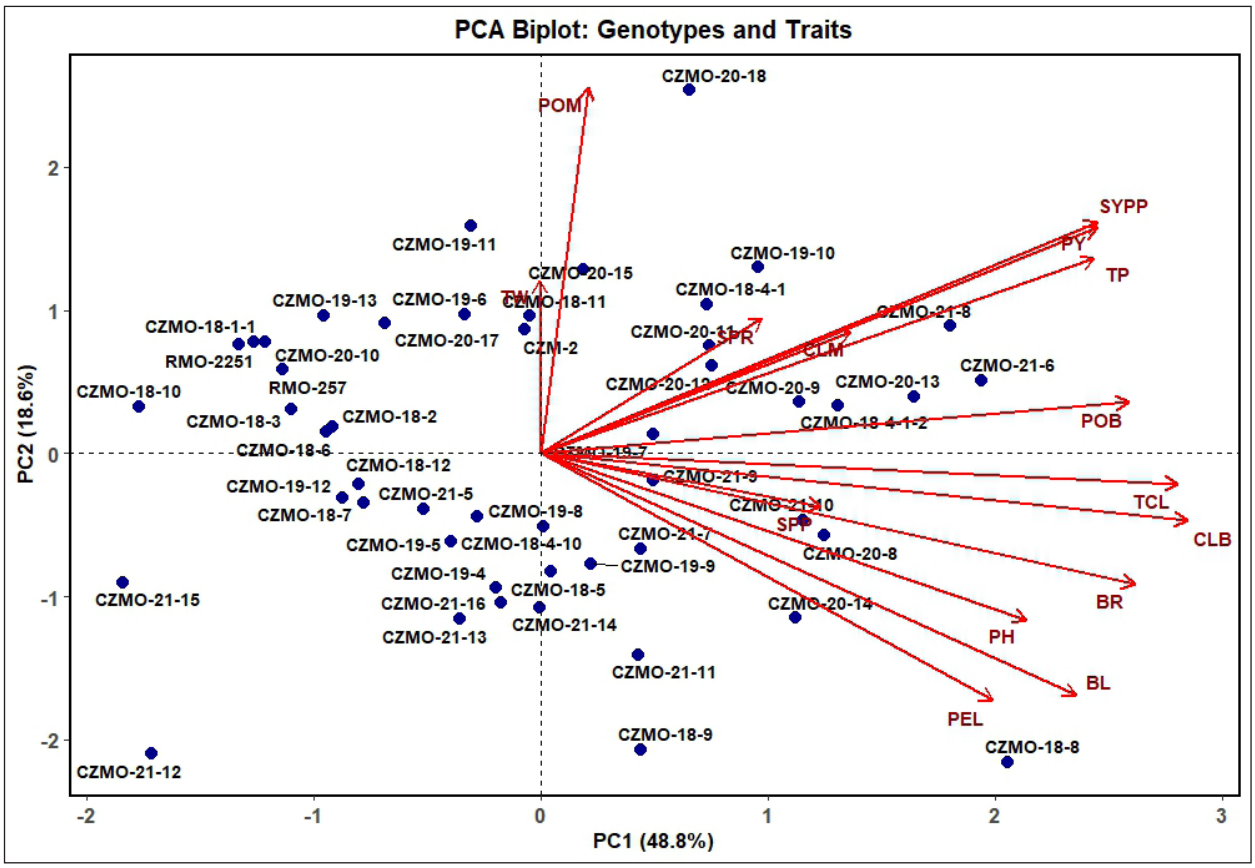


Fig. 4. Scattered Biplot analysis of moth bean genotypes.

These PCA results align with existing literature where reproductive traits, especially pod number on branches, have emerged as critical determinants of yield and genetic diversity in moth bean and other legumes (Chaudhari *et al.*, 2021; Kohakade *et al.*, 2017; Nainu *et al.*, 2020). The concentration of variability in fewer principal components suggests that breeding programs can effectively focus on these major traits to enhance yield and adaptability (Rajora *et al.*, 2012). The differentiation of genotypes based on pod distribution and seed size in PC2 supports the possibility of improving seed traits independently of yield components, consistent with the reported genetic independence of seed size and yield. Minor components capturing peduncle length and seed-to-pod ratio further add to the understanding of subtle yet meaningful genetic variation within germplasm. Overall, PCA provides a comprehensive view of the multivariate genetic architecture of moth bean traits, confirming that reproductive output on branches and associated architectural traits are pivotal targets for breeding to improve yield performance and genetic diversity.

