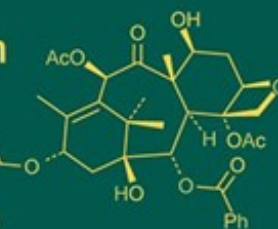
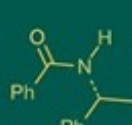


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Multivariate analysis of genetic divergence among rice genotypes using Mahalanobis D^2 statistic

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ABSTRACT

The present investigation was carried out to assess the genetic divergence among 28 rice (*Oryza sativa* L.) genotypes, including released varieties and traditional landraces of Chhattisgarh, using the Mahalanobis D^2 statistic and to identify suitable parents for hybridization. The genotypes were evaluated in a randomized block design with three replications during kharif 2025-26 at the College of Agriculture and Research Station, Mahasamund. Six characters were recorded: days to 50 per cent flowering, stem length, panicle length, effective tillers per plant, 1000-seed weight and grain yield. Genotypes differed highly significantly for all six characters and Wilks' λ showed that they also differed when the characters were taken together. Of the 378 pairwise D^2 values, 365 were significant at the 1 per cent level, so divergence was widespread in the collection. Tocher's method grouped the genotypes into six clusters. One cluster held eighteen genotypes, while three clusters had only one genotype each. The widest gap was between the cluster of the aromatic landrace Jaldubi and the cluster of CG Barani Dhan-1, whereas genotypes within the large cluster were closely related. Days to 50 per cent flowering contributed most to divergence (38.10 per cent), followed by grain yield (25.40 per cent) and stem length (24.07 per cent). In canonical variate analysis, the first two functions explained 87.82 per cent of the variation among genotypes, which agreed with the clustering. Crosses between the high-yielding genotypes Zinco rice, Mahamaya, Chandrasini and Swarna and the highly divergent landrace Jaldubi could help broaden the genetic base of rice breeding in the region.

Keywords: Rice, Genetic divergence, Mahalanobis D^2 , Tocher's clustering, Canonical variate analysis, Multivariate analysis

Introduction

Rice (*Oryza sativa* L.) is the main food crop of Chhattisgarh. The state holds one of the largest active rice germplasm collections in the world and is a rich source of genetic diversity for the crop (Burman *et al.*, 2019) [1]. Raising yield through breeding depends on finding parents that are genetically different, because crosses between closely related genotypes seldom give heterosis or transgressive segregants. The traditional and aromatic landraces still grown in the state, together with the released varieties, offer a wide base in which such parents can be found (Patel *et al.*, 2015) [2].

Comparing genotypes one character at a time gives an incomplete and sometimes misleading picture, because it ignores the correlations among characters. Mahalanobis (1936) [3] solved this with the D^2 statistic, which weights the difference between two mean vectors by the inverse of their pooled dispersion matrix. The distance obtained does not depend on the units of measurement or on the correlations among characters. Rao (1952) [4] later described how to compute D^2 in biometric work, along with Tocher's method for sorting populations into compact groups that lie far from each other.

Used together, D^2 and Tocher's clustering let a breeder group germplasm objectively, see which genotypes are farthest apart and find which characters drive the separation. A univariate comparison cannot give this information. The present study was therefore carried out to measure divergence among 28 rice genotypes grown in the Mahasamund region of Chhattisgarh using the D^2 statistic, to test the significance of the distances and to use Tocher's clustering to identify the most divergent genotypes as parents for future hybridization.

Materials and Methods

The experiment was conducted during kharif 2025-26 at the Research cum Instructional Farm, College of Agriculture and Research Station, Mahasamund, Indira Gandhi Krishi Vishwavidyalaya, Raipur (21°06' N, 82°06' E; 318 m above mean sea level). The site receives 1,200–1,600 mm of annual rainfall, mostly from

the south-west monsoon. Twenty-eight rice genotypes, made up of released varieties and traditional aromatic and fine-grain landraces (Table 1), were grown in a randomized block design with three replications.

