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CLUSTER ANALYSIS OF COWPEA GENOTYPES BASED ON YIELD AND COMPONENT TRAITS

Gangadhara Doggalli^{1*}, Sanjeev K. Deshpande¹, B.R. Patil¹, S. A. Ashtaputre² and M.G. Hegde³

¹Department of Genetics and Plant Breeding, University of Agricultural Sciences, Dharwad, India -580005

²Department of Plant Pathology, University of Agricultural Sciences, Dharwad, India -580005

³Department of Agricultural Entomology, University of Agricultural Sciences, Dharwad, India -580005

*Corresponding author E-mail: gangadhar0195@gmail.com

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ABSTRACT

Genetic diversity assessment is a prerequisite for effective parent selection in crop improvement programmes. The present study aimed to evaluate the genetic divergence among cowpea [*Vigna unguiculata* (L.) Walp. subsp. *unguiculata*] genotypes based on yield and yield-contributing traits using multivariate analysis. Seventeen diverse cowpea genotypes were evaluated during summer 2024 at the University of Agricultural Sciences, Dharwad, in a randomized block design with four replications. Observations were recorded on nine quantitative characters, including phenological, yield and yield-attributing traits. Genetic divergence was assessed using Mahalanobis' D² statistics, and genotypes were grouped following Tocher's method. The analysis grouped the genotypes into six distinct clusters, indicating the presence of substantial genetic variability independent of pedigree or origin. Cluster I was the largest, comprising five genotypes, while the remaining clusters contained two to three genotypes each. Intra-cluster distances ranged from 8.33 to 19.85, with Cluster III exhibiting the lowest and Cluster VI the highest intra-cluster divergence. The maximum inter-cluster distance was observed between Cluster I and Cluster VI, followed by Cluster I and Cluster III, suggesting wide genetic divergence among these clusters. Genotypes belonging to widely separated clusters are expected to generate greater variability and superior segregants upon hybridization. The results highlight the potential of using genetically diverse parents from distant clusters to enhance yield improvement in cowpea. Overall, the study provides valuable insights into genetic diversity patterns and offers a scientific basis for parent selection in cowpea breeding programmes.

Keywords : Cowpea, genetic divergence, Mahalanobis' D² analysis, cluster analysis, yield and yield-related traits, parent selection.

Introduction

Cowpea [*Vigna unguiculata* (L.) Walp. subsp. *unguiculata*] is an important grain legume cultivated widely in the tropical and subtropical regions due to its nutritional value, adaptability and role in sustainable farming systems (Timko *et al.*, 2007; Boukar *et al.*, 2019). It serves as a major source of dietary protein, minerals and fodder, particularly in resource-poor regions (Gonçalves *et al.*, 2016). Agronomically, cowpea is valued for its drought tolerance, nitrogen-fixing ability and suitability for intercropping and marginal soils (Carvalho *et al.*, 2017). However, despite its importance, cowpea productivity remains

low in many countries, including India, owing to the cultivation of traditional landraces, limited genetic improvement and vulnerability to biotic stresses such as storage pests, especially bruchid beetles (*Callosobruchus* spp.), which cause severe post-harvest losses (Deshpande *et al.*, 2011; Tripathi *et al.*, 2019).

The improvement of cowpea for yield stability and stress resistance largely depends on the effective utilization of genetic diversity available within the species (Timko *et al.*, 2007; Fatokun *et al.*, 2012). Cowpea possesses considerable variability for agronomic, morphological and resistance traits, yet this diversity remains underexploited in breeding

programmes (Deshpande *et al.*, 2018; Doggalli *et al.*, 2024). Systematic assessment of genetic diversity among genotypes is therefore essential to identify genetically divergent parents for hybridization (Swarup *et al.*, 2021). Morphological characterization using quantitative and qualitative traits remains a reliable approach for assessing diversity, as these traits are stable, heritable and easily measurable. Evaluating genetic divergence allows breeders to select superior and diverse genotypes that can generate broad variability and transgressive segregants in subsequent generations, thereby enhancing breeding efficiency (Deshpande *et al.*, 2018).

