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Assessment of Genetic Divergence in Sorghum [*Sorghum bicolor* (L.) Moench] using Mahalanobis D² Statistics

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Abstract

Genetic divergence among genotypes can be measured using Mahalanobis D² statistics, which are widely used to classify genotypes into clusters based on multivariate traits. The present investigation was carried out during the *kharif* season of 2023 at the Instructional Research Farm, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, to assess genetic divergence

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in sorghum [*Sorghum bicolor* (L.) Moench]. The experimental material comprised 60 sorghum genotypes and three standard checks, which were evaluated in an augmented design. Fourteen quantitative traits, including days to 50 per cent flowering, days to maturity, plant height, yield components, protein content and grain yield per plant, were recorded. Genetic divergence was estimated using Mahalanobis D^2 statistics, and genotypes were grouped using Tocher's method. The 63 entries were classified into fourteen clusters, indicating considerable genetic variability among the material studied. Clusters III and IV each contained the highest number of genotypes, while clusters VIII to XIV were monogenotypic, suggesting the presence of distinct genotypes. Inter-cluster distances were generally higher than intra-cluster distances, indicating substantial divergence among clusters. The highest inter-cluster distance was observed between clusters II and IX, followed by clusters IX and XIV, suggesting that genotypes from these clusters may be useful in hybridisation programmes. Cluster mean analysis showed that clusters XIV, V and X had higher grain yield per plant, while cluster VII recorded the highest biological yield per plant. Grain yield per plant contributed the highest proportion to total divergence, followed by plant height, harvest index and biological yield per plant. The results suggest that selecting genetically diverse genotypes with desirable mean performance may help identify promising parents for hybridisation and selection in sorghum breeding.

Keywords: Sorghum; Sorghum bicolor; genetic divergence; Mahalanobis D^2; Tocher's method; cluster analysis; augmented design; grain yield; biological yield; harvest index.

1. Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is an important cereal crop grown in arid and semi-arid regions because of its adaptability to drought and high temperatures (Doggett, 1988). It ranks among the major cereals globally and serves as a source of food, fodder and industrial raw materials (Kumar *et al.*, 2011). It is also used for animal feed, biofuel production and brewing purposes (Taylor *et al.*, 2006). Sorghum is believed to have originated in Africa, particularly in Ethiopia and Sudan, and later spread to different parts of the world, resulting in diverse landraces and cultivars (Harlan & de Wet, 1972; House, 1985). India is considered a secondary centre of diversity with substantial genetic variability (Murty, 1983). Recent field evaluation of sorghum under rain-fed conditions has also treated days to flowering, plant height, grain yield and harvest index as key growth and yield-component traits, thereby supporting their relevance for productivity-oriented assessment (Abdelrahman *et al.*, 2025).

The assessment of genetic divergence helps in identifying diverse parents for breeding programmes and increases the chances of obtaining superior recombinants (Arunachalam, 1981). Genetic divergence among genotypes can be measured using Mahalanobis D^2 statistics, which are widely used to classify genotypes into clusters based on multivariate traits (Mahalanobis, 1936; Rao, 1952). Recent sorghum studies using Mahalanobis D^2 statistics, principal component or cluster-based analyses, and SNP-based diversity assessment have further emphasised the value of multivariate and genomic approaches for identifying divergent or breeding-relevant germplasm (Navyashree *et al.*, 2024; Ahmad *et al.*, 2025; Kasule *et al.*, 2024). This method provides useful information about genetic relationships and assists in parent selection (Murty & Arunachalam, 1966). In sorghum, significant variability has been reported for yield and its component traits, such as plant height, flowering time and grain yield (Reddy *et al.*, 2008). These traits are influenced by both genetic and environmental factors, making their analysis important for crop improvement (House, 1985). The use of genetically diverse parents enhances the chances of obtaining heterotic hybrids and transgressive segregants (Arunachalam, 1981). Despite the availability of diverse germplasm, yield improvement in sorghum remains limited, highlighting the need for systematic genetic analysis (Dahlberg, 2000). However, evidence is still needed for the present set of 60 sorghum genotypes and three checks under the Udaipur *kharif* environment, particularly because grain yield, fodder-related traits and protein content were evaluated jointly in this material. Therefore, the present study aims to assess genetic divergence among sorghum genotypes and identify suitable parents for effective breeding programmes.

