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MAHALANOBIS' D²-BASED GENETIC DIVERGENCE AND WARD'S CLUSTERING OF FINGER MILLET [*Eleusine coracana* (L.)] GENOTYPES IN A MULTI-LOCATION TRIAL

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ABSTRACT

Genetic divergence among twenty-four finger millet [*Eleusine coracana* (L.)] genotypes, comprising advanced breeding lines, released cultivars and checks, was assessed using Mahalanobis' generalized distance. The genotypes were evaluated in an alpha lattice design with three replications at six locations (Almora, BAIF Nashik, Gaya, Palem, Ranichouri and Vizianagaram) during *kharif*, 2025 and best linear unbiased estimates across locations for nine quantitative traits formed the basis of the divergence analysis. Hierarchical clustering of the distance matrix by Ward's minimum variance method grouped the genotypes into five clusters: Clusters I and IV were the largest (six genotypes each), followed by Cluster II (five genotypes), Cluster III (four genotypes) and Cluster V (three genotypes). Days to maturity (16.33%), days to 50% flowering (13.63%) and plant height (12.25%) contributed most to the total genetic divergence, together accounting for more than 42% of it. Intra-cluster distance ranged from 9.17 (Cluster I) to 22.60 (Cluster V), whereas inter-cluster distance was greatest between Clusters IV and V (23.69) and least between Clusters I and II (15.20), identifying Clusters IV and V as the most divergent pair. Cluster III recorded the most favourable cluster means for grain yield per plot and stover weight, together with earliness and reduced plant height. Crosses between genotypes of the divergent Clusters IV and V and between the high-yielding Cluster III and Cluster V, are suggested for generating desirable segregants in future finger millet improvement programmes.

Key words : Finger millet, *Eleusine coracana*, Genetic divergence, Mahalanobis' distance, Ward's clustering, Trait contribution.

Introduction

Finger millet, *Eleusine coracana* (L.) Gaertn., is a self-pollinated allotetraploid small millet cultivated extensively in the semi-arid tropics of Africa and South Asia. Once regarded mainly as a subsistence crop, it has recently gained recognition as a nutrient-dense "superfood" because of its high calcium, dietary fibre and polyphenol contents and its marked tolerance of drought, poor soils and low-input management (Kaur *et al.*, 2024). In spite of this renewed attention, the genetic base used in finger millet breeding programmes remains narrow compared with that of the major cereals.

Assessing the genetic divergence present in the available germplasm is the logical first step in any hybridization-based improvement programme, because crosses between genetically distant parents are more likely to yield useful transgressive segregants than crosses between closely related ones (Bharathi *et al.*, 2023). Mahalanobis' generalized distance (D^2) (Mahalanobis', 1936) remains the most widely used multivariate tool for

measuring such divergence in crop germplasm. It is usually combined with a clustering procedure that groups genotypes into homogeneous classes and it also allows the percentage contribution of each character to the total divergence to be estimated (Singh, 1981); together, these outputs identify both the breeding groups present and the traits chiefly responsible for separating them. Tocher's method has been the clustering approach most often applied in earlier finger millet divergence studies, whereas Ward's minimum variance method provides an alternative hierarchical approach that, in foxtail millet, has been reported to partition the same distance matrix differently while still capturing real genetic divergence (Babu and Ayesha, 2019).

The present study was therefore undertaken to assess the genetic divergence among twenty-four finger millet genotypes evaluated at six locations during *kharif* 2025, using Mahalanobis' D², Ward's minimum variance clustering and the contribution of individual characters to divergence, with the aim of identifying genetically divergent and agronomically promising genotypes as parents for future hybridization programmes.

Materials and Methods

The experimental material comprised twenty-four finger millet genotypes (coded G1–G24; Table 1): fifteen test entries (advanced breeding lines) and nine checks, including the released cultivars GPU 67, PR 202, VL 376, CFMV 1, CFMV 2, KMR 316, GPU 48, KMR-630 and RAU 8. The genotypes were evaluated in an alpha lattice design with three replications at six locations: Almora, Uttarakhand (29.5973°N, 79.660°E); BAIF Nashik, Maharashtra (19.90°N, 73.23°E); Gaya, Bihar (24.7969° N, 85.0039°E); Palem, Telangana (16.5156° N, 78.2491°E); Ranichouri, Uttarakhand (30.3207° N, 78.407°E) and Vizianagaram, Andhra Pradesh (18.1039° N, 83.394°E), during *kharif* 2025. Each plot consisted of four rows, each 4 m long, with a spacing of 30 cm × 10 cm; sowing was done by hand dibbling and two hand weedings were carried out as intercultural operations.