Data were recorded on days to 50 per cent flowering, stem length (cm), panicle length (cm), effective tillers per plant, 1000-seed weight (g) and grain yield (q/ha). Each character was tested by analysis of variance and all six were tested together by Wilks' λ (Wilks, 1932) [5]. Genetic divergence was then estimated by the Mahalanobis D^2 statistic (Mahalanobis, 1936) [3] as:

$$D^2 = (\bar{x}_1 - \bar{x}_2)'S^{-1}(\bar{x}_1 - \bar{x}_2)$$

where $\bar{x}_1$ and $\bar{x}_2$ are the mean vectors of two genotypes and S is the pooled within-genotype

variance-covariance matrix. D^2 is related to Hotelling's T^2 (Rao, 1973) [6] and, unlike Euclidean

distance, is not affected by units of measurement or correlations among characters (Atchley, 1980) [7]. Each of the 378 pairwise D^2 values was tested against the chi-square distribution with six degrees of freedom.

The contribution of each character to divergence was calculated by the ranking method (Singh *et al.*, 1977) [8]. Genotypes were grouped by Tocher's method (Rao, 1952) [4] and the grouping was checked by hierarchical clustering (unweighted pair-group method with arithmetic mean) and the cophenetic correlation coefficient (Sokal and Rohlf, 1962) [9]. Canonical variate analysis was also performed after checking multivariate normality with a chi-square quantile plot. All analyses were done in Microsoft Excel and R version 4.6.1 (R Core Team, 2026) [10].

Results and Discussion

Genotypes differed highly significantly ($P < 0.01$) for all six characters (Table 2). The low coefficients of variation (1.33–6.24 per cent) showed good experimental precision, similar to earlier rice trials (Shinde *et al.*, 2022; Singh *et al.*, 2021) [11, 12]. Wilks' λ was also significant ($\lambda = 1.00 \times 10^{-7}$; $F = 28.93$; $P < 0.001$; Table 3), confirming that genotypes differed when all characters were considered together. Pairwise D^2 values ranged from 1.22 to 2210.49. Of the 378 pairs, 365 were significant at the 1 per cent level and the remaining 13 were not, so divergence was present across almost the whole collection. Nidhi *et al.* (2025) [13] found much the same in a comparable set of rice genotypes. The highest values were between Vikram-TCR and Jaldubi (2210.49) and between Poornima and Jaldubi (2186.90), which makes Jaldubi the most distinct genotype.

The largest share of divergence came from days to 50 per cent flowering (38.10 per cent). Grain yield (25.40 per cent) and stem length (24.07 per cent) came next and 1000-seed weight added

10.32 per cent. Panicle length and effective tillers per plant together gave less than 2.2 per cent (Table 4, Fig. 1). The leading character often varies between germplasm sets (Missio *et al.*, 2007) [14]; in other studies, for example, grain breadth and 100-grain weight were the main contributors (Mounika *et al.*, 2020; Thakare *et al.*, 2025) [15, 16]. Tocher's method grouped the genotypes into six clusters (Table 5, Fig. 2). Cluster II was the largest with 18 genotypes. Cluster I had four and cluster III had three, whereas clusters IV, V and VI held only Indira Sugandhit Dhan-1, CG Barani Dhan-1 and Jaldubi, respectively. Clustering was not related to release status or origin, a pattern Burman *et al.* (2019) [1] and Hema Sai *et al.* (2022) [17] also observed in rice.

Intra-cluster D^2 ranged from 66.65 (cluster I) to 150.42 (cluster III) and was much lower than inter-cluster D^2 (Fig. 3). Clusters V and VI were the most distant ($D^2 = 1824.07$). Cluster VI was in fact the farthest from every other cluster, with D^2 of 1726.04 from cluster II and 1317.54 from cluster III. At the other end, clusters I and IV were the closest ($D = 13.51$). Nivedha *et al.* (2024) [18] reported similarly wide inter-cluster distances in rice.