Clustering of cowpea genotypes based on multivariate analyses plays a crucial role in understanding the extent and pattern of genetic divergence (Mahalanobis, 1936). Grouping genotypes into distinct clusters enables the identification of genetically distant parents, which is particularly valuable for developing high-yielding and resilient cultivars (Murthy and Arunachalam, 1966). Genotypes belonging to widely separated clusters are more likely to produce superior recombinants due to the accumulation of favourable alleles (Arunachalam, 1981). Cluster analysis thus provides a scientific basis for parent selection and breeding strategy formulation, especially for complex traits such as yield and resistance to biotic stresses (Doggalli *et al.*, 2024; Fasahat *et al.*, 2016). In this context, the present study focuses on assessing genetic diversity and clustering of cowpea genotypes to identify promising parental lines that can be effectively utilized in crop improvement programmes.

Materials and Methods

The present investigation was carried out during summer 2024 at the Department of Genetics and Plant Breeding, University of Agricultural Sciences, Dharwad. This experiment material comprised of thirteen diverse genotypes including. The experimental materials of cowpea were sown in Randomized Block Design in four replications. For the recording of observations, five plants of each genotype were randomly selected in each replication excluding the border plant. Data was recorded on 9 quantitative characters at specific growth stages when they were fully expressed. The observations were recorded for 9 characters *viz.*, Days to fifty per cent flowering, Days to maturity, Number of clusters per plant, Number of pods per cluster, Number of pods per plant, Pod length (cm), Number of seeds per pod, Hundred seed weight (g) and Seed yield per plant (g). The D^2 analysis was done by using the method as suggested by Mahalanobis.

Results

Clustering of cowpea genotypes based on yield and yield-related traits

The genetic divergence among the seventeen cowpea genotypes was quantified using Mahalanobis' D^2 statistics, and the genotypes were grouped using Tocher's method of clustering. Based on the relative genetic distances computed from the pooled mean values of yield and yield-related traits, the genotypes were classified into six distinct clusters (Table 1). The formation of multiple clusters of varying size clearly demonstrated the presence of substantial genetic diversity among the genotypes evaluated, which is a prerequisite for effective selection and hybridization in crop improvement programmes.

Cluster I emerged as the largest cluster, comprising five genotypes, namely EC-724153, KBC-5, KBC-6, DC-16 and RC-101. The aggregation of a higher number of genotypes within this cluster indicates a close genetic relationship among them, suggesting similarity in the expression of the traits considered for divergence analysis. Such grouping may be attributed to shared genetic background, common ancestry or parallel selection for similar agronomic traits. The presence of a large cluster also implies comparatively lower intra-cluster genetic distances among these genotypes.

Cluster II consisted of two genotypes, TPTC-29 and C-152, which formed a distinct group separate from Cluster I. The placement of these genotypes in an independent cluster reflects moderate genetic divergence, indicating that they differ appreciably from the genotypes in other clusters for one or more yield-related traits. Similarly, Cluster III comprised three genotypes, *viz.*, KBC-8, KBC-9 and EC-738126. The grouping of these genotypes suggests closer mutual similarity, while their separation from Clusters I and II indicates distinct genetic behaviour across the evaluated traits.

Cluster IV included two genotypes, GC-3 and COCP-7, which exhibited sufficient genetic similarity to be grouped together, yet were divergent from the genotypes in other clusters. Cluster V, represented by EC-724157 and EC-724160, formed another independent cluster, indicating their unique genetic constitution. The occurrence of small clusters containing two genotypes highlights the presence of genotypes with comparatively distinct trait combinations, which may not be widely represented among the remaining genotypes.

Cluster VI comprised three genotypes, namely DCS 47-1, DC-18 (DC-15 × Phule 0540) and DC-15.

