



Genetic Divergence for Grain Quality and Agro-morphological Traits in Pearl Millet

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Abstract: Calcium and magnesium deficiencies have severe health consequences in adults and children, especially in developing countries. Being naturally endowed with higher levels of grain minerals (Fe, Ca, Zn, Mg, P, and K), protein, carbohydrates, and fibre, pearl millet is considered as a potential crop to address micronutrient deficiency. Fifty germplasm lines were assessed for genetic divergence using randomized block design during kharif (Rainy) 2020 at CCSHAU, Hisar. Observations were taken for eight quantitative characters, and grain Ca and Mg were determined for each genotype using ICP-MS. D² analysis and hierarchical cluster analysis utilising the UPGMA method were applied to ascertain the degree of diversification and relative proportion of each component trait towards the overall divergence. Both clustering approaches arranged genotypes into six clusters, signifying that there is of substantial genetic variation among 50 genotypes of pearl millet. Based on the ranking method used in D² analysis, the grain Ca content displayed highest diversity, followed by grain yield per plant and dry fodder yield per plant. In both clustering methods, clusters with the genotypes HR-1038, HRI-115, and GP-227 had the highest mean performance for grain Ca and Mg content, whereas clusters with the genotypes HR-607, HR-101, HI-1012, HI-1013, and HTP03/13-927 had the lowest mean performance. Regarding grain yield per plant, clusters containing the genotypes GP-141, GP-176, GP-181, EBL-12-237, and ICMR09888 performed exceptionally well.

Key words: D² analysis, genetic divergence, grain minerals, hierarchal cluster and pearl millet.

Pearl millet [*Pennisetum glaucum* (L.) R. Br.], a C₄ grass, is an extensively cross-pollinated diploid (2n = 2x = 14) with high photosynthetic efficiency and biomass production capacity (Varshney *et al.*, 2017). Pearl millet thrives well in regions with prolonged drought, poor soil fertility and excessive temperature. Due to the plant's tolerance for challenging growing conditions, it can be cultivated in regions where other cereal crops, such as maize and sorghum, would not persist. The crop is grown for both grain and fodder purpose in the Indian subcontinent and semi-arid regions of Africa (Devos *et al.*, 2006). However, in the United States (US) and Europe, pearl

millet is cultivated as a livestock fodder and feed crop. It is produced on approximately 30 m ha in more than 30 countries, with the greater portion of this area in Africa (approximately 18 mha), Asia (>10 mha), and the Americas (>2 mha) (Gupta *et al.*, 2015). In India, pearl millet is grown on an area of 7.65 mha, with production and productivity of 10.8 mt and 1,420 kg ha⁻¹, respectively, in 2020-21 (Indiastat, 2023). It is primarily cultivated by resource-poor farmers in arid and semi-arid regions, especially in the states of Rajasthan, Maharashtra, Gujarat, Uttar Pradesh, and Haryana, representing for almost 95% of the land under cultivation.

A healthy human necessitates over 40 essential nutrients, comprising vitamins, proteins, micronutrients, macronutrients and lipids to meet the metabolic requirements of body. Insufficient intake of any of these will lead to unfavourable metabolic disturbances, resulting in illness, deprived health, stunted growth in children and significant economic losses to society. A quick study of children in India found that 18.6% of newborns, 34.6% of children below 3 years of age, and 62.5% of adolescent females are affected by malnutrition and mineral deficiencies, resulting in annual losses of \$2.5 billion. A lack of dietary choices and monotonous cereal-based diets have been reported to cause mineral deficiency. Calcium and magnesium deficiencies have a negative impact on health. Calcium and magnesium are required for muscle contraction, transmission of nerve impulses, bone growth, cell signalling, and blood coagulation (FAO, 2002). Calcium and magnesium inadequacy is prevalent among adults and children, especially in developing nations (Combs and Nielsen, 2009). Genetic biofortification of traditional crops is presently one of the most sustainable as well as successful method being used to combat the prevalent problem of mineral and protein malnutrition (Miller and Welch, 2013 and Pfeiffer and McClafferty, 2007). Pearl millet is a potential crop that can help to alleviate micronutrient malnutrition due to its naturally high levels of grain minerals (Fe, Ca, Zn, Mg, P, and K), protein (8-19%), starch (62.8 to 70.5 g 100 g⁻¹), fibre (1.2 g 100 g⁻¹), and vitamin B-complex in comparison to all other staple cereal food crops (Velu *et al.*, 2007; Nambiar *et al.*, 2011).