Cluster VI (Jaldubi) had the tallest plants (160.03 cm), the longest panicles (28.50 cm) and the latest flowering (122.33 days), but the fewest tillers (6.00) and the lowest yield (27.50 q/ha) (Table 6). Cluster II had the highest mean yield (48.42 q/ha) and included Zinco rice, Mahamaya and Chandrahasini. Swarna, another good yielder, fell in cluster III (45.00 q/ha).

The hierarchical dendrogram agreed with the Tocher grouping and its cophenetic correlation of 0.880 was above the 0.7 needed for a good fit (Sokal and Rohlf, 1962) [9].

In canonical variate analysis, the first two functions explained 87.82 per cent of the variation, with 76.48 per cent from the first. The first function contrasted days to 50 per cent flowering (-0.876), stem length (-0.881) and panicle length (-0.794) with effective tillers per plant (0.803), 1000-seed weight (0.835) and grain yield (0.837). It thus separated tall, late types from early, high-yielding ones. The plot of genotypes on the first two functions (Fig. 4) matched the Tocher clusters, with Jaldubi at the negative extreme.

Conclusion

The 28 rice genotypes tested at Mahasamund showed wide and mostly significant genetic divergence. Days to 50 per cent flowering, grain yield and stem length together accounted for 87.57 per cent of it. Tocher's method formed six clusters that were not related to genotype origin. Jaldubi was the most divergent genotype, while cluster II, the largest and highest-yielding group, held productive genotypes such as Zinco rice, Mahamaya and Chandrahasini. Canonical variate analysis agreed with the clustering and showed that phenology and plant stature were the main axes of separation. Crosses between the high-yielding genotypes (Zinco rice, Mahamaya, Chandrahasini and Swarna) and the divergent Jaldubi, CG Barani Dhan-1 and Indira Sugandhit Dhan-1 should combine genetic distance with good agronomic performance. These crosses deserve testing in future rice hybridization programmes, in line with the value of agro-morphological diversity studies for parent selection in rice (Patil *et al.*, 2025) [19]. Even so, parents should be chosen on both divergence and desirable traits, not on divergence alone.

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1980;29(2):161-76.

1962;11(2):33-40.

Tables

Table 1. Treatment mean squares and coefficient of variation for six characters in 28 rice genotypes (RBD, 3 replications)

Character	Treatment MS (df = 27)	Error MS (df = 54)	CV (%)
Days to 50% flowering	252.99**	1.68	1.33
Stem length (cm)	1100.95**	5.87	2.16
Panicle length (cm)	7.55**	0.21	1.85
Effective tillers per plant	6.37**	0.35	6.24
1000-seed weight (g)	20.66**	0.23	2.14
Yield (q/ha)	178.56**	1.34	2.62

** Significant at $P < 0.01$.

(continued on next page)

Table 2. Test of the genotype effect by Wilks' lambda criterion

Source	df	Wilks' λ	Approx. F	Pr(>F)
Replication	2	0.7887	1.03	0.4288
Treatment	27	1.00×10^{-7}	28.93	< 0.001**

