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Evaluation of genetic diversity among barnyard millet genotypes (*Echinochloa esculenta* L.)

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ABSTRACT

Genetic diversity studies for barnyard millet genotypes were conducted at Botany Farm, Government College of Agriculture, Pune during kharif 2025 season. Total 40 genotypes were evaluated for 11 different characters during the investigation in Randomized Block Design (RBD) with three replications using D^2 Statistics. The Mahalanobis D^2 analysis revealed considerable genetic variability among the evaluated genotypes. The forty genotypes were analysed and divided into fourteen clusters. Cluster II was the largest cluster with 13 genotypes, followed by Cluster I and Cluster IV which had 8 genotypes each. Remaining Clusters III, V, VI, VII, VIII, IX, X, XI, XII, XIII, XIV had contain only one genotype each indicating wide divergence from other clusters. Cluster IV showed the maximum intra-cluster distance (11.30). The maximum inter-cluster distance was observed in between the Cluster VI and cluster XII (24.16) indicated the heterogenous behaviour. The minimum inter-cluster distance was noticed in between Cluster III and Cluster V (6.20) indicated proximity among the genotype with each other. The characteristics that contributed most to overall divergence were grain zinc content, grain iron content, 1000-grain weight, grain yield per plant, spikelet length. Based on the divergence classes, genotypes, viz., KIBMG-22-4, KOPBM-23-25, KIBMG-226, P. Barti-1 (C), KOPBM-23-13, KIBMG-22-9, KOPBM-23-18 and KIBMG-22-5 were identified as promising diverse genotypes and may be utilized as parents in future breeding programmes.

Keywords: Cluster, RBD, Genotypes, Divergence, Etc.

Introduction

Barnyard millet (*Echinochloa frumentacea* L.) is an important small millet crop grown for food and fodder. It is a short-duration crop and can perform well under low moisture and unfavourable environmental conditions (Amasiddha *et al.*, 2023) [1]. Barnyard millet is also considered nutritionally valuable because it contains protein, dietary fibre, minerals and other important nutrients (Renganathan *et al.*, 2020) [2]. In India, it is mainly cultivated in different regions where millets are grown as food crops. However, compared with major cereal crops, barnyard millet has received less attention in crop improvement programmes (Amasiddha *et al.*, 2023) [1].

The available barnyard millet germplasm shows considerable variation for growth, yield and nutritional traits. Such variation is useful for developing improved varieties with desirable characters (Manimekalai *et al.*, 2018; Nehru *et al.*, 2023) [3, 4]. Genetic diversity is important in plant breeding because diverse genotypes can provide useful parents for developing superior breeding material. The study of genetic divergence helps to determine the amount of variation present among genotypes and to identify genetically diverse groups. Mahalanobis D^2 analysis and cluster analysis are commonly used to study genetic divergence and classify genotypes into different clusters. Considerable genetic diversity among barnyard millet genotypes has been reported through D^2 analysis and other characterization methods (Nehru *et al.*, 2023) [4]. Recent studies have also reported wide variation for agronomic and nutritional traits in barnyard millet germplasm, indicating good scope for its use in crop improvement (Amulya *et al.*, 2026) [5]. Therefore, the present study was conducted to assess the genetic diversity among forty barnyard millet genotypes using important agro-morphological and nutritional characters and to identify diverse genotypes that can be useful in future breeding programmes.

Materials and methods

Forty genotypes of barnyard millet were evaluated for eleven characters, namely, days to 50 per cent flowering, days to maturity, plant height, number of tillers per plant, panicle length, spikelet length, 1000-grain weight, protein content, grain iron content, grain zinc content, and grain yield per plant. The experiment was conducted at the Botany Farm, Government College of Agriculture, Pune, during the kharif 2025 season.

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The genotypes were sown in a Randomized Block Design (RBD) with three replications. All recommended agronomic and cultural practices were followed to ensure proper crop growth. Observations were recorded from five randomly selected plants for each genotype and were used for statistical analysis.

Genetic divergence among the genotypes was estimated using Mahalanobis D^2 statistic (Mahalanobis, 1936) [6], based on the multi-variate data of eleven characters. The genetic diversity of the forty genotypes was evaluated using these traits. To classify the parental genotypes according to their level of divergence, the method proposed by Arunachalam *et al.* (1984) [7] was followed, which grouped the divergence values into four divergence classes (DC).

