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MULTI TRAIT ANALYSIS REVEALS SUBSTANTIAL DIVERSITY IN PEARL MILLET [*Pennisetum glaucum* (L.) R. Br.] INBRED LINES

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ABSTRACT

The strength of any ongoing breeding programme thoroughly depends on the presence of genetic variation at both morphological and molecular level. In present study, total 36 pearl millet genotypes were evaluated for different morphological characters along with grain Fe and Zn contents. High estimates of coefficient of variation inclusive of high heritability and genetic advance as per cent of mean was marked for dry fodder yield per plant, grain yield per plant, Fe and Zn contents. This suggested that the selection based on these traits will be effective in improving breeding material. Further correlation analysis showed a highly significant correlation between grain Fe and Zn content which signifies simultaneous improvement in the two traits. Grain yield per plant showed non significant negative correlation with Fe and Zn thus suggesting improvement in nutrient value without sacrificing yield. A set of 64 SSRs was also used for molecular diversity assessment. A significant positive correlation was observed among number of alleles, Polymorphic Information Content (PIC) and number of repeats in the SSR motifs. Across the linkage groups, the mean PIC varied from 0.48 (LG 3) to 0.76 (LG 2). Mean alleles per locus and overall PIC obtained was 7.20 and 0.68 respectively. Presence of ample variation at morpho-genetic level signifies their use as parents in the out crossing programmes to obtain new improved hybrids with desired traits.

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1 Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a highly cross pollinated C₄ monocot species belonging to the family Poaceae. It has a relatively small genome (2n=2x=14) with a DNA content of 1C=2.36 pg (Martel et al., 1997). An estimated 30 million ha is cultivated with pearl millet in the arid and semi-arid tropical regions of Asia, Africa and Latin America (Yadav & Rai, 2013). India is the largest producer of pearl millet in Asia, both in terms of area (about 9 million ha) and production (8.3 million tons) with an average productivity of 930 kg/ha (AICPMIP, 2017). It is a crop of area characterized with low and erratic rainfall (200-600 mm), high temperature, high salinity or low pH and impoverished infertile soils. Because of its tolerance to harsh growing conditions, it can be grown in areas where other cereal crops, such as wheat, maize or rice would not survive. Also, high protein and oil content, gluten free nature and richness in vitamin B (especially niacin, B₆, folic acid), calcium, potassium, magnesium and other essential micronutrients have made its production popular from being a neglected 'orphan' crop earlier.

Knowledge for the extent of genetic variation among breeding material (germplasm, breeding lines & inbred lines) is essential to understand the pattern of diversity and evolutionary relationship among them. This is also helpful to efficiently use germplasm in crop improvement program (Wu et al., 2007; Thiyaagu et al., 2011). Pearl millet has been found to exhibit a wide range of morphological variability for several traits such as plant height, days to 50% flowering, panicle length and width, grain yield and nutritional value (Haussmann et al., 2006; Bhattacharjee et al., 2007; Stich et al., 2010; Bashir et al., 2014). The assessment of genetic diversity on the basis of morphological characteristics alone might not provide accurate information. It is due to the restricted number of morphological traits evaluated, environmental influence and development-specific trait expression. All these factors thus necessitate application of molecular markers for having a better view of genetic diversity present among the breeding material.

In pearl millet, the first application of molecular markers was creation of genetic linkage map using Restriction Fragment Length Polymorphism (RFLP) (Liu et al., 1994). Later, sequence independent PCR based markers like RAPD, ISSR, AFLP and microsatellite probes have been used for genetic diversity studies (Chowdari et al., 1998; vomBrocke et al., 2003; Yadav et al., 2007; Govindaraj et al., 2009). Meanwhile, development of SSR markers (Allouis et al., 2001; Qi et al., 2001) and their use in genetic diversity and gene mapping studies succeeded (Mariac et al., 2006; Chandra-Shekhra et al., 2007). Microsatellite markers have been adjudged as more

effective and reliable DNA markers for such studies (Kapila et al., 2008) because of their abundance in genome, multi allelism, genome specificity, even distribution, high polymorphism, easy detection, high-throughput, highly reproducible and co-dominantly inherited behavior (Hernandez et al., 2002).

Worldwide serious and widespread human health problems have been recognized due to dietary deficiency of mineral micronutrients such as iron and zinc (WHO, 2002; Welch & Graham, 2004). Micronutrient malnutrition increases mortality & morbidity rates and health-care costs, reduces labor productivity, and thus impacts on national developmental efforts (Darnton-Hill et al., 2005; Stein, 2010). The developing and under-developed countries of Africa and Asia are worst affected by malnutrition. These regions grow pearl millet as staple for dietary energy. Genetic resources of pearl millet are untapped and need attempts to improve the iron and zinc content. Discerning the genetic diversity of these traits and characterizing genotypes with advent of conventional as well as by molecular markers will be useful to identify contrasting parental materials to enhance heterozygosity or to optimize the genetic heterogeneity in a hybrid population and enhance yield stability along with nutrient value in variable and changing climates (Haussmann et al., 2007; Hausmann et al., 2012). Keeping all this in mind, present study was conducted to understand genetic diversity existing at morpho-biochemical and molecular level among pearl millet inbred lines.

2 Materials and methods

2.1 Raising of crop

Seeds of 36 pearl millet genotypes (Table 1) were obtained from Bajra section, Department of Genetics and Plant Breeding, CCSHAU, Hisar. The crop was grown in Randomized Block Design with two replications in two environments [normal-sown (NS) and late-sown(LS)] at the research area of Bajra section with a plot size 2 rows x 4m x 0.5m and 10-12 cm intra-row spacing. The research site is located at 29° 17'N latitude and 75° 47'E longitude at an altitude of 215.2 meters above mean sea level in the subtropical climatic zone of India and a sandy-loam soil type. All recommended agronomic practices were followed for raising a good crop. Seven characters were selected for morphological studies namely, days to 50% flowering, plant height, ear length, ear diameter, 1000 grain weight, grain yield per plant and dry fodder yield per plant. All these traits were measured on individual plant and the data was analyzed as means of 5 individual plants from each line. On harvest, open-pollinated grains were used for analysis of Fe and Zn content using X-ray Fluorescence Spectroscopy (XRF) technique at ICRISAT (Paltridge et al., 2012).

