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Shiva Kumar KAssistant Professor, AICRP on
Groundnut, MARS, UAS,
Raichur, Karnataka, India**Rajanna B**Associate Professor,
Department of Genetics and
Plant Breeding, College of
Agriculture, UAS, Raichur,
Karnataka, India**Praveen Kumar B**AICRP on Pigeonpea, Zonal
Agricultural Research Station
(ZARS), Kalaburagi,
Karnataka, India**Raghavendra BT**Associate Professor,
Department of Plant
Pathology, College of
Agriculture, Hagari,
Karnataka, India**Lokesh GY**Professor, Department of
Genetics and Plant Breeding,
College of Agriculture, UAS,
Raichur, Karnataka, India**Corresponding Author:****Shiva Kumar K**Assistant Professor, AICRP on
Groundnut, MARS, UAS,
Raichur, Karnataka, India

Unraveling genetic diversity and trait associations through multivariate analysis in recombinant inbred lines of groundnut (*Arachis hypogaea* L.)

Shiva Kumar K, Rajanna B, Praveen Kumar B, Raghavendra BT and Lokesh GY

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Abstract

The present study assessed genetic divergence and trait contribution among 270 recombinant inbred lines (RILs) of groundnut derived from the reciprocal crosses TMV-2 x TMV-2-NLM and TMV-2-NLM x TMV-2. The experiment was conducted during spring/summer 2025-26 at AICRP on Groundnut, MARS, UAS, Raichur, in an augmented block design. Ward's D² analysis grouped the genotypes into seven clusters, indicating substantial genetic variability within the population. Cluster III recorded superior mean performance for mature pods per plant, hundred-pod weight, shelling percentage and kernel yield, while Cluster VII showed the highest harvest index. The maximum inter-cluster distance was observed between Clusters III and IV, suggesting wide genetic divergence and the potential for effective hybridization. Trait contribution analysis identified kernel yield, pod yield and mature pods per plant as the major contributors to total divergence. PCA showed that the first four principal components explained 78.17% of the total variation, with PC1 primarily associated with yield traits and PC2 with phenological traits.

Keywords: Groundnut, recombinant inbred lines, genetic divergence, mahalanobis d², principal component analysis, yield traits

Introduction

Groundnut (*Arachis hypogaea* L.) is one of the most important oilseed and food legume crops worldwide, valued for its high oil and protein content as well as its contribution to food security and agricultural sustainability. Continuous genetic improvement is essential to enhance productivity, stability and adaptability under changing climatic conditions. The availability of sufficient genetic diversity within breeding populations forms the basis of effective crop improvement, enabling breeders to identify divergent parents for hybridization and exploit heterosis for the development of superior cultivars (Saini *et al.*, 2020; Danalakoti *et al.*, 2024) ^[1, 2].

Recombinant inbred lines (RILs) are valuable genetic resources because of their high level of homozygosity and genetic stability, making them suitable for evaluating complex quantitative traits across environments and for identifying superior breeding materials (Kumar *et al.*, 2024) ^[3]. Assessment of genetic divergence among RILs facilitates the selection of genetically diverse parents capable of generating broad variability and transgressive segregants in subsequent generations (Kulheri *et al.*, 2023; Yami & Abteew, 2025) ^[4, 5].

Multivariate statistical techniques such as Mahalanobis D² statistics, hierarchical cluster analysis and principal component analysis (PCA) are indispensable tools for characterizing genetic diversity and understanding relationships among multiple quantitative traits simultaneously. These approaches classify genotypes into distinct groups based on overall similarity; identify the traits contributing most to genetic divergence and assist in selecting diverse parental combinations for crop improvement (Umadevi *et al.*, 2023; Srivastava *et al.*, 2025) ^[6, 7, 8]. PCA further reduces data dimensionality and identifies the key traits responsible for the observed variation, thereby improving the efficiency of genotype evaluation and selection (Bhatt *et al.*, 2025; Hariharan *et al.*, 2025) ^[11, 12].

Several studies have demonstrated the effectiveness of multivariate analyses in identifying genetically diverse and agronomically superior groundnut genotypes,

while also highlighting the importance of other traits like pod yield, kernel yield, harvest index and pod weight in determining overall productivity (Rajanna *et al.*, 2024; Sonaniya *et al.*, 2025; Umadevi *et al.*, 2025) ^[13, 14, 8]. Moreover, integrating multivariate analysis with recombinant inbred populations provides a robust framework for understanding complex trait architecture and genotype x environment interactions, thereby enhancing selection accuracy and accelerating genetic gain (Rathnakumar *et al.*, 2020; Rani *et al.*, 2024) ^[15, 16].

