DOI : 10.58993/ijh/2025.82.2.4

Indian J. Hortic. 82(2), June 2025: 147-154

Genetic diversity and cluster analysis of dolichos bean (*Lablab purpureus* L.) in the Bundelkhand region using multivariate statistical techniques

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ABSTRACT

Dolichos bean is a nutritionally and agronomically important legume with significant genetic variability. This study assessed the genetic diversity among 21 genotypes from the Bundelkhand region using Principal Component Analysis (PCA) and hierarchical clustering under a Randomized Block Design (RBD). PCA revealed that the first three principal components accounted for 67.85% of the total variance, with PC1 contributing 32.41%, PC2 21.73%, and PC3 13.71%. Hierarchical clustering grouped genotypes into five distinct clusters, with the highest inter-cluster distance (5698.57) observed between cluster 4 and cluster 5, indicating high genetic divergence. Cluster 5 exhibited superior pod yield traits, while cluster 3 recorded the highest dry matter content (42.47%) and seed weight (26.73 g), making them valuable for breeding programs. These findings highlight the genetic diversity in dolichos bean and provide valuable insights for breeding high-yielding and stress-tolerant cultivars. The study supports sustainable genetic improvement programs in India, particularly in the Bundelkhand region.

Key words: Genotypes, heatmap, ward's clustering, PCA, tocher clustering, crop improvement.

INTRODUCTION

Indian bean, botanically known as *Lablab purpureus* L., is also referred to as Bonavist or Egyptian bean. It belongs to the family Fabaceae and has a chromosome number of 2n = 22. It is one of the most ancient crops known for its food and fodder value. It is an important crop grown throughout the country. Indian bean is indigenous crop because of its origin in India. It is a perennial herbaceous crop but cultivated as an annual bushy and pole erect or climbing type. Wild species is mostly perennial in nature. Fruit is a type of legume pod variable in shape size and colour. Indian bean is primarily grown for green pods used for vegetable purposes. It is a rich source of protein, minerals, vitamins, carbohydrates and fiber. Protein content is found approximate 4% in green pods and 20-25% in dry pods (Ibrahim *et al*., 9). Among vitamins and minerals, it contains vitamin C around 5 mg per 100 g, vitamin B1 0.1 mg per 100 g and vitamin A 312 IU, whereas in minerals potassium content is around 262 mg per 100 g, calcium 210 mg per 100 g, iron 1.7 mg per 100 g carbohydrates content is 6.7 g per 100 g (Gopalan *et al*., 8). Water soluble polysaccharides such as rhamnose, xylose, arabinose, galactose, glucose, uronic acid, and unidentified carbohydrates and proteins are found in its seeds (Basu *et al*., 4). It is having highest nutrients index as compare to the french bean. Indian bean is an important leguminous vegetable crop grown throughout the country and distributed in Madhya Pradesh, Maharashtra, Andhra Pradesh, Tamil Nadu and North Eastern states. Karnataka accounts for approximately 92% of the total production of Indian bean in India. Despite the existence of a wide genetic base, this crop has not received adequate attention from geneticists and breeders for systematic improvement. (Magalingam *et al*., 11). Despite of being originated in India, very little work has been done in the field of yield and quality aspect of Indian bean. Augmenting the current low on-farm productivity (0.5 t ha^{-1}) to potential level (2.0 t ha^{-1}). Singh *et al*. (17) suggested broadening the genetic base of Indian bean cultivars through enhanced use of diverse germplasms accessions.

The yield potential of Indian bean is low, which is mainly due to the non-availability of desirable high yielding, disease and insect resistant varieties and poor management practices. Hence, the high yield potential and quality are the main targets for effective breeding programme in Indian bean. For any crop improvement programme, it is essential that sufficient

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variability exist for economically important traits in germplasm. Many researchers have repeatedly emphasized the importance of genetic diversity in selecting the parents for recombination breeding in crops. Genetic diversity plays a pivotal role in plant breeding and crop improvement programs. A diverse genetic pool allows breeders to select high-yielding, disease-resistant, and climate-resilient genotypes. However, limited genetic information on dolichos bean has restricted its potential for systematic breeding and genetic enhancement. Therefore, an in-depth genetic diversity assessment is essential for identifying superior genotypes and developing high-performing cultivars. Various multivariate statistical methods are commonly used to assess genetic diversity, including Ward's Minimum Variance Clustering, Principal Component Analysis (PCA), and Tocher's clustering method. Ward's clustering classifies genotypes based on genetic similarity, forming distinct groups that facilitate hybrid selection. PCA simplifies complex datasets by reducing dimensionality, emphasizing the most significant agronomic traits that contribute to genetic variation. These multivariate approaches are useful for evaluating genetic diversity and categorizing germplasm (Uyeda *et al*., 25). Among these techniques, PCA is widely used to analyze multiple variables by transforming them into principal components that are uncorrelated. This method helps in characterizing germplasm, visualizing differences among individuals, and determining their contribution to total genetic variation (Singh *et al*., 19).