Table 1 Pearl millet genotypes used in the present study

S. No.	Genotypes	S. No.	Genotypes
1.	HMS 48B	19.	AC-04/7
2.	HMS 14B	20.	H 2302
3.	HMS 58B	21.	72-2-2/98K-1
4.	HMS 53B	22.	HTP 93/109
5.	HMS 55B	23.	HTP 98-2
6.	HMS 43B	24.	H 78/711
7.	HMS 60B	25.	H 10/4-7
8.	HMS 7B	26.	H 10/10-2
9.	HMS 44B	27.	HTP 94/54
10.	HMS 22B	28.	HTP 98/3
11.	HMS 16B	29.	HTP 92/80
12.	HMS 38B	30.	TCH 26-1
13.	HMS 37B	31.	H 94/46
14.	HMS 26B	32.	HBL 11
15.	HMS 30B	33.	AC-04/13
16.	H 72-2-2	34.	H 12/1009
17.	H 12/009	35.	99 HS 139
18.	H 12/008	36.	99 HS-23

(1-15 = B-lines and 16-36 = R-lines); #All these lines have been developed at CCSHAU

2.2 DNA extraction and PCR assay

Young and fresh leaves of 30 days old plants were used for DNA extraction. Genomic DNA was isolated using CTAB extraction method of Saghai-Marouf et al. (1984) with little modifications. RNase treatment was given to purify the extracted DNA. Isolated DNA from each genotype was run in 0.8% agarose gel and analyzed using UV spectrophotometer (Elico Ltd., India). A total of 71 SSR primer pairs (Imperial Life Sciences, USA) comprising of genomic as well as EST-SSRs were selected on the basis of previous studies (Allouis et al., 2001; Qi et al., 2001; Budak et al., 2003; Qi et al., 2004; Mariac et al., 2006; Yadav et al., 2007; Senthilvel et al., 2008) to study molecular diversity among genotypes encompassing at least two SSR loci from each linkage group, representing the whole nuclear genome. PCR amplifications were performed using G-storm thermocycler in 20 µl reaction mixture containing 1 µl 50 ng DNA, 1 µl DMSO, 1.25X PCR buffer, 0.25 mM dNTPs, 0.125 µM forward and reverse primers, 2.5 U taq DNA polymerase. Touch-down amplifying program for PCR cycles was used which consisted of initial denaturation at 94°C for 3 min, 5 cycles of denaturation at

94°C for 45 sec, annealing at 46-61°C (depending on primer) for 1 min, extension at 72°C for 45 sec; then 20 cycles consisting of denaturation at 94°C for 45 sec, annealing at 44-59°C for 1 min, extension at 72°C for 45 sec and a final extension at 72°C for 10 min. Amplified PCR products (10 µl) were separated on ethidium bromide stained 6% polyacrylamide gel (C.B.S. Scientific, USA) at 220V for 3 hours in 0.5X Tris- Borate EDTA buffer. The DNA bands were visualized and photographed under UV light using gel documentation system (UVP, USA). The experiments were repeated at least twice to confirm the results and check for the reproducibility of the amplified products.

2.3 Statistical and Molecular Data Analysis

All statistical analysis of recorded phenotypic data was performed using OPSTAT software (Sheron et al., 1998 available at <http://www.hau.ac.in>). Cluster analysis of morphological characters as well as Fe and Zn content was done based on Ward's Minimum Variance method using IndoStat software (developed by IndoStat service, Hyderabad). For molecular analysis clearly resolved bands were scored for presence (1) or absence (0). The size (in nucleotide base pairs) of the most intensely amplified reproducible and unambiguous bands for each microsatellite marker was determined based on its migration relative to a standard DNA marker (20 or 100 bp G-biosciences DNA ladder). Multiple alleles were inferred whenever a given marker produced more than one cluster of bands. The binary data matrix generated was then used to calculate pair-wise similarity coefficient for all possible pairs of genotypes using Jaccard's similarity coefficient (Jaccard, 1908). 'Simqual' subprogram of software NTSYS-pc version 2.02e (Rohlf, 2000) was used to generate similarity matrix. Cluster analysis was done using 'SAHN' sub-program and dendrogram was then constructed based on UPGMA algorithm. Furthermore, matrix was subjected to Principal Component Analysis (PCA) for the three principal components using 'Eigen' subprogram to generate a 2-D and 3-D representation of genetic relationship among the genotypes. WinBoot software program (Yap & Nelson, 2002) was also used to verify the clusters obtained by NTSYS-pc.

3 Results and Discussion

3.1 Estimates of Variability

The analysis of variance revealed that the mean sum of squares showed highly significant differences among the genotypes for the various characters studied in two environments (data not shown). The estimates of variability parameters for all the characters are shown in the Table 2. A wide range of differences for Genotypic Coefficient of Variation (GCV) was observed which varied from 7.54 for days to 50% flowering to 39.19 for dry fodder yield per plant in NS and 7.70 for days to 50% flowering to 41.19 for dry

Table 2 Estimates of variability parameters for different characters in thirty-six pearl millet genotypes grown during *kharif*

S. No.	Character	Mean $\pm$ SE (d)		Range		GCV		PCV		Heritability (%)		Genetic advance		Genetic advance (% of mean)	
		NS	LS	NS	LS	NS	LS	NS	LS	NS	LS	NS	LS	NS	LS
1	Days to 50% Flowering	53.10 $\pm$ 0.73	50.20 $\pm$ 0.142	42.50-60.50	39.00-57.50	07.54	07.70	07.66	08.21	96.76	88.14	08.11	07.48	15.27	14.90
2	Plant height (cm)	145.50 $\pm$ 07.27	127.00 $\pm$ 07.14	83.70-221.00	53.50-205.50	21.46	23.94	22.03	24.59	94.87	94.78	62.66	60.96	43.06	48.02
3	Ear length (cm)	19.80 $\pm$ 01.56	18.00 $\pm$ 01.32	12.80-32.00	13.60-27.50	17.40	15.89	19.11	17.51	82.96	82.43	06.48	05.36	32.66	29.72
4	Ear diameter (cm)	2.20 $\pm$ 00.16	2.00 $\pm$ 00.22	01.58-02.70	01.45-02.60	12.87	13.08	14.76	16.94	76.05	59.59	00.50	00.42	23.12	20.79
5	1000 grain weight (g)	7.90 $\pm$ 00.75	7.40 $\pm$ 01.31	03.92-12.65	03.74-12.25	19.78	19.76	21.89	26.46	81.64	55.77	02.93	02.26	36.82	30.40
6	Grain yield per plant (g)	21.10 $\pm$ 02.38	14.20 $\pm$ 02.30	07.23-33.15	04.40-24.20	34.84	34.44	36.61	38.07	90.55	81.81	14.44	09.10	68.29	64.16
7	Dry fodder yield per plant (g)	59.80 $\pm$ 06.22	43.10 $\pm$ 06.04	16.80-120.00	17.04-86.80	39.19	41.19	40.55	43.51	93.42	89.63	46.66	34.63	78.04	80.33
8	Fe content (ppm)	53.57 $\pm$ 09.41	59.00 $\pm$ 06.34	29.00-93.50	33.50-108.00	34.46	34.22	38.67	35.87	79.39	91.04	33.88	39.69	63.24	67.26
9	Zn content (ppm)	40.39 $\pm$ 07.40	42.13 $\pm$ 06.65	23.00-81.00	23.00-75.00	29.79	31.97	34.98	35.65	72.53	80.39	21.11	24.87	52.26	59.04