2. Materials and Methods

To assess genetic divergence in sorghum [*Sorghum bicolor* (L.) Moench], the present investigation was conducted during the *kharif* season of 2023 at the Instructional Research Farm, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, India. The experimental site is geographically located at 24°35′ N latitude and 73°42′ E longitude, at an altitude of about 579 m above mean sea level. The region falls under the sub-humid southern plain and Aravalli hill zone of

Rajasthan and is characterised by a subtropical climate. The average rainfall during the cropping season was approximately 650–700 mm, most of which was received from the southwest monsoon.

The experimental material comprised 60 diverse sorghum genotypes along with three standard checks, namely CSV-23, CSV-17 and PRJ-1. The experiment was laid out in an augmented design. Each genotype was grown in a single row of 3 m length with a spacing of 45 cm between rows and 15 cm between plants. All recommended agronomic practices and plant protection measures were followed to raise a healthy crop.

Observations were recorded on fourteen quantitative traits, namely days to 50 per cent flowering, days to maturity, plant height (cm), number of leaves per plant, stem girth (cm), flag leaf length (cm), panicle length (cm), panicle width (cm), 100-seed weight (g), biological yield per plant (g), harvest index (%), dry fodder yield per plant (g), protein content (%) and grain yield per plant (g).

The calculated D^2 values were used to assess the degree of genetic diversity among genotypes based on multiple traits (Mahalanobis, 1936). The statistical analysis was carried out using standard biometrical procedures (Singh & Chaudhary, 1985) with the help of software packages such as R (version 4.0 or above) and Microsoft Excel.

The genotypes were grouped into different clusters following Tocher's method, as described by Rao (1952). In this method, clustering was performed by arranging D^2 values in ascending order and grouping genotypes such that the average intra-cluster distance was minimised compared with inter-cluster distances. Initially, the two genotypes with the lowest D^2 value were grouped into the first cluster, and subsequently, additional genotypes were included based on minimum average D^2 values. This procedure was repeated until all genotypes were assigned to their respective clusters.

3. Results and Discussion

The analysis of genetic divergence using Mahalanobis D^2 statistics grouped the 60 sorghum genotypes along with three checks into fourteen distinct clusters (Table 1), indicating the presence of substantial genetic variability among the experimental material. The distribution of genotypes into different clusters revealed that Clusters III and IV contained the maximum number of genotypes (13 each), followed by Cluster I (12), while Clusters VIII to XIV were monogenotypic, each consisting of a single genotype. The formation of a large number of clusters with uneven distribution of genotypes suggests the existence of considerable genetic diversity among the genotypes studied. Similar clustering patterns indicating high variability in sorghum have also been reported by Verma & Biradar (2022) and Pugahendhi *et al.*, (2023). The presence of monogenotypic clusters (VIII to XIV) indicates that these genotypes are highly divergent from others and may possess unique genetic makeup. Such genotypes are of particular importance in breeding programmes, as they can be used for creating heterosis (Arunachalam, 1981).

The clustering pattern revealed that genotypes were not grouped according to their geographical origin, indicating that genetic divergence is not necessarily related to geographical distribution but rather to genetic constitution and selection pressure. Similar findings have been reported in sorghum by Kavithamani *et al.*, (2019) and Prasad & Biradar (2017).

Clusters III and IV, which had the highest number of genotypes, indicate that these genotypes share a relatively close genetic relationship among themselves. In contrast, the presence of solitary genotypes in Clusters VIII to XIV suggests that these genotypes are genetically distant from others and may serve as potential parents in hybridisation programmes. The existence of significant genetic divergence among genotypes indicates ample scope for selection and crop improvement. The genotypes present in distantly related clusters are expected to produce higher heterosis and a wider spectrum of variability in segregating generations (Falconer & Mackay, 1996; Verma & Biradar, 2022). Therefore, crosses between genotypes belonging to clusters with high inter-cluster distances would be more promising for obtaining superior recombinants.