Observations were recorded for nine quantitative traits: days to 50% flowering (DFF), days to maturity (DM), plant height at maturity (PH), number of productive tillers per plant (NPT), finger length (FL), number of fingers per panicle (NFPP), 1000-grain weight (TGW), grain yield per plot (GYLD) and stover weight (SW). Genotype means across the six locations were obtained as best linear unbiased estimates (BLUEs) for each trait.

All statistical analyses, including the computation of BLUEs, Mahalanobis' D² (Mahalanobis', 1936) and cluster analysis, were performed in R software. The BLUE-based D² distance matrix was subjected to agglomerative hierarchical clustering by Ward's minimum variance method (Ward, 1963) using the *hclust* function and the dendrogram was cut to partition the genotypes into five clusters. Cluster means, intra-cluster D² and inter-cluster D² were computed for each cluster and the percentage contribution of each of the nine traits to the total divergence was estimated following the method of Singh (1981).

Results

Clustering pattern of 24 finger millet genotypes based on Mahalanobis' D²

Ward's minimum variance clustering of the BLUE-based Mahalanobis' D² matrix resolved the twenty-four genotypes into five non-overlapping clusters (Fig. 1, Table 1). Clusters I and IV were the largest, with six genotypes each, followed by Cluster II with five genotypes and Cluster III with four genotypes; Cluster V was the smallest, with three genotypes.

Genotypes derived from the same breeding series were distributed across different clusters instead of being grouped together. For example, entries of the 'ICF X

Table 1 : Clustering pattern of 24 finger millet genotypes based on Mahalanobis' D² (Ward's minimum variance method).

Cluster	No. of genotypes	Genotype code	Designation
I	6	G1, G3, G6, G10, G13, G24	ICF X 201015-91-1-3, ICF X 201015-9-1-1-8, ICF X 201002-8-11-1-9, ICFV 231038, ICFV231014, GPU 48
II	5	G2, G7, G8, G9, G20	ICF X 201015-9-1-1-26, ICF X 201002-8-11-1-15, ICF X 201015-9-1-1-30, ICFV 231036, CFMV 2
III	4	G4, G11, G12, G21	ICFV 231034, ICFV231013, ICFV231006, KMR 316
IV	6	G5, G14, G16, G17, G22, G23	ICF X 211011-1-23-1-6, ICFV231008, GPU 67, PR 202, RAU8, KMR-630
V	3	G15, G18, G19	ICFV231005, VL 376, CFMV 1

G, genotype code; genotype codes G1–G24 are as defined in Materials and Methods.

Table 2 : Percentage contribution of individual traits to total genetic divergence (Mahalanobis' D^2).

Trait	Contribution (%)
Days to maturity (DM)	16.33
Days to 50% flowering (DFF)	13.63
Plant height at maturity (PH)	12.25
Stover weight (SW)	11.58
Grain yield per plot (GYLD)	10.94
Finger length (FL)	9.89
No. of fingers per panicle (NFPP)	9.78
1000-grain weight (TGW)	8.34
No. of productive tillers per plant (NPT)	7.27

D^2, Mahalanobis' generalized distance; trait abbreviations are given in parentheses.

Table 3 : Intra-cluster (diagonal, bold) and inter-cluster D^2 values among the five clusters.

Cluster	I	II	III	IV	V
I	**9.17**	15.20	17.03	17.47	21.17
II	15.20	**12.29**	19.31	18.01	22.85
III	17.03	19.31	**13.58**	19.80	23.67
IV	17.47	18.01	19.80	**15.24**	23.69
V	21.17	22.85	23.67	23.69	**22.60**

D^2, Mahalanobis' generalized distance; I–V, clusters. Intra-cluster values are on the diagonal (bold).