NS- Normal-sown environment; LS- Late-sown environment

fodder yield per plant in LS. Phenotypic Coefficient of Variation (PCV) varied from 7.66 for days to 50% flowering to 40.55 for dry fodder yield per plant in NS and 8.21 for days to 50% flowering to 43.51 for dry fodder yield per plant in LS. The presence of wide range of GCV and PCV for all the characters indicated the presence of considerable amount of variability among the genotypes. GCV and PCV values were highest for dry fodder yield per plant in both the environments followed by grain yield per plant then Fe & Zn content indicating availability of sufficient variation and thus, scope for genetic improvement through selection for all these traits. Days to 50% flowering exhibited least GCV and PCV in both the environments as also studied by Lakshmana et al. (2010), Choudhary et al. (2012) and Kumar et al. (2014). A higher PCV of characters than their corresponding GCV was observed in present study. This suggested the role of environment in the expression of all these characters. Kumar et al. (2014) also observed higher PCV than GCV for the studied characters.

A wide range for heritability in broad sense, genetic advance and genetic advance in terms of per cent of mean was noticed in both the environments. Estimates of heritability in broad sense ranged from 72.53% for Zn content to 96.76% for days to 50% flowering and 55.77% for 1000 grain weight to 94.78% for plant height in NS and LS respectively. Range for genetic advance in terms of per cent of mean varied from 15.27 and 14.90 for days to 50% flowering to 78.04 and 80.33 for dry fodder yield per plant in NS and LS respectively. High estimates of coefficient of variation along with moderate to high heritability and genetic advance as per cent of mean for dry fodder yield per plant, grain yield per plant, Fe

and Zn contents are indicative of additive gene action for these characters. These traits are thus more reliable for effective selection in improving the plant performance. High heritability and genetic advance per cent of mean was also reported by Bhoite et al. (2008) and Bind et al. (2015) for dry fodder yield per plant and grain yield per plant suggesting their reliability for effective selection. Ear length, ear diameter and 1000 grain weight had moderate heritability and genetic advance which indicated both additive and non-additive gene effects that makes use of reciprocal recurrent selection. In the present study, higher values of heritability and lower values of genetic advance and variability were observed for days to 50% flowering, signaling towards the presence of non-additive gene actions. The most favorable breeding strategy for improvement in such traits can be the selection at later stages.

3.2 Correlation Coefficient analysis

Phenotypic and genotypic correlation coefficient analysis was carried out to assess the association between various traits (Table 3). Fe content (ppm) showed a significant positive correlation with 1000 grain weight at genotypic ($r = 0.282$; $p = 0.05$) level and dry fodder yield per plant at both phenotypic ($r = 0.278$; $p = 0.05$) and genotypic ($r = 0.282$; $p = 0.05$) level in LS. A significant negative correlation of Fe content with plant height at genotypic ($r = -0.269$; $p = 0.05$) level and with ear length at both phenotypic ($r = -0.266$; $p = 0.05$) and genotypic ($r = -0.315$; $p = 0.01$) level was observed in NS. Zn content showed a significant positive correlation with 1000 grain weight at genotypic ($r = 0.272$; $p = 0.05$) level in NS and at phenotypic ($r = 0.240$; $p = 0.05$) as well as at genotypic ($r = 0.398$; $p = 0.01$) level in LS. Zn content also exhibited significant positive

Table 3: Phenotypic and genotypic correlation coefficients among nine characters in thirty-six pearl millet genotypes grown during *kharif*

Character			Days to 50% flowering	Plant height (cm)	Ear length (cm)	Ear diameter (cm)	1000 grain weight (g)	Grain yield (g)	Dry fodder yield (g)	Fe (ppm)	Zn (ppm)
Days to 50% flowering	NS	P	1.000								
		G	1.000								
	LS	P	1.000								
		G	1.000								
Plant height (cm)	NS	P	-0.240*	1.000							
		G	-0.250*	1.000							
	LS	P	-0.283*	1.000							
		G	-0.293*	1.000							
Ear length (cm)	NS	P	-0.098NS	0.459**	1.000						
		G	-0.108NS	0.463**	1.000						
	LS	P	-0.155NS	0.430**	1.000						
		G	-0.210NS	0.465**	1.000						
Ear diameter (cm)	NS	P	-0.072NS	0.122NS	0.249*	1.000					
		G	-0.081NS	0.134NS	0.227NS	1.000					
	LS	P	-0.044NS	0.257*	0.426**	1.000					
		G	-0.053NS	0.317**	0.522**	1.000					
1000 grain weight (g)	NS	P	-0.034NS	0.324**	0.209NS	0.495**	1.000				
		G	-0.053NS	0.332**	0.236*	0.544**	1.000				
	LS	P	-0.327**	0.513**	0.242*	0.289*	1.000				
		G	-0.461**	0.640**	0.289*	0.247*	1.000				
Grain yield (g)	NS	P	-0.121NS	0.295*	0.495**	0.322**	0.334**	1.000			
		G	-0.128NS	0.292*	0.496**	0.328**	0.365**	1.000			
	LS	P	-0.372**	0.644**	0.472**	0.395**	0.577**	1.000			
		G	-0.445**	0.723**	0.565**	0.509**	0.707**	1.000			
Dry fodder yield (g)	NS	P	-0.007NS	0.331**	0.320**	0.278*	0.425**	0.717**	1.000		
		G	0.001NS	0.331**	0.307**	0.276*	0.475**	0.712**	1.000		
	LS	P	-0.363**	0.533**	0.308**	0.212NS	0.528**	0.757**	1.000		
		G	-0.416**	0.558**	0.340**	0.234*	0.652**	0.802**	1.000		
Fe (ppm)	NS	P	-0.016NS	-0.217NS	-0.266*	0.171NS	0.217NS	0.125NS	0.058NS	1.000	
		G	-0.024NS	-0.269*	-0.315**	0.223NS	0.216NS	0.141NS	0.071NS	1.000	
	LS	P	-0.170NS	-0.012NS	-0.078NS	0.070NS	0.166NS	0.002NS	0.278*	1.000	
		G	-0.180NS	-0.016NS	-0.035NS	0.166NS	0.282*	0.031NS	0.282*	1.000	
Zn (ppm)	NS	P	-0.050NS	-0.153NS	-0.372**	0.109NS	0.224NS	0.198NS	0.053NS	0.873**	1.000
		G	-0.062NS	-0.208NS	-0.469**	0.144NS	0.272*	0.212NS	0.070NS	0.899**	1.000
	LS	P	-0.267*	0.113NS	-0.152NS	-0.101NS	0.240*	0.006NS	0.252*	0.847**	1.000
		G	-0.322**	0.127NS	-0.139NS	-0.078NS	0.398**	0.105NS	0.308**	0.914**	1.000