This study aims to evaluate the genetic diversity among dolichos bean genotypes using these clustering techniques. The findings will aid in selecting suitable parents for breeding programs, ultimately contributing to the development of high-yielding and stress-tolerant varieties. While India has a rich collection of indigenous dolichos bean strains, particularly in the northern regions, limited efforts have been made toward their genetic improvement in terms of yield and quality. Therefore, this research focuses on assessing the genetic variability among 21 Indian bean genotypes, which exhibit differences in morphological traits. Understanding this variability is essential for any genetic improvement program, as it serves as a foundation for developing superior cultivars.

MATERIALS AND METHODS

The experiment was conducted in the vegetable research farm of RLBCAU, Jhansi during 17 August 2020-2021. The experimental site is situated at 25.31° N latitude and 78.33° E longitude at an altitude of 227 m above mean sea level. The experimental material 21 germplasms of Indian bean. All the germplasms are locally collected from different regions of Bundelkhand and Sultanpur district of UP which is seen in Table 1.

It was observed that soil of the experimental plot was sandy loam having pH 6.7 which depicts the acidic nature of experimental soil. The organic carbon content of soil was 2.03 g/kg of soil and electrical conductivity was 0.4dS/m. The genotypes were sown to different sub-plots in each replication randomly following the principle of RBD. Each genotype was sown by dibbling method and two to three seeds were sown per hill at distance 100x 75 cm (row to plant) and subplot diameter 4x2 m (length × width). The plots were irrigated immediately after sowing. After a span of 25 days weak and non-vigorous seedlings were thinned out and one good seedling was left per hill. Throughout the raising of a crop, it

Table 1. Genotypes of dolichos bean and their source of collection.

S. No.	Genotype's name	Location of collection	S. No.	Genotype's name	Location of collection
1.	RLBDL-S-1	Sultanpur	12.	RLBDL-S-9	Sultanpur
2.	RLBDL- S-1-1	Sultanpur	13.	RLBDL-S-10	Sultanpur
3.	RLBDL-S-1-2	Sultanpur	14.	RLBDL-S-11	Sultanpur
4.	RLBDL-S-2	Sultanpur	15.	RLBDL-S-12	Sultanpur
5.	RLBDL-S-3	Sultanpur	16.	RLBDL-S-13	Sultanpur
6.	RLBDL-S-4	Sultanpur	17.	RLBDL-S-14	Sultanpur
7.	RLBDL-S-4-5	Sultanpur	18.	RLBDL-J-1	Jhansi
8.	RLBDL-S-5	Sultanpur	19.	RLBDL-J-2	Jhansi
9.	RLBDL-S-6	Sultanpur	20.	RLBDL-J-3	Jhansi
10.	RLBDL-S-7	Sultanpur	21.	RLBDL-J-4	Jhansi
11.	RLBDL-S-8	Sultanpur			

Note: RLBDL-S-6: Rani Lakshami Bhai Dolichus bean – Sultanpur - 6, RLBDL - J - 1: Rani Lakshami Bhai Dolichus bean - Jhansi - 1 became observed that aphids and pod borers are the primary pests of Indian bean. Insecticide (granular) was used with water spray form to minimize insects and pests for example Aldicarb 10G @ 10-15 kg per hectare at the time of sowing was effective for aphids. Chlorpyriphos 25 EC @ 1.5 percent spray was carried out to govern the pod borer prevalence (Singh *et al.*, 18). The anthracnose and rust disease become determined in the crop. Wettable Sulphur @ 3g per liter was sprayed to manipulate rust ailment. The observations were recorded from 5 randomly selected plants from each genotype in each replication. The data recorded on 16 quantitative and 5 quality characters *viz*., leaves length (cm), width of leaves (cm), days to first flowering, days to 50% flowering, length of inflorescence (cm), number of flower per inflorescence, number of pod per inflorescence, pod length (cm), pod width (cm), days to first pod harvesting, duration of picking, pod per cluster, 10 pod weight (cm), number of seed per pod, pod yield per plant (kg), pod yield per plot (kg) total soluble solid (^{0}Brix), dry matter content, chlorophyll content (mg/100g), vitamin C content (mg/100g) and protein content (%).