The clustering of DC-18 along with its parental line DC-15 indicates the retention of parental genetic characteristics in the derived genotype, while the inclusion of DCS 47-1 suggests partial similarity in yield and yield-related traits. The formation of this cluster reflects a distinct genetic grouping, clearly separated from the remaining clusters.

Overall, the distribution of seventeen cowpea genotypes into six clusters of varying sizes suggests that genetic diversity was largely independent of pedigree or origin and was instead influenced by the collective performance of yield and yield-contributing traits. The existence of both large and small clusters signifies differential levels of genetic divergence among genotypes. Such divergence is particularly important from a breeding perspective, as genotypes belonging to widely separated clusters are expected to yield superior segregants upon hybridization due to the accumulation of favourable alleles. Hence, the observed clustering pattern provides valuable information for the identification of genetically diverse parents for yield improvement in cowpea.

Intra- and inter-cluster distances among cowpea genotypes

The estimates of average intra- and inter-cluster distances among the six clusters of cowpea genotypes, computed using Mahalanobis' D^2 statistics, are presented in Table 2. The diagonal values represent intra-cluster distances, while the off-diagonal values indicate inter-cluster distances. The magnitude of these distances provides a measure of genetic diversity both within and between clusters for yield and yield-related traits.

The intra-cluster distances ranged from 8.33 to 19.85, indicating varying degrees of homogeneity among the clusters. Cluster III recorded the lowest intra-cluster distance (8.33), suggesting a high degree of genetic similarity among the genotypes grouped within this cluster. This implies that the genotypes in Cluster III were relatively uniform in their expression of yield and yield-attributing traits. In contrast, Cluster VI exhibited the highest intra-cluster distance (19.85), followed by Cluster II (19.21), indicating greater genetic variability among the genotypes within these clusters. Higher intra-cluster distances reflect the presence of genotypes with relatively diverse trait expression, despite being grouped within the same cluster.

The inter-cluster distances revealed considerable variation, reflecting differing degrees of genetic divergence between clusters. The highest inter-cluster distance was observed between Cluster I and Cluster

VI (24.36), followed by Cluster I and Cluster III (23.82), and Cluster II and Cluster III (22.00). Such high inter-cluster distances indicate wide genetic divergence between the genotypes belonging to these clusters, suggesting the presence of substantial variability for yield and yield-related traits. Crosses involving genotypes from these widely separated clusters are expected to generate greater heterosis and transgressive segregants due to the accumulation of diverse and complementary alleles.

Moderate inter-cluster distances were observed between Cluster III and Cluster IV (20.43), Cluster III and Cluster V (18.36), and Cluster II and Cluster V (17.66), indicating appreciable but comparatively lower divergence. On the other hand, relatively lower inter-cluster distances, such as those observed between Cluster I and Cluster V (15.36), Cluster III and Cluster VI (12.36), and Cluster IV and Cluster V (12.35), suggest closer genetic affinity between the respective clusters. Genotypes from such clusters are expected to be less divergent and may contribute comparatively lower variability if used as parents in hybridization programmes.

Overall, the wide range of inter-cluster distances in comparison to intra-cluster distances highlights the presence of substantial genetic diversity among the cowpea genotypes studied. The greater divergence between certain cluster pairs indicates that genetic diversity was efficiently captured through the clustering approach. From a breeding perspective, the selection of parents from clusters separated by maximum inter-cluster distances, particularly Cluster I with Cluster VI and Cluster I with Cluster III, would be desirable for the development of high-yielding and genetically diverse segregating populations.

Discussion

Genetic divergence and clustering of cowpea genotypes

The clustering of seventeen cowpea genotypes into six distinct groups using Mahalanobis' D^2 statistics and Tocher's method clearly revealed the presence of substantial genetic divergence among the genotypes evaluated. Genetic divergence analysis based on quantitative traits is a reliable approach for assessing variability, as it captures the cumulative effects of multiple yield and yield-related attributes. The existence of wide divergence among genotypes is a prerequisite for the success of any crop improvement programme, as it enhances the probability of obtaining superior recombinants through hybridization.