Results

Table I shows, assessment of genetic diversity over eleven quantitative characters revealed pairwise D^2 values ranging from 38.44 to 583.70. This wide dispersion in distance values reflects considerable diversity across the evaluated accessions. The genotypes KIBMG-22-4 and KOPBM-23-35 had the greatest D^2 value (583.70), with a corresponding (D) value of 24.16. Genotypes KOPBM-23-18 from Cluster III and KIBMG-22-16 from Cluster V recorded the lowest inter-cluster distance, with a D^2 estimate of 38.44 ($D = 6.20$).

Cluster analysis is used to assess the intra- and inter-cluster genetic diversity and helps in identifying suitable parents for crop improvement. It groups different genotypes into distinct clusters based on the similarities observed for the characters under study. Genotypes belonging to the same cluster show greater similarity, whereas those placed in different clusters are more diverse. Thus, a greater difference between two clusters indicates higher genetic diversity among the genotypes.

The corresponding (D) values ranged from 0.00 to 11.30, whereas the intra-cluster (D^2) values varied from 0.00 to 127.69. Cluster IV had the largest intra-cluster distance (127.69; $D = 11.30$), followed by Cluster II (111.93; $D = 10.58$) and Cluster I (69.05; $D = 8.31$). Due to their single genotype composition, the remaining clusters displayed zero intra cluster distance. The inter-cluster distances showed significant variation. There was more genetic difference between Cluster VI and Cluster XII (583.70; $D = 24.16$), Cluster IV and Cluster XI (575.52; $D = 23.99$), and Cluster I and Cluster XI (572.64; $D = 23.93$).

The maximum intra cluster distance observed in cluster IV (11.30) followed by cluster II (10.58) and cluster I (8.31). Other clusters such as cluster III, cluster IV, cluster V, cluster VI, cluster VII, cluster VIII, cluster IX, cluster X, cluster XI, cluster XII, cluster XIII, cluster XIV showed 0.00 intra cluster distance.

The maximum inter cluster distance was recorded between cluster VI and cluster XII (24.16) followed by cluster IV and cluster XI (23.99), cluster I and cluster XI (23.93), cluster I and cluster XII (22.64). More the difference between the clusters more the genetic variability indicated different genetic constitution among the genotypes.

The minimum inter-cluster distance was noticed in between Cluster III and Cluster V (6.20), followed by Cluster V and Cluster VII (7.42), Cluster III and Cluster IX (8.11), Cluster III and Cluster VII (8.16) indicated proximity among the genotype with each other. The more distance present in the clusters more diverse genotypes in cluster which can be easily exploited for crop improvement programme. The lower distance between clusters represented similarities in genotypes in the clusters.

Cluster I was recorded the maximum inter cluster distance with Cluster XI (23.93), followed by Cluster XII (22.64), Cluster VIII (22.50), Cluster XIV (18.41), Cluster IX (18.32), Cluster XIII (18.16), Cluster II (16.81), Cluster V (16.55), Cluster VII (16.35), Cluster III (15.35), Cluster IV (12.76), Cluster X (12.49), Cluster VI (11.59).

Whereas, Cluster II showed the highest inter cluster distance from Cluster XII (19.68) followed by Cluster VI (16.61), Cluster VII (16.38), Cluster XIV (16.36), Cluster XI (16.05), Cluster IV (15.96), Cluster IX (15.21) and others.

Cluster VIII was recorded from Cluster IV (18.49) followed by Cluster X (16.37), Cluster XIV (15.12), Cluster VI (15.06), Cluster XI (14.08), Cluster IV (13.54), Cluster XIII (12.18), Cluster XII (10.56), Cluster VII (8.16), Cluster IX (8.11) and Cluster V (6.20).

Cluster IV recorded the maximum distance from Cluster XI (23.99) followed by Cluster VIII (21.13), Cluster XIV (19.33), Cluster XIII (18.39), Cluster VI (18.28), Cluster VII (17.35), Cluster V (16.02), Cluster IX (15.78) and Cluster X (14.00).

Cluster V recorded the maximum distance from Cluster VIII (16.39) followed by Cluster X (15.06), Cluster VI (14.49), Cluster XII (12.27), Cluster XIV (11.15), Cluster XIII (11.07), Cluster XI (10.21), Cluster IX (9.86) and Cluster VII (7.42).

Cluster VI showed maximum inter cluster distance with Cluster XII (24.16) followed by Cluster VIII (22.04), Cluster IX (18.90), Cluster XIV (18.78), Cluster XI (18.66), Cluster (17.88), Cluster (15.59) and Cluster VII (12.46).