Due to its highly cross-pollinated nature and diverse adaptation to an extensive range of

environmental conditions, pearl millet displays an enormous degree of variability at both the genotypic and phenotypic levels (Ramya *et al.*, 2017). The accessibility, evaluation, and exploitation of genetic diversity and distance between genotypes contribute to the formation of heterotic groups, which assist in the selection of parents for subsequent crossing. And the cross among substantially different parents will result in greater heterosis rather than a cross between closely related parents (Birchler *et al.*, 2010). In recent decades, significant breeding efforts have centred on the yield potential of cultivars with inherent genetic diversity. No major research has been conducted on the variability in the nutritional content of pearl millet grains except for variability in Fe and Zn. The genetic resources of pearl millet are largely unexplored and additional efforts are required to enhance the grain's calcium, magnesium, copper, potassium, and manganese content. Determining the genetic diversity of grain minerals, yield, and agro-morphological characters will aid in the identification of contrasting parents, to achieve the greatest possible amount of heterozygosity, and the attainment of yield stability and nutrient value in diverse and changing climates (Hausmann *et al.*, 2012). The pattern of genetic relation and distance between and within accessions can be explored using multivariate analysis techniques. Among various multivariate analyses, genetic divergence in the present study was measured using clustering analyses, such as D² analysis and hierarchical clustering. The D² analysis is a potential and distinctive tool for phenotypic diversity assessment by classifying genetically different clusters of genotypes and selecting key parameters that contribute to the overall variation of the germplasm. The hierarchical clustering with average linkage, also known as UPGMA (Unweighted pair-group method utilising arithmetic averages), employs arithmetic means for the construction of dissimilarity measures and classifies the genotypes into various groups. Several breeding lines from two distinct heterotic pools (seed parents and restorer parents) are continuously exploited at ICRISAT and other private and public sector organisations. The aforementioned facts suggest that pearl millet has enormous potential to overcome malnutrition and the objective of this study was to determine the genetic diversity

for various agro-morphological traits, grain calcium and magnesium content among 50 pearl millet genotypes.

Material and Methods

Fifty germplasm lines (designated maintainer and restorer lines of medium to late maturity) of pearl millet were assessed in randomized block design with two replications at the Bajra Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar, during the kharif (rainy) season of 2020-21. Geographically, Hisar is located at an elevation of 215.52 m MSL (mean sea level) at 29.1504° N, 75.7057°E. The texture of the soil at the site of study is sandy loam. It is situated in a semi-arid sub-tropical territory on the outer edges of the south-west (SW) monsoon region and has a tropical monsoonal climate with a mean annual precipitation of approximately 452 mm. Each genotype was planted in a 4 m long, 45 cm-wide single-row plot. The plant-to-plant distance was 10 cm to 12 cm. Prescribed agronomic practises were followed to produce a healthy crop. Data were collected on eight quantitative characters, such as days to 50% flowering, panicle diameter, panicle length, plant height, number of productive tillers per plant, dry fodder yield per plant, 1000-seed weight, and grain yield per plant, on five randomly selected competitive plants in each replication. Days to 50% flowering (days) were recorded when the main panicles of 50% of the plants in the row exhibited complete stigma emergence on a row-by-row basis.

Determination of mineral content

The open-pollinated grains of all genotypes were well cleaned to remove any dust and other contaminants. A known amount (0.2 g) of grain sample were weighed and placed into a dry, clean and labelled digestion tube. Seven ml of a diacid mixture of HNO₃ and H₂O₂ in a ratio of 5:2 was pipetted in each digestion tube. The samples were then digested in a closed microwave digestion system with the following settings: a rise time of 20 minutes to reach 170°C, a hold time of 25 minutes at 170°C, and a cooling time of 15 minutes to reach 50°C. The samples were then cooled to ambient temperature, vented, and opened, after which the solution became transparent. After cooling, the solution was diluted to a final volume of 25 ml by adding ultra-

deionized water. The diluted solution was filtered with 25 mm diameter, 0.2 µm pore size Whatman syringe filters, and the calcium and magnesium concentrations were determined using ICP-MS (Inductively Coupled Plasma Mass Spectrometry, Thermo Fisher Scientific). Elements' concentrations were determined using calibration curves and expressed in mg kg⁻¹. All the reagents, standards, blanks, and samples were made with ultra-deionized water and ultra-high-purity commercial acids (Sigma-Aldrich and Merck).

Statistical analysis

The statistical study of replicated data was carried out using INDOSTAT version 7.1 software. Mahalanobis (1936) introduced D² statistics, and Rao (1952) put forward its application for the evaluation of genetic diversity in plant breeding. The Euclidean distance (using Ward's minimum variance method) was utilised to calculate the pair-wise genetic dissimilarity index. The percentage contribution of characters to genetic divergence was estimated as suggested by Singh and Chaudhary (1985).

