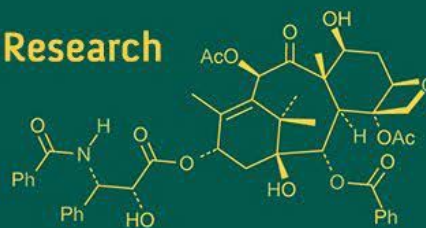
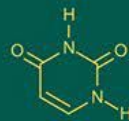
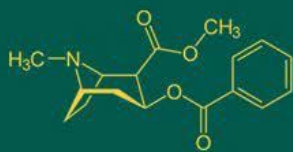


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Unravelling genetic divergence and trait architecture governing yield in Pearl Millet

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Abstract

Understanding genetic diversity and trait interrelationships is essential for the systematic improvement of pearl millet (*Pennisetum glaucum* (L.) R. Br.), a climate-resilient cereal cultivated predominantly in arid and semi-arid regions. The present study aimed to unravel the extent of genetic divergence and to elucidate the trait architecture governing yield among a diverse set of pearl millet germplasm. Thirty-seven genotypes were evaluated in a randomized complete block design with three replications, and data were recorded on twenty-one agro-morphological and yield-related traits at 30 and 60 days after sowing. Multivariate statistical tools, including Mahalanobis' D^2 analysis, Tocher's clustering, and principal component analysis (PCA), were employed to quantify genetic diversity and identify key traits contributing to variability. Significant multivariate differences among genotypes confirmed the presence of substantial genetic divergence. The germplasm was grouped into six distinct clusters, reflecting structured genetic variation and indicating the availability of genetically diverse parental lines. Analysis of inter- and intra-cluster distances revealed a wide divergence between specific clusters, suggesting strong potential for heterosis and transgressive segregation through strategic hybridization. Trait contribution analysis indicated that early-stage structural attributes, particularly stem diameter at 30 days after sowing, along with grain yield and leaf width, played a major role in determining genetic divergence. PCA extracted five principal components with eigenvalues greater than unity, of which the first three accounted for a large proportion of total variation, highlighting the combined influence of growth vigour, biomass accumulation, and yield-related traits on genetic differentiation. The concordance between clustering and PCA results validated the robustness of the multivariate approach. The study identified genetically diverse and agronomically promising germplasm lines like MP7888, PROAGRO9001, LG70304, 86M95, RHUTA ICMV221, GHB732, MS20248, BALWANNBH4903, DHANSHAKTI, and 8695 may be regarded as elite and good germplasm lines that can be effectively utilized in breeding programmes aimed at yield enhancement, genetic base broadening, and long-term sustainability of pearl millet improvement efforts.

Keywords: Pearl millet, *Pennisetum glaucum*, genetic diversity, agro-morphological traits

Introduction

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is one of the most important cereal crops cultivated in arid and semi-arid regions of Asia and Africa, where it serves as a primary source of food, fodder, and livelihood for resource-poor farming communities (Yadav & Rai, 2013) [18]. Its exceptional tolerance to drought, heat, and low soil fertility makes it uniquely suited to marginal environments in which other cereals, such as maize or wheat, fail to produce reliable yields. Beyond yield stability, pearl millet grains are rich in micronutrients such as iron and zinc, contributing significantly to nutritional security and the mitigation of hidden hunger in developing regions (Kamble, 2022) [6]. The sustainability of pearl millet production systems heavily depends on the availability and effective implementation of genetic diversity. As a highly cross-pollinated crop with protogynous flowering, pearl millet naturally harbours substantial intra- and inter-population variability, which provides a critical buffer against biotic and abiotic stresses (Kaushik, 2019) [8]. Genetic diversity enables breeders to identify complementary parental lines, exploit heterosis, and develop hybrids and varieties with improved yield, resilience, and nutritional traits. Molecular and morphological studies consistently demonstrate the presence of wide genetic divergence among released hybrids,

landraces, and breeding lines, confirming the continued availability of exploitable variability for crop improvement (Ambawat *et al.*, 2020) ^[1].

Genetic diversification has played an integral part in counterpoising pearl millet output by lowering vulnerability to disease epidemics and environmental changes. The need to preserve wide diversity in breeding operations has been highlighted by historical experiences, such as the collapse of resistance in hybrids due to restricted genetic bases (Yadav & Rai, 2013) ^[18]. Therefore, conservation and systematic evaluation of diverse germplasm collections are fundamental prerequisites for long-term genetic gain and climate-resilient agriculture.