Cluster VII observed maximum distance from Cluster VIII (21.80) followed by Cluster X (18.82), Cluster XII (16.83), Cluster XIV (14.47), Cluster IX (12.97) and Cluster VIII (12.49).

Cluster VIII observed maximum distance from Cluster XIII (22.23) followed by Cluster IX (17.59), Cluster XII (16.38), Cluster XI (16.22), Cluster XIV (15.18) and Cluster X (13.64).

Cluster X recorded maximum distance from Cluster XI (21.06) followed by Cluster XII (19.68), Cluster XIII (17.37) and Cluster XIV (12.09).

Cluster XI observed maximum distance from Cluster XIII (17.35) followed by Cluster XII (16.44) and Cluster XIV (15.00).

Cluster XII showed highest distance from Cluster XIV (18.43) followed by Cluster XIII (17.64).

Cluster XIII recorded maximum distance from Cluster XIV (11.03).

Genotypes with similar characteristics are grouped together in the same cluster, irrespective of their geographical origin. In contrast, genotypes belonging to different clusters show greater variation from one another, even when they originate from the same geographical region.

Cluster means showed wide range of genetic variation for most of characters represented in Table III.

Days to 50% flowering had a cluster mean between 47.66 and 63.66 days. In the range of days to 50% flowering, the genotypes grouped in Cluster V (47.66 days) were the earliest, followed by Cluster XIV (48.00 days), Clusters VII and XI (48.33 days), and Cluster X (49.33 days).

The days to maturity cluster mean ranged between 101.66 and 109.66 days. Cluster V genotypes matured first (101.66 days), followed by Cluster III, VI, IX, XIII (103.33 days) and Cluster VIII (109.66 days) genotypes.

The range of the plant height cluster mean was 117.80 to 141.70 cm. Cluster IV had the lowest plant height (117.80 cm), followed by Cluster I (121.92 cm). Cluster VIII had the highest plant height (141.70 cm), followed by Cluster XI (140.11 cm) and Cluster XIV (138.63 cm).

The cluster mean for number of tillers per plant ranged from 4.40 to 6.20. The highest number of tillers per plant was recorded in Cluster VIII (6.20), followed by Cluster XI (6.06) and Cluster XIII (5.93), whereas the lowest was observed in Cluster IV (4.40).

The panicle length cluster mean varied between 20.36 and 29.33 cm. Cluster XI had the longest panicle (29.33 cm), followed by Cluster VIII (28.66 cm) and Cluster II (26.75 cm). Cluster XIV had the shortest panicle (20.36 cm).

The spikelet length cluster means varied between 2.86 & 3.85 cm. Cluster VIII had the tallest spikelet (3.85 cm), followed by Cluster XIV (3.80 cm) and Cluster X (3.60 cm), and Cluster IV had the shortest (2.86 cm).

Cluster XI (3.98 g) had the highest 1000-grain weight, followed by Cluster VIII (3.83 g) and Cluster II (3.68 g), while Cluster XIII (2.96 g) had the lowest 1000-grain weight. The cluster mean for 1000-grain weight ranged from 2.96 to 3.98 g.

Grain protein content exhibited considerable variation among the evaluated groups, with cluster means spanning from 8.70% to 11.36%. The maximum mean value was registered in Cluster XIV (11.36%), closely followed by Cluster XIII (11.08%) and Cluster X (10.88%). While, the minimum grain protein content was documented in Cluster VI (8.70%).

Wide variation was observed for grain iron content across the clusters, with group means varying from 4.32 to 11.13 mg. The maximum grain iron concentration was documented in Cluster XII (11.13 mg), followed by Cluster VIII (8.96 mg) and Cluster IV (8.79 mg), while the lowest level was manifested by genotypes grouped under Cluster VI (4.32 mg).

Evaluation of grain zinc content revealed cluster means varying between 2.73 and 3.81 mg. The maximum concentration was noted in genotypes belonging to Cluster VII (3.81 mg), followed closely by Cluster XI (3.78 mg) and Cluster XIII (3.75 mg), whereas the minimum performance was documented in Cluster X (2.73 mg).

With respect to grain yield per plant, cluster means varied between 19.81 g and 29.77 g. The highest individual cluster performance was

registered by Cluster XI (29.77 g), followed in descending order by Cluster XIV (28.68 g) and Cluster VIII (28.37 g), whereas genotypes belonging to Cluster IV exhibited the minimum value of 19.81 g.