Cluster analysis

The use of Mahalanobis D^2 statistics in this study effectively captured the multivariate genetic divergence among moth bean genotypes (Fig. 5), consistent with previous legume breeding research (Harsh Deep *et al.*, 2021; Kanavi *et al.*, 2019). These studies reported similar clustering patterns in lentil and green gram germplasm, confirming the method's robustness in legume genetic diversity assessment. The cluster analysis revealed clear genetic divergence using Mahalanobis D^2 distances. The Moore–Penrose generalized inverse was applied to address the singularity of the covariance matrix (condition number > 1e12). It was essential for handling covariance matrix singularity, a noted solution in high-dimensional trait analyses (Ben-Israel and Greville, 2003). Hierarchical clustering with Ward's minimum variance method grouped the genotypes into five distinct clusters (Table 3), indicating substantial genetic diversity within

Table 2. Eigenvalues, variability explained by principal components, and rotated component matrix of moth bean genotypes

PCs	EV	PV	PH	BR	BL	CLM	POM	CLB	POB
PC1	7.316	48.8	0.263	0.322	0.29	0.168	0.025	0.35	0.319
PC2	2.792	18.6	-0.232	-0.183	-0.337	0.17	0.511	-0.093	0.072
PC3	1.547	10.3	-0.25	0.143	0.062	-0.574	-0.294	-0.037	0.337
PC4	1.226	8.2	-0.119	-0.069	0.099	0.161	-0.132	-0.008	-0.165
PC5	0.756	5	0.035	-0.125	-0.015	0.357	0.03	0.096	-0.119
PC6	0.503	3.4	-0.163	-0.077	-0.072	-0.246	-0.096	0.009	0.064
PC7	0.354	2.4	0.645	0.086	0.134	-0.257	0.44	-0.303	-0.08
PC8	0.22	1.5	-0.532	0.191	0.202	0.134	0.299	-0.292	-0.036
PC9	0.113	0.8	0.084	-0.863	0.104	-0.159	-0.009	0.082	0.127
PC10	0.086	0.6	0.102	-0.069	0.321	0.101	-0.325	-0.286	-0.235
PCs	TCL	TP	PEL	SPP	PY	TW	SYPP	SPR	
PC1	0.345	0.3	0.245	0.152	0.302	-0.001	0.302	0.12	
PC2	-0.043	0.271	-0.344	-0.074	0.315	0.241	0.323	0.188	
PC3	-0.165	0.193	0.013	-0.492	0.076	0.068	0.104	0.235	
PC4	0.031	-0.2	0.308	0.081	-0.119	0.656	-0.042	0.559	
PC5	0.166	-0.104	0.036	-0.538	-0.081	-0.522	-0.022	0.471	
PC6	-0.049	0.025	-0.161	0.651	-0.039	-0.409	0.027	0.516	
PC7	-0.325	0.089	0.114	-0.037	-0.083	-0.003	-0.046	0.251	
PC8	-0.225	0.069	0.563	0.065	0.048	-0.237	0.033	-0.1	
PC9	0.036	0.143	0.374	0.014	0.085	-0.039	0.074	-0.115	
PC10	-0.227	-0.32	-0.162	0.006	0.477	-0.07	0.465	-0.018	

PH-Plant Height (cm); BR-Number of Branches; BL-Branch length(cm); CLM-Clusters on main stem; POM-Number of Pods on main stem; CLB- Number of clusters on branches; POB-Pods on branches; TCL-Total clusters per plant; TP-Total pods per plant; PEL- Peduncle length(cm); SPP- Number of seeds per pod; PY-Pod yield per plant (g); TW-test weight(g); SYPP- Seed yield per plant(g); SPR-Seed to pod ratio.

the panel. Cluster I included 19 genotypes, incorporating the checks and genotypes like CZMO-18-1-1, CZMO-18-2, and CZMO-20-18. Cluster II consisted of 22 genotypes, such as CZMO-18-4-1, CZMO-20-8, and CZMO-21-11. Cluster III contained six genotypes including CZMO-18-5, CZMO-19-9, and CZMO-21-12. Clusters IV and V were each represented by a single, highly divergent genotype: CZMO-18-8 and CZMO-18-9, respectively.

This clustering pattern demonstrated that genotypes within the same cluster shared similar multivariate trait profiles, while genotypes in different clusters were more genetically divergent. Ward's minimum variance method further enhanced cluster reliability by minimizing within-cluster variance, as supported by clustering studies in mung bean (Raghavan *et al*., 2022) and winged bean (Bhadmus *et al*., 2023).