Software SPSS version 26 was used to carry out the AHC (Agglomerative Hierarchical Clustering) method, in which genotypes were grouped utilising the method of average linkage between groups, also known as UPGMA (unweighted paired group method using arithmetic averages), which is suggested as the best and most widely used method (Romesburg, 1990). In this investigation, City Block distance (also known as Manhattan distance) was utilised to determine the relative distances between and within the various clusters.

Results and Discussion

In this study, genetic divergence was assessed using clustering techniques such as D² analysis and hierarchical clustering. Table 1 displays the grouping of genotypes into distinct clusters for both analyses. D² estimates the extent of diversification and gives the relative percentage of each component trait to the overall divergence. Figure 1 depicts the dendrogram for the D² cluster analysis using Ward's minimum variance method. On the basis of D² value, the experimental material was clustered into six main clusters using ten

Table 1. Distribution of genotypes in both cluster analyses

Cluster Number	As per D ² cluster analyses		As per Hierarchal clustering	
	Number of genotypes	Name of genotypes	Number of genotypes	Name of genotypes
Cluster 1	8	HR-607, HTRC, HR-101, HI-1012, HI-1013, HTP03/13-927, ICMV91059, HBL-1124	9	HR-101, HR-607, HR-117, HI-1012, HI-1013, HMC-94-4, HMC-94-2, HBL-21-10, HTP03/13-927
Cluster 2	12	HI-1037, GP-224, H78/711, HB-65, HR-104, HB-1150, HB-1002-2, HR-1026, HB-1002-1, EMRL-14/243, GP-223, HBL-21-5	12	HBL-114, HI-112, HTRC, HBL-21-5, HBL-1124, HB-1002-2, HB-116, HRC-1171, HR-128, HRI-13, HRE-19, SGP-10-107-2
Cluster 3	3	HMC-94-2, SGP-10-107-1, HI-112	12	ICMV91059, HB-1150, HB-65, HR-104, HMC-94-2, GP-141, GP-176, GP-80, EBL-12-237, ICMR09888, ICRI1485, SGP-10-107-1
Cluster 4	13	HR-117, HBL-21-10, HMC-94-4, HMC-94-28, GP-141, GP-181, GP-176, EBL-12-237, GP-219, EMRCT-14/103, ICMR09888, HMC-283, HB-116	10	HB-1002-1, HR-1026, HR-1032, HR-119, EMRL-14/243, HMC-283, ICRI1499, GP-219, GP-223, GP-224
Cluster 5	7	HBL-114, SGP-10-107-2, HR-1032, ICRI1499, GP-227, HR-1038, HRI-115	6	HI-1037, HR-108, HRI-115, H78/711, EMRLT-14/103, GP-227
Cluster 6	7	HRC-1171, HRE-19, HR-128, ICRI1485, HRI-13, HR108, HR-119	1	HR-1038

quantitative characteristics. Out of six clusters, cluster IV contain the greatest number of genotypes of thirteen, followed by cluster II with twelve, cluster I with eight, cluster V and cluster VI with seven each, and cluster III with three. Patterns of different clusters and allocation of breeding lines to distinct clusters demonstrated the existence of divergence among experimental lines. Five of the nine B (maintainer) lines were in cluster II (HBL-21-5, HB1002-1, HB-1150, HB1002-2, and HB-65), followed by group IV with two B- lines (HBL-21-10, HB-116) and one each in groups I

(HB-1124) and V (HB-114). This demonstrated the clear divergence among maintainer lines (Kaushik *et al.*, 2018).

Inter-cluster distances were found to be greater than intra-cluster distances (Table 2; Figure 2). The cluster with the maximum intra-cluster distance was cluster V (3.712), followed by cluster II (3.549), and cluster IV (3.381), VI (3.370), III (3.136) and cluster I (3.010). The maximum inter cluster distance was found between cluster IV and cluster V (5.963), followed by cluster IV and cluster VI

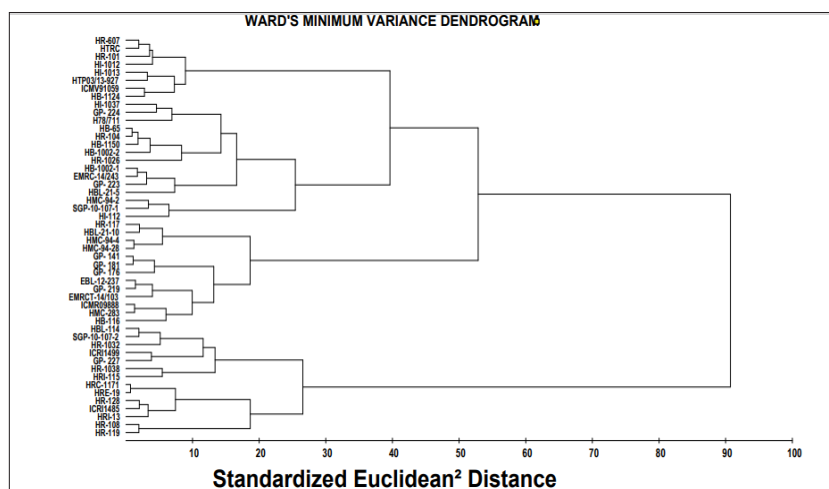


Fig. 1. Dendrogram representing the D² cluster analysis using ward's minimum variance method in pearl millet genotypes for grain nutritional and agro morphological traits.