201015' series were placed in Cluster I (G1 and G3) and Cluster II (G2 and G8).

Percentage contribution of individual traits to total genetic divergence

Days to maturity accounted for the largest share of the total divergence (16.33%), followed by days to 50% flowering (13.63%) and plant height at maturity (12.25%); together, these three traits explained more than 42% of the total divergence among the genotypes. Stover weight and grain yield per plot contributed 11.58% and 10.94%, respectively, whereas the number of productive tillers per plant contributed the least (7.27%) (Table 2).

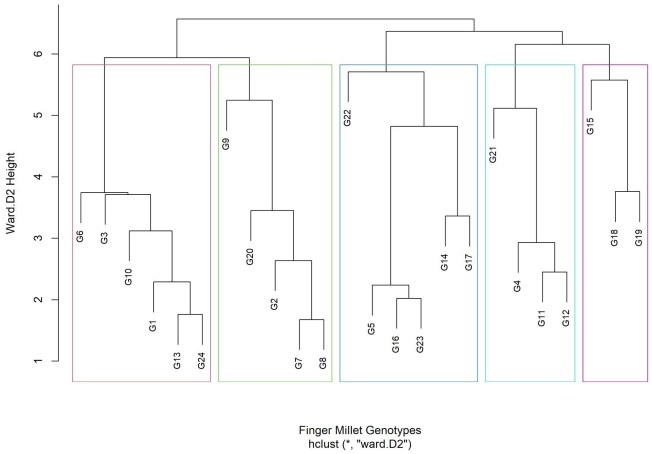


Fig. 1 : Dendrogram of the twenty-four finger millet genotypes based on Mahalanobis' D^2 (Ward's minimum variance method).

Intra-cluster and inter-cluster D^2 values among the five clusters

Intra-cluster D^2 (diagonal values, Table 3) ranged from 9.17 in Cluster I, the most homogeneous group, to 22.60 in Cluster V, which was the most heterogeneous despite being the smallest cluster. Inter-cluster D^2 ranged from a minimum of 15.20 between Clusters I and II to a maximum of 23.69 between Clusters IV and V; Clusters III and V (23.67) formed an almost equally divergent pair.

Cluster mean values for nine quantitative traits (BLUE scale)

Cluster III had the highest mean grain yield per plot (1346.3 g plot^{-1}) and stover weight (2515.6 g plot^{-1}), together with the earliest maturity (116.48 days), the shortest plants at maturity (89.06 cm) and the highest number of productive tillers per plant (2.77) among the five clusters (Table 4). This cluster comprised ICFV 231034, ICFV231013, ICFV231006 and KMR 316. Cluster V had the earliest flowering (76.65 days) and the highest number of fingers per panicle (8.21) and its finger length (8.01 cm) was second only to that of Cluster II (8.51 cm). Cluster II recorded the longest crop duration

Table 4 : Cluster mean values for nine quantitative traits (BLUE scale).

Cluster	DFF	DM	PH	NPT	FL	NFPP	TGW	GYLD	SW
I	81.94	118.92	98.95	2.56	7.52	6.84	3.13	960.4	1973.9
II	83.92	122.42	101.01	2.63	8.51	7.27	3.14	1129.9	1950.9
III	78.58	116.48	89.06	2.77	7.20	6.72	2.94	1346.3	2515.6
IV	82.62	118.19	96.59	2.75	7.41	6.95	2.77	1079.9	1994.8
V	76.65	117.35	95.03	2.47	8.01	8.21	2.83	1117.9	2064.9

DFF, days to 50% flowering (days); DM, days to maturity (days); PH, plant height at maturity (cm); NPT, number of productive tillers per plant; FL, finger length (cm); NFPP, number of fingers per panicle; TGW, 1000-grain weight (g); GYLD, grain yield per plot (g plot^{-1}); SW, stover weight (g plot^{-1}). Values are cluster means of best linear unbiased estimates (BLUEs).

(122.42 days) and the greatest plant height at maturity (101.01 cm).