*,** Significant at $P=0.05$ and $P=0.01$ levels respectively; P-Phenotypic correlation coefficient values;

G- Genotypic correlation coefficient values; NS- Non Significant

correlation with dry fodder yield at both phenotypic ($r = 0.252$; $p = 0.05$) and genotypic ($r = 0.308$; $p = 0.01$) levels in LS. A highly significant positive correlation between Fe and Zn content in the two environments at phenotypic ($r = 0.873$; $p = 0.01$, $r = 0.847$; $p = 0.01$) as well as genotypic ($r = 0.899$; $p = 0.01$, $r = 0.914$; $p = 0.01$) levels was observed in this study. Earlier studies on pearl millet by Velu et al. (2007, 2008a, 2008b), Gupta et al. (2009), Govindaraj et al. (2012, 2013) and Rai et al. (2012, 2013, 2015) also showed a highly significant and positive correlation between the levels of Fe and Zn contents. Similar relationship between these two mineral elements has also been reported in other cereals such as finger millet (Upadhyaya et al., 2011), sorghum (Kumar et al., 2009, 2013), maize (Oikeh et al., 2003, 2004), rice (Anandan et al., 2011) and wheat (Garvin et al., 2006; Velu et al., 2011). This positive correlation could be due to common and overlapping quantitative trait loci (QTL) for grain Fe and Zn contents as reported in pearl millet (Kumar, 2011), wheat (Peleg et al., 2009; Singh et al., 2010), rice (Stangoulis et al., 2007) and common bean (Blair et al., 2009; Cichy et al., 2009).

In order to realize maximum impact of micronutrient-rich cultivars, the micronutrients must be delivered in high-yielding cultivars with farmer's preferred traits such as large seed size and yield. Fe content showed a significant but weak positive correlation with 1000 grain weight at only genotypic ($r = 0.282$; $p = 0.05$) level in LS. Zn content also showed positive correlation with 1000 grain weight at genotypic ($r = 0.272$; $p = 0.05$) level in NS and at phenotypic ($r = 0.240$; $p = 0.005$) as well as genotypic ($r = 0.398$; $p = 0.01$) levels in LS. It is in concordance with correlation observed by Velu et al. (2008b) and Kanatti et al. (2014) in pearl millet hybrids. A significant moderate to high correlation was also observed by Velu et al. (2007, 2008b) in their studies. However, a non significant correlation observed between Fe content and 1000 grain weight at both levels in NS and at phenotypic level in LS is in concordance with results of Velu et al. (2008a), Gupta et al. (2009), Govindaraj et al. (2012), Rai et al. (2012, 2013, 2015) and Kanatti et al. (2014). Early maturity is another farmer preferred trait that enables the crop to escape terminal drought stress in short season environments and permits double cropping. Correlation observed between Fe and days to 50% flowering was non significant at both the levels in both the environments. Similar results were observed in studies of Velu et al. (2008a) and Rai et al. (2012, 2015). However, study of Velu et al. (2008b) reported negative correlation between Fe and days to 50% flowering. Non significant correlation observed between Zn content and days to 50% flowering at both the levels in NS is similar to the results of Velu et al. (2008a, 2008b) and Rai et al. (2012, 2015). None of the earlier studies presented significant negative correlation as observed in this study between Zn content and days to 50% flowering at phenotypic ($r = -0.267$; $p = 0.05$) as well as genotypic ($r = -0.332$; $p = 0.01$) levels in LS.

For any biofortification programme, an important aspect is to take care that nutrient value enhancement should not be at the cost of grain yield. Therefore, a correlation study was conducted between grain yield per plant and Fe as well as Zn content. A non significant correlation was observed both for Fe and Zn content with grain yield per plant at phenotypic as well as genotypic level in the two environments. This is supported by the studies of Gupta et al. (2009), Rai et al. (2012) and Kanatti et al. (2014). Studies have also observed significant but weak to moderate negative correlation between Fe and grain yield (Kanatti et al., 2014; Rai et al., 2012, 2013). Similarly, significant and weak negative correlation was observed between Zn and grain yield (Kanatti et al., 2014). Traits namely, plant height, ear length, ear diameter, 1000 grain weight and dry fodder yield per plant exhibited significant positive correlation at both the levels with grain yield per plant. The positive correlation of grain yield per plant with these characters implies that improving one or more of these traits could result in higher grain yield for pearl millet.

3.3 Morphological cluster analysis

Morphological characters and Fe as well as Zn content were used for cluster analysis to estimate genetic divergence between genotypes with an aim to improve yield along with quality. The cluster analysis is helpful in selection of genotypes for their further use in the hybrid breeding programme and creation of greater variability to broaden genetic base. All the 36 genotypes were clustered into 5 clusters at a Standard Euclidean Square distance of 15 based on Ward's Minimum Variance method (Figure 1a and 1b). This suggests that significant diversity is present in the experimental material for the characters studied including nutritional parameters. Similar level of diversity for morphological traits was observed by Ramya et al. (2017) and Abdulkakeem et al. (2019) in pearl millet genotypes. The members of clusters in two environments were nearly same with minor differences (Table 4). According to the cluster pattern of NS, cluster 1 and 3 were largest consisting of 11 genotypes each followed by cluster 4 (7 genotypes) and cluster 5 (5 genotypes). Smallest cluster was 2 with only 2 genotypes. Whereas cluster pattern of LS had cluster 2 as the largest with 11 genotypes followed by cluster 1 (9 genotypes), cluster 5 (7 genotypes), cluster 3 (5 genotypes) and cluster 4 (4 genotypes). The intra and inter cluster distances for the two environments are given in Table 5. Range for diversity within cluster varied from 2.75 to 4.43 in NS and 2.36 to 3.97 in LS. A maximum difference among the genotypes within the same cluster was shown by cluster 2 (4.43) and cluster 3 (3.97) indicating high divergence among the genotypes of the clusters, whereas lowest was exhibited by cluster 4 (2.75) and cluster 1 (2.36) in NS and LS respectively. Cluster 2 and 3 had maximum inter cluster distance of 6.32 in NS whereas cluster 1 and 3 showed a value 6.07 in LS.