Despite recent advances in groundnut breeding, information on the extent of genetic divergence and trait contribution among advanced recombinant inbred lines remains limited. Therefore, the present study was undertaken to assess genetic divergence using Mahalanobis D² statistics, characterize variation through principal component analysis and identify genetically diverse and superior recombinant inbred lines.

Material and Method

The study was conducted during spring/summer 2025-26 at All India Coordinated Research Project on Groundnut, MARS, UAS, Raichur, Karnataka (16° 12'N R 77° 21' E; 484 m MSL), located in North Eastern Dry Zone (Zone II) with a semi-arid climate and clay-loam soils. The experimental material comprised 270 recombinant inbred lines (RILs) derived from the reciprocal crosses TMV-2 x TMV-2-NLM and TMV-2-NLM x TMV-2, along with five check varieties, namely TMV-2, Dh-256, ICGV-91114, KDG-128 and K-6. The RILs were advanced through the single pod descent method to ensure genetic uniformity and homozygosity. The experiment was laid out in an Augmented Block Design with six blocks and each RIL was planted in a single 5-m row at spacing of 30x10 cm following recommended agronomic practices.

Data were recorded on yield per plant and related traits, and genetic divergence was estimated using D² statistics and principal component analysis (PCA). All analyses were performed using GRAPES and RStudio software.

Genetic Divergence

Ward's D² analysis revealed substantial genetic divergence among the recombinant inbred lines (RILs), indicating the presence of considerable genetic variability that can be effectively exploited in groundnut improvement programmes. The dendrogram generated from Euclidean distances (Fig. 1) grouped the genotypes into seven distinct clusters, reflecting a broad genetic base within the population. Similar clustering patterns have been reported in groundnut by Danalakoti *et al.* (2024) ^[2] and Sonaniya *et al.* (2025) ^[14], who emphasized that genetically diverse clusters provide opportunities for selecting superior parental combinations.

The unequal distribution of genotypes among the clusters further confirmed the heterogeneous nature of the RIL population. Cluster II contained the largest number of genotypes, indicating greater genetic similarity among its members, whereas Cluster VII with only 15 genotypes represented a relatively distinct genetic group. Cluster mean analysis identified Cluster III as the most promising, recording superior values for mature pods per plant, hundred-pod weight, shelling percentage and kernel yield. Similarly, Cluster VI exhibited favourable performance for kernel yield and shelling percentage, while Cluster VII recorded the highest harvest index, indicating greater assimilate partitioning efficiency. These findings suggest that genotypes from these clusters possess complementary traits that could be effectively utilized in breeding programmes.

The intra and inter-cluster distances (Table 1) further demonstrated the magnitude of genetic divergence among the clusters. The lowest intra-cluster distance observed in Cluster IV indicated greater genetic homogeneity, whereas the highest value in Cluster III reflected relatively broader variation among its constituent genotypes. The maximum inter-cluster distance between Clusters III and IV followed by Clusters II and III indicated wide genetic divergence. According to Kulheri *et al.*, (2023) ^[4] and Umadevi *et al.*, (2025) ^[8] crosses involving highly divergent parents are more likely to produce superior heterotic combinations and broad transgressive segregants because of the accumulation of favourable alleles from genetically diverse backgrounds. Therefore, genotypes belonging to Clusters II, III and IV may serve as valuable parental lines for broadening the genetic base and improving yield potential, as also suggested by Abady *et al.* (2021) ^[17] and Sonaniya *et al.* (2025) ^[14].

Trait contribution analysis (Table 2) revealed that kernel yield, pod yield, and mature pods per plant contributed most to the total genetic divergence, indicating that these characters were primarily responsible for differentiating the RILs. Similar observations by Rajanna *et al.*, (2024) ^[13] demonstrated that these yield-related traits are reliable selection indices for identifying superior genotypes in groundnut breeding programmes. The pronounced divergence between Clusters III and IV therefore provides an excellent opportunity for strategic hybridization aimed at combining desirable yield attributes and generating genetically diverse progenies.

