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Genetic Diversity among Advanced Breeding Lines for Yield and Yield Contributing Traits along with Grain Micronutrients in Pearl Millet

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Abstract: In pearl millet the magnitude of genetic diversity among 184 advanced breeding lines and four check varieties was determined using Mahalanobis D^2 statistics. Based on D^2 values, the advanced breeding lines were grouped into nine clusters. Among the nine clusters, cluster I was largest, with 78 lines, followed by cluster IV (36) and cluster II (31). Genotype Fe & Zn 115-2-1 (cluster VII) and WSBLT 59-2 (cluster V) had the greatest D^2 distance between them measuring 1091.086, whereas, genotype HTRLT 2-2 (cluster VII) and WSBLT 59-2 (cluster V) showed second highest D2 distance of 1051.169, followed by D^2 distance of 1043.287 between genotype Fe & Zn 115-2-1 (cluster VII) and WSBLT 51-2 (cluster V). Iron content contributed the most to the total divergence. The highly divergent pairs which were identified in the present study can be crossed for creating variability and isolating better 'R' lines as well as 'B' lines.

Key words: Diversity, iron content, grain yield, D2 distance, clusters.

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Pearl millet [*Pennisetum glaucum* (L.) R. Br.] belongs to the family poaceae (Graminae). It is a highly cross-pollinated crop with protogynous flowering and an anemophilous mechanism, which fulfils one of the essential biological requirements for hybrid development. Pearl millet is a C4 plant and an annual tillering diploid (2n=2x=14) crop. It is often considered to be a "Super cereal" due to its rapid growth, high photosynthetic efficiency, balanced nutritional profile and tolerance to extreme climatic conditions.

India is the largest producer of pearl millet in the world, with 43.3% of the world's area and 42% of the world's production and covers about 30% of the global area and production of the crop. The country produces 10.86 mt of pearl millet grains annually, with a productivity of 1420 kg ha^{-1} over an area of 7.65 mha (Anonymous, 2021). Pearl millet is mainly cultivated in the states of Rajasthan, Punjab, Maharashtra, Gujarat, Madhya Pradesh, Karnataka, Andhra

Pradesh, Uttar Pradesh and Tamil Nadu. In Karnataka, pearl millet is one of the major *kharif* crops, grown over an area of 0.222 mha with an annual production of 0.275 mt with a mean productivity of 1241 kg ha^{-1} (Anonymous, 2021).

Micronutrient malnutrition is increasingly being recognized as a serious public health problem affecting more than half of the population worldwide, with pre-school children and women in developing countries being the most vulnerable. Improving essential nutrient content in staple food crops through biofortification breeding can overcome the micronutrient malnutrition problem. Biofortification of varieties is cost effective and sustainable way to address the nutritional issue (Mahalingam *et al.,* 2019). The most widespread of these are dietary deficiencies for iron (Fe), zinc (Zn) and vitamin A (Kumar *et al.,* 2019; Mahalingam *et al.,* 2019). In India, ongoing breeding programs have one of the major objective of increasing Fe and Zn levels in pearl millet grain; and presently more than 0.07 mha area is under biofortified varieties and more are under process of development and release (Mahalingam *et al.,* 2019). The Fe and Zn content in pearl millet is largely governed by additive genetic control, general combining ability of parental lines used in hybrid development has shown highly significant positive correlation for increased Fe and Zn content (Mahalingam *et al.,* 2013).

The presence of variability is essential to the successful improvement of any genetic character in a crop. To examine the extent of genetic variability among breeding material it is important to understand the diversity pattern and the evolutionary relationship between them. The genetic diversity in the germplasm of a breeding program affects the potential genetic gain through selection. Information about genetic diversity also permits the classification of germplasm into heterotic groups, which is particularly important in hybrid breeding. Even though the genetic mechanisms that explain heterosis are not fully understood, it is well documented that crosses between unrelated and consequently genetically distant parents, show greater hybrid vigour than crosses between closely related parents (Stuber, 1994; Hallauer, 1999). It is generally agreed that genetically diverse parents will show the maximum heterosis and offer the maximum chance of isolating transgressive segregants.

Material and Methods

The experiment was carried out at two locations *viz.*, Regional Agricultural Research Station, Vijayapur and Agricultural Research Station, Mudhol, during *kharif*, 2021-22. The research station at Vijayapur is situated in the northern dry zone (Zone 3) of Karnataka at 16°49′ N latitude, 75°43′ E longitude and 593 m above MSL and the average annual rainfall was 600.8 mm. Mudhol is also located in the northern dry zone (Zone 3) of Karnataka, situated at 16°20′ N latitude, 75°15′ E longitude and 544 m above MSL.

