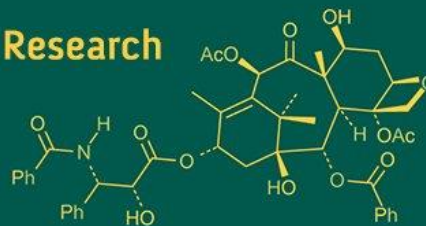


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Genetic variability and genetic diversity studies for biofortified lines in pearl millet [*Pennisetum glaucum* (L.) R.]

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Abstract

Improving essential nutrient content in staple food crops through biofortification breeding can overcome the micronutrient malnutrition problem. Genetic improvement depends on the availability of genetic variability in the primary gene pool. This study was aimed to ascertain the magnitude of variability and diversity in a 27 biofortified lines along with three checks *Viz.*, ICTP-8203, VPMV-9 and Dhanashakti and predict pearl millet biofortification prospects for essential micronutrients. The ANOVA showed highly significant differences among the genotypes for all the characters studied, indicating the presence of sufficient variability in the experimental material. The Phenotypic Coefficient of Variation (PCV) was slightly higher than the respective Genotypic Coefficient of Variation (GCV) for all the characters under study indicated less influence of environment on the genotypes in expression of traits. High heritability with high genetic advance as percent of mean for thousand seed weight, iron content and zinc content. This indicates the lesser influence of environment in the expression of the characters and may be governed by additive gene action. Hence, these traits are likely to respond to direct simple selection.

Based on the relative magnitude of D^2 values resulted through Tocher's method the genotypes were grouped in three clusters. Out of three clusters, Cluster I was the largest and included 28 genotypes while the other clusters *i.e.*, cluster II and III consisted of single genotypes each. This suggested the existence of high degree of diversity among the genotypes. The trait thousand seed weight contributed maximum towards genetic divergence. The intra cluster distance was recorded in cluster I. The highest inter-cluster distance was observed between cluster I and III followed by the distances between cluster II and III and cluster I and cluster II indicate the substantial diversity among the genotypes in these clusters.

Keywords: Pearl millet, biofortification, genetic variability, heritability, genetic advance, genetic diversity, iron, zinc, mahalanobis D^2 analysis

Introduction

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is a climate-smart and nutritionally dependable cereal crop serving several millions of people in sub-Saharan Africa and Asia. About 2 billion people across the world suffer from micronutrient deficiencies. While India is the largest producer of pearl millet, recent official data indicate that 67% of children under the age of 5 and 52.2% of pregnant women are anemic and 35.5% of children of the same age group are stunted Anon., 2019 (NFHS-5 2019-2021). Diets deficient in iron (Fe) and zinc (Zn) which are important micronutrients, are the major cause of anemia and stunting, respectively. Pearl millet, being naturally gifted with relatively higher proportions of grain iron (Fe) and zinc (Zn) compared to all other staple cereal food crops, is considered as a potential crop to combat micronutrient malnutrition.

Pearl millet is very rich in calories, proteins (6-15%), fat (5-6%), carbohydrates (60-72%), fiber (1-1.8%) and minerals with less HCN than other crops making it a highly palatable and nutritious crop. Micronutrient malnutrition has become a major global health concern and it is particularly prevalent among those living in poor nations who consume a diet high in cereals like wheat and rice. Millets are nutritious grains that can help to fight against this malnourishment. Among all millets, pearl millet is a particularly abundant source of minerals like iron (Fe) and zinc (Zn) content and essential amino acids (Anuradha *et al.*, 2018) [4]. In comparison to other cereals and vegetables, it is also the least expensive source of Fe and Zn.

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Of all food crops, pearl millet grain has the highest caloric content per 100 grams, with the majority of those calories coming from lipids, carbohydrates and proteins. High protein content (11.6%), low lignin content, high dry matter yield, ease of digestion with possesses less oxalic acid which is an antinutritional component are important qualities of pearl millet feed.