Principal Component Analysis

Principal component analysis (PCA) revealed that the phenotypic variability among the recombinant inbred lines was largely explained by a few principal components. The first four principal components accounted for 78.17% of the total variation, indicating that most of the genetic diversity could be effectively summarized by these components. The scree plot showed a sharp decline in eigenvalues after the fourth component, confirming that subsequent components contributed relatively little to the total variation. Similar results have been reported by Umadevi *et al.*, (2023) ^[6] and Bhatt *et al.*, (2025) ^[11] in groundnut.

The first principal component (PC1) explained 32.60% of the total variance and was predominantly associated with kernel yield, pod yield, mature pods per plant, and shelling percentage indicating that this component primarily represented the yield potential of the genotypes. Consequently, genotypes with higher PC1 scores may be considered superior for yield improvement. PC2 accounting for 18.05% of the variation, was mainly influenced by days to maturity and days to 50% flowering reflecting variation in crop phenology. Similar associations between phenological traits and the second principal component were reported by Hariharan *et al.* (2025) ^[12].

The third principal component (PC3) explained 15.66% of the total variation and was mainly influenced by hundred-pod weight, hundred-kernel weight, and harvest index, indicating the importance of seed size and assimilate partitioning. PC4 contributing 11.86% of the variation, represented biomass partitioning and additional yield-related variation, suggesting that these traits also contributed to the overall diversity of the RIL population.

The loading pattern of the principal components further demonstrated that yield-related traits were the major contributors to PC1, whereas phenological traits primarily influenced PC2. Comparable findings were reported by

Danalakoti *et al.* (2024) [2] and Umadevi *et al.* (2023) [6], who also observed that yield components account for a substantial proportion of the genetic diversity in groundnut. The PCA biplot (figure 2) further supported these relationships by revealing strong positive associations among kernel yield, pod yield, shelling percentage, and mature pods per plant, while plant height, hundred-kernel weight, and harvest index

contributed more prominently to the subsequent principal components. These results indicate that the evaluated RIL population possesses substantial variability for economically important traits and provides valuable genetic resources for parent selection and future groundnut improvement programmes.

Table 1: Average intra (bold) and inter cluster distance (D^2) values of RIL population of Groundnut

Cluster	I	II	III	IV	V	VI	VII
I	2.46358	3.38538	5.09102	2.39413	3.4425	4.263	3.34965
II		2.29449	5.81609	1.97997	2.51312	3.44785	3.44533
III			2.96834	6.06268	4.53381	3.73967	3.7513
IV				1.95856	2.6687	4.07063	3.72913
V					2.53244	3.0398	3.00304
VI						2.42536	3.06276
VII							2.57739

Table 2: Percent contribution of Eleven characters to genetic divergence in RIL population of Groundnut

Source	Percent contribution	Times ranked first
Days to 50 percent flowering	10.11	552
Days to maturity	10.16	555
Plant height (cm)	6.66	364
Number of matured pods per plant	10.69	584
Hundred pod weight (g)	4.61	252
Hundred kernel weight (g)	5.15	281
Shelling percentage (%)	9.53	520
Harvest index (%)	7.75	424
Biological yield per plant (g/plant)	4.63	253
Pod yield per plant (g/plant)	14.71	803
Kernel yield per plant (g/plant)	15.95	871

