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Molecular Characterization and Genetic Diversity Analysis of Released Hybrids and Varieties of Pearl Millet [*Pennisetum glaucum* (L.) R. Br.]

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Authors' contributions

This work was carried out in collaboration among all authors. Author SA designed and executed the study, performed the statistical analysis, wrote the protocol and wrote the whole manuscript. Authors RM, VK and RCM supported in conducting the experiment. Authors SA and RM managed the literature searches and recorded feedback analyses of the study. Author CTS edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Pearl millet is a climate-resilient crop which is most widely grown in the arid and semi-arid tropics of Asia and Africa over 26 mha. It is a highly nutritious cereal crop and rightly termed as nutricereal. This crop requires low inputs and delivers high cost-effective benefits. Development of high yielding hybrids is the major target of pearl millet researchers globally. The understanding of genetic diversity is very important and must for developing superior hybrids and crop improvement programs. In the present study, we evaluated the diversity among 30 different released hybrids and varieties of pearl millet using 125 Simple Sequence Repeat (SSR) markers. Out of these, 61 polymorphic SSRs were reported giving 191 alleles with an average of 3.13 alleles per primer. Polymorphic Information Content (PIC) varied from 0.33 to 0.76 with an average of 0.55 PIC value. The cluster analysis based on these SSR markers categorized the genotypes into four major clusters viz., I, II, III, IV with similarity coefficient ranging from 0.58 to 0.73. The results depicted that

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sufficient genetic variability exists among the different hybrids and varieties used in the study which can further prove useful for pearl millet improvement programs. The study also reveals that SSR markers are proficient and may be used efficiently for genetic diversity studies in pearl millet. It is also anticipated that findings of this study may be further used for DNA fingerprinting and varietal identification.

Keywords: Diversity estimation; microsatellites; molecular analysis; pearl millet; varietal identification.

1. INTRODUCTION

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a broadly cultivated, climate-resilient, nutritious cereal grown in arid and semi-arid regions of Africa and South Asia. It contributes mainly for around half of the total global production of millets [1]. It is the essential and important fraction of traditional farming systems and is cultivated since thousands of years. It is being consumed by humans due to its more nutritive value in comparison to other cereals. It has huge yield potential and both forage and grain are vital for the espousal of improved cultivars. Pearl millet is drought tolerant, highly photosynthetic efficient crop with high dry matter production capacity and can even sustain on less fertile soils having poor water and nutrient holding capacity. Such types of features make it a highly desirable crop among farmers under changing climatic scenario [2]. It has significant amount of genetic diversity due to its ample distribution all over the world, high adaptability towards harsh environments, cross pollination mechanism and protogynous flowering [3]. Although pearl millet has a very efficient energy production system but its genetic improvement is lesser as compared to other major cereals giving lesser yields. This lower productivity is mainly due to inadequate genetic improvement and accessibility of improved hybrids and varieties besides agronomic and socioeconomic production limitations [4]. Identification of superior genotypes and genetic diversity estimation are the major goals of any crop improvement programme. Several studies have been carried out in various crops to assess genetic diversity for augmenting the genetic base of parental lines to develop superior cultivars [5-8]. Availability, assessment, and exploitation of genetic diversity are quite useful and have been used in pearl millet as well to develop new cultivars and heterotic group. Cultivars of pearl millet have been generated from a narrow gene pool and thus there is a high need to study genetic diversity to strengthen current breeding programs in pearl millet which will put forward the potential for their use to improve pearl millet hybrids and open-pollinated varieties [9]. Subsequently, analysis of genetic relationships in pearl millet is of utmost importance as it will give information of genetic diversity, which can be further used for several breeding applications, conservation of genetic resources and ultimately to hasten its genetic enhancement for agronomical and nutritional traits.

Improved and superior climate-smart pearl millet cultivars can be developed using innovative breeding strategies to meet out the increasing demands of food for growing population [10]. Hybrid breeding has led to a progressive yield improvement, especially in India and is the most significant approach for pearl millet improvement. Morphological characterization is mainly used by researchers to select superior genotypes but different studies revealed that morphological markers are not appropriate for traits exhibiting lower heritability and greatly affected by environmental conditions. Such constraints gave rise to the evolution of molecular markers which can not only be used to distinguish various germplasm accessions, but can also characterize and estimate genetic distances among different groups of genotypes ultimately enhancing the power of conventional plant breeding and genetics methods [11]. Thus, molecular markers put forward significant advantages over morphological markers for assessment of genetic diversity. Since past several years, various molecular markers were used to analyze genetic diversity among different cultivars and land races of pearl millet [12-17].

Restriction Fragment Length Polymorphism (RFLP) markers were first time used in pearl millet to create the first genetic linkage map. After this, several molecular makers including RFLPs, Sequence Tagged Sites (STSs), Amplified Fragment Length Polymorphism (AFLPs), genomic SSRs, Single-Strand Conformation Polymorphism (SSCPS) and genic SSRs were developed and applied in pearl millet improvement studies [14]. Later, high throughput platforms like Diversity Arrays Technology (DArT) [14], Genotyping-by-sequencing (GBS) [17], Single Nucleotide Polymorphism (SNPs) [18] were developed and used for profiling

genome-wide nucleotide variations in pearl millet. Although nowadays, Next Generation Sequencing (NGS)-based single-nucleotide polymorphisms have become the marker of choice but still SSRs seem to be largely useful for estimating diversity, defining heterotic groups and DNA fingerprinting. Among the different DNA markers used earlier for estimation of genetic diversity in cultivars and land races of pearl millet, SSRs are the markers of choice and more consistent owing to several features like multi-allelism, codominant inheritance, genome specificity, cost effectiveness, even distribution throughout the genome, high polymorphism, technically simple method, automation and easy detection, requirement of common lab equipments etc. [8,19]. Further, they can be swapped amid laboratories and are vastly transferable among populations and hence have been extensively used for analyzing genetic diversity and germplasm characterization, identification of genotypes, DNA fingerprinting, estimation of genetic distances among populations and defining heterotic groups for inbred lines [20]. Thus, keeping all this in view, the current study was aimed to investigate the nature and extent of genetic variance among released hybrids/varieties of pearl millet using SSR markers and take initiative for genomic studies in pearl millet under (ICAR) Indian Council of Agricultural Research - All India Coordinated Research Program (AICRP) on Pearl millet because database on various morphological parameters for these hybrids/varieties is already available but till now no molecular database is existing so that it can be further used in pearl millet improvement programs.