Figure 1a Standardized Euclidean² distance based dendrogram of thirty-six pearl millet genotypes under normal-sown conditions

Figure 1b Standardized Euclidean² distance based dendrogram of thirty-six pearl millet genotypes under late-sown conditions

Useful recombinants can be generated for hybridization by selecting lines from these clusters. Minimum inter cluster distance was observed between cluster 1 and 4 (3.95) and between cluster 2 and 4 (3.79) in NS and LS respectively. Thus crossing of genotypes from these clusters may be avoided as it may not produce a high amount of heterotic expression in F_1 and broad range of variability in F_2 segregating population.

There is a considerable difference among all the clusters as revealed by cluster means for the 9 characters studied. Cluster wise mean and over all cluster mean for the characters are presented in Table 6. Cluster mean performance for grain yield and other contributing characters was highest for cluster 1 and cluster 3 in NS and LS respectively while moderately high for cluster 5 in both the environments. Also, from the present data, it

Table 4 Clustering of thirty-six pearl millet genotypes based on different morphological and biochemical parameters

Cluster	Number of genotypes		Name of genotypes
Cluster 1	NS	11	HMS 48B, HMS 30B, 72-2-2/98k-1, HTP 93/109, HTP 98-2, H 10/4-7, H 10/10-2, HTP 98/3, HTP 92/80, TCH 26-1 and AC-04/13
	LS	9	HMS 58B, HMS 55B, HMS 7B, HMS 22B, HMS 38B, HMS 37B, HMS 26B, HBL 11 and 99 HS 139
Cluster 2	NS	2	HMS 44B and HTP 94/54
	LS	11	HMS 48B, HMS 43B, HMS 30B, AC-04/7, HTP 98-2, H 10/4-7, HTP 92/80, TCH 26-1, H 94/46, AC-04/13 and 99 HS-23
Cluster 3	NS	11	HMS 58B, HMS 43B, HMS 7B, HMS 22B, HMS 37B, HMS 26B, AC-04/7, H 78/711, H 94/46, HBL 11 and 99 HS 139
	LS	5	HMS 44B, H 78/711, H 10/10-2, HTP 94/54 and HTP 98/3
Cluster 4	NS	7	HMS 14B, HMS 53B, HMS 55B, HMS 60B, HMS 16B, HMS 38B and 99 HS-23
	LS	4	HMS 14B, HMS 53B, HMS 60B and 72-2-2/98K-1
Cluster 5	NS	5	H 72-2-2, H 12/009, H 12/008, H 2302 and H 12/1009
	LS	7	HMS 16B, H 72-2-2, H 12/009, H 12/008, H 2302, HTP 93/109 and H 12/1009

Table 5 Average Inter and Intra cluster distances of five clusters based on Ward's Minimum Variance method

	Cluster 1		Cluster 2		Cluster 3		Cluster 4		Cluster 5	
	NS	LS	NS	LS	NS	LS	NS	LS	NS	LS
Cluster 1	2.76	2.36	4.33	3.98	4.33	6.07	3.95	4.18	4.84	5.29
Cluster 2			4.43	2.58	6.32	3.88	5.54	3.79	6.15	4.30
Cluster 3					3.09	3.97	4.03	4.79	5.09	4.97
Cluster 4							2.75	2.88	4.03	4.41
Cluster 5									3.46	3.02

Diagonal values are intra cluster distances

Table 6 Mean values of various clusters for different morphological and biochemical parameters

	DT50%		PH (cm)		EL (cm)		ED (cm)		1000GW (g)		GY/P (g)		DFY/P (g)		Fe (ppm)		Zn (ppm)	
	NS	LS	NS	LS	NS	LS	NS	LS	NS	LS	NS	LS	NS	LS	NS	LS	NS	LS
Cluster 1	51.18	52.72	169.53	97.68	22.17	15.63	2.29	1.76	8.32	5.68	27.41	7.58	71.15	21.73	43.32	45.56	33.23	34.61
Cluster 2	58.50	50.36	188.00	138.22	26.15	17.85	2.47	2.06	11.73	7.44	26.41	17.12	93.90	49.00	43.50	47.36	33.25	33.09
Cluster 3	54.23	47.60	136.60	163.00	18.17	22.160	1.88	2.30	6.44	9.46	24.06	19.66	39.01	57.18	41.95	50.80	33.95	38.20
Cluster 4	54.50	50.00	106.46	97.87	19.30	20.87	2.34	2.34	7.80	6.30	23.12	12.49	54.62	36.31	68.14	77.87	46.93	45.37
Cluster 5	50.90	48.57	150.00	137.79	16.52	16.75	2.09	1.86	9.15	8.90	18.03	15.11	74.10	55.18	85.30	89.64	64.00	66.93

DT 50%= Days to 50% flowering, PH= Plant height, EL= Ear length. ED= Ear diameter, 1000GW= 1000 Grain weight, GY/P= Grain yield per plant, DFY/P= Dry fodder yield per plant, Fe = Iron and Zn = Zinc

is evident that the mean value of Fe and Zn is highest in cluster 5 (85.30 ppm Fe and 64.00 ppm Zn in NS and 89.64 ppm Fe and 66.93 ppm Zn in LS). This signifies that the cluster 5 is composed of high Fe and Zn genotypes consistent in two environments. Exploitation of heterosis can only be done by utilizing parents with maximum genetic divergence. This increases spectrum of

variability in the segregating generation and a more pronounced heterotic effect can be achieved. Therefore, genotypes from cluster 5 will serve as potential parents for enhancing grain Fe and Zn content with simultaneous improvement in yield and its associated characters. Also, days to 50% flowering mean is less in this cluster that means hybrids which can escape terminal drought

can be produced by crossing genotypes belonging to this cluster.

3.4 Molecular marker analysis

Sixty-four primer pairs selected out of 71 primer pairs amplified a total of 461 alleles. The number of alleles per locus varied from 2 (ICMP 3016, ICMP 3018, XCUMP 0017, XCUMP 0019 and PSMP 20176) to 27 (PSMP 2008) with a mean of 7.20 alleles per locus (Table 7). This value is much higher than the value of mean alleles per locus reported earlier i.e. 5.7 (Budak et al., 2003), 3.07 (Sumanth et al., 2013) and 3.0 (Singh et al., 2013) but lesser than the value 16.4 as obtained by Stich et al. (2010). The value obtained is comparable with value 6.26 (Kapila et al., 2008) and 8.1 (Nepolean et al., 2012). Fourteen primers amplified alleles in range of 11 to 16. The highest number of alleles (27) was obtained using primer PSMP 2008 with a dinucleotide type of repeat [(GT)₃₇]. A value more than

27 number of alleles has also been reported at individual loci by Saghai-Marooof et al. (1994) (37 alleles in barley) and Rongwen et al. (1995) (26 alleles in soybean) suggesting high levels of polymorphism in plant SSRs. Primers Xpsmp2070 and Xpsmp 2218 have also been reported to amplify more than 20 alleles by Nepolean et al. (2012). Gupta et al. (2015) observed up to 40 alleles amplified by primer Xpsmp 2218 while 23 to 40 alleles per locus were amplified by primers Xpsmp 2068, Xpsmp 2079 and Xpsmp 2218. Polymorphic Information Content (PIC) value in present study ranged from 0.14 (PSMP 2227) to 0.95 (PSMP 2008) with an average of 0.68 which is near to value 0.77 as observed by Bashir et al. (2015) but higher than 0.58 (Kapila et al., 2008; Nepolean et al., 2012) and 0.44 (Budak et al., 2003; Singh et al., 2013). Primer PSMP 2008 with highest PIC was found to be highly informative of all the primers surveyed. Figure 2 shows the allelic polymorphism among 36 pearl millet genotypes at PSMP 2201 locus.