Table 2. Estimates of intra (diagonal bolded) and inter (non-diagonal non-bolded) cluster distances in pearl millet genotypes for grain nutritional and agro morphological traits by D^2 method

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6
Cluster 1	3.010	4.175	4.788	4.175	4.945	4.139
Cluster 2		3.549	4.432	4.448	4.480	4.641
Cluster 3			3.136	4.718	4.769	4.885
Cluster 4				3.381	5.963	5.060
Cluster 5					3.712	4.285
Cluster 6						3.370

(5.060) and cluster III and cluster VI (4.885). The lowest inter cluster distance was realised among two cluster pairs i.e. cluster I and cluster II and cluster I and cluster IV, which was 4.175. Higher inter-cluster distance than intra-cluster distance, indicated similarity and limited genetic variation within a cluster. A smaller intra cluster distance suggested that the genotypes within the cluster should be closely related and less divergent in their traits (Malik *et al.*, 2017; Kaushik *et al.*, 2018; Rasitha *et al.*, 2020). The genotypes within the clusters can be subjected to further evaluation for morphological trait uniformity and general combining ability in order to produce synthetics and composites by combining four to ten lines. The genotypes from the two most divergent clusters (IV and V) could be utilised for determining specific combining ability (sca), as potential parents for producing new hybrids and to find superior recombinants in F_2 (Sankar *et al.*, 2014; Santos *et al.*, 2017; Ramya *et al.*, 2017). A greater distance between clusters also results in a broader spectrum of variability in the segregating population used for selection. It was also found that genotypes in the same cluster or those that had low genetic divergence and relatively small statistical distances were a greater probability to yield the desired heterotic

effects in the population as due to of crossing if they complement some important weaknesses of each other, as opposed to those including genotypes with wide genetic differences and falling in separate clusters (Dave and Joshi, 1995; Kalagare *et al.*, 2022)

The extent of genetic diversity between the genotypes was also determined by the substantial variability in cluster means for various characters. The cluster-specific and overall cluster-specific means for each character are given in Table 3. Cluster I had the maximum mean for days to 50% flowering (64.750) and the lowest mean for grain calcium and magnesium content (76.780 and 1,281.955). Cluster II contains the genotypes with the greatest mean for number of productive tillers per plant (2.150) and the lowest mean for panicle diameter (24.471). Cluster III had the lowest mean number of days to 50% flowering (54.667), indicating early maturation that allowed the cultivars to survive terminal drought (Sumathi *et al.*, 2016; Rasitha *et al.*, 2020). Cluster IV was distinguished by genotypes with the greatest mean panicle length (24.058), panicle diameter (28.827), plant height (170.254), dry fodder yield per plant (64.563), 1000-seed weight (10.392) and grain yield per plant (30.168) (Fig. 3). Cluster V had the highest mean grain magnesium content

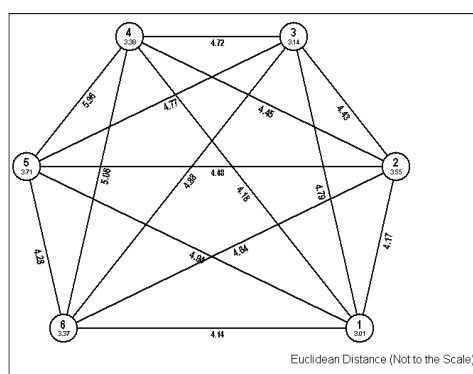


Fig. 2. Intra- and inter-cluster distance for the six groups of fifty germplasm lines of pearl millet using D^2 clustering method.

Table 3. Cluster mean of pearl millet genotypes and percentage contribution to total variability for grain nutritional and agro morphological traits by D² method

Character	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Contribution %
Days to 50% flowering	64.750	62.958	54.667	63.346	59.429	61.571	0.90%
Panicle length (cm)	21.013	18.983	19.700	24.058	17.143	23.057	6.04%
Panicle diameter (mm)	27.931	24.471	25.700	28.827	26.907	27.500	0.57%
Productive tillers (No./plant)	1.688	2.150	1.900	1.858	1.986	1.414	7.27%
Plant height (cm)	155.438	163.833	144.833	170.254	137.286	127.714	10.20%
Dry fodder yield plant ⁻¹ (g)	40.434	50.320	55.798	64.563	26.369	30.207	12.08%
1000-seed weight (g)	9.552	9.272	8.253	10.393	7.851	9.527	0.33%
Grain Ca content (mg kg ⁻¹)	76.780	147.260	118.117	118.528	181.035	181.287	39.59%
Grain Mg content (mg kg ⁻¹)	1281.955	1827.639	1529.138	1580.265	1957.081	1642.232	6.78%
Grain yield/plant (g)	16.384	19.719	29.952	30.168	11.863	13.952	16.24%

