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Molecular characterization of pearl millet [*Pennisetum glaucum* (L.) R. Br] inbreds using microsatellite markers

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Abstract: Studies on genetic diversity in *Pennisetum* germplasm are the promising opportunities for the use of un-domesticated materials for improving pearl millet varieties. DNA based markers have now emerged as a potential genomic tool for estimation of genetic diversity among various cultivars and varietal identification. In present study, genetic diversity among 49 stay green inbreds of pearl millet was studied using simple sequence repeats (SSRs). Twenty nine polymorphic SSR primers, identified after initial screening of 70, were used to study diversity among these lines. A total of 108 alleles were amplified, collectively yielding unique SSR profiles for all the 49 inbreds. The average number of SSR alleles per locus was 3.72, with a range from 2 to 13. Polymorphic information content (PIC) values of various SSR loci across all the 49 inbreds ranged from 0.14 to 0.87 with an average of 0.51 per locus. This indicated sufficient diversity among the 49 pearl millet inbreds and total 5 out of 29 polymorphic SSR loci, namely Xpsmp2070, Xpsmp2001, Xpsmp2008, Xpsmp2066, Xpsmp2072 revealed PIC values above 0.70, can be considered highly useful for differentiation of pearl millet inbred lines. The lowest PIC value (0.47) for linkage group 7 showed comparatively conserved nature of this linkage group A dendrogram obtained using WARD's minimum variance method further delineates 49 inbreds into 8 major clusters, and the clustering pattern corroborated with their pedigree and characteristics traits.

Keywords: Pearl millet, Genetic diversity, PIC, SSR Marker, Dendrogram

INTRODUCTION

Pearl millet [*Pennisetum glauccum* (L.) R. Br.] is a summer annual crop originating from Africa, from where it was introduced into other regions of the world with diverse agro-climatic conditions that is, from the hot area of Africa (the tropical zone) to the hot area of temperate zones. Therefore a large number of diversity is found within and among pearl millet cultivars. Due to its highly out-crossing breeding behavior, pearl millet was originated from several independent domestication events and wide range of stressful environmental conditions, in which it had been traditionally cultivated. Pearl millet exhibits a tremendous amount of diversity at both phenotypic and genotypic levels (Liu *et al*., 1994). Estimation of genetic diversity and identification of superior genotypes are some of the prime objectives of any crop improvement programmes. Highly diverse genotypes or accessions can be utilized as parents in hybridization programmes to produce superior varieties/hybrids. Therefore there is a need to evaluate available genotypes for their genetic diversity (Sumathi and Vinodhana, 2014).

Pearl millet is a C_4 monocot species belonging to the *Poaceae* family and has a relatively small diploid genome (2n =2x =14) with a DNA content of 2.36 pg. It is a highly cross-pollinated crop with protogynous flowering nature which fulfils one of the essential biological requirements for hybrid development. Pearl millet is one of the major cereals grown, primarily for grain production, on more than 29 m ha in the arid and semi-arid tropical regions of Africa and Asia (Kannan *et al*., 2014). India is the largest producer of pearl millet in Asia and occupied an area of 7.95 m ha with production of 8.79 mt and productivity 1106 kg/ha (Anonymous, 2014). Pearl millet is drought tolerant and 'high energy' coarse cereal with high starch (70%) in dry grain, protein (10-12%) and 5-7% fat. Adaptability to diverse environmental conditions has made it a preferred crop in areas where other crops like maize or wheat would not survive. It is primarily cultivated for grain, but is also a valuable source of fodder.. The energy density of pearl millet is relatively high, arising from its higher oil content relative to maize, wheat or sorghum (Hill and Hanna, 1990). Pearl millet contains 27 to 32% more protein than maize, higher concentrations of essential amino acids, twice the ether extract, and higher gross energy than maize (Ejeta *et al*., 1987). Genetic diversity studies in pearl millet germplasm offer possibilities for their use in improving pearl millet open-pollinated varieties and hybrids. Molecular markers are helpful in diversity analysis as they are not influenced by environment and provide more accurate results. DNA based molecular markers have been suc-