Seven monogenotypic clusters (VIII, IX, X, XI, XII, XIII and XIV) deserve special attention because they represent unique and genetically distinct genotypes. These genotypes can be effectively used in breeding programmes to broaden the genetic base and improve yield and other desirable traits. A similar observation has been reported by Reddy & Pitha (2024) in sorghum.

The average intra- and inter-cluster distances based on Mahalanobis D^2 values revealed considerable genetic diversity among the sorghum genotypes (Table 2). The intra-cluster distance was lowest in Cluster II (32.1), indicating close genetic similarity among genotypes within this cluster, whereas relatively higher intra-cluster distances in Clusters III (44.8), IV (44.3) and V (46.4) suggested moderate variability within these groups. In contrast, the inter-cluster distances were markedly higher than the intra-cluster distances, confirming substantial genetic divergence among clusters. The maximum inter-cluster distance was observed between Cluster II and Cluster IX (386.5), followed by Cluster IX and Cluster XIV (374.4) and Cluster II and Cluster VII (356.1), indicating wide genetic divergence among genotypes of these clusters (Pugahendhi *et al.*, 2023; Prasad & Biradar, 2017).

The magnitude of inter-cluster distance plays a vital role in selecting parents for hybridisation programmes. Genotypes belonging to clusters with maximum inter-cluster distance are expected to produce higher heterosis and wider variability in segregating generations (Arunachalam, 1981; Falconer & Mackay, 1996). Therefore, crosses involving genotypes from highly divergent clusters, such as IX, XIV, II and VII, would be more effective for developing superior recombinants. In contrast, clusters with lower inter-cluster distance indicate genetic similarity and are less useful for generating variability. The presence of several monogenotypic clusters further indicates the uniqueness of these genotypes, which can be effectively used in breeding programmes to broaden the genetic base (Guleria *et al.*, 2021; Kavithamani *et al.*, 2019).

The cluster mean values for fourteen quantitative traits revealed considerable variability among the sorghum genotypes (Table 3). Days to 50 per cent flowering ranged from 52.4 days (Cluster XI) to 72.8 days (Cluster XIV), while days to maturity varied from 88.8 days (Cluster XI) to 122.5 days (Cluster XIV), indicating the presence of both early- and late-maturing genotypes. Plant height was maximum in Cluster XIV (199.0 cm), followed by Cluster V (197.1 cm), whereas the minimum was observed in Cluster VIII (145.4 cm). Cluster VII recorded the highest number of leaves per plant (12.0), while Cluster X showed the maximum panicle width (9.0 cm). The highest 100-seed weight was observed in Cluster XIV (3.1 g), indicating bold-seeded genotypes. These variations in yield-contributing traits reflect the presence of substantial genetic variability among clusters (Verma & Biradar, 2022).

Mean performance based on multivariate analysis for yield and related traits indicated that Cluster XIV recorded the highest grain yield per plant (106.3 g), along with the maximum harvest index (53.9%) and protein content (11.6%), followed by Cluster V (103.0 g) and Cluster X (100.3 g). Cluster VII exhibited the highest biological yield (521.2 g), whereas Cluster IX showed the lowest harvest index (10.8%). Clusters VI and XIII also performed well for dry fodder yield (328.4 g and 324.8 g, respectively). The superior performance of Clusters XIV, V and X for grain yield and associated traits suggests that genotypes belonging to these clusters can be effectively used as potential parents in breeding programmes. Selection of genotypes from clusters with high mean values for yield and its components, along with genetic divergence, is expected to enhance breeding efficiency and the development of superior varieties (Pugahendhi *et al.*, 2023).

Table 1. Grouping of 60 Sorghum genotypes with 3 checks into fourteen clusters

Cluster	Name of genotypes	Number of genotypes
I	SU1638, SU1640, SU1622, SU1630, SU1456, SU1634, SU1629, SU1583, SU1529, SU1519, CSV23, PRJ1	12
II	SU1612, SU1616, SU1631	3
III	SU1557, SU1561, SU1582, SU1591, SU1569, SU1609, SU1602, SU1570, SU1587, SU1632, SU1573, SU1539, SU1637	13
IV	SU1618, SU1620, SU1572, SU1628, SU1639, SU1611, SU1575, SU1633, SU1615, SU1610, SU1635, SU1571, CSV17	13
V	SU1606, SU1607, SU1580, SU1605, SU1581	5
VI	SU1598, SU1600, SU1597, SU1592, SU1596	5
VII	SU1604, SU1608, SU1636, SU1550, SU1577	5
VIII	SU1625	1
IX	SU1574	1
X	SU1599	1
XI	SU1619	1
XII	SU1601	1
XIII	SU1594	1
XIV	SU1579	1