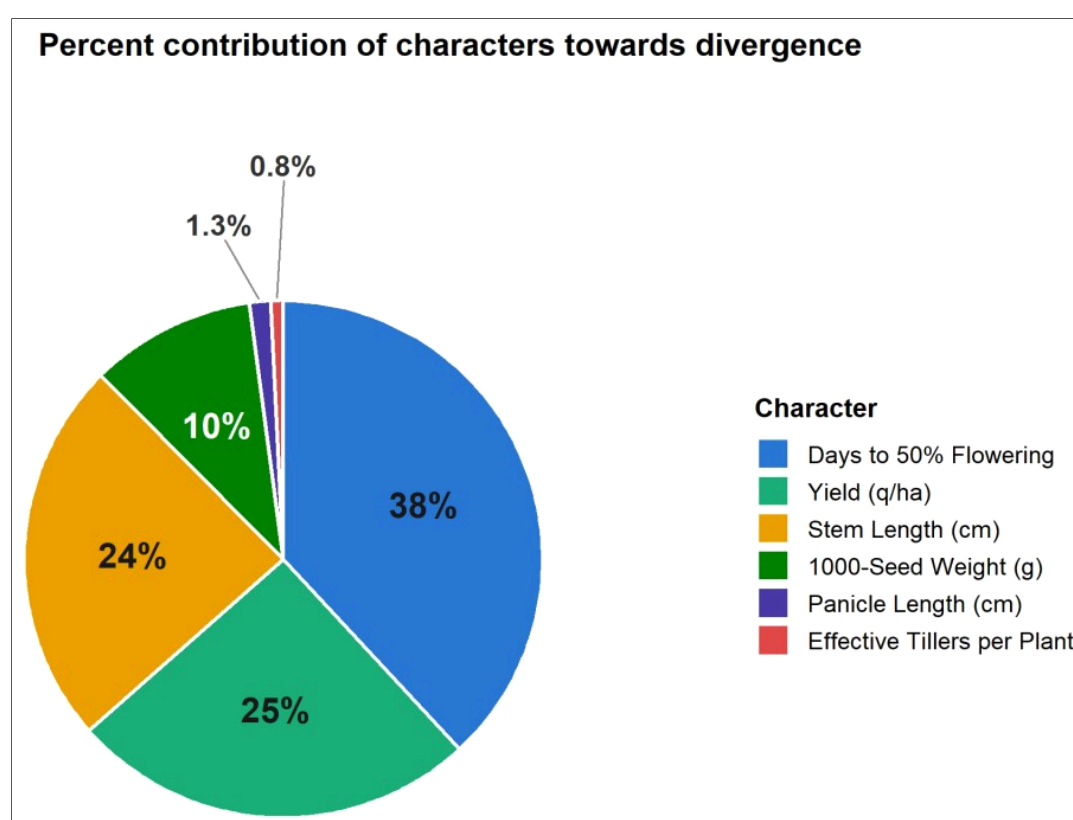
** Significant at $P < 0.01$.

Table 3. Contribution of the six characters to total divergence

Character	Times ranked first (of 378 pairs)	Contribution (%)
Days to 50% flowering	144	38.10
Yield (q/ha)	96	25.40
Stem length (cm)	91	24.07
1000-seed weight (g)	39	10.32
Panicle length (cm)	5	1.32
Effective tillers per plant	3	0.79

Table 4. Composition of the six clusters formed by Tocher's method

Cluster	No. of genotypes	Genotypes (codes)
I	4	G7, G8, G15, G27
II	18	G1, G2, G3, G5, G6, G10, G11, G12, G16, G17, G19, G21, G22, G23, G24, G25, G26, G28
III	3	G4, G9, G20
IV	1	G13
V	1	G14
VI	1	G18