Table 7 Amplification results of 64 SSR primers

S. No.	SSR locus	Linkage group (LG)	SSR motif	Amp. Range (bp)	Allele No.	PIC (B- & R- line)
1	PSMP 2008	5	(GT) ₃₇	120-1000	27	0.95 (0.93; 0.94)
2	PSMP 2027	7	(GT) ₃₁	238-410	11	0.84 (0.79; 0.85)
3	PSMP 20	NA	NA	150-200	7	0.78 (0.79; 0.76)
4	PSMP 2059	2	(AC) ₁₁	115-125	6	0.78 (0.82; 0.74)
5	PSMP 2084	4	(AC) ₄₂	228-1000	13	0.89 (0.80; 0.90)
6	PSMP 2087	7	(AC) ₁₀	120-140	5	0.72 (0.67; 0.74)
7	PSMP 2090	1	(CT) ₁₂	150-330	7	0.82 (0.67; 0.75)
8	PSMP 2201	2	(GT) ₆	368-505	11	0.81 (0.73; 0.84)
9	PSMP 2224	7	(TG) ₁₀	130-185	7	0.78 (0.77; 0.79)
10	PSMP 2227	3	(GT) ₇	195-220	3	0.14 (0.12; 0.16)
11	PSMP 2229	5,7	(GT) ₅	220-600	4	0.42 (0.39; 0.44)
12	PSMP 2232	1,2	(TG) ₈	230-380	6	0.75 (0.74; 0.74)
13	PSMP 2233	5	(TG) ₉	250-900	8	0.81 (0.73; 0.83)
14	PSMP 2237	2	(GT) ₈	205-500	14	0.89 (0.87; 0.86)
15	PSMP 2246	1	(TG) ₇	245-390	5	0.47 (0.31; 0.53)
16	PSMP 22	NA	NA	160-220	5	0.70 (0.73; 0.67)
17	PSMP 2263	7	(AG) ₃₃	200-260	7	0.82 (0.63; 0.79)
18	PSMP 2270	6	(GA) ₂₆	150-180	7	0.61 (0.55; 0.64)
19	PSMP 2271	7	(GA) ₁₁	180-240	5	0.67 (0.39; 0.70)
20	PSMP 2273	1	(GA) ₁₂	155-240	6	0.65 (0.44; 0.59)
21	PSMP 2274	5,7b	(GA) ₁₃	230-350	9	0.74 (0.66; 0.78)
22	ICMP 3016	6	(CA) ₁₇	550-600	2	0.50 (0.50; 0.50)
23	ICMP 3017	1	(CAG) ₇	178-240	11	0.84 (0.70; 0.84)
24	ICMP 3018	NA	(CATG) ₄	210-225	4	0.71 (0.68; 0.72)

S. No.	SSR locus	Linkage group (LG)	SSR motif	Amp. Range (bp)	Allele No.	PIC (B- & R- line)
25	ICMP 3019	NA	(CGTA) ₄	190-210	2	0.49 (0.44; 0.50)
26	ICMP 3020	NA	(CGTG) ₅	195-200	3	0.19 (0.38; 0.00)
27	ICMP 3029	4	(GCA) ₆ (GCA) ₅	216-250	8	0.80 (0.76; 0.79)
28	ICMP 3050	6	(TA) ₈	210-260	5	0.50 (0.12; 0.61)
29	ICMP 3056	2	(TGG) ₅	135-155	3	0.48 (0.39; 0.53)
30	ICMP 3088	1	(TCC) ₈ (TCTA) ₄	145-1000	15	0.86 (0.87; 0.74)
31	ICMP 10	NA	NA	300-1100	16	0.82 (0.78; 0.83)
32	XCUMP 005	NA	(CTG) ₉	148-195	9	0.76 (0.52; 0.84)
33	XCUMP 006	NA	(TATG) ₉	152-900	11	0.86 (0.85; 0.86)
34	XCUMP 009	NA	(CTC) ₆	152-160	5	0.63 (0.59; 0.65)
35	XCUMP 0016	NA	(CT) ₉	190-1000	14	0.85 (0.85; 0.84)
36	XCUMP 0017		(AAG) ₇	105-110	2	0.18 (0.00; 0.27)
37	XCUMP 0018	NA	(GCA) ₇	220-800	6	0.80 (0.73; 0.73)
38	XCUMP 0019	NA	(AGCG) ₅	220-300	2	0.47 (0.48; 0.47)
39	CTM 8	7	(CT) ₈ (CT) ₁₁	240-400	5	0.76 (0.76; 0.77)
40	CTM 10	3	(CT) ₂₂	160-230	12	0.88(0.82; 0.89)
41	CTM 21	2	(CT) ₂₄	180-300	8	0.82 (0.76; 0.80)
42	CTM 25	5	(CT) ₃₄	230-900	9	0.79 (0.78; 0.79)
43	CTM 26	NA	(CT) ₁₀	250-300	6	0.74 (0.69; 0.76)
44	CTM 27	1	(CT) ₇₁	320-700	8	0.80 (0.72; 0.76)
45	CTM 55	NA	(CT) ₂₅	710-800	4	0.74 (0.55; 0.69)
46	CTM 58	NA	(CT) ₂₄	280-900	12	0.88 (0.81; 0.89)
47	CTM 59	NA	(CT) ₁₁	261-265	3	0.59 (0.64; 0.53)
48	CTM 60	NA	(CT) ₂₃	120-950	3	0.62 (0.58; 0.64)
49	PGIRD 46	NA	(CTC) ₆	90-98	4	0.48 (0.34; 0.53)
50	PGIRD 49	NA	(CGG) ₆	190-400	12	0.84 (0.81; 0.83)
51	PGIRD 50	NA	(TGT) ₆	150-225	7	0.82 (0.81; 0.81)
52	PGIRD 54	NA	(GT) ₅	114-174	11	0.85 (0.85; 0.85)
53	PSMP 2001	5	(CT) ₈ (CA) ₄₈	205-380	7	0.76 (0.74; 0.73)
54	PSMP 2030	1	(CA) ₁₁ (GA) ₁₀	118-500	12	0.75 (0.73; 0.75)
55	PSMP 2043	NA	(CA) ₁₃ (GA) ₆	190-750	5	0.68 (0.69; 0.64)
56	PSMP 2076	4	(AC) ₁₅	150-170	2	0.48 (0.30; 0.49)
57	PSMP 2080	NA	(AC) ₁₄	180-240	7	0.79 (0.73; 0.79)
58	PSMP 2085	5	(AC) ₁₁	170-240	6	0.71 (0.57; 0.71)
59	PSMP 2208	5	(GT) ₁₀	250-380	4	0.43 (0.38; 0.47)
60	PSMP 2248	6	(TG) ₁₀	160-220	4	0.63 (0.64; 0.61)
61	PSMP 2249	3	(GT) ₇	130-240	6	0.76 (0.75; 0.73)
62	PSMP 2267	3	(GA) ₁₆	210-235	3	0.14 (0.12; 0.16)
63	PSMP 2275	6	(AAG)	260-400	6	0.68 (0.75; 0.60)
64	ICMP 3002	6	NA	220-350	4	0.61 (0.54; 0.65)