Grain production remains an essential concern in pearl millet breeding, though selection based solely upon yield is intrinsically constrained because yield is a complicated trait controlled by numerous interrelated factors and greatly impacted by environmental factors. Empirical studies consistently show that genotypes with similar yield performance may differ substantially in underlying yield components, adaptation traits, and stress tolerance, which cannot be captured through univariate selection approaches (Shekhawat *et al.*, 2022) ^[16]. Yield-specific screening also risks dipping the genetic foundation of breeding populations, thus reducing long-term progress and increasing sensitivity to pressures. Traits like plant height, tiller number, panicle structure, and micronutrient content frequently contribute more to genetic divergence than grain yield itself, according to several divergence studies in pearl millet. Consequently, dependence on yield directly may ignore genetically distinctive parents that deliver valuable adaptation or quality traits essential for future breeding goals.

Multivariate divergence analysis provides a robust framework for overcoming these limitations by simultaneously considering multiple traits and their interrelationships. In pearl millet, methods including principal component analysis (PCA), cluster analysis, and Mahalanobis D^2 statistics have been frequently used to measure genetic diversity and find divergent paternal lines (Amudha, 2024) ^[2]. These strategies reliably show that genetic divergence is multifaceted, with variability among germplasm collections being driven by distinct sets of characteristics.

Importantly, multivariate analytical approaches facilitate the identification of parental lines based not solely on mean performance but on comprehensive genetic divergence, thereby increasing the likelihood of generating superior segregants and exploiting heterotic potential in hybrid combinations. Research combining yield, morphological characteristics, and micronutrient content demonstrates that it is possible to increase productivity and nutritional quality at the same time without suffering negative negotiations. Therefore, the adoption of multivariate divergence-based selection constitutes a critical strategy for attaining balanced

genetic advancement, as it not only secures immediate yield improvements but also underpins the long-term sustainability of pearl millet breeding programs. The present investigation aimed to identify genetically diverse and agronomically superior genotypes that could be effectively utilized as potential parents in hybridization programs for sustained yield improvement and adaptability in pearl millet.

Materials and methods

Experimental Material and Field Evaluation

The experimental material comprised 37 pearl millet germplasm lines. The trial was conducted using a Randomized Complete Block Design (RCBD) with three replications. Each entry was grown in plots following recommended agronomic practices to maintain a healthy and competitive plant population

Traits observation

Observations were recorded for a comprehensive set of morphological and yield-related traits. To capture the dynamic growth and development of the crop, data for these quantitative characters were specifically documented at 30 and 60 days after sowing (DAS). To collect mean values for statistical processing, five competitive plants per replication were chosen at random for each trait.

Statistical Tools for Diversity Analysis

Genetic divergence is precisely quantified using Mahalanobis's D^2 statistics (1936), as suggested by C.R. Rao (1952) ^[14], which begins by obtaining a set of uncorrelated linear combinations through the pivotal condensation of the common dispersion matrix of correlated variables. These relationships are used to transform the mean values of different genotypes for various characters into uncorrelated mean values. The specific genetic distance (D^2) is then calculated. Following this calculation, genotypes are organized into group constellations via Tocher's method, applying the criterion that genotypes within the same cluster must exhibit a smaller average D^2 value than those in different clusters. The process concludes by determining intra- and inter-cluster genetic distances, which are computed as the square root of the average D^2 values derived from all possible genotype combinations within or between those clusters.

Result and Discussion

Genetic Divergence and Trait Architecture Analysis

The assessment of genetic divergence among the 35 pearl millet genotypes using Mahalanobis' D^2 statistics revealed significant differences for the combined effect of the 21 characters studied (Figure1). The significance of this multivariate divergence was confirmed by Wilk's criterion ($\chi^2=36830.077$ for 714 degrees of freedom), which justified the extension of the study into D^2 and cluster analyses.