Furthermore, with the aim of integrating millets into the mainstream, harnessing their nutritionally advantageous characteristics and encouraging their cultivation, India declared 2018 as 'National Year for Millets' to promote millets for nutrition security. Millet renamed as 'Nutri-Cereals' by GOI, Gazette Notification issued on 13-4-2018 (Anon., 2018). United Nations Food and Agriculture Organization have declared 2023 as the 'International Year of Millets'. Notably, the Minimum Support Price (MSP) for pearl millet saw an increase to Rs. 2500 per quintal, a rise from the preceding years MSP of Rs. 2250. This adjustment led to the highest profits for farmers involved.

Despite decades of investment to address this issue, India still has one of the worst rates of child malnutrition in the world. India is ranked 111 out of 121 countries in the Global Hunger Index (Anon., 2023) which takes into account factors like child stunting, wasting and death. In order to improve its situation, India must take the lead in the biofortified crop sector and millets may be a good option going forward.

Biofortification of millets is one of the strategies to combat hunger through the genetically enhancing major food crops to produce more nutrients. It is a multidisciplinary strategy that seeks to address the persisting micronutrient issue by utilizing the full potential of agricultural development and nutrition science.

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 experimental material consisted of 27 parents *viz.*, VJP Fe & Zn-1, VJP Fe & Zn-2, VJP Fe & Zn-3, VJP Fe & Zn-4, VJP Fe & Zn-5, VJP Fe & Zn-6, VJP Fe & Zn-7, VJP Fe & Zn-8, VJP Fe & Zn-9, VJP Fe & Zn-10, VJP Fe & Zn-11, VJP Fe & Zn-12, VJP Fe & Zn-13, VJP Fe & Zn-14, VJP Fe & Zn-15, VJP Fe & Zn-16, VJP Fe & Zn-17, VJP Fe & Zn-18, VJP Fe & Zn-19, VJP Fe & Zn-20, VJP Fe & Zn-21, VJP Fe & Zn-22, VJP Fe & Zn-23, VJP Fe & Zn-24 and VJP Fe & Zn-25 and 3 checks *Viz.*, VPMV-9 (Local check), ICTP-8203 (Local check) and Dhanashakti (National check).

The experiment was carried out at the Regional Agricultural Research Station, Vijayapura during summer 2022-23, which is situated in the Northern Dry Zone (Zone 3) of Karnataka at 16° 49' N latitude, 75° 43' E longitude and 593 m above mean sea level and the average annual rainfall was 594.4 mm

Twenty-seven parents and three checks were assessed using a Randomised Complete Block Design in two replications with plot size, two rows of three meter length. The spacing between rows was 45 cm and the distance between plants was 15 cm. Gap filling and thinning operation was carried out at 15 and 21 days after seedling emergence respectively to maintain desirable number of plants and complete package was for raising the crop.

From each entry, five random competitive plants were selected and tagged (no border plants were selected). The mean of the plants was computed and taken for analysis. The following observations were recorded: *Viz.*, days to 50 percent flowering, plant height (cm), SPAD chlorophyll meter readings at flowering and maturity stage, number of productive tillers per plant, panicle length (cm), panicle girth (cm), grain yield (kg ha⁻¹), dry fodder yield (kg ha⁻¹), thousand seed weight (g), Iron content(ppm) and Zn content (ppm).

Results and Discussion

The analysis of variance for yield and its components studied in the 27 parents with three checks were tabulated in Table 1. The mean sum of squares for treatments was significant for all traits studied. In contrast, the mean sum of squares for replication was non-significant for all the traits under study.