**Figure 1.** Percentage contribution of the six characters to total divergence

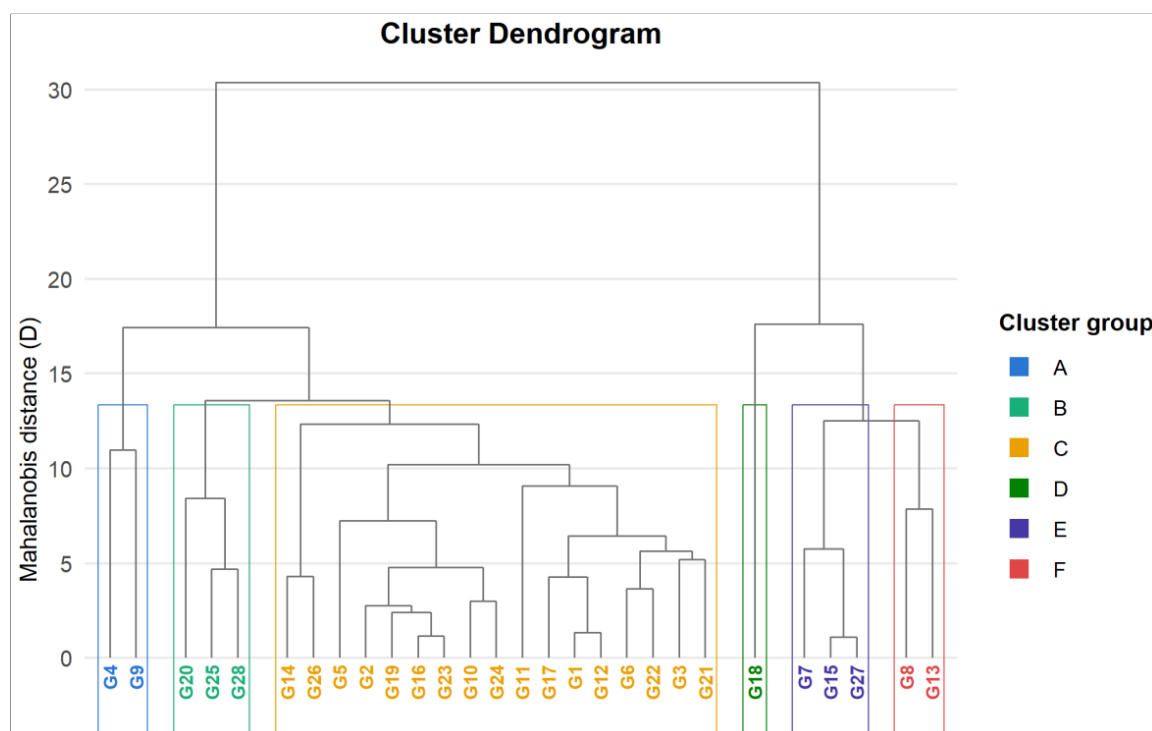


Figure 2. Dendrogram of the 28 rice genotypes obtained by hierarchical clustering of Mahalanobis distances, boxed into six groups corresponding to the Tocher clusters