Both qualitative and quantitative morphological traits were analyzed to evaluate the genetic diversity among the genotypes. For multivariate analysis, the XLSTAT software (Addinsoft, www.xlstat.com) was employed. Principal Component Analysis (PCA) was conducted to classify genotypes into groups and determine the principal axes and attributes that significantly influenced variation based on the similarity matrix. To visualize the genetic relationships, two-dimensional scatter plots were generated using the first two principal components, which accounted for the highest proportion of variance. Additionally, Agglomerative Hierarchical Clustering (AHC) was performed using Ward's minimum variance method and squared Euclidean distances to measure dissimilarity among genotypes.

RESULTS AND DISCUSSION

Hierarchical clustering using Ward's Minimum Variance Method grouped the 21 dolichos bean genotypes into distinct clusters based on their genetic similarity. The dendrogram revealed five major clusters, indicating substantial genetic diversity among the genotypes (Fig. 1).

The dendrogram revealed that genotypes RLBDL-J-2 and RLBDL-J-3 formed a closely related pair, suggesting a high degree of genetic similarity between them. Similarly, RLBDL-S-4-5 and RLBDL-S-7 were clustered together, followed by RLBDL-J-1 as an independent entity, indicating moderate divergence from other genotypes. RLBDL-S-6 and RLBDL-S-9 were grouped together, while RLBDL-S-2 and RLBDL-S-3 showed strong genetic association. Clusters containing RLBDL-S-1, RLBDL-S-1-1, RLBDL-S-4, and RLBDL-S-1-2 exhibited moderate genetic distances, suggesting potential for intra-cluster selection and breeding (Kiran *et al.*, 10). The more genetically distinct genotypes, such as RLBDL-J-4 and RLBDL-S-12, were positioned at greater distances from the rest, indicating their suitability for hybridization programs aimed at maximizing genetic gain. The dendrogram confirms significant genetic variability among dolichos bean genotypes, which is essential for crop improvement programs. The clustering pattern suggests that genotypes from distant clusters can be selected as parents in breeding programs to enhance heterosis and develop superior cultivars.

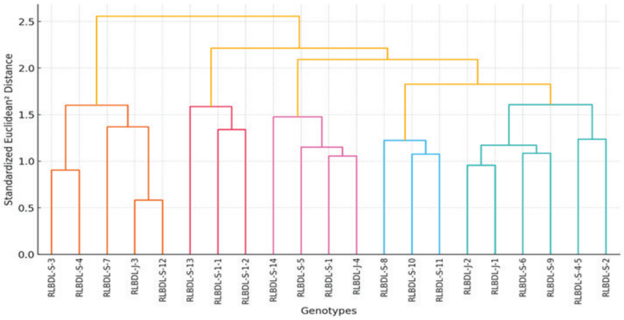

Fig. 1. Ward's minimum variance dendrogram hierarchical clustering of genotypes based on euclidean squared distances.

The inter-cluster distance matrix provides insights into the genetic divergence among dolichos bean genotypes, highlighting variations in genetic relationships (Fig. 2). The heatmap represents the genetic distances between different clusters, where higher values (depicted in red) indicate greater genetic divergence, while lower values (depicted in blue) suggest genetic similarity. The analysis reveals significant genetic variability among the genotypes, with the highest genetic distance (5698.57) observed between cluster 4 and cluster 5, indicating that these clusters are the most genetically divergent (Gangadhara *et al.*, 5). This suggests that genotypes from these clusters could be useful for hybridization programs aiming to enhance heterosis. Conversely, Cluster 1 had the lowest intra-cluster distance (1520.87), signifying a high degree of genetic similarity among its genotypes (Verma *et al.*, 23; Nayak *et al.*, 14). Such a cluster is ideal for stabilizing desirable traits within a breeding program. The moderate genetic distances observed between cluster 1 and cluster 2 (3477.68) and cluster 3 and cluster 4 (2963.35) suggest that these clusters have sufficient genetic diversity to be considered for recombination