Table 2. Average inter and intra cluster distance based on corresponding D^2 values in Sorghum

Cluster	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
I	**36.9**	162.8	106.0	82.3	79.5	131.0	199.4	162.8	228.4	106.1	124.4	69.4	198.3	156.7
II		**32.1**	260.2	94.5	195.0	262.4	356.1	320.4	386.5	201.3	57.5	132.4	346.9	79.6
III			**44.8**	175.5	105.4	98.5	106.0	74.5	135.7	131.8	220.8	149.0	118.0	244.5
IV				**44.3**	119.7	184.9	270.3	234.9	300.7	134.4	65.1	67.4	263.9	99.7
V					**46.4**	85.9	181.7	165.8	226.5	58.3	171.5	82.2	162.4	159.4
VI						**40.4**	137.5	139.1	188.9	75.2	238.3	135.2	95.8	224.0
VII							**40.8**	64.4	68.3	199.4	317.8	238.7	75.1	333.5
VIII								**0.0**	67.0	190.0	277.2	210.1	109.1	309.3
IX									**0.0**	248.5	342.7	275.4	126.6	374.4
X										**0.0**	186.7	79.4	162.9	155.8
XI											**0.0**	112.6	316.5	108.1
XII												**0.0**	219.4	110.4
XIII													**0.0**	312.9
XIV														**0.0**

Table 3. Mean values of different characters for 60 Sorghum genotypes with 3 checks grouped into 14 clusters

Cluster	Days to 50 percent flowering	Days to maturity	Plant height (cm)	Number of leaves per plant	Stem girth (cm)	Flag leaf length (cm)	Panicle length (cm)	Panicle width (cm)	100-seeds weight (gm)	Biological yield per plant (gm)	Harvest index (%)	Dry fodder yield per plant (gm)	Protein content (%)	Grain yield per plant (gm)
I	59	99.8	157.4	10	1.6	35.3	28.3	6.5	2.4	327.5	26.6	239.2	9.7	85.5
II	58.3	98.4	160.8	10.7	1.6	37.2	27.4	7.7	2.5	168.9	45.8	237	10.8	84.6
III	60.2	102.2	164.7	11.2	1.6	39.6	28	7.8	2.4	425.1	20	244	9.1	87.4
IV	59	99.7	161.4	10.4	1.6	36.5	26.4	7	2.5	255.7	34.9	239.6	9.4	87.8
V	70.6	115.9	197.1	10.8	2	37.8	29	7.9	2.8	348.2	30	285.2	11	103
VI	67.6	113.7	185	11.2	1.9	35.1	29.6	8.2	2.4	409.6	24.5	328.4	9.1	98.5
VII	63.3	107.5	174.4	12	1.7	42.3	28.8	8.3	2.7	521.2	17.9	258.6	9.4	95.6
VIII	56.1	94.5	145.4	10.9	1.5	38	32.4	6.5	2.3	486.9	17.8	227	8.5	81.3
IX	53.8	91.2	147.5	10.1	1.5	36.3	26.6	7.3	2.2	552.6	10.8	219.5	8.4	78.4
X	68.8	115.5	188.2	12.5	1.9	39.2	27.3	9	2.4	339.6	29.7	333.8	9.1	100.3
XI	52.4	88.8	149.4	12	1.5	41.4	26.8	8.7	2.3	210.9	34.3	210.5	8.5	75.8
XII	58.8	98.5	161.2	8.7	1.6	26.7	34.5	6.4	2	287.6	29.8	288.8	10.9	85.3
XIII	66.8	112.5	182.8	10.7	1.8	33.3	31.3	7.8	2.3	501.6	19.9	324.8	7.2	97.3
XIV	72.8	122.5	199	9.7	2	34.8	27.5	7.1	3.1	193.6	53.9	287.9	11.6	106.3