Figure 2 Ethidium bromide stained 6% polyacrylamide gel showing allelic polymorphism among 36 pearl millet genotypes at PSMP 2201 locus. Lane L₁=100bp ladder, L₂= 20 bp ladder, C= negative control and 1 to 36 represents HMS 48B, HMS 14B, HMS 58B, HMS 53B, HMS 55B, HMS 43B, HMS 60B, HMS 7B, HMS 44B, HMS 22B, HMS 16B, HMS 38B, HMS 37B, HMS 26B, HMS 30B, H 72-2-2, H 12/009, H 12/008, AC-04/7, H 2302, 72-2-2/98K-1, HTP 93/109, HTP 98-2, H 78/711, H 10/4-7, H 10/10-2, HTP 94/54, HTP 98/3, HTP 92/80, TCH 26-1, H 94/46, HBL 11, AC-04/13, H 12/1009, 99 HS 139 and 99 HS-23

SSR loci XCUMP 0017 and ICMP 3020 were monomorphic for B- and R-lines, respectively. The PIC value for B-lines varied from 0.12 (PSMP 2227 and PSMP 2267) to 0.93 (PSMP 2008) with an average of 0.63 whereas for R-lines it ranged from 0.16 (PSMP 2227 and PSMP 2267) to 0.94 (PSMP 2008) and an average of 0.68 (Table 7). In both kinds of lines same SSR loci are exhibiting lowest and highest PIC. Nepolean et al. (2012) have reported average PIC values for B- and R-lines as 0.46 and 0.58, respectively and Gupta et al. (2015) observed values 0.56 and 0.70 for B- and R-lines, respectively. Higher numbers of alleles were revealed by R-lines (407) than B-lines (357). Mean alleles per locus were 5.58 (B-lines) and 6.36 (R-lines). More number of alleles amplified in R-lines can be credited to the broader genetic base used in the development of these lines and the differences in sample size (21 R-lines vs. 15 B-lines). Previous studies have also reported more diversity in R-lines as compared to B-lines. Nepolean et al. (2012) observed occurrence of 284 alleles in 115 R-lines and 214 alleles in 98 B-lines using 38 SSRs. Similarly, Gupta et al. (2015) reported 329 alleles in 193 R-lines and 237 alleles in 186 B-lines using a set of highly polymorphic 28 SSRs.

The high number of alleles and PIC value obtained could be credited to polyacrylamide gel having higher resolving power than agarose gel electrophoresis and to a high number (i.e. 67% of total primers amplified) of dinucleotide SSR primers used on a small number of samples in the study. The overall size of PCR amplified products ranged from 90 bp (PGIRD 46) to 1100 bp (ICMP 10). The molecular size difference between the smallest and the largest allele at a SSR locus varied from 4 bp (CTM 59) to 880 bp (PSMP 2008). More than two alleles also called Surnumary bands were observed in this study which can be explained as duplication of some loci as also discussed in earlier studies or EST belonging to multi gene families (Devos et al., 2000; Budak et al., 2003; Mariac et al., 2006). As the genotypes used were inbred lines, still occurrence of more than one allele at loci can be explained on the

basis that pearl millet is a highly out crossing crop with higher chances of contamination from foreign pollen and this residual heterozygosity at loci demands more inbreeding along with maintenance breeding. This also signifies the importance of molecular markers in elucidating the heterozygosity which cannot be determined solely by morphological data.

A significant positive correlation was observed among number of alleles, PIC and number of repeats in the SSR motifs (Table 8). Highly significant positive correlation between number of alleles and PIC ($r = 0.69$, $p = 0.01$) implies that alleles can be indirectly used to assess PIC. Similarly, significant positive correlation values for number of repeats in SSR motifs with number of alleles ($r = 0.30$, $p = 0.05$) and PIC ($r = 0.30$, $p = 0.05$) signifies selection of repeats while taking in consideration the size of repeats for such studies. Similar associations have been reported by other studies as well (Huang et al., 2002; Ni et al., 2002; Kapila et al., 2008; Sumanth et al., 2013). However, according to Budak et al. (2003) the degree of polymorphism did not correlate with the number of repeats in the microsatellites but were stated to be correlated with the mutation rate.

Few of the studies have conducted genetic diversity evaluation across linkage groups. Genetic diversity analysis on the whole genome basis was done by using molecular markers from all 7

Table 8 Correlation between PIC, Number of alleles and Number of repeats

	PIC	Number of alleles	Number of repeats
PIC	1	0.695**	0.303*
Number of alleles	0.695**	1	0.296*
Number of repeats	0.303*	0.296*	1

*,** Significant at P= 0.05 and P= 0.01 levels respectively

Figure 3 Mean allele number and PIC of different linkage groups

linkage groups (LG). This provided difference existing in the genetic makeup throughout the genome. In the present study, mean PIC varied across the linkage groups from 0.48 (LG 3) to 0.76 (LG 2) (Figure 3). Kapila et al. (2008) observed lowest PIC value for LG 6 (0.239), concluding evolutionary more dynamic nature of this linkage group. In present study, however lowest PIC value is observed for LG 3 (0.48) and LG 6 showed a moderate value of 0.59 explained on the basis that number of markers belonging to LG 3 used were low (4 markers). LG 2 and LG 1 displaying high values of PIC and mean allele number suggests presence of a high level of genetic variation at these linkage groups.