2. MATERIALS AND METHODS

2.1 Plant Material

Plant material comprised of a total of 30 genotypes including popular released hybrids and varieties of pearl millet developed under Indian Council of Agricultural Research-All India Coordinated Research Program on Pearl Millet, Jodhpur, India. The molecular marker analysis was performed at PC Unit, ICAR-AICRP on Pearl millet, Jodhpur during 2019-20.

2.2 Genomic DNA Isolation and Quantification

DNA extraction was done from fresh and young leaves of 12 days old plants of 30 genotypes following CTAB method along with some modifications without using liquid nitrogen as described in other study [21] and quantified on 0.8 % agarose gel using the standard λ DNA.

2.3 Molecular Characterization

A total of 125 SSR primers were used for PCR amplification and study of molecular diversity among 30 pearl millet genotypes. The sequence of these PSMP series genomic SSR markers were obtained from the previous studies [22-24]. For PCR reaction, DNA was diluted accordingly to make available final concentration of 10 ng/µl and amplification reactions were performed in a volume of 10 µl containing 10 mM Tris HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, , 200 mM each dNTP, 0.4 µM 10-mer primer mix, 1 unit Taq NA polymerase (Bangalore GeNei, India) and 10 ng of DNA. Amplifications were carried out in a 96-well thermal cycler (Agilent Technologies). PCR programme constituted of 1 cycle of 5 min at 94°C for initial denaturation followed by 35 cycles of 30s at 94°C for denaturation, 30 s of 55°C for annealing and 1 min at 72°C for primer extension. Finally, a step of final extension was carried out for 10 mins at 72°C followed by hold at 4°C. The PCR products were analyzed on 2.5% agarose gel.

2.4 Diversity Analysis and Dendrogram Construction

Each band represented a genetic locus and the variations in the bands were scored manually as presence (1) and absence (0) of bands using gel photographs. Only the clear, unambiguous amplicons were scored and their sizes were estimated using 50 bp DNA ladder (HiMedia) as standard. Based on Jaccard's similarity coefficients, cluster analysis was performed among the genotypes using UPGMA and SAHN-clustering algorithms of NTSYS-PC (Numerical Taxonomy System, Version 2.02e NTSYS-pc, version 2.02e (Applied Biostatistics) software. Polymorphism Information Content (PIC) was calculated as, Polymorphism Information Content (PIC) =Σ $(1-P^2i)/n$, where n is the number of band positions analyzed in the set of accessions and P^2i is the frequency of i^{th} allele.

3. RESULTS AND DISCUSSION

3.1 Molecular Characterization and SSR Analysis

In the current study, a total of 30 popular pearl millet hybrids and varieties were used for molecular characterization using 125 SSRs. Of

these 125 primers used here, 93 SSRs amplified products of varying sizes ranging from 100 to 780 bp and 32 (25.6 %) were monomorphic (Table 2) and 61 (48.8%) were polymorphic (Tables 1 and 2). Figs. 1 and 2 show the amplification profile of 30 genotypes using primer pairs PSMP 2070 and PSMP 2218. Thus, a good amount of polymorphic markers were obtained which could be helpful for genotype identification, germplasm management and genetic diversity assessment and further introgress the genes underlying them to desirable genetic backgrounds. A total of 191 alleles were obtained in this study and the number of alleles per locus varied between 2 to 6 (Table 1) with an average of 3.13 alleles. These values are similar to 3.4 alleles per locus and 3.1 alleles per locus as observed by other researchers [25,26], respectively. But, they were relatively lower than 2-18 alleles (6.8 alleles per locus), 4.62 alleles per primer and 12.5 alleles per locus as observed by different investigators in other studies [12,27,28], respectively. Such observations could be attributed to diverse world collection of germplasm. Similar results regarding effectiveness of SSR markers in monitoring genetic diversity have also been reported by other investigators [12,15,27,29-31]. Several reports have been observed on estimation of genetic diversity among parental lines of pearl millet on the basis of molecular profiling [14,15,16,11,17,27]. But, this study is the first report on molecular characterization and genetic diversity analysis of hybrids and varieties which will provide molecular database for the existing hybrids and varieties and will be helpful for developing genomic studies and DNA fingerprinting for pearl millet hybrids and varieties. Molecular characterization has various purposes like management of genetic resources, identification and characterization of new genotypes, revealing genetic relationships among breeds/varieties, utilizing association of traits and markers and analysis of population structure [19]. PCR-based markers are largely preferred for genotypic characterization because of their simplicity in use, nondistractive nature and requirement of small quantity of DNA. SSRs provide unique allelic profiles or DNA fingerprints thus can effectively and precisely establish genotypic identity. They also have better distinguishing power than RFLP markers and can reveal genetic relations and pedigree of the inbred lines more effectively. Thus, SSR markers are the most preferred and proficient markers owing to their capability to detect multiallelic loci, simple to use, co-dominance, higher reproducibility, high polymorphism with enormous ability to differentiate the genotypes [27]. Previously, SSRs have been used to assess crop germplasm and genetic diversity in several species such as rice, olive, rye, sugarcane, grape, Brassica etc. [19]. SSR markers possess various advantages in comparison to SNPs in diversity analysis and over and again SSR data can be more useful in defining pedigrees than SNP data. PIC values can range between 0 and 0.5 because of bi-allelic nature of SNPs while it can go above 0.5 in case of SSRs due to their mutli-allelic nature. Although in the present genomic era, SNP markers are gaining popularity and are considered as markers of choice but SSR markers will persist to be useful and favourable because of several advantages [19,32]. In several studies, in-depth genotyping revealed by SNPs is not much essential and in such cases SSRs are an appropriate choice as they can be used for larger expansion of sample size without increasing much cost. On the other hand, existing SSR data can be easily used and integrated along with new studies. Thus, SSR markers are the most excellent and of preference for small scale laboratories having limited facilities and budget in comparison to SNPs [33].