Discussion

The distribution of genotypes from the same breeding series across different clusters suggests that pedigree or common line origin alone does not adequately explain the genetic relationships among them and it underlines the value of assessing overall multivariate divergence from the combined variation in the traits studied. A similar lack of correspondence between origin and cluster membership was reported by Bharathi *et al*. (2023) in a 105-genotype finger millet study, in which germplasm from the same source location was scattered across several clusters; it reflects the general observation that clustering outcomes depend on overall multi-trait similarity rather than on parentage.

The prominent contribution of days to maturity, days to 50% flowering and plant height at maturity indicates that differences in crop duration, flowering behaviour and plant stature were the main sources of genetic differentiation among the genotypes. Comparable results have been documented in finger millet, where flowering- and maturity-related traits were identified as important contributors to divergence (Bharathi *et al*., 2023; Kumari and Singh, 2015; Soe *et al*., 2022). A comparable assessment of genetic variability has also been reported in foxtail millet (Babu and Ayesha, 2019).

From a breeding standpoint, selecting parents on the basis of divergence alone is likely to pair genotypes that differ mainly in phenology and height; it is therefore advisable to combine such pairs with genotypes selected for grain yield per plot and stover weight (Table 4), so that hybridization targets yield improvement rather than maturity divergence alone.

A maximum inter-cluster distance involving the smallest cluster (Cluster V), as observed here, parallels the findings of Kumari and Singh (2015) in a 22-genotype finger millet study and of Soe *et al*. (2022) in Uttarakhand hill germplasm, in which the most divergent cluster pairs likewise did not coincide with the largest clusters. The recurrence of this pattern in independent finger millet germplasm sets supports the view that cluster size and genetic distinctness are not necessarily related and that a small cluster can still represent a genetically distinct breeding pool.

Cluster-wise performance identified Cluster III as the most agronomically promising group. Its genotypes combined an early, short-statured, high-tillering plant type with superior grain and stover yield, a combination generally favoured in finger millet breeding for lodging resistance and for mechanised harvesting without loss of productivity. A similar pattern of cluster differentiation for yield potential was observed by Kumari and Singh (2015), who reported a cluster with desirable earliness and yield-related characteristics among the five clusters studied. Likewise, Soe *et al*. (2022) showed that certain clusters in finger millet germplasm from the Himalayan region had higher genetic advance for grain and biological yield, which emphasizes the importance of cluster-wise evaluation in identifying promising genotypes for crop improvement.

Following the D²-based parent selection principle applied by Bharathi *et al*. (2023) and Nagaraja *et al*. (2023) in their finger millet divergence studies, crosses between genotypes of Cluster IV (ICF X 211011-1-23-1-6, ICFV231008, GPU 67, PR 202, RAU 8 and KMR-630) and Cluster V (ICFV231005, VL 376 and CFMV 1), the most divergent pair ($D^2 = 23.69$), are expected to maximize the genetic distance between parents and thereby the likelihood of recovering desirable transgressive segregants. However, D² reflects overall multi-trait dissimilarity, which in this study was driven chiefly by maturity duration and plant height at maturity (Table 2) and it does not by itself guarantee the magnitude of heterosis in the progeny. Combining divergence-based pairing with the demonstrated yield performance of Cluster III, for example by crossing Cluster III genotypes with the almost equally divergent Cluster V ($D^2 = 23.67$), therefore offers a more targeted hybridization strategy than relying on the maximum inter-cluster distance alone, because it joins genetic divergence with *per se* performance for grain yield per plot and stover weight.

Conclusion

Ward's minimum variance clustering of BLUE-based Mahalanobis' D² distances grouped twenty-four finger millet genotypes, evaluated at six locations during *kharif* 2025, into five genetically distinct clusters of unequal size. Days to maturity, days to 50% flowering and plant height at maturity together accounted for more than 42% of the total divergence, identifying maturity duration and plant stature as the principal drivers of genetic differentiation in this germplasm set. Clusters IV and V were the most divergent pair ($D^2 = 23.69$) and are recommended as parents for hybridization aimed at generating transgressive segregants, whereas Cluster III, which combined high grain yield per plot and stover weight with earliness and reduced plant height at maturity, was identified as the most promising donor group for direct use or as one parent in targeted crosses with the genetically distant Cluster V.

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