The results pertaining to genetic parameters *viz.*, range, mean, Phenotypic Coefficient of Variation (PCV), Genotypic Coefficient of Variation (GCV), broad sense heritability (h^2_{bs}), genetic advance (GA) and genetic advance as percent over mean (GAM) were computed to identify the magnitude of variability existing in the thirty genotypes, are shown in Table 2 and Fig. 1 and 2

The trend of the phenotypic Coefficient of Variation (PCV) and Genotypic Coefficient of Variation (GCV) for all the characters remained same. The Phenotypic Coefficient of Variation (PCV) was higher than the Genotypic Coefficient of Variation (GCV) for all the characters under study. The PCV and GCV can be split into high ($\geq 20\%$), medium (10-20%) and low (0-10%) as indicated by Sivasubramanian and Menon (1973) [18]. Low (0-10%) values of GCV and PCV were obtained for characters like Days to 50 percent flowering, panicle girth, SPAD chlorophyll reading at flowering and maturity which indicates the narrow difference between PCV and GCV showed that there exists a least influence of environment on these characters. Medium (10-20%) values of GCV and PCV were obtained for the characters like plant height, grain yield, dry fodder yield, thousand seed weight and iron content, which confirmed the existence of moderate variability within this parental population. Similar results were reported by other workers in pearl millet *viz.*, Lakshmana *et al.* (2009) [13], Kumar *et al.* (2014) [12], Salih *et al.* (2014) [24], Aung (2020) [7] and Athoni *et al.* (2022) [5].

Wide differences between PCV and GCV implicit their susceptibility to environmental fluctuation, whereas narrow differences between PCV and GCV suggested their relative resistance to environmental alterations and any selection pressure operated on this character may help to realize improvement at early generation. The estimate of GCV and

PCV alone is not very helpful in determining the heritable portion. Further, heritability in conjunction with genetic advance is more effective and reliable in predicting the resultant effect of selection and based on that, selection procedures can be evaluated. The estimates of heritability (broad sense) are categorised as high ($\geq 60\%$), medium (30-60%) and low (0-30%) as given by Robinson (1949) [17]. The results of the present study on heritability for twelve characters suggested that the traits like Days to 50 percent flowering, plant height, dry fodder yield, thousand seed weight, iron content and zinc content showed high heritability and remaining traits are categorised under medium heritable except number of productive tillers. Similar results were reported by other workers in pearl millet by Varu *et al.* (2005) [21], Bhoite *et al.* (2008) [8], Govindaraj *et al.* (2010) [9], Lakshmana *et al.* (2009) [13], Kumar *et al.* (2014) [12], Salih *et al.* (2014) [24], Aung (2020) [7] and Athoni *et al.* (2022) [5].

The estimates of genetic advance did not project the actual genetic gain that has been attained in relation to the *per se* performance which obviously is not uniform indifferent populations or even in the same population. Therefore, the expected genetic advance as percent of mean (GAM) was calculated. According to Johnson *et al.* (1955) [11], genetic advance categorised as percentage of mean values can be split in to low (0-10%), moderate (10-20%) and high ($\geq 20\%$). High heritability with high values of genetic advance as percent of mean ($\geq 20\%$) were obtained for character like thousand seed weight, iron content and zinc content. This indicates the lesser influence of environment in the expression of the characters and may be governed by additive gene action. Hence, these traits are likely to respond to direct simple selection. Aung (2020) [7] and Athoni *et al.* (2022) [5].

High heritability coupled with high values of genetic advance as percent of mean (10-20%) were obtained for characters like thousand seed weight, iron content and zinc content. This indicates the lesser influence of environment in the expression of these characters and may be governed by additive gene action and traits can be easily fixed. Hence, these traits are likely to respond to direct simple selection. Similar results were reported by other workers in pearl millet by Varu *et al.* (2005) [21], Bhoite *et al.* (2008) [8], Lakshmana *et al.* (2009) [13], Govindaraj *et al.* (2010) [9], Vinodhana *et al.* (2013) [22], Aung (2020) [7] and Athoni *et al.* (2022) [5]. The moderate values of genetic advance as percent of mean (10-20%) were obtained for characters like days 50 percent flowering, plant height, panicle length, grain yield and dry fodder yield. The low estimate of genetic advance as a percentage of the mean was obtained for panicle girth, number of productive tillers, SPAD chlorophyll meter reading at flowering and maturity.