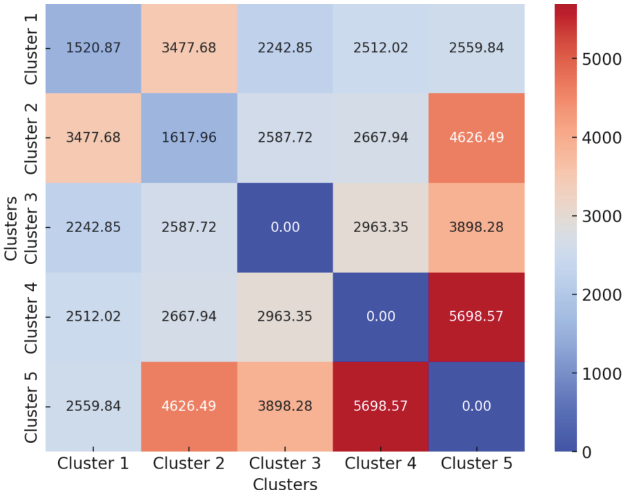

Fig. 2. Inter- and intra-cluster distance heatmap visualizing the genetic distance between clusters.

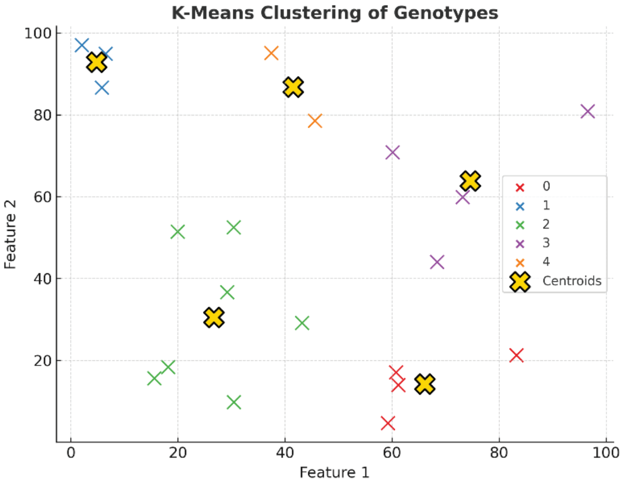

Fig. 3. K-Means clustering distribution of genotypes based on agronomic traits.

Note: 0-4 – number of clusters

and hybridization efforts (Verma *et al*., 23; Nayak *et al*., 14). Cluster 5 recorded the highest values for pod yield traits, including total pod weight (31.25 g) and fresh pod weight (37.30 g), making it an ideal candidate for yield improvement programs. Cluster 3 exhibited the highest dry matter content (42.47%) and seed weight (26.73 g), suggesting its potential utility in biomass production (Pawar *et al*., 15; Kiran *et al*., 10). Cluster 4 demonstrated high chlorophyll content (67.50) and moderate seed yield, making it suitable for stress tolerance and seed production. Clusters 1 and 2 showed balanced agronomic performance, including moderate pod yield, seed weight, and biochemical traits, which could be valuable for breeding programs aimed at overall crop improvement (Gangadhara *et al*., 5). The inter- and intra-cluster distance heatmap highlights significant genetic variation among dolichos bean genotypes. The presence of genetically distant clusters suggests that crossing highly divergent clusters, such as cluster 4 and cluster 5, could maximize heterosis and improve yield potential. Additionally, selecting genetically stable clusters, such as cluster 1, can help in breeding programs focused on trait stabilization. Fig. 2 effectively visualizes the genetic relationships among clusters, offering valuable insights for future genetic improvement strategies.