The identification of unique single-genotype clusters (IV and V) underscores the presence of novel genetic resources. Such divergent genotypes are valuable donors for trait introgression, a strategy emphasized by Vaggar *et al*. (2022) and others for broadening genetic bases in legumes.

Crossing genotypes from genetically distant clusters, particularly between clusters I and IV/V or II and IV, would likely maximize heterosis and produce transgressive segregants. The inclusion of checks in Cluster I provided a genetic baseline for comparison, aiding breeders in identifying superior genotypes relative to known varieties (Sachan and Parveen, 2025). Ultimately, this combination of Mahalanobis D^2 distances and Ward's clustering offers a rigorous framework for parental selection in moth bean breeding, facilitating yield improvement and broadening the crop›s genetic diversity, in line with earlier grain legume breeding initiatives (Yadav *et al*., 2025; Ram *et al*., 2020).

Proposed PCA-based selection indices

Three selection indices were developed using principal component analysis to facilitate

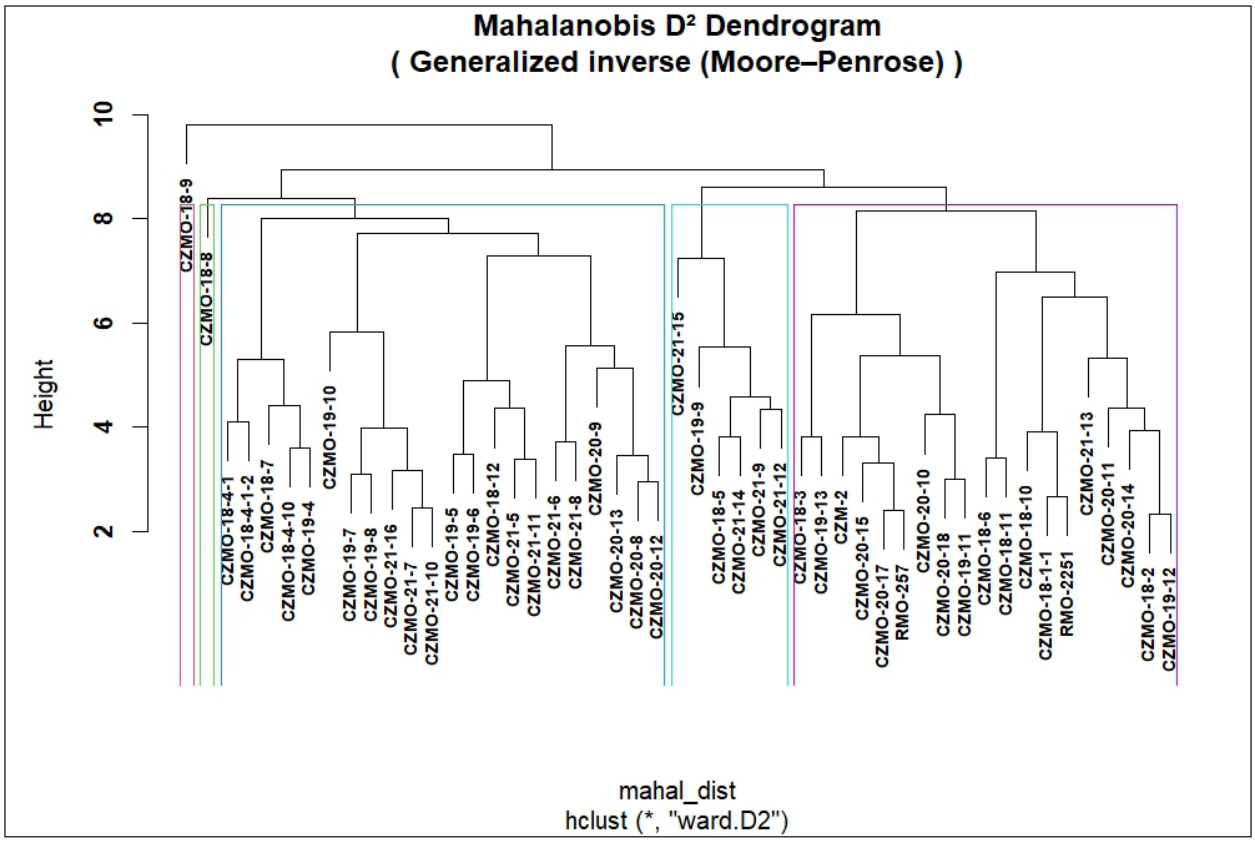


Fig. 5. Dendrogram showing clustering pattern of genotypes.

efficient multi-trait improvement in moth bean (Table 4). The Yield Index, based on PC1 which accounts for 45.7% of the total variability, emphasizes reproductive traits including clusters and pods on branches, total clusters, total pods, pod yield, and seed yield per plant. Trait weights in this index are proportional to their respective PC1 loadings, giving priority to total pods and seed yield, thereby enabling the selection of high-yielding genotypes with improved productivity (Debnath *et al.*, 2022; Jain *et al.*, 2023).