(181.287) and the lowest mean panicle length (17.143), dry forage yield per plant (26.369), 1000-seed weight (7.851) and grain yield per plant (11.863) (Table 2). Cluster VI had the highest mean for grain Ca content (181.287) and the lowest for number of productive tillers per plant (1.414) and plant height (127.714). From findings of cluster mean values, it was evident that cluster IV played a major role in yield and yield-attributing traits. And as cluster VI and cluster V contain genotypes with higher Ca and Mg content (Figure 4), these could be used to increase mineral content in future plant breeding programs. These results are consistent with the outcomes of Shanmuganathan *et al.* (2006) regarding the number of productive tillers per plant, plant height, and panicle diameter; Govindaraj *et al.* (2011) for panicle

length and plant height; and Sumathi *et al.* (2016) for grain yield per plant and 1000 seed weight.

Grain Ca content contributed the greatest percentage to total variation (39.59%), followed by grain yield per plant (16.24%), dry fodder yield per plant (12.08%), plant height (10.20%), number of productive tillers per plant (7.27%), grain Mg content (6.78%), panicle length (6.04%), days to 50% flowering (0.90%), panicle diameter (0.57%), and 1000-seed weight (0.33%). The Ca content, grain yield per plant, dry fodder yield per plant, plant height, number of productive tillers per plant, and grain Mg content accounted for nearly 90% of the variability (Fig. 5), showing the potential for selection of these characteristics in the given experimental

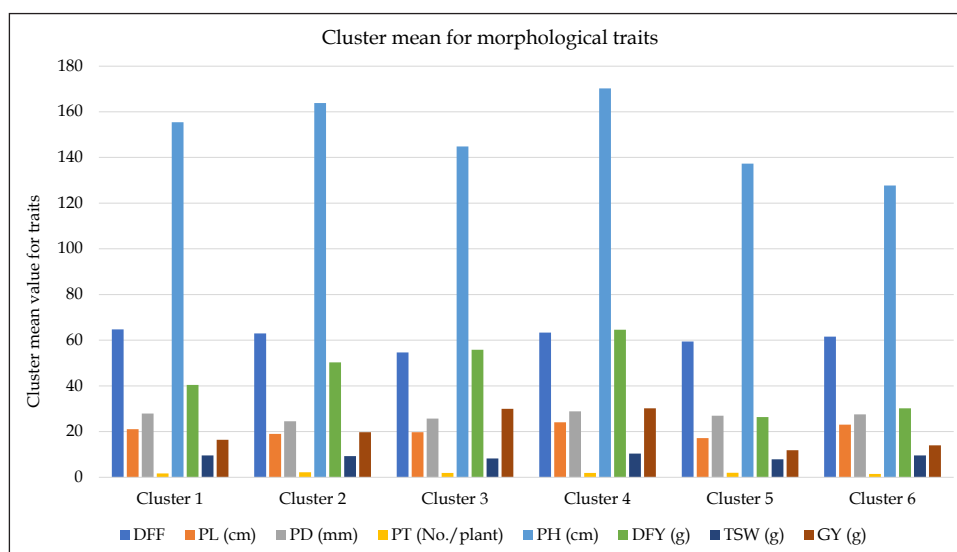


Fig. 3. Cluster mean for morphological traits using D² clustering method (DFF- Days to 50% flowering, PL- Panicle length, PD- panicle diameter, PT- Number of productive tillers, PH- Plant height, DFY- Dry fodder yield per plant, TSW- Thousand seed weight, GY- Grain yield per plant).

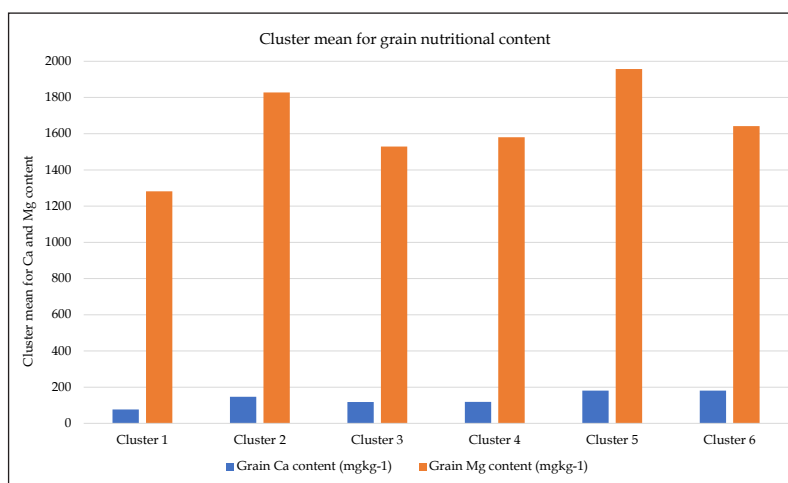


Fig. 4. Cluster mean for grain nutritional content using D^2 clustering method.