The polymorphic information content (PIC) value measures polymorphism for a marker locus as it calculates informativeness of markers and measures the diversity of alleles. It takes into account the number of expressed alleles as well as their relative frequencies to assess the discriminatory power of a locus. PIC index evaluates the intensity of gene variation and a PIC value of ≥0.5 is an indication of higher diversity, while PIC ≤ 0.25 depicts lower diversity and PIC value between 0.25 and 0.5 is indicator of intermediate diversity [34]. Here, in this study, PIC values ranged from 0.33 (PSMP 2059) to 0.76 (PSMP 2203) (Table 1) with an average of 0.55. In some of the previous reports, PIC values varying between 0.02 to 0.97 were reported [31,27,35-37]. An average PIC value of 0.55 observed in the present study is similar to 0.56 and 0.58 reported by other investigators [27,29], respectively. However, it was lesser than average PIC value of 0.671 recorded in a study using Indian rice germplasm [36] while higher than 0.37 PIC value reported when investigating the reliability of the RAPD technique for sesame (*Sesamum indicum* L.) germplasm [38] and 0.41 and 0.43 as observed in other studies on rice germplasm [35,39], respectively. Out of 61 markers, 30 markers (49.2%) had PIC value > 0.5 indicating that these were highly informative

and the most useful markers for differentiating these hybrids and varieties. Markers having PIC values of 0.5 or above are believed to be extremely valuable in discriminating the genotypes and useful for molecular genetic diversity studies [40]. A high PIC value between 0.65 and 0.75 were recorded in 27.9 % (17 SSR) markers (Table 1). Marker PSMP2203 gave the highest PIC value (0.76) followed by PSMP2072 (0.75), PSMP3032 (0.74) and PSMP2001, PSMP3017, PSMP2066 (0.73) revealing that PSMP2203 is the most informative and best marker for identification and diversity estimation of these pearl millet genotypes followed by PSMP2072, PSMP3032, PSMP2001, PSMP3017 and PSMP2066 markers while the lowest PIC value 0.33 was for maker PSMP 2059 indicating it as the least powerful marker. High PIC values may be observed due to the use of large number of informative markers [40]. Similar reports were observed in several other studies in different crops like pearl millet [12,27,28,29], rice [30,36] and brassica [31].

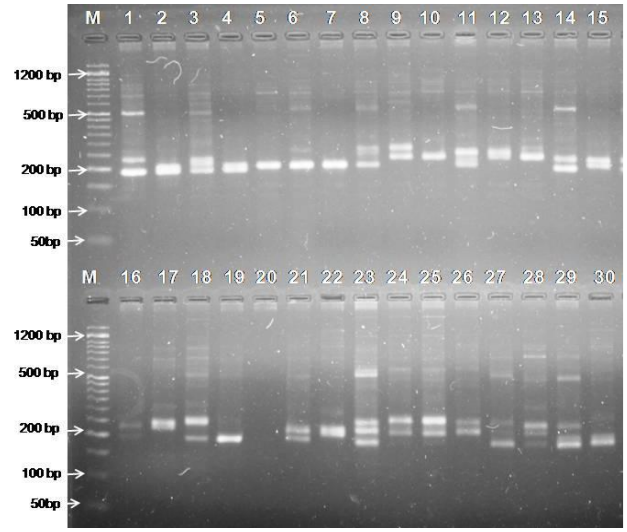

Fig. 1. Agarose gel showing amplification profiles of pearl millet hybrid/varieties using the primer PSMP 2070. Lane M-50 bp ladder, Lane 1-30 pearl millet hybrids/varieties

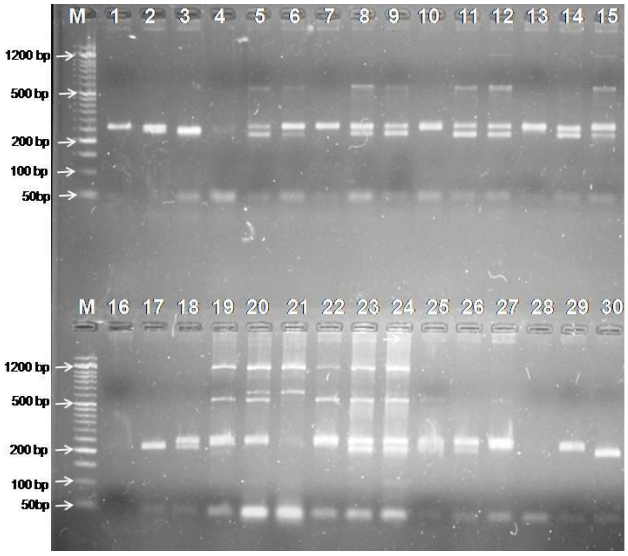

Fig. 2. Agarose gel showing amplification profiles of pearl millet hybrid/varieties using the primer PSMP 2218. Lane M-50 bp ladder, Lane 1-30 pearl millet hybrids/varieties

Table 1. Polymorphic SSR primers used for genetic diversity analysis among *Pennisetum glaucum* genotypes