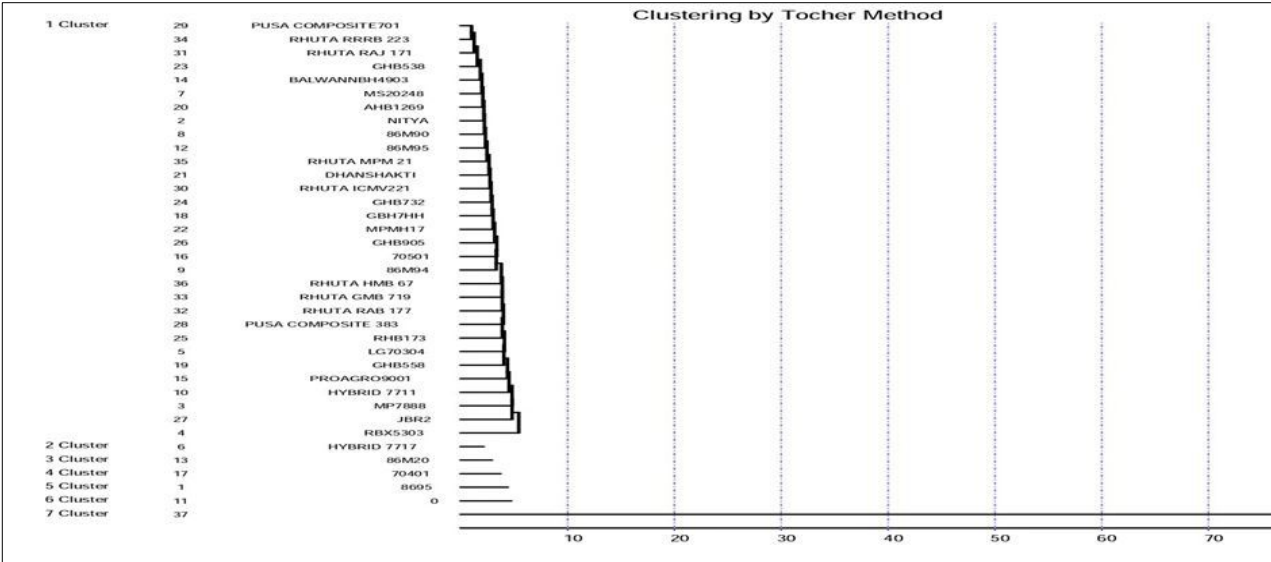


Figure 1: Ward’s Minimum Variance Dendrogram Depicting Genetic Relationships among Pearl Millet Germplasm

Group Constellation and Clustering Pattern

The 37 germplasm lines were organized into six distinct clusters based on their genetic relatedness (Table 1). The distribution of genotypes was relatively uniform, with Clusters II, III, IV, V, and VI containing the majority of the entries, while Cluster I functioned as a smaller, distinct group. This clustering pattern aligns with the findings of

earlier researchers who reported that pearl millet germplasm often partitions into distinct groups ranging from three to ten clusters, depending on the diversity of the base material (Figure2). Specifically, the formation of six clusters is in close agreement with studies by Kalagare *et al.*, 2022 and Amudha, 2024 [5, 2].

Table 1: Cluster Composition of Pearl Millet Germplasm Based on Tocher Method

Cluster	Number of Genotypes	Genotypes
I	7	MP7888, PROAGRO9001, RBX5303, LG70304, HYBRID 7711, 86M94, 70401
II	6	GHB558, RHB173, JBR2, PUSA COMPOSITE 383, RHUTA GMB 719, Unnamed
III	6	HYBRID 7717, GHB538, PUSA COMPOSITE 701, RHUTA RRRB 223, RHUTA HMB 67, 86M20
IV	8	MS20248, 86M90, BALWANNBH4903, NITYA, AHB1269, DHANSHAKTI, MPMH17, RHUTA RAB 177
V	8	86M95, RHUTA ICMV221, GHB732, RHUTA MPM 21, 70501, GHB905, GBH7HH, RHUTA RAJ 171
VI	1	8695