Genetic diversity

Mahalanobis D^2 is a powerful method for assessing genetic

divergence among various genotypes enabling the identification of genetically distinct parents and the determination of the relative contribution of each component trait.

In this current study, the classification of genotypes into clusters was performed using Mahalanobis D^2 statistics and Tocher's method. The 30 pearl millet genotypes were categorized into three clusters indicating a significant level of divergence within the studied material. Presented in Table 3. Cluster I was the largest and included 28 genotypes while the other clusters *i.e.*, cluster II and III consisted of single genotypes each. These findings align with similar results reported in previous studies by Sumathi *et al.* (2010) [20], Athoni *et al.* (2016) [6] and Rachana *et al.* (2023) [16].

In this current investigation, only cluster I (with an intra-cluster distance of 93.36) displayed evidence of genetic divergence among the genotypes within the cluster suggesting that some diversity still existed among them. In contrast, the remaining clusters did not exhibit any intra-cluster distance since each of these clusters contained only a single genotype. The substantial intra-cluster distance in cluster I indicate the presence of genetic diversity among the genotypes grouped within this cluster. Consequently, there is an opportunity for the exchange of genes among the genotypes within this cluster. Selection within such clusters could be carried out based on the highest mean value for the desired trait.

The highest inter-cluster distance was observed between clusters I and III (285.74) followed by the distances between cluster II and III (141.99) and cluster I and cluster II (136.53) (Table 4). These findings indicate the substantial diversity among the genotypes in these clusters. Selecting parents from these clusters could prove beneficial in generating greater variability in segregating generations. The wide range of D^2 values underscores the potential for significant diversity through inter-cluster hybridization.

In the current study, the highest contribution to genetic diversity was attributed to thousand seed weight (23%) followed by days to 50 percent flowering (19.6%), plant height (17.2%), Iron content (7.5%), grain yield (5.5%), panicle girth (5%), panicle length (4.3%), dry fodder yield (4.2%), zinc content (3.7%), SPAD chlorophyll meter readings at flowering (3.7%), SPAD chlorophyll meter readings at maturity (3.5%) and number of productive tillers per plant (3%). displayed in Table 5 and Fig 3. These findings suggest that these characteristics offer significant potential for the selection of desired genotypes. Similar result was obtained by Yadav (2019) [23], Pujar *et al.* (2020) [15] and Rachana *et al.* (2023) [16]. Table 6 displays 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 I had the highest ranking (rank 1), followed by cluster II (rank 2) and cluster III (rank 3).

Table 1: Analysis of variance (Mean sum of squares) for biofortified restorers with respect to morpho-phenological characters in pearl millet

Source	Df	DFF	PH	PL	PG	NPT	SCMF	SCMM	GY	DFY	TW	Fe	Zn
Replication	1	0.82	88.82	5.77	0.10	0.03	12.33	1.91	4740.72	36579.82	1.00	28.57	29.54
Treatments	29	27.97**	484.12**	10.35**	0.11**	0.19**	19.98**	14.30**	237612.546**	1281554.01**	4.40**	117.63**	50.00**
Error	29	1.92	53.40	2.70	0.04	0.04	8.14	5.86	72543.62	310928.21	7.61	26.67	12.02
S. Em. $\pm$		0.98	5.17	1.16	0.13	0.12	2.02	1.71	190.45	394.29	0.36	3.65	2.45
CD @ 5%		2.83	14.95	3.36	0.39	0.34	5.84	4.95	550.86	1140.44	1.05	10.56	7.09
CD @ 1%		3.82	21.14	4.53	0.52	0.46	7.87	6.67	742.40	1536.99	1.41	14.23	9.56
CV %		2.48	5.11	7.60	6.86	9.41	6.02	6.00	11.11	9.33	4.67	9.22	6.98

*Significant at $p=0.05$, **Significant at $p=0.01$

DFF - Days to 50 percent flowering, 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), DFY - Dry Fodder Yield (kg/ha), TW - 1000 grain weight (g), Fe - Iron content (ppm), Zn - Zinc content (ppm), S.E.m. $\pm$ -Standard error means, CD-Critical difference, CV - Coefficient of Variation and Df - Degrees of Freedom.