At the time of sowing, recommended dose of fertilizer @ 50:25:0 kg ha^{-1} of NPK was applied. Thinning operation was carried out at 21 days after seedling emergence to maintain desirable number of plants. The soil type and climatic conditions are well suited for pearl millet cultivation. At maturity, five random plants were selected from each line and observations were recorded for thirteen traits *viz.,* days to 50% flowering, days to maturity, plant height (cm), panicle length (cm), panicle girth (cm), number of productive tillers per plant, SPAD chlorophyll meter reading at flowering, SPAD chlorophyll meter reading at maturity, grain yield (kg ha^{-1}), dry fodder yield (kg ha^{-1}), 1000 grain weight (g), iron content (μg g^{-1}) and zinc content (μg g^{-1}). Grain Fe and Zn densities were analyzed in an Energy-Dispersive X-Ray Fluorescence Spectrometry machine (ED-XRF), installed in the Pearl Millet Breeding Program at ICRISAT, Patancheru.

Pooled ANOVA over the locations (Vijayapur and Mudhol) were performed and adjusted mean values over pooled data were subjected for estimation of genetic diversity. Statistical analysis was carried out by the Windostat software.

Results and Discussion

The analysis of variance was carried out using data recorded for thirteen different morpho-phenological and yield-contributing traits along with grain micronutrients (Fe and Zn). The results revealed existence of a high and significant variation among the advanced breeding lines of pearl millet and scope of selection for genetic enhancement of pearl millet

Table 1. Range and mean values for morpho-phenological and yield contributing traits in pearl millet advanced breeding lines across two locations

Traits	Mean	Range
Days to 50% flowering	48	39 – 55
Days to maturity	89	81 – 94
Plant height (cm)	142	107-172
Panicle length (cm)	21.5	14.6 – 31.7
Panicle girth (cm)	2.7	2.1 – 3.7
Number of productive tillers per plant	2.2	1.4 – 3.0
SPAD chlorophyll meter reading at flowering	55	44 – 63
SPAD chlorophyll meter reading at maturity	50	37 – 58
Grain yield (kg ha^{-1})	2379	1323 – 3495
Dry fodder yield (kg ha^{-1})	7291	5194 – 12843
1000 grain weight (g)	10.4	7.8 – 14.6
Iron content (μg g^{-1})	63.14	39.83 – 98.96
Zinc content (μg g^{-1})	37.25	26.71 – 57.98

for almost all the traits studied. The range was comparatively high for the traits *viz.,*dry fodder yield (kg ha^{-1}), grain yield (kg ha^{-1}), iron content (μg g^{-1}), zinc content (μg g^{-1}), plant height (cm), 1000 grain weight (g) and panicle length (cm) (Table 1). The mean value for Fe content varied from 39.83 μg g^{-1} to 98.96 μg g^{-1} with a general mean of 63.14 μg g^{-1}. Furthermore, for Zn content, ranged was from 26.71 μg g^{-1} to 57.98 μg g^{-1} with the mean of 37.25 μg g^{-1}. Similar result was obtained by Yadav, (2019) (mean value for Fe content varies from 29.7 μg g^{-1} to 106.1 μg g^{-1} with a general mean of 50.6 μg g^{-1} and the mean value for Zn content varies from 19.2 μg g^{-1} to 96.7 μg g^{-1} with a general mean of 38.6 μg g^{-1}) and Pujar *et al.,* 2020 (Fe content varied from 35 μg g^{-1} to 116 μg g^{-1}, with an average grain Fe density of μg g^{-1}, whereas Zn varied from 21 μg g^{-1} to 80 μg g^{-1} with an average grain Zn density of 46 μg g^{-1}).

Mahalanobis's D^2 is a potent technique of measuring genetic divergence among different genotypes, which helps to select genetically divergent genotypes and determines the relative proportion of each component trait. In the present investigation, based on Mahalanobis's D^2 statistics and Tocher's method, the genotypes were grouped into different clusters. The 184 advanced breeding lines of pearl millet along with four checks were grouped into nine clusters. This suggested the presence of a high degree of divergence in the material studied (Fig. 1).