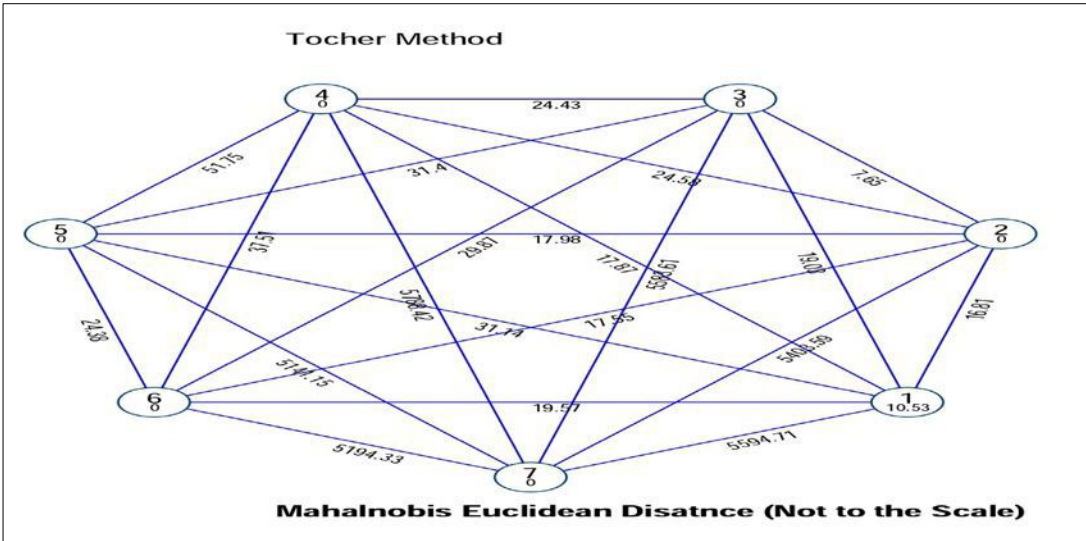


Fig 2: Graphical representation of genetic divergence among pearl millet

Average Intra- and Inter-Cluster Distances

The statistical "distance" within and between clusters provided a measure of internal consistency and external divergence. The maximum intra-cluster distance was recorded in Cluster I (106.79), followed by Cluster IV

(89.77) and Cluster II (84.20) (Table 2). A higher intra-cluster distance suggests significant internal genetic variation among the genotypes within that specific group. Conversely, Cluster V exhibited the minimum intra-cluster distance (66.63), indicating it was the most internally

homogeneous group. The inter-cluster D^2 values revealed substantial diversity between the groups. The maximum inter-cluster distance (250.75) was observed between Cluster I and Cluster V, followed by the distance between Cluster I and Cluster VI (217.33). In genetic terms, the high distance between Cluster I and V identifies these groups as the most genetically divergent, suggesting that hybridization between genotypes from these two clusters could lead to a high heterotic response and the recovery of superior transgressive segregants. The minimum inter-cluster distance (101.89) was found between Cluster IV and Cluster V, identifying them as the most closely related groups in the panel.

Table 2: Inter and Intra-Cluster D^2 Distance Matrix among Pearl Millet Germplasm (Tocher Method)

Cluster	I	II	III	IV	V	VI
I	10.53	16.81	19.03	17.87	31.14	19.57
II	16.81	0.00	7.65	24.58	17.98	17.55
III	19.03	7.65	0.00	24.43	31.40	29.87
IV	17.87	24.58	24.43	0.00	51.75	37.51
V	31.14	17.98	31.40	51.75	0.00	24.38
VI	19.57	17.55	29.87	37.51	24.38	0.00

Relative Contribution of Traits Toward Genetic Divergence

The analysis identified the specific quantitative traits that act as the primary drivers of genetic divergence. Stem diameter at 30 DAS (28.07%) contributed the most significantly to the total genetic diversity. Other major contributors included: i) Grain yield per plant (17.00%); ii) Leaf width at 60 DAS (14.45%); iii) Number of leaves at 60 DAS (11.76%); iv) Number of leaves at 30 DAS (10.08%). Traits such as plant height at 30 DAS (5.04%) and internode length at 60 DAS (7.73%) provided more moderate contributions. Interestingly, while some literature identifies biological yield (Rajpoot *et al.*, 2023) [13] or test weight (Kumar *et al.*) as the highest contributors to divergence in their respective populations, this study highlights early-stage stem architecture (stem diameter at 30 DAS) as the critical factor governing the trait architecture of this specific pearl millet panel.

Principal Component Analysis (PCA) and Trait Relationships

The Principal Component Analysis (PCA) served to validate the D^2 clustering results, demonstrating that the observed genetic divergence was not randomly distributed but followed a structured pattern dictated by the primary principal components (Choudhary *et al.*, 2021) [3]. The analysis extracted five principal components (PCs) with eigenvalues greater than unity. Among these, PCA I was the most dominant, with an eigenvalue of 15.64, accounting for 74.47% of the total variation. This indicates that a major proportion of genetic variability among the genotypes is explained by the first principal component alone. When combined with PCA II (7.20%) and PCA III (5.22%), the first three principal components together explained 86.89% of the cumulative variation, suggesting that these components adequately represent the overall diversity structure of the germplasm. This multivariate approach confirms that the uniqueness of these inbred lines is driven by a combination of early morphological traits and final yield components (Kandarkar *et al.*, 2024) [7].

Table 3: Eigenvalues and Percentage of Variance Explained by Principal Components

Principal Component	Eigenvalue	Variance Explained (%)	Cumulative Variance (%)
PC I	15.6379	74.47	74.47
PC II	1.5125	7.20	81.67
PC III	1.0955	5.22	86.89
PC IV	1.0304	4.91	91.79
PC V	0.5452	2.60	94.39

N.B.- The first three principal components together accounted for 86.89% of the total variability, indicating that they adequately explain the genetic divergence present in the pearl millet germplasm.