Table 2: Estimates of genetic variability in parental lines for morpho-phenological characters in pearl millet

Sl. No.	Characters	Mean	Range		GCV (%)	PCV (%)	Heritability % (broad sense)	Genetic advance	Genetic advance over mean (%)
			Min	Max					
1.	Days to 50 percent flowering	56	51	63	6.46	6.92	87.15	6.94	12.43
2.	Plant height (cm)	143	77	163	10.25	11.48	79.71	26.93	18.86
3.	Panicle length (cm)	21.62	16.1	26.2	9.05	11.82	58.61	3.08	14.27
4.	Panicle girth (cm)	2.78	2.4	3.3	6.70	9.59	48.84	0.27	9.65
5.	Number of productive tillers per plant	3.04	2.6	3.5	4.75	10.54	20.32	0.13	4.41
6.	SPAD chlorophyll meter reading at flowering	47.38	40.6	52.3	5.13	7.91	42.09	3.25	6.86
7.	SPAD chlorophyll meter reading at maturity	40.31	34.1	45.5	5.10	7.88	41.89	2.74	6.80
8.	Grain yield (kg ha ⁻¹)	2425.06	1651.85	3244.44	11.85	16.24	53.22	431.74	17.80
9.	Dry fodder yield (kg ha ⁻¹)	5975.30	3888.88	7222.22	11.66	14.93	60.95	1120.39	18.75
10.	Thousand seed weight (g)	10.96	8.1	14.3	13.13	13.94	88.69	2.79	25.46
11.	Iron content (ppm)	56.02	41.7	69.4	12.04	15.16	63.04	11.03	20.69
12.	Zinc content (ppm)	49.70	39.0	56.9	8.77	11.20	61.23	7.02	21.13

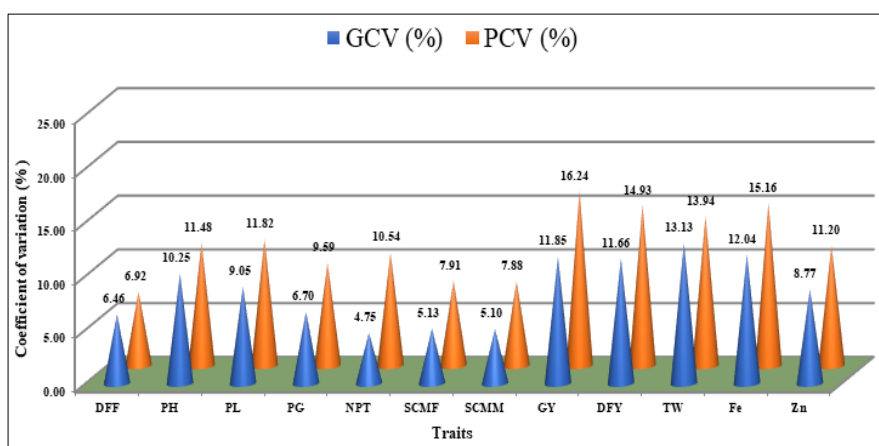


Fig 1: Estimates of GCV and PCV for morpho-phenological and yield contributing traits