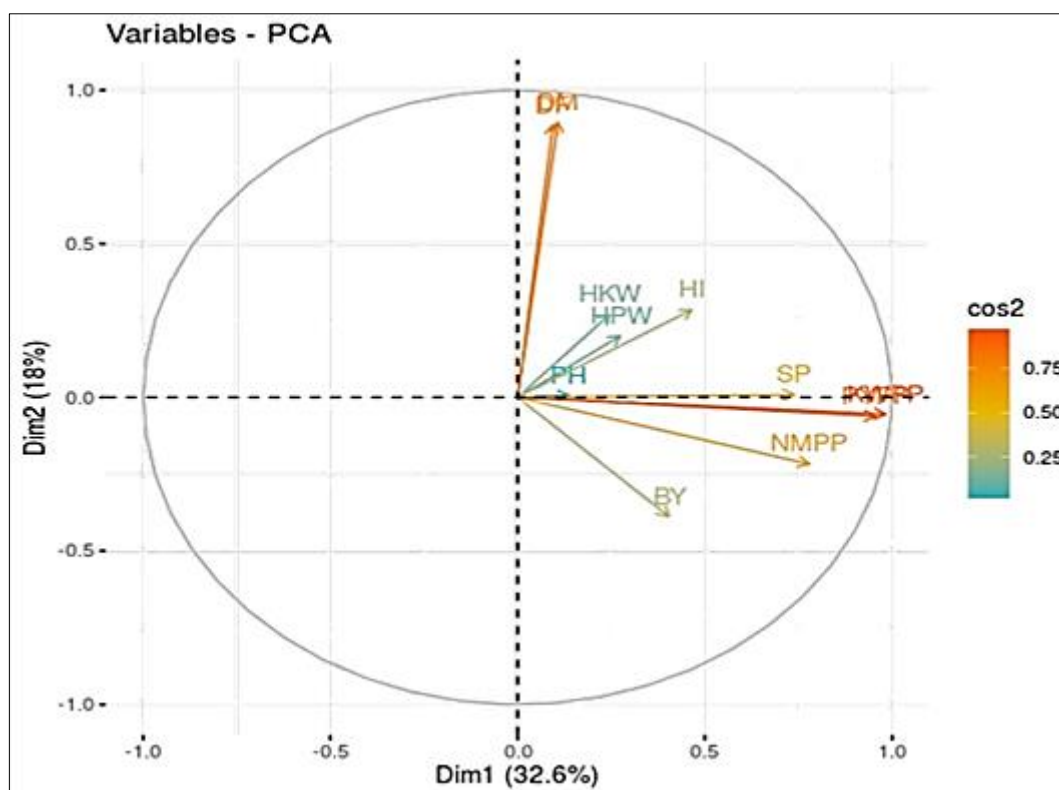


Fig 1: PCA Biplot showing variation among traits.

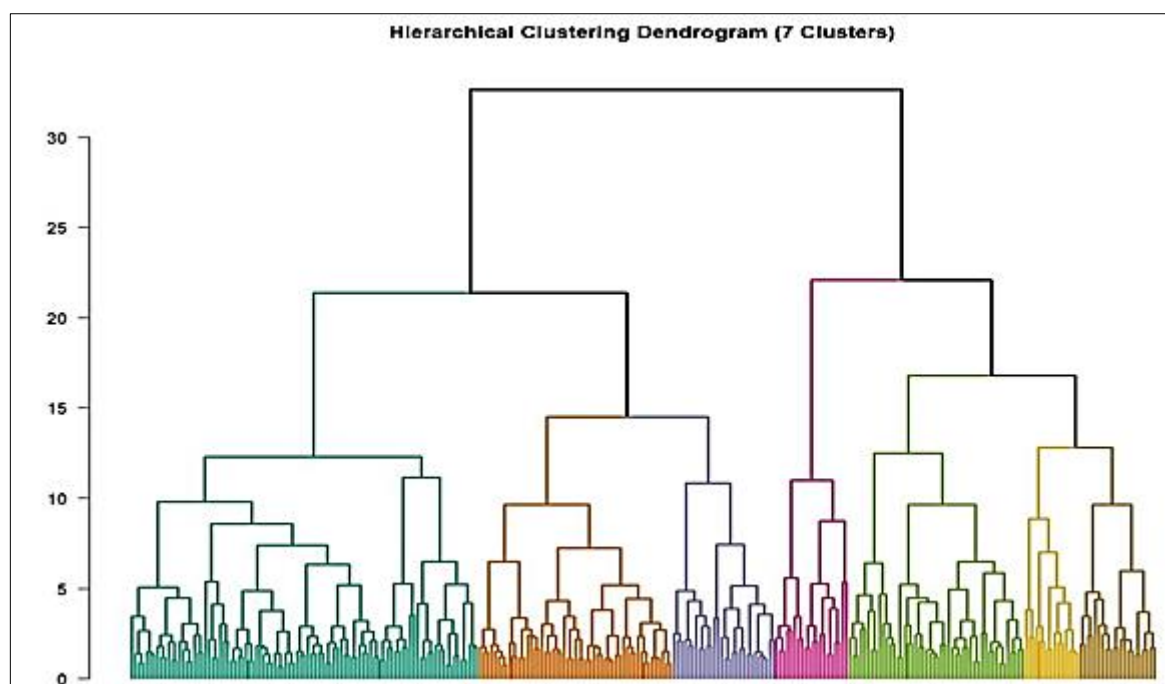


Fig 2: Dendrogram of Groundnut RILs based on phenotypic

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