The K-means clustering method grouped genotypes based on their agronomic and yield-related traits (Fig. 3). The K-means clustering analysis grouped dolichos bean genotypes into five clusters based on genetic similarity. The number of genotypes per cluster ranged from three to ten, with each cluster exhibiting distinct agronomic characteristics. The within-cluster sum of squares (SS) values indicated varying levels of genetic diversity within each group, with cluster 5 (210.5855 SS) showing the highest intra-cluster variability, followed by cluster 2 (208.7302 SS), suggesting potential for further selection within these clusters. The cluster means for key agronomic traits highlighted significant differences among the groups. Cluster 1 exhibited the highest pod yield per plant (6.35 kg) and pod width (1.86 cm), making it a valuable group for yield improvement (Gangadhara *et al*., 5). Cluster 5 recorded the highest pod yield per 10 pods (76.30 g), total soluble solids (12.044 °Brix), and chlorophyll content (16.81 mg/100 g), suggesting its potential for both nutritional and biomass improvement. Cluster 4 had the highest number of seeds per pod (8.35) and the highest dry matter content (40.48%), indicating its suitability for seed production and processing. The genotypic distribution within the clusters revealed close genetic relationships among some genotypes. Cluster 1 contained RLBDL-S-1, RLBDL-S-1-1, RLBDL-S-2, and RLBDL-S-3, showing genetic similarity among these accessions. Cluster 2 grouped RLBDL-S-1-2, RLBDL-S-5, and RLBDL-S-8, forming a distinct subgroup. Cluster 3 included RLBDL-S-4, RLBDL-S-10, and RLBDL-J-4, indicating a mix of genotypes with varying agronomic traits. Cluster 4 had RLBDL-S-6, RLBDL-S-9, RLBDL-S-12, and RLBDL-S-13, while Cluster 5 contained the largest number of genotypes, including RLBDL-J-1, RLBDL-J-2, and RLBDL-J-3, suggesting a diverse genetic pool (Kiran *et al*., 10).

Overall, the K-means clustering analysis confirmed significant genetic diversity among dolichos bean genotypes, with clusters 5 and 4 showing the highest variability in pod yield, seed number, and biochemical traits (Singh *et al*., 19).

These results suggest that crossing genotypes from diverse clusters (Cluster 5 × Cluster 4) could enhance heterosis and yield improvement. The identified clusters provide a basis for breeding programs aimed at improving yield potential, seed quality, and stress tolerance in dolichos bean.

Cluster 5 exhibited the highest pod yield (76.3 g per 10 pods) and the highest chlorophyll content (16.81%), making it an excellent selection for breeding programs focusing on high-yielding and photosynthetically efficient varieties. Cluster 4 had the highest number of seeds per pod (8.35), suggesting its potential use in seed production and improvement programs. These results emphasize that different clusters exhibit specific desirable traits, which can be strategically utilized for targeted trait improvement in dolichos bean breeding.

PCA was conducted to identify the key agronomic traits contributing to genetic variation among the dolichos bean genotypes (Mofokeng *et al*., 13). The first three principal components (PCs) explained 54.36% of the total variance, highlighting the most significant factors influencing genetic diversity (Kiran *et al*., 10).

The PCA analysis identified three principal components (PCs) that explained 54.36% of the total variation in the dataset (Fig. 4) (Gerrano *et al*., 6,7; Vijaykumar *et al*., 24). PCA revealed that PC1 accounted for 22.33% of the total variation, with pod length, pod width, and days to flowering being the most influential traits, indicating their significance in genetic differentiation (Bharati *et al*., 3). PC2 explained 19.28% of the variation, primarily driven by the number of flowers per inflorescence and pods per cluster, highlighting the importance of reproductive traits in genetic diversity (Gangadhara *et al*., 5). PC3 contributed 12.74% of the total variance, with dry matter content and protein estimation playing a major role, emphasizing their relevance in nutritional quality assessment. These findings suggest that breeding programs should prioritize genotypes exhibiting high pod yield and superior reproductive traits, as identified in PC1 and PC2, while also considering the nutritional improvements associated with PC3 (Adu *et al*., 1; Singh *et al*., 22).

The PCA biplot further confirmed that genotypic variation is strongly influenced by morphological and reproductive traits, emphasizing the importance of multi-trait selection in breeding programs (Fig. 5). The PCA biplot illustrates the influence of various traits on the principal components. Pod length has the highest positive contribution, indicating its strong role in explaining the variation in the dataset (Shibli *et al*., 21). In contrast, pod width, days to 50% flowering, and pods per cluster show negative contributions, suggesting their importance in a different dimension of variation. Chlorophyll has minimal influence, staying close to zero, while protein and vitamin C contribute positively, with protein showing a stronger effect. This analysis highlights the key traits driving diversity in the dataset, providing valuable insights for selection and breeding strategies (Sharifi *et al*., 20; Akshaya *et al*., 2).

The 3D PCA score plot visualized genotype distribution based on principal component values. Genotypes RLBDL-S-1, RLBDL-J-2, and RLBDL-J-3 were positioned at extremes in PCA space, suggesting that they are genetically unique and could serve as distinct parental lines in hybridization programs (Fig. 6). Genotypes with higher pod yield per plant clustered separately, confirming that PCA effectively differentiates high-yielding genotypes from lower-yielding ones.