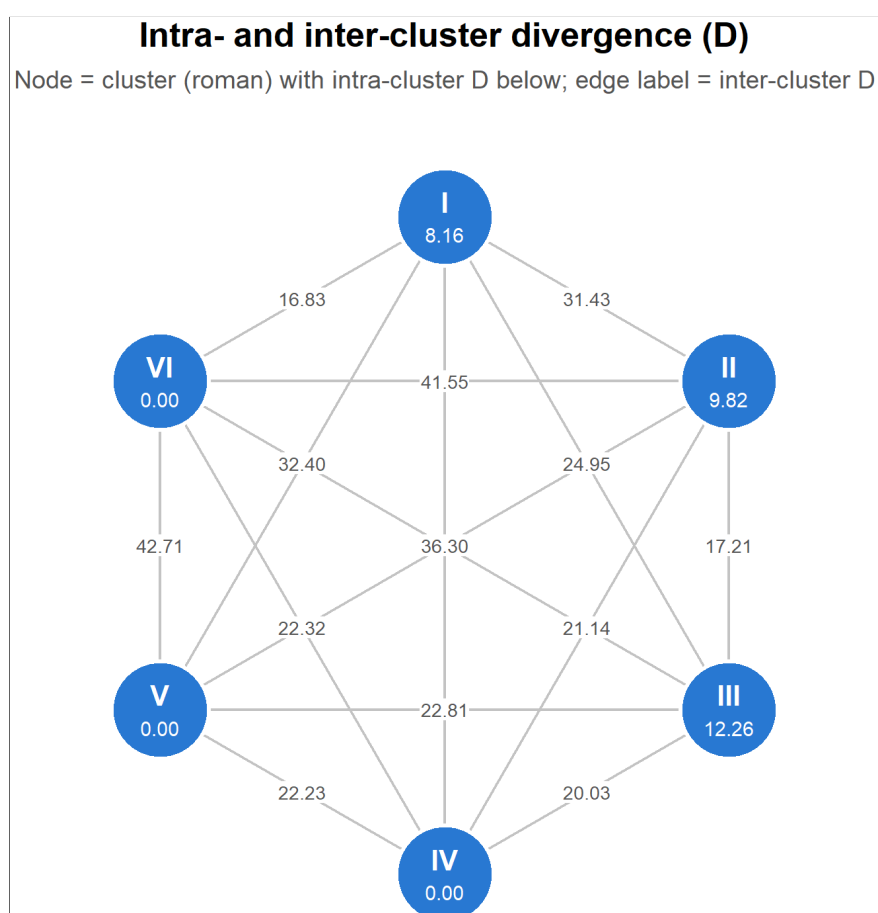


Figure 3. Diagrammatic representation of intra- (node) and inter-cluster (edge) Mahalanobis distance (D) among the six clusters

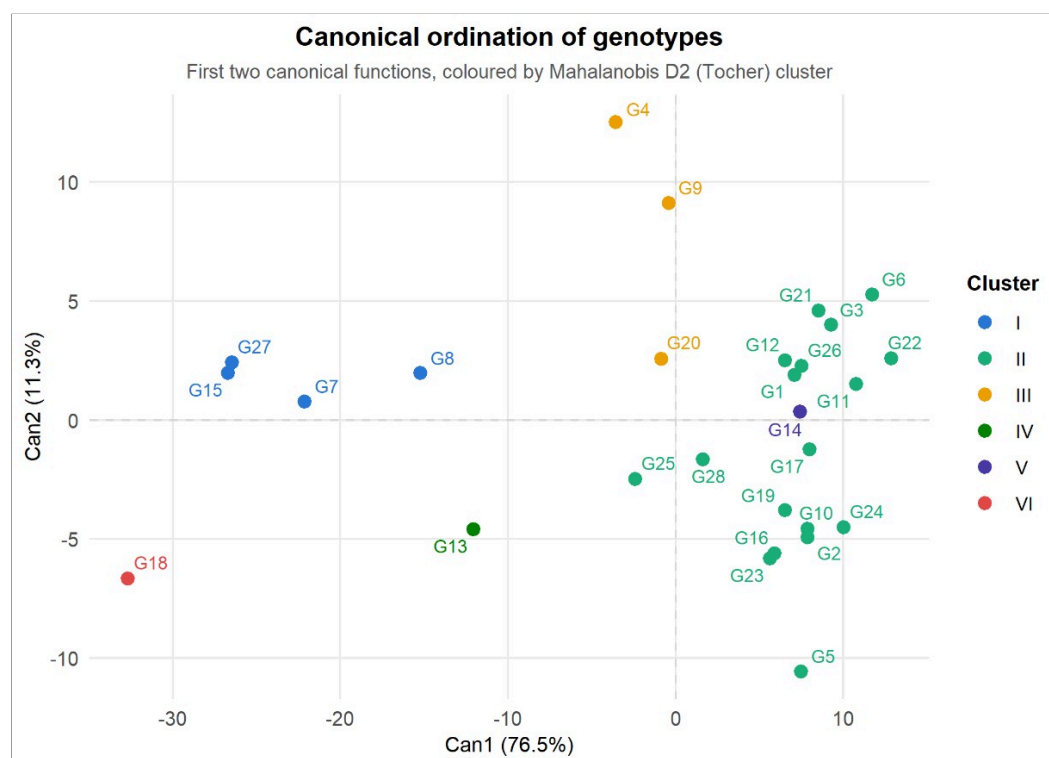


Figure 4. Canonical ordination of the 28 genotypes on the first two canonical functions, coloured by Tocher cluster

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