S. No.	Name of Primer	Forward Primer Sequence (5' – 3')	Reverse Primer Sequence (5' – 3')	Product range (bp)	No. of alleles amplified	PIC
1.	PSMP 2203	GAACTTGATGAGTGCCACTAGC	TTGTGTAGGGAGCAACCTTGAT	170-460	5	0.76
2.	PSMP 3032	AGGTAGCCGAGGAAGGTGAG	CAACAGCATCAAGCAGGAGA	180-400	4	0.74
3.	PSMP 2027	AGCAATCCGATAACAAGGAC	AGCTTTGGAAAAGGTGATCC	250-280	2	0.50
4.	PSMP 2275	CCAGTGCCTGCATTCTTGGC	GCATCGAATACTTCATCTCA	270-300	2	0.47
5.	PSMP 3017	CACCAAACAGCATCAAGCAG	AGGTAGCCGAGGAAGGTGAG	200-550	4	0.73
6.	PSMP 3080	CAAACAGCATCAAGCAGGAG	GCGTAGACGGCGTAGATGAT	220-500	3	0.63
7.	PSMP 2072	GAAATCTACACAAGGGTCTCCA	GTACGGAGCAATGACATCTGAA	150-155	2	0.38
8.	PSMP 2225	CCGTACTGATGATACTGATGGTT	TGGGAGGTAAGCTCAGTAGTGT	220-570	4	0.69
9.	PSMP 2070	ACAGAAAAAGAGAGGCACAGGAGA	GCCACTCGATGGAAATGTGAAA	200-700	5	0.72
10.	PSMP 2076	GGAATAGTATATTGGCAAAATGTG	ATACTACACACTGTAAGCATTGTC	150-160	2	0.47
11.	PSMP 2084	AATCTAGTGATCTAGTGTGCTTCC	GGTTAGTTTGTTTGAGGCAAATGC	180-500	5	0.65
12.	PSMP 2078	CATGCCCATGACAGTATCTTAAT	ACTGTTCGGTTCCAAAATACTT	140-220	3	0.62
13.	PSMP 2063	GAGCACATGAAATAGGAAGCAG	AAGGTAGTTATAGTTAGCTTGATC	150-450	4	0.69
14.	PSMP 2204	TGCCTTCTTGACTATGTTTTCC	AGATATGGCGAACGTGAGGAG	150-220	2	0.52
15.	PSMP 2205	AGGTGCTCACGAGCTGTAAGAG	AGCAAGACACTATTTTACCATC	180-250	2	0.46
16.	PSMP 2210	CAATGATGACCGTAATCTGGGTG	GGGCAAGATATGTGAAATCAAG	190-450	5	0.60
17.	PSMP 2211	CTGCATGACGTGTGACCAATACC	AACAAATCAGCACCAGCCTCC	250-780	4	0.70
18.	PSMP 2215	CCACGTCATTAGAGTAATCCGAG	ACTCAAATCCCAATCTTGAATC	130-210	3	0.42
19.	PSMP 2216	GTATGTAGGTGATGGTTAGTTCG	AGTAGAAGGATGGAGTCATACAG	140-160	2	0.36
20.	PSMP 2218	CTCTGTAAGTTCCTGGTGCTCAA	TCAGGCCAGTAACACATCTCAA	250-550	4	0.62
21.	PSMP 2001	CATGAAGCCAATTAGGTCTC	ACCATCTGACTTGTTCTTATCC	200-400	6	0.73
22.	PSMP 2074	CACCTAGACTCTACACAATGCAAC	AATATCAAGTGATCCACCTCCCAA	250-260	2	0.50
23.	PSMP 2089	TTCGCCGCGGCTACATACTT	TGTGCATGTTGCTGGTCATT	200-700	6	0.71
24.	PSMP 2008	GATCATGTTGTCATGAATCACC	ACACTACACCTACATACGCTCC	190-700	5	0.65
25.	PSMP 2018	CGCAAGACATTTTAGTATCACC	ACAGTCATCCTCAGTCGTCC	150-200	2	0.43
26.	PSMP 2019	TGTGCCACAGCTTGTTCCTC	CAAGCAGCCAGTTCCTCATC	200-250	2	0.50
27.	PSMP 2030	ACCAGAGCTTGGAAATCAGCAC	CATAATGCTTCAAATCTGCCACAC	125-450	3	0.62
28.	PSMP 2043	TCATATTCTCCTGTCTAAAACGTC	ACAAATCGTACAAGTTCCACTC	190-620	4	0.72
29.	PSMP 2056	ACCTGTAGCTTCAAAATTCAAAAA	AATTCAGTGTGATTTCGATGTTGC	120-250	3	0.42
30.	PSMP 2059	GGGGAGATGAGAAAACACAATCAC	TCGAGAGAGGAACCTGATCCTAA	125-300	3	0.63
31.	PSMP 2060	AGTTATAATGTATGTGCGACACG	TACCACAATTTCAATATACATGGC	160-500	4	0.69
32.	PSMP 2064	ACCGAATTAAAGTCATGGATCG	TTGATTCTTCTGACACAAATGAG	90-150	4	0.68
33.	PSMP 2066	ATATTAGAGCATTGCATCGC	GCATAGCAGCATACAGCAGCAA	190-580	5	0.73
34.	PSMP 2072	GAAATCTACACAAGGGTCTCCA	GTACGGAGCAATGACATCTGAA	150-600	6	0.75
35.	PSMP 2077	GCCAATATTATTCCCAAGTGAACA	CTCTTGGTTGCATATCTTTCTTTT	150-160	2	0.48
36.	PSMP 2080	CAGAATCCCCACATCTGCAT	TGCAACTGAGCGAAGATCAA	190-210	2	0.49

S. No.	Name of Primer	Forward Primer Sequence (5' – 3')	Reverse Primer Sequence (5' – 3')	Product range (bp)	No. of alleles amplified	PIC
37.	PSMP 2088	AAGAAGCCACCAGCACAAAA	TGCATGAAAGTAGAGGATGGTAAA	150-350	3	0.52
38.	PSMP 2085	GCACATCATCTCTATAGTATGCAG	GCATCCGTCATCAGGAAATAA	130-180	2	0.49
39.	PSMP 2086	CGCTTGTTTTCCTTTCTTGCTGTT	CCTTCTCAGATCCTGTGCTTTCTT	100-140	2	0.48
40.	PSMP 2087	GGAACAGACTCCATACCTGAAA	TACCTGCCTGTGCTGTTAGT	130-140	2	0.50
41.	PGIR D5	CAACCCAACCCATTATACTTATCTG	GCAACTCTTGCCTTTCTTGG	150-155	2	0.50
42.	PGIR D7	CGGAGACGCACTAGACTTGG	CCGGATGCTCACTTCCTTAT	100-160	2	0.43
43.	PGIR D12	ACTCGTTCGGATGCACTTCT	CGGGGAAGAGACAGGCTACT	140-200	2	0.50
44.	PGIRD13	CAGCAGCGAGAAGTTTAGCA	GCGTAGACGGCGTAGATGAT	200-270	3	0.67
45.	PGIR D19	TGAGGACCGAGAAGAAGCAT	CAACACCCAACAGAAACTGAA	225-500	3	0.48
46.	PGIR D21	GCTATTGCCACTGCTTCACA	CCACCATGCAACAGCAATAA	130-160	2	0.51
47.	PGIR D25	CGGAGCTCCTATCATTCCAA	GCAAGCCACAAGCCTATCTC	160-165	2	0.50
48.	PSMP 2066	ATATTAGAGCATTGCATCGC	GCATAGCAGCATACAGCAGCAA	140-145	2	0.42
49.	PSMP 2213	CCCAAAAGAACCACACCCAC	GTTGATGCTACTGCTCGTTTG	130-270	3	0.47
50.	PSMP 2220	GCATCCTTCACCATTCAAGACA	TGGGAAACAGAATGGAGAAAAGAG	150-350	4	0.61
51.	PSMP 2222	TGGCTTCCAGACTAATCATCAC	TTATTTTAGCGGCGAGATTGAC	200-350	2	0.44
52.	PSMP 2223	CATGCTTCTTCTTCTTTTGTAACC	CAGCTCTTTGATCTCACTACAC	190-250	2	0.49
53.	PSMP 2048	TGAATTGGGAATAAAGGAGACC	ACGTGTGCCTGCTTTTAGTAAC	200-450	5	0.68
54.	PSMP 2063	GAGCACATGAAATAGGAAGCAG	AAGGTAGTTATAGTTAGCTTGATC	150-350	3	0.39
55.	PSMP 2078	CATGCCCATGACAGTATCTTAAT	ACTGTTCGGTTCCAAAATACTT	160-350	2	0.34
56.	PSMP 2079	AGCCGAAGGCTAATCAACAA	GTGGTCAGCAGCAGATGTAA	250-450	3	0.59
57.	PSMP 2081	CTGTGCTGTCATTGTTACCA	TCAGATCACCTATTACTTTCCCT	170-300	2	0.46
58.	PSMP 2059	GGGGAGATGAGAAAACACAATCAC	TCGAGAGAGGAACCTGATCCTAA	100-350	3	0.33
59.	PSMP 2074	AGGACTGTAGGAGTGTGGACAA	CCAGACCTACCAGTGAATGAGA	140-500	4	0.47
60.	PSMP 2212	GATTGGATGGCAGTGCTTG	CAAACCAGCCATCAACAACCAG	250-460	3	0.54
61.	PSMP 2221	TTGCCGTCAGCAATGTGCCT	CCGAAGTGCCCAGTGCCCAA	220-400	2	0.36