The Tocher clustering method classified the dolichos bean genotypes into five distinct clusters,

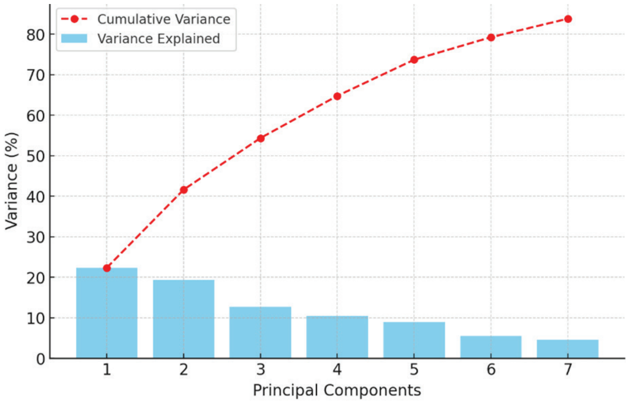

Fig. 4. PCA variance explained plot showing the percentage of total variability explained by each principal component (PC).

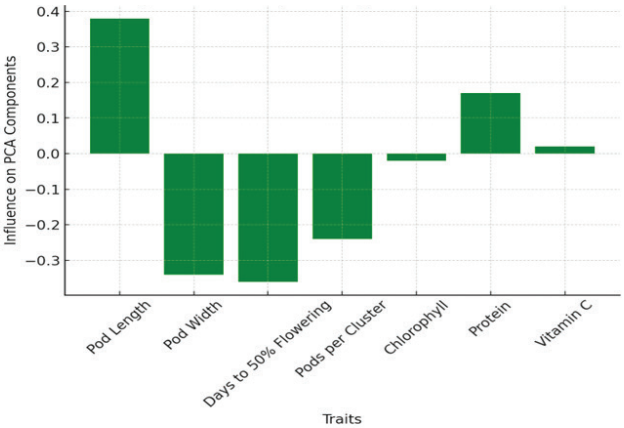

Fig. 5. PCA Biplot highlighting the contribution of key traits to genetic diversity.

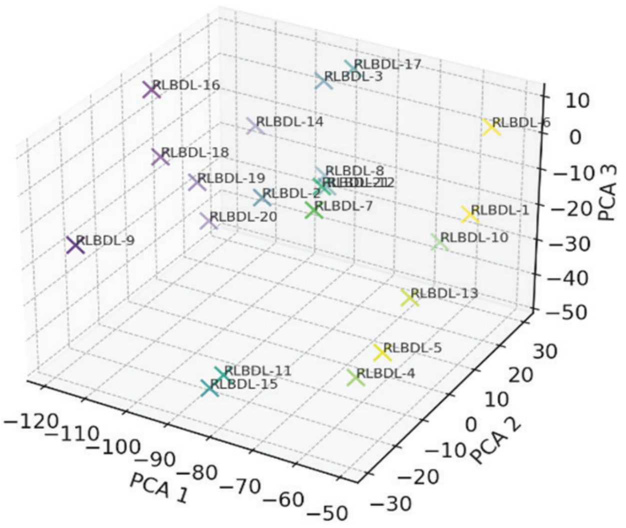

Fig. 6. 3D PCA Score Plot displaying the distribution of genotypes in PCA space based on their genetic variation.