Cluster I was the largest and comprised of 78 advanced breeding lines, followed by cluster IV (36), cluster II (31), cluster V (16), cluster VI (15), cluster III (6), cluster VII (3) and cluster VIII (2), whereas cluster IX had only one line (1). The intra-cluster distance was highest for cluster VII (88.46), followed by cluster IV (81.47) and cluster VIII (67.05), indicating that some genetic divergence still existed among the genotypes within the clusters (Table 2). The high intra-cluster distance manifests the presence of genetic diversity between the genotypes that were grouped in the same cluster. Hence, there is scope for gene exchange among the genotypes within a cluster. Selection within such clusters might be conducted based on the maximum mean value for the desired character. The highest inter cluster distance was shown between cluster V and VII (854.36), followed by cluster V and IX (570.75) and cluster V and VI (448.29), indicating the presence of wide diversity between the genotypes of the different

Table 2. Intra (diagonal and bold) and Inter cluster average D^2 values in pearl millet advanced breeding lines across two locations (Tocher's method)

Clusters	I	II	III	IV	V	VI	VII	VIII	IX
I	43.69	86.51	115.07	67.66	108.74	197.01	471.00	104.6	339.24
II		39.77	71.85	98.02	254.84	82.43	242.84	93.11	213.47
III			35.42	131.08	271.38	112.76	289.29	128.27	112.95
IV				81.47	146.84	196.00	462.00	123.50	340.65
V					55.74	448.29	854.36	248.42	570.75
VI						57.74	139.96	123.33	198.20
VII							88.46	332.91	299.27
VIII								67.05	351.69
IX									0