Table 2. Summary of SSR primers used to amplify the genomic DNA of 30 *Pennisetum glaucum* genotypes

Markers	No. of markers
Number of markers used	125
Number of amplified markers	93
Number of non- amplified markers	32
Number of polymorphic markers	61
Number of monomorphic markers	32
Size of amplified products (bp)	100-780
Percent polymorphism	48.8%
Total number of alleles	191
Average no. of alleles per primer	3.13

3.2 Diversity Analysis and Dendrogram Construction

Pearl millet has a remarkable amount of diversity at both genotypic and phenotypic levels. Genetic variation has much importance and its evaluation and relationship in breeding material can have significant impact in the crop improvement programs as characterization and knowledge of genetic diversity is highly helpful for development of commercial hybrids [2]. The genetic relationships among the genotypes used in the current study were invariably according to the available pedigree data. The cluster analysis based on SSRs categorized the genotypes into four main clusters viz., I, II, III, IV with similarity coefficient ranging from 0.58 to 0.73 (Fig. 3) which are similar to those reported in other pearl millet collections [27,41,42] and sorghum [25]. Cluster I contained fifteen genotypes and grouped together at similarity index of 0.58. In this cluster, early maturing pearl millet hybrids/varieties viz. RHB 177, Dhanshakti, HHB 67 Imp, PB 1756, HHB 272, RHB 223, MPMH 21 clustered together while HHB 299, MPMH 17, AHB 1269, XMT 1497, GHB 558, DHBH 1397 which are medium maturing grouped together. This cluster can be further subdivided into three sub-clusters- Ia, Ib, Ic. Sub-cluster Ia included three hybrids HHB 299, MPMH 17 and RHB 177 which are specific for A_1 zone grouped together indicating that they can be used for developing drought tolerant pearl millet hybrids for drier parts of Rajasthan. Here, hybrids HHB 299 and MPMH 17 showed closer relationship with each other at minimal genetic distances of 0.68. Sub-cluster Ib contained AHB 1269, HHB 272, RHB 223, RHB 233, HHB 67 Imp which are specific for A_1 zone while XMT 1497 and Dhanshakti for A zone. Sub-cluster Ic contained hybrids MPMH 21, GHB 558, DHBH 1397 and landrace Chanana Bajra which are suitable for A_1 zone. Thus, different genotypes grouped according to their characteristics and salient features as described in other report [43]. Further, cluster I contains high Fe/Zn hybrids/varieties like Dhanshakti, RHB 233, HHB 299 and AHB 1269 and here, HHB 67 Imp and PB 1756; AHB 1269 and XMT 1497; MPMH 21 and GHB 558 showed closer relationship with each other at minimal genetic distances of 0.72, 0.73, and 0.68 respectively (Fig. 3 & Table 3). Cluster II was obtained at a similarity index of 0.60 containing six genotypes namely Gadhwal ki Dhani-3, Chadi Bajri, Sulkhaniya Bajra, HHB 197, NBH 5061 and HHB 146. In this cluster, all landraces are grouped together which are having low to medium rancidity and suitable for drier parts of Rajasthan while HHB 197 and HHB 146 are suitable for A zone and NBH 5061 for B zone. Gadhwal ki Dhani-3 and Chadi Bajri; Sulkhaniya Bajra and HHB 146 clustered close to each other at minimal genetic distances of 0.70 (Table 3). Cluster III includes six genotypes namely BHB 1202, NBH 5767, PB 1720, PB 180, Nandi 52, HHB 223 at a similarity index of 0.59 and BHB 1202 & HHB 223 are suitable for A_1 zone while NBH 5767 is for B zone and PB 180 is a summer hybrid. Further, BHB 1202 and NBH 5767; PB 180 and Nandi 52 showed closer relationship with each other at minimal genetic distances of 0.66 and 0.69 respectively in this cluster (Table 3). The three genotypes viz. AHB 1200, RHB 234 and HHB 311 are included in cluster IV at a similarity index of 0.67. Here, all three hybrids of medium maturity group and biofortified are grouped together but are entirely separated from other hybrids of medium maturity group. Here, AHB 1200 and RHB 234 clustered together with lowest genetic distance (0.64) while AHB 1200 and HHB 311 showed similarity at 0.69. It has been proved that SSRs can be suitable and efficient tool for molecular characterization of many plant species including pearl millet. Similarly, clustering between genotypes of pearl millet based on molecular markers was also recorded in other studies [11,17,44-46].