demonstrating varying degrees of genetic similarity and divergence. This clustering pattern suggests that genotypes within the same cluster share common genetic traits, whereas those in different clusters exhibit greater variability, making them potential candidates for hybridization programs. Cluster 1 contained the highest number of genotypes, including RLBDL-J-2, RLBDL-J-3, RLBDL-J-1, RLBDL-S-14, RLBDL-S-1-2, RLBDL-S-10, RLBDL-S-5, RLBDL-S-11, and RLBDL-S-7 (Nayak *et al.*, 14). This grouping indicates a moderate level of genetic similarity among these accessions. Cluster 2, which also had a large number of genotypes, included RLBDL-S-1, RLBDL-S-1-1, RLBDL-S-3, RLBDL-S-2, RLBDL-S-6, RLBDL-S-9, RLBDL-S-13, RLBDL-S-4, and RLBDL-S-12 (Rai *et al.*, 16). This cluster exhibited moderate genetic diversity, suggesting that genotypes within this group could be useful for stabilizing desirable traits through selection. Cluster 3 consisted solely of RLBDL-J-4, indicating that this genotype is genetically distinct from the others. Similarly, cluster 4 contained RLBDL-S-4-5, while cluster 5 included RLBDL-S-8, both of which were highly distinct from the rest of the genotypes (Shibli *et al.*, 21). The genetic distinction of these genotypes suggests that they possess unique traits, making them potential candidates for crossbreeding with genotypes from other clusters to maximize heterosis. The clustering heatmap further supports these findings, as it illustrates the genetic similarity between clusters based on a numerical scale. Clusters 1 and 2 displayed the highest genetic similarity (9.70), while Clusters 3 and 4 exhibited the lowest similarity (0.45), indicating significant genetic distance (Nayak *et al.*, 14). The results suggest that hybridization between genetically distant clusters, such as cluster 3 and cluster 4, could introduce novel genetic variation and enhance agronomic traits. The Tocher clustering approach thus confirms the presence of substantial genetic diversity among dolichos bean genotypes, reinforcing its significance in selecting genetically distant parents for crop improvement programs. The placement of these genotypes in separate clusters suggests that they have unique genetic characteristics, making them potential candidates for crossbreeding with genotypes from other clusters to maximize heterosis. The Tocher clustering results confirm significant genetic variation among dolichos bean genotypes, highlighting the potential for selecting genetically distant parents for hybridization. The presence of highly distinct genotypes in clusters 3, 4, and 5 suggests that crossing these with genotypes from clusters 1 and 2 could enhance genetic variability and lead to the development of improved cultivars with superior agronomic traits (Fig. 7).

The Mahalnobis Euclidean distance matrix confirmed that cluster 5 and cluster 4 had the highest genetic distance (5698.57), suggesting potential for maximizing heterosis and genetic variability through hybridization. Cluster 1 had the lowest intra-cluster distance (1520.87), confirming genetic uniformity within this group (Meena *et al.*, 12). These results indicate that crossing genotypes from highly divergent clusters (Cluster 5 × Cluster 4) could introduce novel genetic combinations and improve hybrid vigour.

This study highlights the presence of substantial genetic variability among dolichos bean genotypes, which can be effectively utilized in breeding

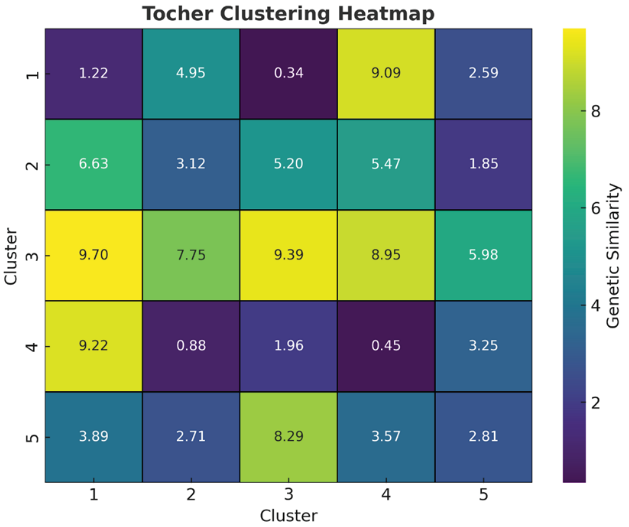

Fig. 7. Tocher clustering heatmap depicting the genetic relationships among clusters.

programs. PCA identified key traits influencing genetic differentiation, while Tocher clustering confirmed genetic groupings. The combination of PCA and clustering methods provides a robust framework for selecting diverse parental lines for breeding. Future research incorporating molecular markers and genome-wide association studies can further refine genetic diversity assessments and marker-assisted selection strategies.

AUTHORS' CONTRIBUTION

Writing - original draft, Formal analysis, Software, Data curation (AS); Investigation, Writing – review & editing, Supervision, Validation (AKP); Writing – review & editing (MP); Formal analysis, Conceptualization, Investigation (GS); Methodology, Investigation, Writing – review & editing (ALO); Writing - review & editing, Methodology (RN); Investigation, Visualization (SA); Conceptualization, Investigation, Resources (DK); Methodology, Resources, Validation (AKS).

DECLARATION

The authors have declared that no competing interests exist.

AKNOWLEDGEMENT

The authors thank RLBCAU for their invaluable assistance in implementing the experiment.

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(Received : April, 2025; Revised : June, 2025;
Accepted : June, 2025)