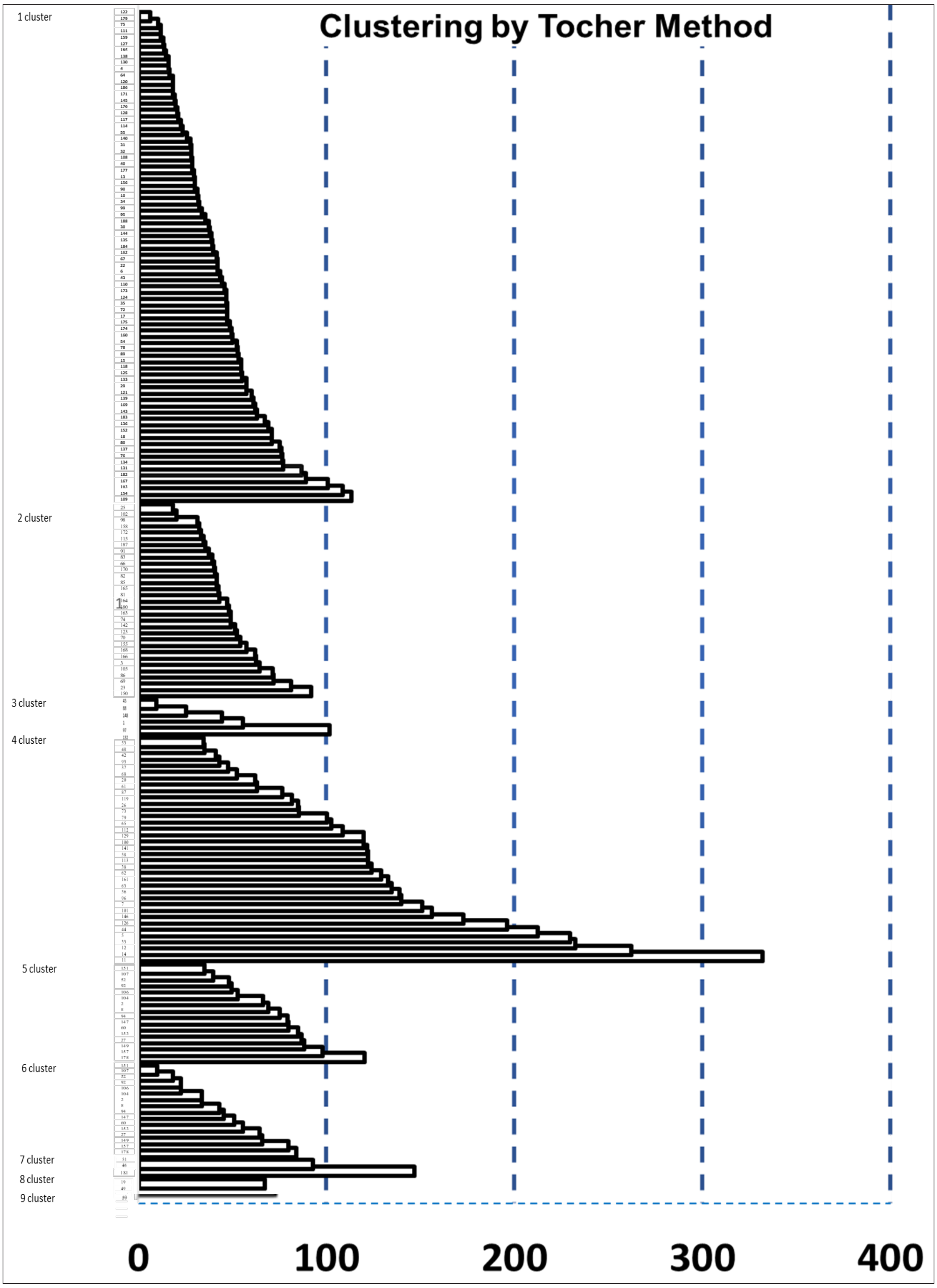

Fig. 1. Dendrogram showing distribution of pearl millet advanced breeding lines into different clusters.

clusters. Selection of parents from these groups would be useful to obtain greater variability in segregating generations. The results are presented in Table 2.

The highest contribution to genetic diversity was by the trait iron content (45.3%) followed by zinc content (16.2%), days to 50% flowering (8.3%), dry fodder weight (5.7%), grain yield (4.7%) and 1000 grain weight (4.3%), indicating that these traits could be offered ample scope for selecting desired genotypes (Fig. 2). Similar result was obtained by Athoni (2016), Yadav

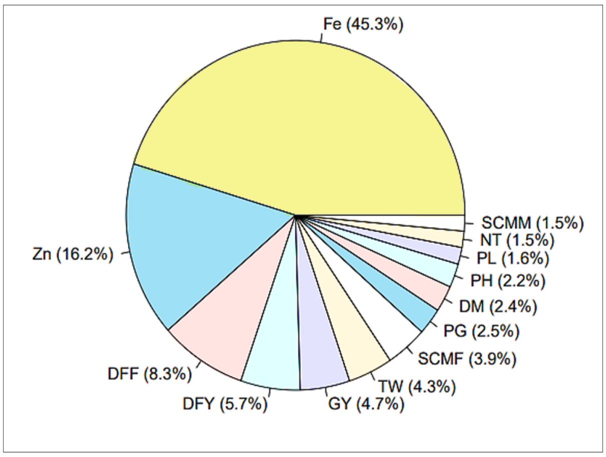

Fig. 2. Contribution of morpho-phenological and yield contributing traits towards genetic divergence in pearl millet advanced breeding lines.

DFF - Days to 50% flowering, DM - Days to maturity, PH - Plant height (cm), PL - Panicle length (cm), PG - Panicle girth (cm), NT - Number of productive tillers per plant, SCMF - SPAD chlorophyll meter reading at flowering, SCMM - SPAD chlorophyll meter reading at maturity, GY - Grain yield (kg ha^{-1}), DFY - Dry fodder yield (kg ha^{-1}), TW - 1000 grain weight (g), Fe - Iron content (µg g^{-1}) and Zn - Zinc content (µg g^{-1}).

(2019) and Pujar *et al.* (2020). Table 3 displays the cluster mean values for each trait in relation to genetic diversity. The cluster mean values for the majority of the investigated traits showed a broad range of variation. Cluster VIII had the highest ranking (rank 1), followed by cluster VII (rank 2) and cluster III (rank 3). Among the clusters, IX registered the lowest ranking (rank 9).

In the present investigation, genotype Fe & Zn 115-2-1 (cluster VII) and WSBLT 59-2 (cluster V) had the greatest D^2 distance, which was measured at 1091.086. HTRLT 2-2 (cluster VII) and WSBLT 59-2 (cluster V) placed second at 1051.169 and Fe & Zn 115-2-1 (cluster VII) and WSBLT 51-2 (cluster V) were third at 1043.287 D^2 distance (Table 4). These genotypes will be helpful in heterotic pool development upon variation of significant heterosis between the specific pair of genotypes. Hybridization programs belonging between these genotypes might be used for exploitation of hybrid vigour and good recombinants.