Trait loadings revealed that PCA I was positively and heavily influenced by growth-related and biomass traits such as plant height (30 and 60 DAS), number of leaves, stem fresh weight, leaf fresh weight, leaf dry weight, and yield per plant. This indicates that PCA I primarily represent general plant vigour, biomass accumulation, and productivity. Genotypes with high positive scores on PCA I can therefore be considered superior for overall growth performance. PCA II showed high positive loadings for internode length (60 DAS), stem diameter (60 DAS), and stem dry weight (30 DAS), while negative loadings were observed for number of tillers and internode length at 30 DAS. This component appears to differentiate genotypes based on structural traits and dry matter partitioning, separating compact from more elongated plant types. PCA III was strongly influenced by stem dry weight at 60 DAS, which showed a very high positive loading, indicating that this component primarily reflects dry biomass accumulation at later growth stages. The remaining components (PCA IV and PCA V), though contributing smaller proportions of variance, further refined the separation of genotypes based on specific morphological attributes such as internode length and leaf width. The PCA score distribution of genotypes demonstrated clear dispersion along the first three axes, confirming the presence of substantial genetic diversity among the pearl millet entries. Genotypes occupying extreme positions along PCA axes are genetically distinct and may serve as promising parents in hybridization programs to exploit heterosis and generate wider segregation in progenies.

The results of this study underscore the existence of substantial genetic variability within the pearl millet germplasm, a prerequisite for successful breeding programs (Gunguniya *et al.*, 2023) [4]. The identification of six clusters provides a systematic roadmap for parent selection. While geographical origin has been a traditional basis for diversity, this study and others suggest that genetic divergence does not always correlate with geographical distribution, but is instead shaped by parentage and selection pressure.

The high inter-cluster distance between Cluster I and V is particularly noteworthy. Genotypes in Cluster I were characterized by distinct morphological values at 30 DAS, whereas Cluster V genotypes showed high values for plant height and yield. Crossing these divergent types provides an opportunity to combine early-stage vigour with late-stage productivity. These findings are supported by Kandarkar *et al.* (2024) [7], who noted that delineating populations into distinct sub-groups facilitates the development of heterotic pools. Prioritizing traits such as stem diameter and leaf width for selection aligns with studies showing that yield improvement in pearl millet is most effectively achieved by

focusing on characters with high contributions to genetic divergence and strong direct effects (Rajpoot *et al.*, 2023) [13].

Conclusion

The present study conclusively established the presence of wide and structured genetic diversity among the evaluated pearl millet germplasm, as revealed through multivariate analyses. The partitioning of genotypes into distinct clusters using Mahalanobis' D^2 statistics indicated substantial inter- and intra-cluster variability, highlighting the non-random nature of genetic divergence within the population. The occurrence of high inter-cluster distances between specific cluster pairs underscores the potential of these genetically divergent groups for use in hybridisation programs aimed at maximising heterosis and recovering superior segregants. Trait contribution analysis further demonstrated that early-stage structural and growth-related attributes, along with grain yield, played a decisive role in shaping overall divergence, emphasizing the importance of these traits in selection strategies. Principal component analysis corroborated the clustering pattern and confirmed that a few principal components accounted for the majority of total variation, reflecting the combined influence of growth vigour, biomass accumulation, and yield determinants on genetic differentiation. Collectively, these findings reinforce the value of multivariate approaches over univariate selection and provide a robust scientific basis for informed parent selection and the development of high-yielding, adaptable, and genetically diverse pearl millet cultivars. In summary, MP7888, PROAGRO9001, LG70304, 86M95, RHUTA ICMV221, GHB732, MS20248, BALWANNBH4903, DHANSHAKTI, and 8695 may be regarded as elite and good germplasm lines from the present investigation. These genotypes are particularly recommended for use in hybridization programmes, heterotic pool development, and broadening the genetic base of pearl millet breeding populations.

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Authors' Contributions: Authors' Contributions:

- **SS:** Data collection, data analysis, writing-original draft, conceptualization.
- **PB:** Data analysis, Writing - review & editing.
- **LS:** formal analysis, data curation, writing-original draft.
- **DY:** Writing - review & editing.

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Research Content

The study presents original research on genetic variability, trait association, and path analysis in pearl millet germplasm under field conditions. The content is unpublished, has not been submitted elsewhere, and does not infringe upon any existing copyright.

Ethical Approval

The research involved plant materials only and complied with institutional and national guidelines for agricultural field experimentation. No human participants or animals were involved; therefore, formal ethical clearance was not required.

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