The Seed Size Index, derived from PC2 explaining 19.2% of the variance, specifically targets pods on the main stem and test weight. Utilizing the loadings as weights allowing breeders to perform independent selection for enhanced seed size and pod distribution without negatively impacting overall yield, which is crucial in maintaining both seed quality and yield (Halimi *et al.*, 2021; Yadav *et al.*, 2023).

The Architecture Index integrates moderate PC1 loadings and minor principal components that capture plant architectural traits such as

Table 3. Clustering of moth bean genotypes using Mahalanobis D2 cluster analysis

Cluster	Number of Genotypes	Genotypes
1	19	CZMO-18-1-1, CZMO-18-2, CZMO-18-3, CZMO-18-6, CZMO-18-10, CZMO-18-11, CZMO-20-11, CZMO-20-14, CZMO-20-15, CZMO-20-17, CZMO-20-18, CZMO-19-11, CZMO-19-12, CZMO-19-13, CZMO-20-10, CZMO-21-13, CZM-2, RMO-257, RMO-2251
2	22	CZMO-18-4-1, CZMO-18-4-10, CZMO-18-4-1-2, CZMO-18-7, CZMO-18-12, CZMO-20-8, CZMO-20-9, CZMO-20-12, CZMO-20-13, CZMO-19-4, CZMO-19-5, CZMO-19-6, CZMO-19-7, CZMO-19-8, CZMO-19-10, CZMO-21-5, CZMO-21-6, CZMO-21-7, CZMO-21-8, CZMO-21-10, CZMO-21-11, CZMO-21-16
3	6	CZMO-18-5, CZMO-19-9, CZMO-21-9, CZMO-21-12, CZMO-21-14, CZMO-21-15
4	1	CZMO-18-8
5	1	CZMO-18-9

Table 4. Selection Indices Derived from Principal Component Analysis for Yield, Seed Size, and Plant Architecture in Moth Bean

Index	PC (variance %)	Key traits	Purpose
Yield index	PC1 (48.8)	Total Clusters (TCL), Pods on Branches (POB), Pod yield (PY), Seed yield (SYPP)	Yield improvement
Seed size index	PC2 (18.6)	Pods on Main stem (POM), test weight (TW)	Enhance seed size & pod distribution
Architecture index	PC1 + minor PCs (moderate)	Plant height (PH), Branch number (BR) and length (BL), Peduncle length (PEL)	Optimize plant architecture & yield stability

plant height, branch number, branch length, and peduncle length. Weighted by trait contributions, this index facilitates the selection of genotypes with favorable plant architecture that indirectly supports yield stability and adaptation (Soni and Modi, 2023; Punia *et al*., 2023).

By assigning greater weight to traits with larger PCA loadings in each index, these indices efficiently capture key genetic variability underlying complex traits and facilitate simultaneous multi-trait selection (Ahlinder *et al*., 2024; Santos *et al*., 2018). Depending on breeding objectives, the indices provide flexibility to prioritize yield improvement, seed size and pod distribution, or plant architecture traits, thereby maximizing genetic gain and overall crop performance in moth bean breeding programs (Debnath *et al*., 2022; Halimi *et al*., 2021).

Conclusions

This study reveals significant genetic variability among 49 moth bean genotypes for key morphometric and yield traits, confirming strong genetic control over branching, reproductive, and yield components. Correlation and PCA analyses identified total pods, seed yield, and reproductive traits on branches as major contributors to yield variability, while test weight was largely independent, enabling simultaneous improvement. The PCA-based multi-trait selection indices: Yield, Seed Size, and Architecture were successfully constructed using trait loadings to efficiently capture the underlying genetic variation and support targeted breeding objectives. Cluster analysis using Mahalanobis D^2 distances and Ward's method further delineated genetically divergent groups, identifying unique genotypes as valuable donors, and demonstrating that crossing between distant clusters can enhance heterosis and transgressive breeding gains. Together, these integrated multivariate and cluster analyses provide a robust framework to select genetically diverse, high-yielding, and well-adapted moth bean genotypes, paving the way for accelerated genetic improvement and yield stability in breeding programs.

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