The contribution of different characters towards total genetic divergence ranged from 0.67 to 14.70 per cent, revealing that grain yield per plant contributed the highest (14.70%), followed by plant height (13.06%), harvest index (10.65%) and biological yield per plant (10.19%) (Table 4), indicating that these traits played a major role in differentiating the genotypes. Moderate contributions were observed for panicle length (9.58%), days to 50 per cent flowering (9.27%) and protein content (7.94%), suggesting their importance in genetic divergence. In contrast, traits such as days to maturity (3.07%), number of leaves per plant (3.69%), 100-seed weight (3.69%), stem girth (4.45%) and flag leaf length (2.25%) contributed less, while dry fodder yield per plant showed the least contribution (0.67%). The higher contribution of yield and its associated traits indicates their greater influence on genetic variability and their usefulness in selection and hybridisation programmes (Arunachalam, 1981; Falconer & Mackay, 1996; Pugahendhi *et al.*, 2023). Therefore, emphasis on these highly contributing traits would enhance the efficiency of breeding programmes for developing superior sorghum genotypes.

Table 4. Per cent contribution of different characters towards total divergence in Sorghum

S. No.	Character name	Per cent contribution
1.	Days to 50 per cent flowering	9.27
2.	Days to maturity	3.07
3.	Plant height	13.06
4.	Number of leaves per plant	3.69
5.	Stem girth	4.45
6.	Flag leaf length	2.25
7.	Panicle length	9.58
8.	Panicle width	6.81
9.	Grain yield per plant	14.70
10.	100 seed weight	3.69
11.	Biological yield per plant	10.19
12.	Harvest index	10.65
13.	Dry fodder yield per plant	0.67
14.	Protein content	7.94

4. Conclusion

The study demonstrated considerable genetic divergence among the evaluated sorghum genotypes and checks, as reflected by their distribution into fourteen clusters based on Mahalanobis D^2 statistics. The presence of several monogenotypic clusters indicated that some genotypes were distinct from the rest of the material and may be useful for widening the genetic base in breeding programmes. Inter-cluster distances were generally greater than intra-cluster distances, confirming the existence of appreciable divergence among clusters. The highest inter-cluster distance was recorded between Clusters II and IX, indicating that genotypes from these clusters may provide useful variability when used in crossing programmes. Cluster mean performance showed that Clusters XIV, V and X had higher grain yield per plant, while Cluster VII showed the highest biological yield per plant. The contribution analysis indicated that grain yield per plant, plant height, harvest index and biological yield per plant were major contributors to total divergence. These traits may therefore be considered important while selecting parents for sorghum improvement. Overall, the findings suggest that combining genetic divergence with desirable cluster mean performance can help identify promising parents for hybridisation and selection in sorghum breeding.

5. Limitations

The study was conducted during a single *kharif* season at one experimental location; therefore, the observed genetic divergence and cluster performance may be influenced by season- and location-specific environmental conditions. The experiment included 60 genotypes and three checks, which provided useful variability; however, broader evaluation across more environments would strengthen the reliability of parent selection. The analysis was based on fourteen quantitative traits, and no molecular marker information was included to support or validate the divergence pattern. Some yield-related traits, including biological yield, dry fodder yield, harvest index and grain yield per plant, require careful verification to ensure numerical consistency before final interpretation. The study also did not present detailed error estimates, adjusted means or significance testing for all traits. Therefore, the results should be interpreted as preliminary evidence of genetic divergence and should be confirmed through multi-location and multi-season evaluation before use in advanced breeding decisions.

Declaration of AI Use

This manuscript was prepared through the combined contributions of all author(s), including contributions to the study design, data, content development, results, interpretation, and related scholarly work. The author(s) acknowledge the use of Grammarly and ChatGPT to assist with grammar checking, language refinement, reference formatting. These AI-assisted tools were not used as authors and did not replace the intellectual contributions or scholarly judgment of the author(s). All AI-assisted outputs, including content, references, and interpretations, were carefully reviewed, revised, verified, and approved by the author(s). The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Competing Interests

Authors have declared that no competing interests exist.

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