cessfully employed in quantification of genetic diversity, genotype identification, delineation and marker assisted selection. These have been effectively utilized for the assessment of genetic diversity as compared to the morphological or biochemical due to their abundance, high level of polymorphism, reproducibility and being independent of the environment. Molecular markers namely RFLP, RAPD, ISSR and SSRs are mostly used, among these microsatellite or SSRs has become the marker of choice for many applications due to their abundance, high level of repeats number, polymorphisms, manifested as the occurrence of the large number of alleles per locus, and co-dominant inheritance has facilitated their extensive use in genome mapping, phylogenetic inference and population genetics. Simple sequence repeats (SSRs) have been found as more reliable because they are highly polymorphic, informative and co-dominant markers which are often broadly applicable since loci are frequently conserved between related species and sometimes genera (Chakauya and Tongoona, 2008). The integration of genomic technologies in pearl millet breeding will improve the efficiency of breeding programs in the development of better cultivars and reduce the time required for cultivar development.

Thus, in the present investigation, SSR markers were used to characterize the pattern of diversity among pearl millet inbreds to identify better inbreds for the development of commercial hybrids in pearl millet.

MATERIALS AND METHODS

Plant materials: Forty nine pearl millet stay green inbreds were selected on the basis of performance and sown in a randomized block design (RBD) with three replications in the field of Bajra section, Genetics and Plant Breeding Department, CCS HAU, Hisar, Haryana (Table 1).

A set of 70 highly polymorphic SSR markers covering different genomic locations were selected for this study (Table 2). The genomic locations for most of the markers were derived from the pearl millet genome map.

Genomic DNA extraction: Genomic DNA was isolated from the young leaves of pearl millet by CTAB (Cetyltrimethylammonium bromide) extraction method as given by Murray and Thompson (1980) and modified by Saghai-Maroof *et al*. (1984) and Xu *et al*. (1994). DNA was subsequently dissolved in appropriate volume of T.E. buffer and samples were stored at -20^{0}C for further use. DNA concentration was determined using the UV spectro-photometer at 260 and 280 nm. PCR amplifications were performed using applied biosystems thermal cycler. The PCR reaction was carried out in a reaction volume of 20 µl containing 10 X PCR buffer, 100 µM each of dNTPs, 0.4 µM of each primer, 1 unit Taq DNA polymerase and 50 ng template DNA. PCR amplification was performed with initial denaturation at 94^{0}C for 5 min followed by 10 cycles of denaturation at 94^{0}C for 25s, annealing at 64^{0}C-54^{0}C (touch-down cycles) for 20 s, and extension at 72^{0}C for 30 s, followed by 40 cycles of denaturation at 94^{0}C for 25s, annealing at 56^{0}C for 20s and exten-

Table 1. List of pearl millet stay green inbred lines used for conduct experiment.

S. No.	Name of inbred line	Pedigree
1.	HSGR-01	SPF 2 98-2
2.	HSGR-02	H 90/4-5 X 77/ 29-2
3.	HSGR-03	HTP 92/5
4.	HSGR-04	(ICMB 92333 X EEBC CI-I)-5 -B-B
5.	HSGR-05	K-560-2 X(J 834-7 X 700544 -7-2-1)
6.	HSGR-06	HTP 3/14
7.	HSGR-07	VCF 6862/ 98-1
8.	HSGR-08	AC-04 /6
9.	HSGR-09	1210/1
10.	HSGR-10	H 94 / 61-2
11.	HSGR-11	JBV 3 S1 - 44-3 -B -4 –B
12.	HSGR-12	HTP - 07-26
13.	HSGR-13	HTP 07-44
14.	HSGR-14	96 AC – 99
15.	HSGR-15	HMP - 0810 (ICMA 01222 X ICMP 451)
16.	HSGR-16	SPF 2 98-2
17.	HSGR-17	VCF 4 1864
18.	HSGR-18	HTP 92 / 110
19.	HSGR-19	SGP -10 – 110
20.	HSGR-20	HTP -10 -137
21.	HSGR-21	PT - 1-10 -1038
22.	HSGR-22	PT-1-10 – 1043
23.	HSGR-23	TCF 3-10 -3 -2
24.	HSGR-24	TCF 3 -10 - 28 -5
25.	HSGR-25	PT -1 - 10 – 1099
26.	HSGR-26	DMRC - 09 / 11-81 -2
27.	HSGR-27	High Fe JBT / 12 -122
28.	HSGR-28	TPRT / 12- 119
29.	HSGR-29	TCPTA / 12 – 128
30.	HSGR-30	110041
31.	HSGR-31	HPT - 2 - 12 -7
32.	HSGR-32	99 HS – 22
33.	HSGR-33	2305
34.	HSGR-34	MIR 97041
35.	HSGR-35	G 73 - 107 - 05 K -1
36.	HSGR-36	AC-04 /13
37.	HSGR-37	99 HS – 23
38.	HSGR-38	99HS -145
39.	HSGR-39	98 Raj 4
40.	HSGR-40	99 ABL – 5
41.	HSGR-41	{ICMB 91777 X (91777B X HHVBC)} - 6 -B
42.	HSGR-42	HBL – 34
43.	HSGR-43	1660(M.T.)
44.	HSGR-44	HF IT - 1 -129
45.	H 77 / 833 -2-202	
46.	H 77 / 833 -2	
47.	H77 / 29 -2	
48.	HBL - 11	
49.	ICMR 01004	