In the present study, Cluster I emerged as the largest cluster, comprising genotypes EC-724153, DC-

16, KBC-5, KBC-6 and RC-101, indicating close genetic affinity among these genotypes. Such grouping may be attributed to similarities in their genetic background, pedigree relationships or parallel selection for comparable agronomic traits. In contrast, smaller clusters such as Cluster IV (GC-3 and COCP-7) and Cluster V (EC-724157 and EC-724160) represented genetically distinct groups, suggesting unique trait combinations that were not widely shared among the remaining genotypes. The formation of both large and small clusters indicates differential levels of genetic diversity within the germplasm, a pattern commonly reported in cowpea and other self-pollinated crops. Similar clustering behaviour has been reported by Guimaraes *et al.* (2023), who emphasized that genetic divergence is often independent of geographical origin and more strongly influenced by selection history and trait expression.

From a breeding perspective, the distribution of genotypes into widely separated clusters is particularly significant. The greater genetic distance observed between certain clusters, especially between Cluster I and Cluster VI, suggests that crosses involving genotypes from these clusters are likely to generate higher levels of heterosis and broader segregation in subsequent generations. Such crosses increase the likelihood of recovering transgressive segregants by bringing together complementary alleles governing yield and yield-contributing traits (Gangadhara *et al.*, 2024). Conversely, crosses among genotypes within the same cluster may be useful for stabilizing specific desirable traits, although they are expected to produce relatively limited variability. Similar strategies for utilizing inter- and intra-cluster divergence have been advocated in cowpea and other legumes by Gbedevi *et al.* (2021) and Chipeta *et al.* (2025), who highlighted the importance of cluster-based parent selection in designing efficient breeding programmes.

Overall, the clustering pattern obtained in this study not only confirms the presence of substantial genetic diversity among the cowpea genotypes but also provides a practical and scientifically sound basis for parent selection. The identification of genetically divergent clusters offers valuable guidance for initiating hybridization programmes aimed at combining high yield potential with desirable agronomic and resistance traits. Thus, the genetic divergence analysis undertaken in the present investigation holds significant promise for the development of improved cowpea varieties suited to diverse agro-ecological conditions.

Cluster formation and grouping

The classification of cowpea genotypes into six clusters using Mahalanobis' D^2 statistics in conjunction with Tocher's method demonstrated a wide range of genetic divergence among the genotypes studied. The clustering pattern indicated that genotypes exhibiting similarity for yield and yield-related traits were grouped together, whereas those possessing contrasting trait combinations were placed into separate clusters, reflecting the underlying genetic diversity within the germplasm (Table 1).

Among the identified clusters, Cluster V, comprising EC-724157 and EC-724160, was characterized by a favourable combination of early flowering, higher number of pods per plant, bold seed size and superior seed yield, suggesting the accumulation of desirable alleles for key yield components. Cluster VI, represented by DCS 47-1, DC-18 and DC-15, was distinguished by longer pod length and higher pod number per cluster, although the genotypes in this cluster exhibited relatively late maturity. Despite this limitation, Cluster VI constitutes an important source of pod-related traits that can be exploited in improvement programmes. Cluster I, which included five genotypes, was noted for enhanced branching ability as reflected by higher clusters per plant, along with moderate yield performance, indicating its potential utility in improving plant architecture while maintaining yield stability.

The presence of clusters with distinct trait profiles offers significant advantages from a breeding perspective, as it broadens the scope for selecting genetically diverse parents for hybridization. Similar patterns of genetic grouping, where individual clusters exhibit specific trait advantages, have been reported earlier in cowpea by Patil *et al.* (2025) and Viradiya *et al.* (2025), reinforcing the effectiveness of cluster analysis in identifying useful parental material.