Presence of unique alleles (i.e. exclusively present in a genotype) was also observed in this study (Table 9). Out of 461 alleles, 25 alleles were unique to a genotype. A total of 18 primers amplified these genotype-specific alleles. ICMP 3088 amplified maximum number of unique alleles (4) in 3 genotypes. Amplification of unique alleles only in B- and R-lines was by 9 and 7 primers respectively, whereas 2 primers amplified unique bands in both the lines. Out of 36 genotypes, 15 showed presence of unique alleles. Maximum unique alleles (10) were observed in HMS 30 B genotype with maximum number of primers (7) contributing these alleles to this genotype. Fifteen alleles were unique to 6 B-lines and 10 alleles were unique to 9 R-lines. A significant number of unique alleles in both B- and R-lines, suggest that these lines have been derived from diverse genetic base. Nepolean et al. (2012) observed few unique alleles (1 to 3) in 14 B- and 30 R-lines whereas Gupta et al. (2015) found genotype specific alleles in 15 B- and 37 R-lines. The unique allele occurrence can be useful in characterizing the genotype, analysis of genetic purity and distinguishing them from each other in case of any discrepancy. These SSR alleles, along with distinctness, uniformity and stability (DUS) characterization can be used as genetic tags to help protect these lines from possible infringement. STS markers can also be generated from these unique bands easily. Such markers will then serve to detect any alien introgression and be used as DNA fingerprints. Also, may be the presence of these line-specific alleles indicates their association with some distinct trait(s) of that particular line, which needs further research using more genotypes to determine their exact significance.

Table 9 List of genotypes and SSR primers exhibiting unique alleles presence

S. No.	Genotype	Primers	No. of unique bands
1	HMS 48B	ICMP 3088	1
2	HHMS 55B	PSMP 2237	1
3	HMS 43B	XCUMP 006	1
4	HMS 7B	XCUMP0018	1
5	HMS 26B	PGIRD 46	1
6	HMS 30B	PSMP 2246, PSMP 2273, ICMP 3020, ICMP 3088, XCUMP 0016, CTM 27, PGIRD 49	10
7	H 72-2-2	ICMP 3050	1
8	H 12/009	PSMP 2233	1
9	H 12/008	PSMP 2008	1
10	AC-04/7	PGIRD 46	1
11	HTP 98-2	PSMP 2270	1
12	H 78/711	PSMP 20	1
13	H 10/10-2	PSMP 2270	1
14	HTP 92/80	PSMP 2090, ICMP 3088	2
15	H 94/46	ICMP 3056	1

Genetic relatedness of the 36 genotypes calculated ranged from 0.16 (HMS 16B and H 78/711; H 78/711 and H 12/1009) to 0.49 (HMS 16B and HMS 38B) with average pair-wise similarity coefficient 0.28 (i.e. between B- and R-lines). In case of B-lines, genetic similarity estimate was highest between HMS 16B and HMS 38B (0.49) and lowest between HMS 55B and HMS 7B (0.19) with an average value for similarity coefficients 0.32. Whereas for R-lines, the highest value (0.44) was observed between 72-2-2/98k-1 and 99 HS 139 and the lowest value (0.16) obtained between H 78/711 and H 12/1009 with an average 0.27. Average similarity coefficient of B-lines was higher in comparison to R-lines. For prediction of hybrid performance, this information available on genetic distances between the parental lines can be used. New recombinants of B- and R-line can be developed by designing crosses of B-line X B-line and R-line X R-line with greater genetic distance, making use of substantial diversity detected among them.

UPGMA dendrogram formed using NTSYS-pc software explained much of similarity present among the genotypes (Figure 4). The extremes of the dendrogram were occupied by HMS 48B and HTP 93/109, with all other distributed in between. The genotypes clustered into 6 clusters at an arbitrary cut-off of 0.28 similarity coefficient on dendrogram. The 1st cluster formed is the largest,

consisting of 16 genotypes encompassing B-lines and R-lines followed by a out group HMS 37B. 2nd and 3rd clusters comprised of 6 genotypes each (B and R-lines in 2nd cluster and R-lines in 3rd cluster). 4th cluster was made by 2 genotypes (B and R-lines). 5th and 6th cluster consisted of 2 genotypes each, which were R-lines followed by one out group genotype namely, HTP 93/109. The failure of HMS 37B to fall into any cluster states that it is diverse of all B-lines and can be used for the new male sterile line development programme. Among the R-lines, HTP 93/109 was highly diversified from all and can be used as a potential pollinator for the elite CMS lines.

Clustering was also performed by WinBoot software program. Similar clusters were obtained with minor variations after 2000 iterations (data not shown). Some clusters showed high bootstrap values whereas others had low values indicating their chances to fall into another cluster and requirement for more genome coverage by using more number of SSR loci. The validation of clustering pattern on the basis of Win Boot scores for different clusters have been previously performed in various studies pertaining to genetic diversity analysis in crops like rice (Joshi et al., 2000), blackgram (Souframanien & Gopalakrishna, 2004), sorghum (Jaikishan et al., 2013) and green gram (Singh et al., 2014).

Figure 4 UPGMA dendrogram showing relationship among thirty-six pearl millet genotypes based on Jaccard similarity matrix data using 64 SSR primers

Furthermore, Jaccard similarity coefficient matrix was subjected to PCA for the three principal components. 2-D and 3-D plot were generated which clearly explain the relationship among the 36 pearl millet genotypes. The groupings in 2-D (Figure 5) and 3-D scaling (Figure 6) followed the same pattern as depicted in the dendrogram with minor differences. PCA clearly grouped the lines in distinct clusters with some lines interspersed.

Conclusion

Present study with an aim to explore the diversity in the collection of pearl millet genotypes present at CCSHAU, Hisar used molecular markers along with biochemical and morphological characters. SSR markers proved to be effective in diversity assessment and in determining remnant heterozygosity at loci. Selection of contrasting parents for construction of mapping population in order to map QTL for Fe and Zn can be made using this genetic diversity study. The results of the analysis will be helpful in selection of best combination of parents for future breeding programmes to produce new improved hybrids having desired characters like high Fe and Zn as well as good yield and broadening of genetic variability.

Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Figure 5 Two dimensional PCA (Principal Component Analysis) scaling of thirty-six pearl millet genotypes using similarity matrix data of 64 SSR primers

Figure 6 Three dimensional PCA (Principal component analysis) scaling of thirty-six pearl millet genotypes based on 64 SSR primers

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Author Contributions

SS¹ SY RK conceived and designed the experiments. SS¹ performed the experiments and wrote 1st draft. RK carried out the statistical analysis of morphological traits. SS¹ SY H NR involved in statistical analysis of molecular data. SY H SS³ reviewed the manuscript.

Informed consent: “Informed consent was obtained from all individual participants included in the study.”

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