Table 2. List of primers and their sequences used in the present study.

S. No.	Linkage group	Primer name	Sequence (forward)	Sequence (reverse)
1	LG1	Xctm12	GTTGCAAGCAGGAGTAGATCGA	CGCTCTGTAGGTTGAACTCCTT
2	LG1	Xctm27	GTTGCAAGCAGGAGTAGATCGA	CGCTCTGTAGGTTGAACTCCTT
3	LG1	Xicmp3017	CACCAAACAGCATCAAGCAG	AGGTAGCCGAGGAAGGTGAG
4	LG1	Xicmp3032	AGGTAGCCGAGGAAGGTGAG	CAACAGCATCAAGCAGGAGA
5	LG1	Xicmp3080	CAAACAGCATCAAGCAGGAG	GCGTAGACGGCGTAGATGAT
6	LG1	Xipes0009	TTGATCGATCGTCTACGGTT	TATACTCACTCACGGCAGCG
7	LG1	Xipes0042	GATAGAAGCAGATGGGCCTG	CTCGTCATCATTCTCGCCAC
8	LG1	Xipes0079	GTTGGACAGGCGAACGATAC	AGCTCTCCTGCATTTTCGTG
9	LG1	Xipes0098	ATCAAGCTTCATACCCCTGC	CATCTTCTTCATCATCTTTCGC
10	LG1	Xipes0126	CCAGCAGGGAAGTCTTTCAC	AAAGGCGCTTGCTGATTTT
11	LG1	Xipes0139	GTGTATGGTATGCGTGTCCG	GCATTGTGATCGAATAAACTACTG
12	LG1	Xipes0146	CATCAGAATACGGACGCCTT	CATCAGCTTTGGAGTCAGCA
13	LG1	Xipes0197	GTGTTCTTCCGAATCCGTGT	CGCTTTGCATTGAACACAGT
14	LG1	Xipes0203	CCCTCGAAGAGATCGAAGTG	CTGAAACAACAGCCTGCAAA
15	LG1	Xipes0216	AAAGGCAGCAGATCCCTACA	TCTTTCGTTGCATCTGTTCG
16	LG1	Xpsmp2006	GACTTATAGTCACTGGGAAAGCTC	GCTTTAATAACTTTGTGCGTATT
17	LG1	Xpsmp2030	ACCAGAGCTTGGAAATCAGCAC	CATAATGCTTCAAATCTGCCACAC
18	LG1	Xpsmp2069	CCCATCTGAAATCTGGCTGAGAA	CCGTGTTCGTACAAGGTTTTGC
19	LG1	Xpsmp2080	CAGAATCCCCACATCTGCAT	TGCAACTGAGCGAAGATCAA
20	LG1	Xpsmp2090	AGCAGCCCAGTAATACCTCAGCTC	AGCCCTAGCGCACAACACAAACTC
21	LG1	Xpsmp2232	TGTTGTTGGGAGAGGGTATGAG	CTCTCGCCATTCTTCAAGTTCA
22	LG1	Xpsmp2273	AACCCCACCAGTAAGTTGTGCTGC	GATGACGACAAGACCTTCTCTCC
23	LG2	Xctm03	GTCCATCGTCGCCGACGAA	GGATTTGCTAGTTGTGGGCT
24	LG2	Xctm21	ATGCCTCCCACCCCACGTCG	CGTCGCACTAGCCACAGTCA
25	LG2	Xpsmp2050	ATCAAACGGCATCAGACAAC	GGATCTCTTAGTGTGGTGGAGAGC
26	LG2	Xpsmp2059	GGGGAGATGAGAAAACACAATCAC	TCGAGAGAGGAACCTGATCCTAA