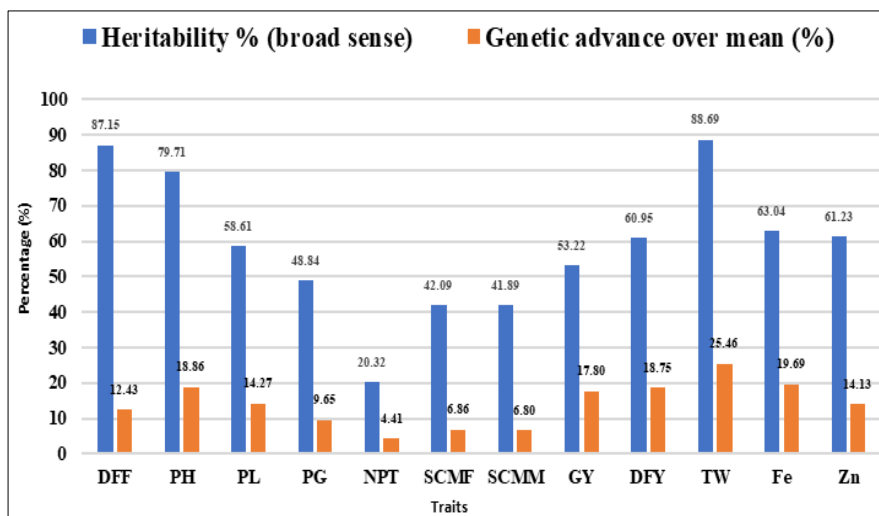


Fig 2: Heritability and genetic advance as percent of mean estimates for morpho-phenological and yield contributing traits

GCV- Genotypic coefficient of variation, PCV - phenotypic coefficient of variation, DFF - Days to 50 percent flowering,

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), DFY - Dry fodder yield (Kg/ha), TW - test weight (g), Fe - Iron content (ppm) and Zn - Zinc content (ppm).

Table 3: Distribution of 27 parents with three checks into different clusters

Cluster No.	No. of genotypes	Genotypes
1	28	VJP Fe & Zn-1 VJP Fe & Zn-2 VJP Fe & Zn-3 VJP Fe & Zn-4 VJP Fe & Zn-5 VJP Fe & Zn-6 VJP Fe & Zn-7 VJP Fe & Zn-8 VJP Fe & Zn-9 VJP Fe & Zn-10 VJP Fe & Zn-11 VJP Fe & Zn-12 VJP Fe & Zn-13 VJP Fe & Zn-14 VJP Fe & Zn-15 VJP Fe & Zn-16 VJP Fe & Zn-17 VJP Fe & Zn-18 VJP Fe & Zn-19 VJP Fe & Zn-20 VJP Fe & Zn-21 VJP Fe & Zn-22 VJP Fe & Zn-23 VJP Fe & Zn-24 VJP Fe & Zn-25 ICMB 8222 ICTP 8203 Dhanashakti
2	1	VJP Fe & Zn-15
3	1	ICMB 94555

Table 4: Intra (diagonal and bold) and Inter cluster average D² values in pearl millet (Tocher's method)

	Cluster 1	Cluster 2	Cluster 3
Cluster 1	93.36	136.53	285.74
Cluster 2		0.0	141.99
Cluster 3			0.0

Table 5: Contribution of morpho-phenological and yield contributing traits towards genetic divergence in pearl millet

Sl. No.	Traits	Contribution percentage (%)
1	Days to 50 percent flowering	19.6
2	Plant height (cm)	17.2
3	Panicle length (cm)	4.3
4	Panicle girth (cm)	5
5	Number of productive tillers per plant	3
6	SPAD chlorophyll meter reading at flowering	3.7
7	SPAD chlorophyll meter reading at maturity	3.5
8	Grain yield (kg/ha)	5.5
9	Dry fodder yield (kg/ha)	4.2
10	Thousand seed weight (g)	23
11	Iron content (ppm)	7.5
12	Zinc content (ppm)	3.7