Table 3. Summary of Nei's (1972) genetic distance among 30 different genotypes of pearl millet

	HHB 299	AHB 1200	Dhans-hakti	RHB 233	RHB 234	HHB 311	AHB 1269	HHB 272	MPMH 21	BHB 1202	RHB 223	RHB 177	HHB 67 Imp.	PB 1756	XMT 1497	NBH 5767	MPMH 17	HHB 197	PB 1720	DHBH 1397	NBH 5061	Gadhwal ki Dhani-3	Chanana Bajra-3	Sulkhaniya bajra	Cha-di bajri	GHB 558	HHB 146	PB 180	Nandi 52	HHB 223
HHB 299	1																													
AHB 1200	0.61	1																												
Dhanshakti	0.63	0.59	1																											
RHB 233	0.60	0.59	0.67	1																										
RHB 234	0.55	0.64	0.58	0.57	1																									
HHB 311	0.55	0.69	0.58	0.56	0.69	1																								
AHB 1269	0.62	0.75	0.65	0.61	0.68	0.68	1																							
HHB 272	0.69	0.61	0.67	0.59	0.59	0.59	0.69	1																						
MPMH 21	0.56	0.57	0.63	0.67	0.66	0.57	0.58	0.65	1																					
BHB 1202	0.65	0.55	0.57	0.57	0.57	0.50	0.64	0.69	0.63	1																				
RHB 223	0.63	0.59	0.64	0.62	0.52	0.59	0.63	0.69	0.59	0.63	1																			
RHB 177	0.63	0.61	0.57	0.65	0.55	0.54	0.58	0.56	0.64	0.65	0.67	1																		
HHB 67 Imp.	0.67	0.63	0.66	0.69	0.63	0.59	0.68	0.66	0.68	0.68	0.71	0.65	1																	
PB 1756	0.65	0.58	0.68	0.69	0.63	0.61	0.62	0.65	0.66	0.64	0.65	0.59	0.72	1																
XMT 1497	0.71	0.62	0.67	0.64	0.58	0.63	0.73	0.70	0.61	0.66	0.68	0.65	0.68	0.66	1															
NBH 5767	0.54	0.58	0.52	0.56	0.52	0.53	0.60	0.60	0.57	0.66	0.60	0.60	0.58	0.59	0.59	1														
MPMH 17	0.68	0.63	0.59	0.61	0.58	0.57	0.61	0.56	0.62	0.67	0.57	0.65	0.65	0.65	0.64	0.63	1													
HHB197	0.58	0.65	0.64	0.66	0.55	0.58	0.63	0.64	0.62	0.59	0.63	0.64	0.66	0.63	0.60	0.53	0.59	1												
PB 1720	0.58	0.61	0.59	0.61	0.54	0.61	0.59	0.63	0.59	0.59	0.61	0.59	0.60	0.61	0.61	0.59	0.62	0.64	1											
DHBH 1397	0.62	0.63	0.63	0.58	0.63	0.60	0.64	0.60	0.55	0.54	0.57	0.54	0.64	0.63	0.63	0.53	0.59	0.56	0.59	1										
NBH 5061	0.67	0.62	0.61	0.63	0.58	0.60	0.65	0.62	0.57	0.61	0.60	0.63	0.69	0.63	0.66	0.54	0.60	0.63	0.59	0.56	1									
Gadhwal ki Dhani-3	0.60	0.59	0.63	0.65	0.54	0.57	0.60	0.64	0.65	0.58	0.53	0.58	0.63	0.59	0.61	0.57	0.59	0.68	0.64	0.61	0.59	1								
Chanana Bajra-3	0.63	0.62	0.66	0.63	0.62	0.55	0.61	0.61	0.63	0.58	0.56	0.59	0.66	0.60	0.64	0.54	0.64	0.65	0.67	0.57	0.59	0.65	1							
Sulkhaniya bajra	0.58	0.57	0.61	0.55	0.53	0.55	0.59	0.56	0.58	0.57	0.61	0.63	0.63	0.57	0.63	0.55	0.61	0.66	0.58	0.58	0.66	0.60	0.61	1						
Chadi bajri	0.55	0.58	0.58	0.56	0.56	0.54	0.64	0.59	0.57	0.55	0.54	0.54	0.61	0.60	0.56	0.55	0.55	0.67	0.54	0.53	0.61	0.70	0.63	0.64	1					
GHB 558	0.65	0.63	0.61	0.59	0.57	0.55	0.63	0.64	0.68	0.67	0.60	0.60	0.68	0.63	0.63	0.61	0.62	0.59	0.59	0.61	0.57	0.59	0.66	0.57	0.62	1				
HHB 146	0.58	0.57	0.68	0.59	0.55	0.58	0.66	0.62	0.57	0.60	0.59	0.56	0.61	0.62	0.68	0.60	0.60	0.60	0.60	0.62	0.62	0.61	0.56	0.70	0.58	0.63	1			
PB 180	0.58	0.55	0.53	0.57	0.48	0.54	0.56	0.59	0.54	0.60	0.58	0.52	0.60	0.59	0.58	0.54	0.57	0.50	0.60	0.55	0.55	0.55	0.56	0.53	0.53	0.67	0.58	1		
Nandi 52	0.60	0.60	0.60	0.60	0.55	0.53	0.59	0.59	0.59	0.60	0.63	0.60	0.60	0.66	0.61	0.61	0.61	0.59	0.63	0.57	0.58	0.59	0.59	0.57	0.57	0.65	0.62	0.69	1	
HHB 223	0.59	0.53	0.57	0.59	0.48	0.52	0.61	0.58	0.55	0.66	0.65	0.61	0.59	0.62	0.64	0.61	0.60	0.58	0.61	0.51	0.58	0.54	0.53	0.62	0.56	0.65	0.65	0.67	0.67	1

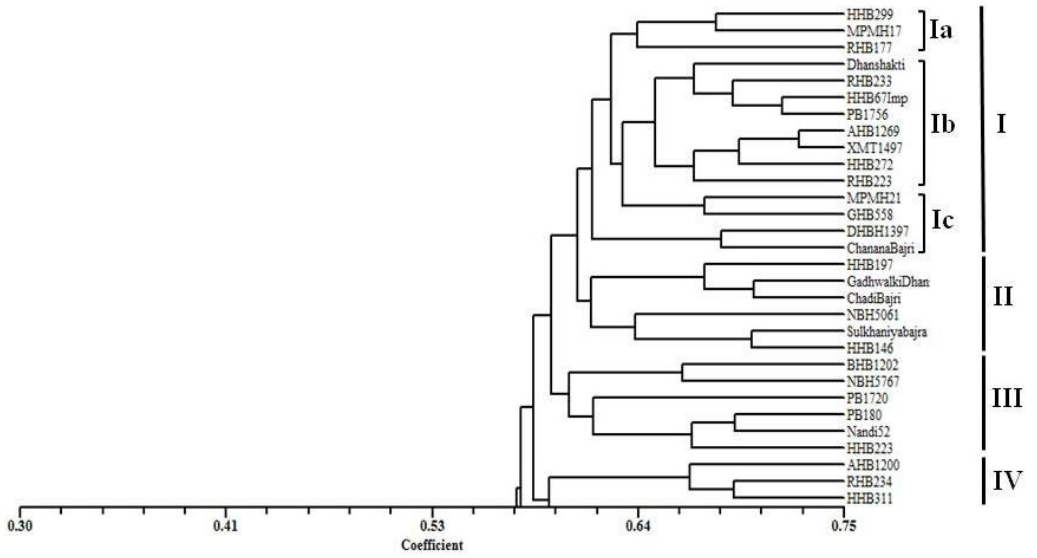

Fig. 3. UPGMA dendrogram showing genetic relationship among pearl millet hybrids/varieties based on Jaccard's similarity coefficients using SSR markers