Overall, hierarchical clustering proved to be an effective approach for distinguishing cowpea genotypes based on their performance for yield and related traits. The clear differentiation among clusters underscores its value in identifying genetically diverse and high-performing genotypes for breeding programmes. Clusters exhibiting superior yield performance can be prioritized for direct selection, while moderately performing or unique clusters may serve as valuable donors for specific trait introgression aimed at broadening the genetic base of cowpea.

Genetic divergence based on intra- and inter-cluster distances

The extent of intra-cluster distance serves as an indicator of the degree of genetic resemblance among genotypes grouped within the same cluster. The estimates presented in Table 2 revealed noticeable variation in both intra- and inter-cluster distances, demonstrating the presence of substantial genetic divergence among the cowpea genotypes studied. In the present investigation, Cluster III exhibited the minimum intra-cluster distance, suggesting a high level of genetic uniformity among its constituent genotypes. Such low intra-cluster divergence indicates limited variability within this cluster. In contrast, Cluster VI recorded comparatively higher intra-cluster distance values, reflecting greater heterogeneity among the genotypes grouped within it. The inter-cluster distance network derived using Tocher's method indicated substantial genetic divergence among the clusters of cowpea parental lines (Fig. 1). Variation in inter-cluster distances reflected differing levels of genetic diversity, with higher distances indicating greater divergence and lower distances suggesting closer genetic similarity among clusters.

The inter-cluster distance analysis revealed pronounced genetic divergence between certain cluster pairs. The maximum inter-cluster distance was observed between Cluster I and Cluster VI, followed by Cluster I and Cluster III. These wide distances indicate that the genotypes belonging to these clusters are genetically diverse and possess contrasting trait combinations (Gangadhara *et al.*, 2024). Hybridization between genotypes from such widely separated clusters is therefore expected to produce superior recombinants due to enhanced genetic variability (Guimaraes *et al.*, 2023). The underlying rationale is that crosses involving genetically distant parents increase the expression of non-additive gene action, thereby improving the probability of favourable allelic interactions, heterosis and transgressive segregation for yield and related traits (Doggalli *et al.*, 2024; Vaggar *et al.*, 2022). Similar observations have been reported by Chipeta *et al.* (2025), who emphasized that crosses between genetically divergent cowpea clusters often result in hybrids with improved yield performance. The minimum spanning tree illustrated the relative genetic relationships among clusters by connecting them through the shortest possible inter-cluster distances (Fig. 2). Clusters linked by shorter distances indicated closer genetic affinity, whereas clusters connected

through longer paths reflected greater genetic divergence among the parental lines.

On the other hand, relatively lower inter-cluster distances were recorded between Cluster V and Cluster IV, as well as between Cluster V and Cluster III, indicating a closer genetic relationship among the genotypes within these clusters. Hybridization among such closely related genotypes may result in limited variability; however, these crosses can be advantageous when the breeding objective is to fix desirable traits or to develop uniform and stable lines. In this context, Nkhoma *et al.* (2020) highlighted that genetically similar genotypes are better suited for varietal stabilization rather than for exploiting heterotic effects.

Conclusion

The present study revealed substantial genetic divergence among the cowpea genotypes evaluated, as evidenced by multivariate analysis based on Mahalanobis' D^2 statistics and clustering using Tocher's method. The grouping of genotypes into distinct clusters with varying intra- and inter-cluster distances confirmed the existence of wide variability for yield and yield-related traits. Such diversity provides a strong foundation for effective parent selection in cowpea improvement programmes. Clusters differed markedly in their trait composition, with certain clusters exhibiting superiority for key yield attributes, while others possessed desirable pod- and seed-related characteristics. The identification of clusters showing maximum inter-cluster divergence highlights the potential of utilizing genetically distant parents to generate superior recombinants through hybridization. Conversely, clusters with low inter-cluster distances may be effectively exploited for trait stabilization and the development of uniform lines.