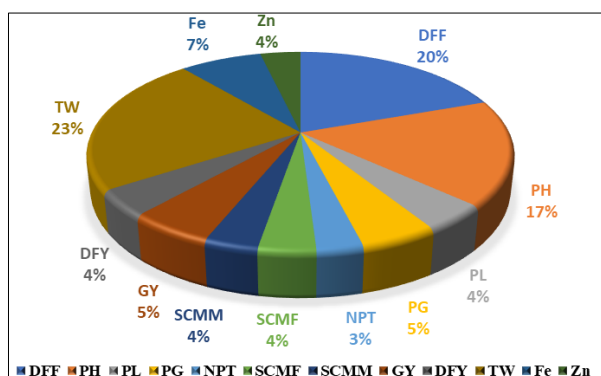


Fig 3: Contribution of morpho-phenological and yield contributing characters towards genetic divergence in pearl millet

DFF - Days to 50 percent flowering, 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), DFY - Dry fodder yield (Kg/ha), TW - test weight (g), Fe - Iron content (ppm) and Zn - Zinc content (ppm).

Table 6: Cluster mean values of parental lines and checks for morpho-phenological and yield contributing traits in pearl millet

Traits	Clusters		
	Cluster 1	Cluster 2	Cluster 3
Days to 50 percent flowering	56 (2)	63 (3)	54 (1)
Plant height (cm)	145 (2)	147 (1)	77 (3)
Panicle length (cm)	21.7 (2)	25.6 (1)	16.1 (3)
Panicle girth (cm)	2.8 (1)	2.8 (1)	2.4 (3)
Number of productive tillers per plant	3.1 (1)	2.9 (2)	2.6 (3)
SPAD chlorophyll meter reading at flowering	47.7 (1)	47.5 (2)	40.6 (3)
SPAD chlorophyll meter reading at maturity	40.4 (2)	41.0 (1)	36 (3)
Grain yield (kg/ha)	2469.048 (1)	1966.667 (2)	1651.852 (3)
Dry fodder yield (kg/ha)	6038.36 (2)	6296.297 (1)	3888.889 (3)
Thousand seed weight (g)	11.14 (1)	8.1 (2)	9 (3)
Iron content (ppm)	57 (1)	41.7 (3)	42.7 (2)
Zinc content (ppm)	50.3 (1)	42.6 (2)	39 (3)
Overall score	17	21	35
Total Rank	1	2	3

Summary and Conclusions

In present investigation estimates of genotypic and phenotypic coefficient of variation indicated that the PCV was higher than the GCV for all the traits studied indicated that all the characters were influenced by the environment. Moderate values of GCV with PCV were recorded for plant height, panicle length, grain yield, dry fodder yield, thousand seed weight, iron content and zinc content. Hence, there is a considerable scope for selection for these traits towards favorable direction. High heritability (broad sense) estimates were obtained for days to 50 percent flowering, plant height, dry fodder yield, thousand seed weight, iron content and zinc content indicating that these traits are heritable and simple selection would be sufficient for these traits to bring genetic improvement in the desired direction. High estimates of heritability coupled with high genetic advance as percent of mean was detected for the trait thousand seed weight, iron content and zinc content revealing that these traits might be governed by additive gene effects and simple selection would lead to fasten genetic enhancement of the genotypes under study.

A genetic diversity study indicated that among various characters studied thousand seed weight contributed maximum to the total divergence while days to 50 percent flowering and plant height were next in order. Based on D² values, 27 parents along with three checks were grouped into three clusters. Among the three clusters, cluster I was the largest with 28 genotypes followed by cluster II and cluster III which has one genotype each. Intra cluster D² values ranged from zero (cluster II and III) to 93.36 (cluster I). Inter cluster D² values ranged from 93.36 to 285.74. The maximum inter cluster distance was recorded between cluster I and III (285.74) followed by cluster II and III (141.99) and cluster I and cluster II (136.53). Hybridization

programmes between these genotypes might be used for exploitation of hybrid vigour and good recombinant types.

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