material (Kumar *et al.*, 2015; Athoni *et al.*, 2016; Sharma *et al.*, 2020). The little contribution of other traits to genetic divergence may be a result of selection for uniformity in these traits, which may have had an eroding effect on genetic diversity (Govindaraj *et al.*, 2011).

The hierarchical cluster analysis resulted in a grouping of six main clusters containing one to fifty genotypes, illustrating their hierarchical relationships and displayed in the form of dendrogram (Fig. 6). The germplasm was agglomeratively grouped, where each genotype at first considered to be a separate cluster which were subsequently combined. Six major clusters illustrated the prevalence of divergence within the lines of germplasm that could be

utilised in future breeding programs. Clusters II and III were the largest of the six clusters, each comprising 12 genotypes. Clusters IV, I, and V had 10, 9, and 6 genotypes, respectively. Cluster VI was the smallest and contained a single genotype. Cluster II contains five of the nine B lines: HBL-114, HBL-21-5, HB-1124, HB-1002-2, and HB-116. Each cluster I (HBL-21-10) and cluster IV (HB-1002-1) had a B line. Two B lines (HB-1150 and HB-65) were contained within Cluster III.

Intra-cluster and inter-cluster distances are enlisted in Table 4. The estimated inter-cluster distance was more than the intra-cluster distance. Cluster V had the highest intra-cluster distance (221.47), followed by cluster

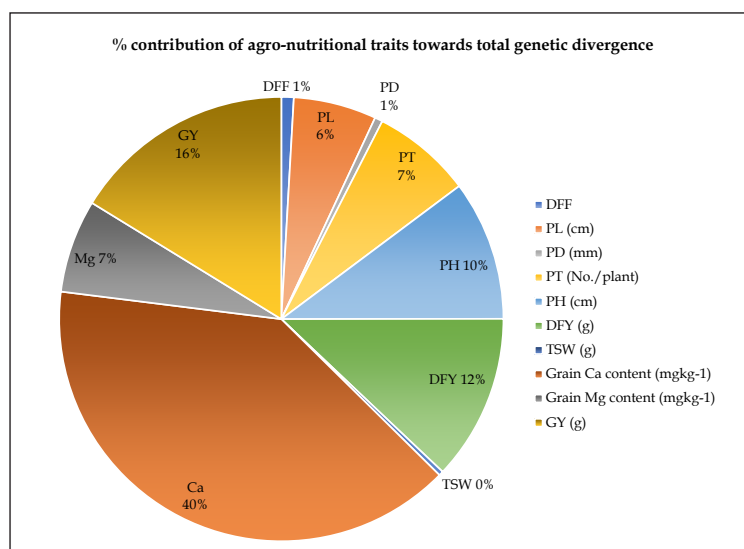


Fig. 5. Per cent contribution of agro-nutritional traits towards total genetic divergence (DFF- Days to 50% flowering, PL- Panicle length, PD- Panicle diameter, PT- Number of productive tillers, PH- Plant height, DFY- Dry fodder yield per plant, TSW- Thousand seed weight, GY- Grain yield per plant, Ca- Grain Ca content, Mg- Grain Mg content).