4. CONCLUSION

In the present study, the pearl millet hybrids/varieties have been successfully characterized and categorized into diverse groups which will be useful to assess the evolutionary relationships with the wild relatives. The results indicated that good genetic variability exists among the different hybrids and varieties and can be further used in pearl millet improvement programs. Here, we could get a good amount of polymorphic SSR markers with high PIC values revealing that SSRs can be efficiently used for genetic diversity studies in pearl millet. These results will be useful in removing the gaps in lineage or selection history, detecting differences in allelic frequencies within genotypes or populations. It will be also fruitful to explore new alleles at various loci of interest and DNA fingerprinting and varietal identification.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Taylor JR, NBTRM. In Millet FS: Pearl. In reference module in food science. Elsevier; 2016.
2. Yadav OP, Rai KN. Genetic improvement of pearl millet in India. Agri. Res. 2013; 2(4):275-292.
3. Singh AK, Rana MK, Singh S, Kumar S, Durgesh K, Arya L. Assessment of genetic diversity among pearl millet [*Pennisetum glaucum* (L.) R Br.] cultivars using SSR markers. Range Manag Agrofor. 2013; 34(1):77-81.
4. Waddington S, Li X, Dixon J, Hyman G, de Vicente MC. Getting the focus right: Production constraints for six major food crops in Asian and African farming systems. Food Secur. 2010;2(1): 27-48.
5. Brar DS, Khush GS. Rice breeding in the genomics era: Perspectives. Agric. Res. J. 2017;54(4):612-619.
6. Kumar S, Parekh MJ, Patel CB, Zala HN, Sharma R, Kulkarni KS, et al. Development and validation of EST-derived SSR markers and diversity analysis in cluster bean (*Cyamopsis tetragonoloba*), J. Plant Biochem. Biotechnol. 2016;25(3):263.
7. Singh BK, Choudhary SB, Yadav S, Malhotra EV, Rani R, Supriya A, et al.

Genetic structure identification and assessment of interrelationships between Brassica and allied genera using newly developed genic-SSRs of Indian mustard (*Brassica juncea* L.). Industrial Crops and Products. 2018;113: 111–120.

8. Bharti R, Kumar S, Parekh MJ. Development of genomic simple sequence repeat (gSSR) markers in cumin and their application in diversity analyses and cross-transferability. Ind. Crops Prod. 2018;111: 158-64.
9. Singh S, Gupta SK. Formation of heterotic pools and understanding relationship between molecular divergence and heterosis in pearl millet [*Pennisetum glaucum* (L.) R. Br.], PLoS One. 2019; 14(5):0207463.
10. Serba DD, Yadav RS. Genomic tools in pearl millet breeding for drought tolerance: Status and prospects. Front Plant Sci. 2016;7:1724.
11. Mohammed HI, Hamza NB. Genetic diversity analysis of forty pearl millet (*Pennisetum glaucum* (L.) R. Br) Accessions from Sudan Using Agronomical Descriptors and DNA Molecular Markers Advances in Bioscience and Biotechnology. 2018;9:322-37.
12. Mariac C, Luong IV, Kapran A, Mamadou F, Sagnard M, Deu J, et al. Diversity of wild and cultivated pearl millet accessions (*Pennisetum glaucum* [L.] R. Br.) in Niger assessed by microsatellite markers, Theor. Appl. Genet. 2006;114:49–58.
13. Govindaraj M, Selvi B, Arun Prabhu D, Rajarathinam S. Genetic diversity analysis of pearl millet (*Pennisetum glauccum* [L.] R. Br.) accessions using molecular markers. African Journal of Biotechnology. 2009; 8(22):6046-6052.
14. Supriya A, Senthilvel S, Nepolean T, Eshwar K, Rajaram V, Shaw R, et al. Development of a molecular linkage map of pearl millet integrating DArT and SSR markers. Theor Appl Genet. 2011;123(2): 239-50.
DOI: 10.1007/s00122-011-1580
15. Radhika Ramya A, Ahamed ML, Srivastava RK. Genetic diversity analysis among inbred lines of pearl millet [*Pennisetum glaucum* (L.) R. Br.] based on grain yield and yield component characters. Int. J. Curr. Microbiol. App. Sci. 2017;6(6):2240-2250.
DOI:https://doi.org/10.20546/ijcmas.2017.606.266
16. Waghmode BT, Sharma KM, Pawar GS, Salunke VD, Kal SP. Genetic diversity and hybrid purity analysis of pearl millet using molecular markers Int. J. Curr. Microbiol. App. Sci. 2018;6:772-79.
17. Kanfany G, D-Serba D, Rhodes D, St. Amand P, Bernardo A, I-Gangashetty P, et al. Genomic diversity in pearl millet inbred lines derived from landraces and improved varieties BMC Genomics. 2020; 21:469.
18. Nadeem MA, Nawaz MA, Shahid MQ, Doğan Y, Comertpay G, Yıldız M, et al. DNA molecular markers in plant breeding: Current status and recent advancements in genomic selection and genome editing. Biotechnol. Biotechnol. Equip. 2018;32(2): 261–85.
19. Abdurakhmonov IY. Introduction to Microsatellites: Basics, trends and highlights: in: Abdurakhmonov IY. (Ed.), microsatellite markers. In Tech Publisher, London, EC3R 6AF, UK. 2016;1-16.
Available:http://dx.doi.org/10.5772/62560
20. Amelework B, Abakemal D, Shimelis H, Laing M. Application of microsatellites in genetic diversity analysis and Heterotic grouping of *Sorghum* and maize in: Abdurakhmonov IY. (Ed.), Microsatellite markers, In Tech Publisher, London, EC3R 6AF, UK. 2016:117-138
Available:http://dx.doi.org/10.5772/65078
21. Supriya A, Kumar R, Singh S**,** Yadav R. An easy, quick and cost effective method of high quality dna extraction from mungbean [*Vigna radiata* (L.) Wilczek] without liquid Nitrogen**.** Int. J. Curr. Microbiol. App.Sci. 2017;6(9):2695-703.
22. Allouis S, Qi X, Lindup S, Gale MD, Devos KM. Construction of a BAC library of pearl millet, [*Pennisetum glaucum* (L.) R. Br.]. Theor. Appl. Genet. 2001;102: 1200-1205.
23. Qi X, Lindup S, Pittaway TS, Allouis S, Gale MD, Devos KM. Development of simple sequence repeat markers from bacterial artificial chromosomes without subcloning. Biotechniques. 2001;31:355-358.
24. Qi X, Pittaway TS, Lindup S, Liu H, Waterman E, Padi FK, et al. An integrated genetic map and a new set of simple sequence repeat markers for pearl millet *[Pennisetum glaucum* (L.) R. Br.]. Theor. Appl. Genet. 2004;109:485-1493.