Conclusion

Genetic diversity present in the set of 184 advanced breeding lines were significantly high for Fe / Zn content, grain and fodder yield, 1000 grain weight, plant height, panicle girth and earliness; hence the genotypes showing maximum D^2 distance in pair wise

Table 3. Cluster mean values for morpho-phenological and yield contributing traits in pearl millet advanced breeding lines across two locations

Clusters	Traits													Total score	Final Rank
	DFF	DM	PH	PL	PG	NPT	SCMF	SCMM	GY	DFY	TW	Fe	Zn		
I	47.07 (4)	88.42 (6)	145.93 (2)	21.23 (4)	2.64 (6)	2.17 (5)	54.07 (4)	45.91 (3)	2399.26 (5)	7123.77 (6)	10.3 (5)	59.52 (8)	35.92 (7)	65	4
II	48.02 (7)	89.46 (9)	142.25 (4)	20.42 (6)	2.59 (7)	2.20 (3)	54.15 (3)	46.71 (2)	2217.17 (7)	6595.19 (7)	9.72 (9)	67.33 (6)	39.18 (5)	75	8
III	46.12 (3)	87.27 (2)	133.23 (8)	20.35 (7)	2.47 (9)	2.18 (4)	53.57 (5)	45.61 (4)	2104.88 (8)	7683.18 (4)	10.96 (2)	69.90 (5)	47.21 (2)	63	3
IV	50.00 (9)	89.17 (8)	139.17 (6)	20.58 (5)	2.76 (3)	2.27 (1)	53.10 (7)	45.07 (5)	2408.62 (4)	7826.69 (2)	11.34 (1)	61.86 (7)	35.53 (8)	66	5
V	47.34 (6)	88.55 (7)	143.66 (3)	21.87 (3)	2.69 (5)	2.21 (2)	53.22 (6)	44.96 (6)	2467.71 (3)	7808.38 (3)	10.27 (6)	45.96 (9)	33.32 (9)	68	6
VI	47.10 (5)	87.76 (4)	136.42 (7)	19.55 (8)	2.73 (4)	2.05 (7)	54.54 (2)	44.51 (8)	2570.17 (2)	7634.57 (5)	10.57 (4)	80.04 (2)	41.83 (4)	69	7
VII	46.07 (2)	87.33 (3)	146.96 (1)	23.40 (2)	2.83 (2)	2.03 (8)	51.97 (8)	44.55 (7)	1916.15 (9)	5356.52 (8)	9.76 (8)	94.22 (1)	43.87 (3)	62	2
VIII	42.05 (1)	84.88 (1)	139.41 (5)	24.65 (1)	3.07 (1)	2.01 (9)	60.60 (1)	49.05 (1)	2956.82 (1)	10998.69 (1)	10.89 (3)	70.79 (3)	36.35 (6)	34	1
IX	49.81 (8)	88.00 (5)	107.01 (9)	16.09 (9)	2.51 (8)	2.10 (6)	46.15 (9)	37.36 (9)	2321.76 (6)	5249.93 (9)	9.77 (7)	70.46 (4)	58.94 (1)	90	9

DFF - Days to 50% flowering, DM - Days to maturity, PH - Plant height (cm), PL - Panicle length (cm), PG - Panicle girth (cm), NPT - Number of productive tillers per plant, SCMF - SPAD chlorophyll meter reading at flowering, SCMM - SPAD chlorophyll meter reading at maturity, GY - Grain yield (kg ha^{-1}), DFY - Dry fodder yield (kg ha^{-1}), TW - 1000 grain weight (g), Fe - Iron content (µg g^{-1}) and Zn - Zinc content (µg g^{-1}).
Figures in the parenthesis, indicate the ranks based on cluster mean.
Total score is the summation of rank numbers for all characters, based on which final rank is indicated.

Table 4. Top 10 pairwise combination of genotypes with maximum D^2 distance among 184 pearl millet advanced breeding lines and four checks

D^2 distance	Genotype (Cluster V)	Genotype (Cluster VII)
1091.086	WSBLT59-2	Fe & Zn115-2-1
1051.169	WSBLT59-2	HTRLT2-2
1043.287	WSBLT51-2	Fe & Zn115-2-1
1034.921	Fe & Zn41-2-1	Fe & Zn115-2-1
1024.043	Fe & Zn197-2-1	Fe & Zn115-2-1
968.201	Fe & Zn200-2-1	Fe &Zn115-2-1
965.191	Fe & Zn41-2-1	Fe & Zn112-1-1
953.535	Fe & Zn178-2-1	Fe & Zn115-2-1
929.720	Fe & Zn132-1-1	Fe & Zn115-2-1
905.142	WSBLT50-2	Fe & Zn115-2-1

combination can be recombined to obtain more diverse progenies. Recurrent selection for Fe/Zn content along with other major yield contributing traits may result in developing genetic stocks for enhanced Fe/Zn content along with grain & fodder yield. Genotypes from distant cluster should also be inter mated in all possible combination for broadening the genetic base. Recombining highly divergent pairs will offer opportunities for creating variability and isolating better 'R' lines as well as 'B' lines for pearl millet hybrid breeding.

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