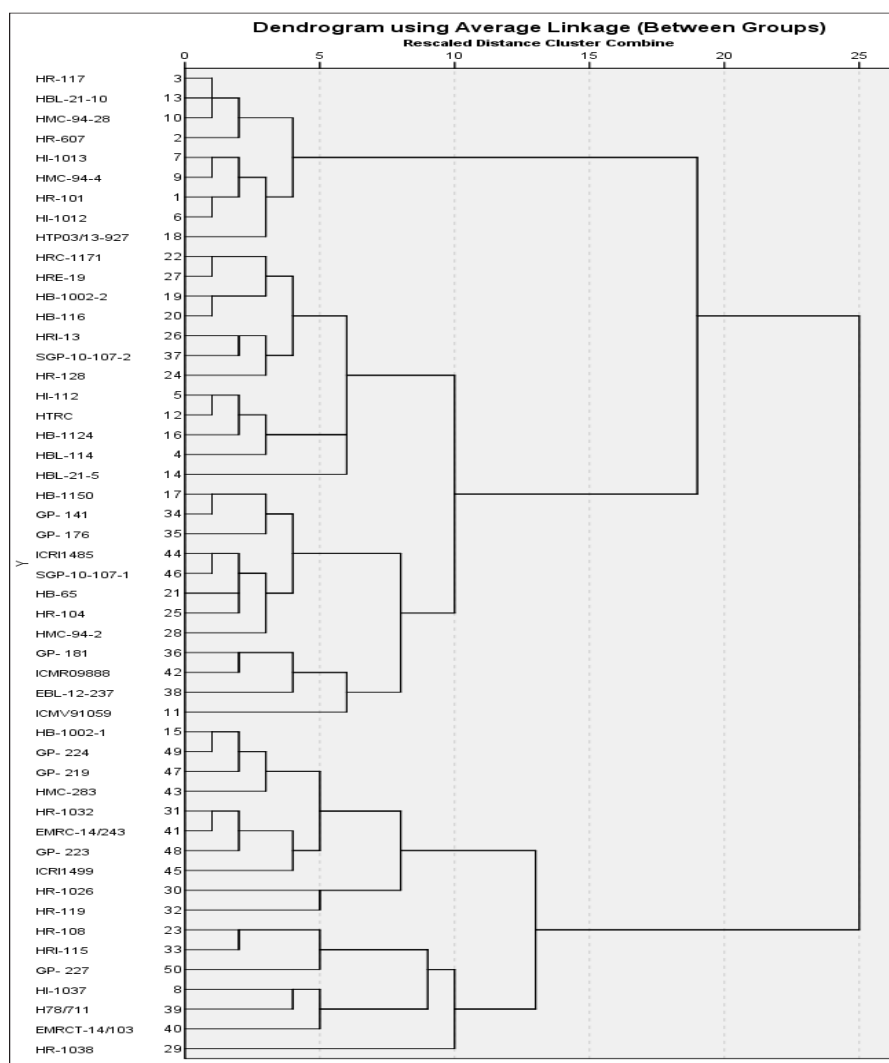


Fig. 6. Dendrogram depicting pearl millet genotypes derived by Average linkage between groups of clustering for grain nutritional and agro-morphological traits.

III (178.99), IV (174.67), II (151.91), and cluster I (111.03). The largest distance between clusters was measured between cluster I and cluster VI (1,398.98), followed by cluster I and cluster V (1283.61), cluster II and cluster IV (1,005), and cluster V and cluster VI (316.75). Intra-cluster distance stated a homogeneity within clusters and a diversity among clusters. The greatest inter cluster distance (cluster I and cluster VI)

explained the variation between the genotypes of two distinct clusters, and these genotypes that had varying mean performance could be used as parents and intercrossed to establish the base population, as given by Vijaylaxmi *et al.* (2019) and Govindaraj *et al.* (2020). Evaluation of genotypes from these clusters for per se performance and specific combining ability will identify the most promising lines and enable

Table 4. Intra- (diagonal) and inter-cluster distance of 50 pearl millet genotypes using Hierarchical cluster analysis

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6
Cluster 1	111.03	473.56	674.40	992.25	1283.61	1398.98
Cluster 2		151.91	321.68	613.14	904.81	1005.00
Cluster 3			178.99	404.15	709.13	842.08
Cluster 4				174.67	407.07	546.12
Cluster 5					221.47	316.75
Cluster 6						0.00

Table 5. Cluster mean values of ten quantitative traits for six clusters revealed by Hierarchical cluster analysis among 50 genotypes

Character	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6
Days to 50% flowering	64.22	61.58	61.00	62.50	61.83	60.00
Panicle length (cm)	22.91	21.13	21.33	20.19	18.98	17.70
Panicle diameter (mm)	28.32	25.03	28.07	26.19	27.75	28.10
Productive tillers (No./plant)	1.63	1.74	1.94	1.99	1.87	2.90
Plant height (cm)	161.37	139.54	162.88	156.05	157.33	126.50
Dry fodder yield/plant (g)	51.29	40.05	56.33	45.46	39.88	18.12
1000-seed weight (g)	9.82	8.85	9.89	9.15	9.40	8.31
Grain Ca content (mg kg ⁻¹)	69.88	126.51	144.32	171.33	182.73	124.40
Grain Mg content (mg kg ⁻¹)	1117.36	1460.07	1657.76	1948.10	2199.51	2365.29
Grain yield/plant (g)	22.79	15.45	26.48	20.54	18.00	8.94

their utilisation as pollinators in developing of hybrids. Similar results were also provided by Drabo *et al.*, 2013; Ghazy *et al.*, 2015; Kiprotich *et al.*, 2015; Nehra *et al.*, 2016; Ton *et al.*, 2022.