25. Shaikh TR. Assessment of hybrid purity in *Sorghum* by using molecular markers, submitted to Vasantrao Naik Marathwada Krishi Vidyapeeth Parbhani-431402 (M.S.) India; 2015.
26. Senthilvel S, Jayashree B, Mahalakshmi V, Kumar PS, Nakka S, Nepolean T, et al. Development and mapping of simple sequence repeat markers for pearl millet from data mining of expressed sequence tags. BMC Plant Biology. 2008;8:119.
27. Kumar S, Hash CT, Singh G, Basava RK, Srivastava RK. Identification of polymorphic SSR markers in elite genotypes of pearl millet and diversity analysis Ecological Genetics and Genomics. 2020;14:100051.
28. Oumar I, Mariac C, Pham JL, Vigouroux Y. Phylogeny and origin of pearl millet (*Pennisetum glaucum* [L.] R. Br) as revealed by microsatellite loci. Theor. Appl. Genet. 2008;117:489–497.
DOI: 10.1007/s00122-008-0793-4
29. Kapila RK, Yadav RS, Plaha P, Rai KN, Yadav OP, Hash CT, et al. Genetic diversity among pearl millet maintainers using microsatellite markers, Plant Breed. 2008;127:33–37.
30. Narshimulu G, Jamaloddin M, Vemireddy LR, Anuradha G, Siddiq E. Potentiality of evenly distributed hyper-variable microsatellite markers in marker-assisted breeding of rice. Plant Breed. 2011;130: 314–320.
DOI: 10.1111/j.1439-0523.2010.01834.x
31. Singh S, Singh VV, Supriya A, Dubey M, Singh D. Screening and estimation of allelic differentiation in Indian Mustard (*Brassica juncea* L.) genotypes based on SSR Markers for background selection. Int. J. Curr. Microbiol. App. Sci. 2017;6(9): 2506-16.
32. Gonzaga ZJ, Aslam K, Septiningsih EM, Collard BCY. Evaluation of SSR and SNP markers for molecular breeding in rice. Plant Breed. Biotechnol. 2015;3:139–52.
DOI: 10.9787/PBB.2015.3.2.139
33. Vieira ML, Santini L, Diniz AL, Munhoz CF. Microsatellite markers: What they mean and why they are so useful. Genet Mol Biol. 2016;39:312–328.
34. Shete S, Tiwari H, Elston RC. On estimating heterozygosity and polymorphism information content value. Theor Popul Biol. 2000;57:265-71.
35. Yadav S, Singh AK, Singh MR, Goel N, Vinod KK, Mohapatra T. Assessment of genetic diversity in Indian rice germplasm (*Oryza sativa* L.): Use of random versus trait-linked microsatellite markers. J Genet. 2013;92:545–557.
36. Tiwari KK, Singh A, Pattnaik S, Sandhu M, Kaur S, Jain S, et al. Identification of a diverse mini-core panel of Indian rice germplasm based on genotyping using microsatellite markers. Plant Breed. 2015; 134(2):164–171.
37. Mahalingam A, Sarawathi R, Ramalingam J. SSR marker diversity analysis for WA cytoplasm based fertility restorer genes of hybrid rice (*Oryza sativa* L.). J Innov Agric. 2016;3(1):19–27.
38. Salazar B, Laurentin H, Davila M, Castillo MA. Reliability of the RAPD technique for *Oryza sativa* analysis of sesame (*Sesamum indicum* L.) from Venezuela. Interciencia. 2006;31:456-460.
39. Shrivastava A, Mishra D, Koutu G, Singh S, Prakash V, Sohgaura N. Assessment of genetic diversity of restorer and maintainer lines of rice using SSR markers. The Ecascan. 2015;9(1–2):265–269.
40. Akkaya MS, Buyukunal-Bal EB. Assessment of genetic variation of bread wheat varieties using microsatellite markers. Euphytica. 2004;135:179-185.
41. Gupta SK, Nepolean T, Sankar SM, Rathore A, Das RR, Rai KN, Hash CT. Patterns of molecular diversity in current and previously developed hybrid parents of pearl millet [*Pennisetum glaucum* (L.) R. Br.]. Am. J. Plant Sci. 2015;6:1697-1712.
42. Sehgal, D, Skot L, Singh R, Srivastava RK, Das SP, Taunk J, et al. Exploring potential of pearl millet germplasm association panel for association mapping of drought tolerance traits. PLoS One. 2015;0(5): 0122165.
43. Satyavathi CT, Khandelwal V, Supriya A, Kumar K**,** Bhanwariya S, Yadav SL, Chand M. Pearl millet: Hybrids and varieties. ICAR-All India Coordinated Research Project on Pearl Millet, Mandor, Jodhpur, India. 2018;142.
44. Shanmuganathan M, Gopalan A, Mohanraj K. Genetic variability and multivariate analysis in pearl millet (*Pennisetum glaucum* (L.) R. Br.) germplasm for dual purpose. J. Agric. Sci. 2006;2(1):73-80.
45. Sathya M, Vinodhana NK, Sumathi P. Hierarchial clustering of pearl millet

(*Pennisetum glaucum* (L.) R.Br) inbreds for morpho-physiological traits. Int. J. Curr. Microbiol. App. Sci. 2013;2(12):647-652.

46. Chaudhary S, Sagar P, Hooda BK, Arya RK. Multivariate analysis of pearl millet data to delineate genetic variation. Forage Res. 2015;40(4):201-208.

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