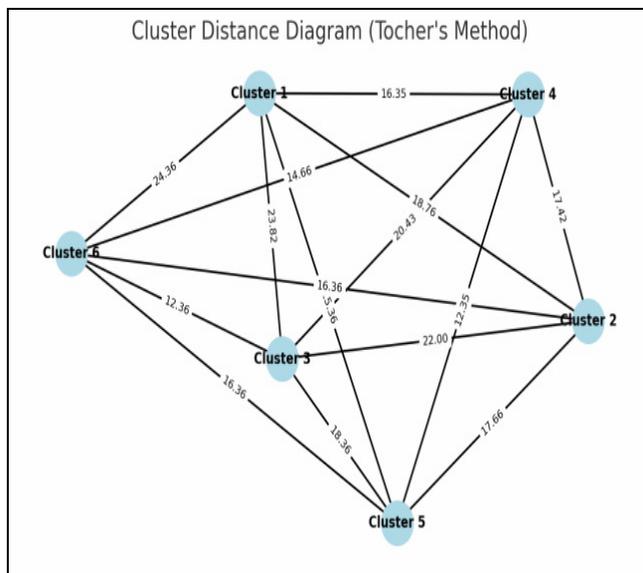
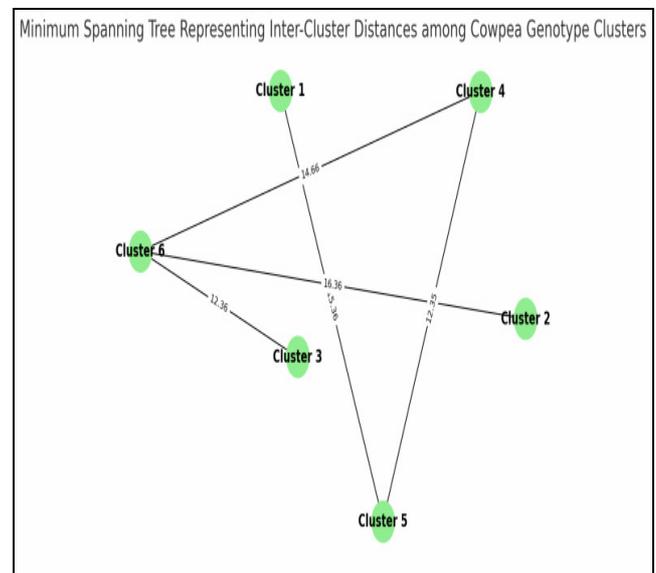
Overall, the combined use of divergence analysis, cluster grouping and graphical representation through minimum spanning tree and boxplots proved effective in discriminating cowpea genotypes based on their phenotypic performance. The insights generated from this study provide valuable guidance for selecting genetically diverse and complementary parents, thereby facilitating the development of high-yielding cowpea genotypes with improved agronomic performance. This approach can be effectively integrated into cowpea breeding programmes aimed at broadening the genetic base and enhancing yield potential.

Table 1: D^2 analysis among Cowpea genotypes collected from diverse eco-system

Cluster 1	EC-724153, DC-16, KBC-5, KBC-6 and RC-101
Cluster 2	TPTC-29 and C-152
Cluster 3	KBC-8, KBC-9 and EC-738126
Cluster 4	GC-3 and COCP-7
Cluster 5	EC-724157 and EC-724160
Cluster 6	DCS 47-1, DC- 18(DC-15 × Phule) and DC-15

Table 2 : Estimates of average intra and inter-cluster distances for yield and yield attributing traits among Cowpea genotypes collected from diverse eco-system

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6
Cluster 1	17.04	18.76	23.82	16.35	15.36	24.36
Cluster 2		19.21	22.00	17.42	17.66	16.36
Cluster 3			8.33	20.43	18.36	12.36
Cluster 4				16.80	12.35	14.66
Cluster 5					12.36	16.36
Cluster 6						19.85

**Fig. 1 :** Inter-cluster distance network derived from Tocher's method of grouping among diverse parental lines of Line x Tester analysis in Cowpea**Fig. 2 :** Minimum spanning tree representing inter-cluster distances among parental lines of Line x Tester analysis in Cowpea.

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