Cluster III had the maximum mean grain yield per plant (26.48 g), dry fodder yield per plant (56.33 g), 1000-seed weight (9.89 g), and plant height (162.88 cm). Cluster VI had the highest grain Mg content (2365.29 mg kg⁻¹) and number of productive tillers per plant (2.90), and the lowest days to 50% flowering (60), panicle length (17.70 cm), plant height (126.50 cm), dry fodder yield per plant (18.12 g), 1000-seed weight (8.31 g), and grain yield per plant (8.31 g). Cluster V has the greatest mean grain Ca content (182.73 mg kg⁻¹), and Cluster VI has the maximum mean grain Mg content (2199.51 mg kg⁻¹). Cluster I had the highest mean panicle length (22.91 cm), panicle diameter (28.32 mm) and days to 50% flowering (64.22 days) and the lowest mean number of productive tillers per plant (1.63), grain calcium (69.88 mg kg⁻¹), and magnesium content (1117.36 mg kg⁻¹). Cluster II had the smallest mean for panicle diameter (25.03 mm) (Table 5). From the analysis of cluster mean values, it was evident that cluster III played the most significant role for high yield and yield component traits, followed by cluster I, exhibiting the importance of these genotypes as potential parents for yield attributing traits. The lines in cluster I could be employed as potential parents to produce enormous panicles with a greater diameter and length. Cluster VI consisted of genotypes with high Mg content, while cluster V contained both high Ca and Mg content genotypes that could be utilised in future crop improvement programs to increase

mineral content. Importantly, the cluster mean for days to 50% blossoming was shortest for cluster VI, followed by cluster III, indicating early maturity. Patterns of distinct clusters and the distribution of B and R- lines in distinct clusters represented the existence of divergence between the lines utilised in this study. Using cluster analysis, Yadav *et al.* (2016); Kumar *et al.* (2020); Pujar *et al.* (2020); Ton *et al.* (2022) also identified genetic diversity in the pearl millet germplasm lines.

In both Mahalanobis' D² statistics and the Agglomerative hierarchical clustering (AHC) method, a total of six clusters are identified, but the classification of genotypes into these clusters differs between the two methods. This is primarily due to the clustering technique and the type of data entered for the cluster analysis. In the case of Mahalanobis' D² statistics, replicated data were utilised as input, whereas for Agglomerative hierarchical clustering (AHC), standardised mean data were utilised to calculate the distances between the various genotypes for characters (Reddy *et al.*, 2022). Calculated Euclidian distances (coefficient) were used for constructing dendrograms using the unweighted pair group method using arithmetic averages (UPGMA). The number of clusters in AHC was determined by the Euclidian distance value used as the dissimilarity coefficient in the dendrograms (Govindaraj *et al.*, 2020). Therefore, the classification of genotypes into distinct clusters varied from one cluster analysis method to another. The D² statistics of Mahalanobis are considered as being more desirable due to its use of replicated data. In the current study, the selection of both cluster analyses is intended to enhance the selection

criteria for the classification of dissimilar parents. Each method has identified distinct genotype groups. In both multivariate analyses, HR-1038, HRI-115, and GP-227 were the most common diverse parents from high performance clusters, in contrast to HR-117, HBL-21-10, and HMC-94-4 from low performance clusters. In both Mahalanobis' D^2 statistics and the AHC method of cluster analysis, the cluster comprising genotypes HR-1038, GP-227, and HRI-115 consistently had the maximum cluster means for grain Ca and Mg content. In both cluster analysis methods, the cluster containing the genotypes GP-141, GP-176, GP-181, EBL-12-237, and ICMR09888 has the maximum mean grain yield per plant. The Ca content, grain yield per plant, dry fodder yield per plant, plant height, number of productive tillers per plant and grain Mg content contributed significantly to total variability. Thus, there is ample opportunity for selection of these characters among the lines evaluated for the purpose of exploiting heterosis in hybrids and finding a broad spectrum of variation in segregating material for both grain nutritional and yield-attributing traits.

Conclusion

The chances of obtaining highly heterotic hybrids and increasing variability for the effective selection of valuable recombinants in crop improvement programs is determined by the extent of divergence between the parents selected. The more diverse the parent selection, the greater the possibility of attaining a large heterotic effect in a hybridization program and attaining a greater quantity of variation in segregating generations (Govindaraj *et al.*, 2011). The genotypes HR-1038, HRI-115, and GP-227 had the maximum mean grain Ca and Mg content, while the genotypes GP-141, GP-176, GP-181, EBL-12-237, and ICMR09888 had the highest grain yield per plant. Parents and hybrids produced must be assessed over different locations to evaluate the general combining ability in order to initiate a successful hybridization program as well as to test hybrid performance in terms of grain nutritional content and grain yield.

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