Table IV. represents the contribution of 11 characters in genetic divergence. Grain zinc content exhibited the highest contribution toward genetic divergence, accounting for 29.90% (ranked first). This was followed by grain iron content with a 23.70% contribution and 1000-grain weight with 12.41%. Together, these three primary traits accounted for 66.01% of the total genetic differentiation among the evaluated genotypes. Among the remaining traits, grain yield per plant (11.64%) and spikelet length (8.81%, 71) displayed moderate contributions to overall diversity. Intermediate-to-low contributions were recorded for grain protein content (3.97%), plant height (3.73%), number of tillers per plant (3.01%), and panicle length (2.21%).

In contrast, phenological traits played a negligible role in discriminating the genotypes, with days to maturity contributing 0.49% and days to 50% flowering recording the lowest contribution of 0.13%.

Table I. Average intra and inter cluster D^2 and D values of 14 clusters formed from 40 Barnyard millet genotypes.

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	x	XI	XII	XIII	XI
I	69.05 (8.31)	282.58 (16.81)	234.09 (15.35)	162.82 (12.76)	273.90 (16.55)	134.32 (11.59)	267.32 (16.35)	485.76 (22.50)	335.62 (18.32)	156.00 (12.49)	572.64 (23.93)	512.56 (22.64)	329.78 (18.16)	338.92 (18.41)
II		111.93 (10.58)	162.05 (12.73)	254.72 (15.96)	160.02 (12.65)	209.45 (16.61)	268.30 (16.38)	158.76 (12.60)	231.34 (15.21)	183.33 (13.54)	257.60 (16.05)	216.97 (14.73)	387.30 (19.68)	267.64 (16.36)
III			0.00 (0.00)	183.33 (13.54)	38.44 (6.20)	226.80 (15.06)	66.58 (8.16)	341.88 (18.49)	65.77 (8.11)	267.97 (16.37)	198.24 (14.08)	111.51 (10.56)	148.35 (12.18)	228.61 (15.12)
IV				127.69 (11.30)	256.64 (16.02)	334.15 (18.28)	314.35 (17.35)	446.47 (21.13)	249.00 (15.78)	196.00 (14.00)	575.52 (23.99)	301.02 (17.35)	338.19 (18.39)	373.64 (19.33)
V					0.00 (0.00)	209.96 (14.49)	55.05 (7.42)	268.63 (16.39)	97.21 (9.86)	226.80 (15.06)	104.24 (10.21)	150.55 (12.27)	122.54 (11.07)	124.32 (11.15)
VI						0.00 (0.00)	155.25 (12.46)	485.76 (22.04)	357.21 (18.90)	243.04 (15.59)	348.19 (18.66)	583.70 (24.16)	319.69 (17.88)	281.56 (16.78)
VII							0.00 (0.00)	475.24 (21.80)	168.22 (12.97)	354.19 (18.82)	156.00 (12.49)	283.24 (16.83)	120.78 (10.99)	209.38 (14.47)
VII								0.00 (0.00)	304.40 (17.59)	186.04 (13.64)	263.08 (16.22)	268.30 (16.38)	494.17 (22.23)	230.43 (15.18)
IX									0.00 (0.00)	265.03 (16.28)	263.08 (16.22)	103.63 (10.18)	110.67 (10.52)	177.68 (13.33)
X										0.00 (0.00)	443.52 (21.06)	387.30 (19.68)	301.71 (17.37)	146.16 (12.09)
XI											0.00 (0.00)	270.27 (16.44)	301.02 (17.35)	225.00 (15.00)
XII												0.00 (0.00)	311.16 (17.64)	339.66 (18.43)
XIII													0.00 (0.00)	121.66 (11.03)
XIV														0.00 (0.00)

Table II. Distribution of 40 genotypes into different cluster.

Cluster no.	Number of genotypes included	Genotypes
I	8	KIBMG-22-6, KOPBM-23-07, KIBMG-22-3, KOPBM-23-20, KIBMG-22-8, KIBMG-22-7, KIBMG-22-1, KIBMG-22-2, KOPBM-23-17, DHBM-93 (C), KOPBM-23-23, KOPBM-23-22,

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Cluster no.	Number of genotypes included	Genotypes
II	13	KOPBM-23-31, KIBMG-22-15, KOPBM-23-24, KOPBM-23-26 KOPBM-23-14, KOPBM-23-32, KOPBM-23-01, KOPBM-23-28, KIBMG-22-12
III	1	KOPBM-23-18
IV	8	KIBMG-22-14, KOPBM-23-08, KIBMG-22-10, KOPBM-23-10, KIBMG-22-11, KOPBM-23-33, KOPBM-23-27, KOPBM-23-06
V	1	KIBMG-22-16
VI	1	KIBMG-22-4
VII	1	KOPBM-23-25
VIII	1	P. Barti-1(C)
IX	1	KOPBM-23-21
X	1	KIBMG-22-13
XI	1	KOPBM-23-13
XII	1	KOPBM-23-35
XIII	1	KIBMG-22-9
XIV	1	KIBMG-22-5

Table III. Cluster means for 11 traits in Barnyard millet.

Clusters	Days to 50 per cent flowering (No.)	Days to maturity (No.)	Plant height (cm)	Numbers of tillers per plant (No.)	Panicle length (cm)	Spikelet length (cm)	1000-grain weight (g)	Grain protein content (%)	Grain Iron content (mg)	Grain Zinc content (mg)	Grain yield per plant (g)
I	51.41	104.16	121.92	4.90	21.95	2.98	3.17	9.89	6.21	2.94	20.72
II	55.46	106.43	136.34	5.55	26.75	3.21	3.68	10.00	8.41	3.05	27.10
III	58.33	103.66	127.46	5.00	23.11	2.91	3.41	9.76	8.36	3.59	23.18
IV	55.00	105.37	117.80	4.40	20.90	2.86	3.09	10.41	8.79	3.04	19.81
V	47.66	101.66	131.53	5.26	23.53	3.23	3.56	10.60	8.13	3.59	24.58
VI	49.66	103.33	132.71	5.33	25.68	3.12	3.60	8.70	4.32	3.21	25.95
VII	48.33	106.00	129.33	5.00	24.21	3.00	3.43	10.03	6.37	3.81	24.35
VIII	55.66	109.66	141.70	6.20	28.66	3.85	3.83	10.15	8.96	2.87	28.37
IX	63.66	103.66	131.86	4.70	22.61	3.23	3.19	8.72	8.78	3.58	24.78
X	49.33	104.00	127.20	5.40	23.56	3.60	3.31	10.88	7.60	2.73	25.18
XI	48.33	107.33	140.11	6.06	29.33	3.50	3.98	10.11	7.23	3.78	29.77
XII	58.66	107.00	131.13	4.93	24.00	3.06	3.41	9.98	11.13	3.56	23.35
XIII	54.66	103.66	123.27	5.93	21.50	3.41	2.96	11.08	6.09	3.75	22.69
XIV	48.00	105.00	138.63	5.33	20.36	3.80	3.35	11.36	7.02	3.32	28.68

Conclusion

Cluster IV showed the maximum intra-cluster distance followed by Cluster II, Cluster I. The maximum inter-cluster distance was observed in between the Cluster IV and cluster XII followed by Cluster IV and XI, Cluster I and Cluster XI, indicated the heterogenous behaviour. The characteristics that contributed most to overall divergence were grain zinc content, grain iron content, 1000-grain weight, grain yield per plant, spikelet length. Based on the divergence classes, genotypes, viz., KIBMG-22-4, KOPBM-23-25, KIBMG-22-6, P.

Barti-1 (C), KOPBM-23-13, KIBMG-22-9, KOPBM-23-18 and KIBMG-22-5 were identified as promising diverse genotypes and may be utilized as parents in future breeding programmes.

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Table IV. Percent share of various characters to genetic divergence.

Sr. No.	Characters	Times ranked 1 st	Contribution (%)
1.	Days to 50 per cent flowering (No.)	1	0.13
2.	Days to maturity (No.)	4	0.49
3.	Plant height (cm)	30	3.73
4.	Number of tillers per plant (No.)	6	3.01

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Sr. No.	Characters	Times ranked 1 st	Contribution (%)
5.	Panicle length (cm)	10	2.21
6.	Spikelet length (cm)	71	8.81
7.	1000-grain weight (g)	100	12.41
8.	Grain protein content (%)	32	3.97
9.	Grain Iron content(mg)	230	23.70
10.	Grain Zinc content (mg)	280	29.90
11.	Grain Yield per plant (g)	16	11.64
	Total	780	100

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