27	LG2	Xpsmp2066	ATATTAGAGCATTGCATCGC	GCATAGCAGCATACAGCAGCAAAA
28	LG2	Xpsmp2068	CAATAACCAAACAAGCAGGCAG	CTTCACTCCCACCCTTTCTAATTC
29	LG2	Xpsmp2072	GAAATCTACACAAGGGTCTCCA	GTACGGCAGAATGACATCTGAA
30	LG2	Xpsmp2077	GCCAATATTATTCCCAAGTGAACA	CTCTTGGTTGCATATCTTTCTTTT
31	LG2	Xpsmp2088	AAGAAGCCACCAGCACAAAA	TGCATGAAAGTAGAGGATGGTAAA
32	LG2	Xpsmp2089	TTCGCCGCTGCTACATACTT	TGTGCATGTTGCTGGTCATT
33	LG2	Xpsmp2237	TGGCCTTGGCCTTTCCACGCTT	CAATCAGTCCGTAGTCCACACCCCA
34	LG3	Xctm10	GAGGCAAAAGTGGAAGACAG	TTGATTCCCGGTTCTATCGA
35	LG3	Xpsmp2056	ACCTGTAGCTTCAAAATTCAAAAA	AATTCAGTGTGATTTCGATGGTTGC
36	LG3	Xpsmp2070	ACAGAAAAAGAGAGGCACAGGAGA	GCCACTCGATGGAAATGTGAAA
37	LG3	Xpsmp2071	TTGCAGTCCCACGAATTATTTG	CTTTGAATTTATAATCCTCATACT
38	LG3	Xpsmp2214	CGCACAGTACGTGTGAGTGAAG	GATTGAGCAGCAAAAACCAGC
39	LG4	Xipes0066	CAACATGTCAAGGAAGTAAAATTGA	GCCTCTTGATACCCAAGATCA
40	LG4	Xipes0114	CGTTGTGTTGAATAATGTCGTACC	CAATAACCAAACGACGGACA
41	LG4	Xipes0186	AGCATATGGCATCCTTTTCG	TTTCAGGCTTGGATTCAATGT
42	LG4	Xipes0208	CGAAGGAGGAGTACGACGAG	TCCACAAGGTGACCTCACTG
43	LG4	Xipes0225	CAAACCTCAAGCTAGGCGAC	CATGCATACACCAGTGCCAT
44	LG4	Xpsmp2008	GATCATGTTGTCATGAATCACC	ACACTACACCTACATACGCTCC
45	LG4	Xpsmp2076	GGAATAGTATATTGGCAAAATGTG	ATACTACACACTGTAAGCATTGTC
46	LG4	Xpsmp2081	CTGTGCTGTCATTGTTACCA	TCAGATCACCTATTACTTTCCCT
47	LG4	Xpsmp2084	AATCTAGTGATCTAGTGTGCTTCC	GGTTAGTTTGTTTGAGGCAAATGC
48	LG4	Xpsmp2085	GCACATCATCTCTATAGTATGCAG	GCATCCGTCATCAGGAAATAA
49	LG4	Xpsmp2086	CGCTTGTTTTCCTTTCTTGCTGTT	CCTTCTCAGATCCTGTGCTTTCTT
50	LG4	Xpsmp3029	ATCGATCTGTTCCACCCAGT	GGACTGGTACTGCTGCTGCT
51	LG5	Xpsmp2001	CATGAAGCCAATTAGGTCTC	ACCATCTGACTTGTTCTTATCC
52	LG5	Xpsmp2064	ACCGAATTAAAGTCATGGATCG	TTGATTCTTCTGACACAAATGAG
53	LG5	Xpsmp2078	CATGCCCATGACAGTATCTTAAT	ACTGTTCGGTTCCAAAATACTT
54	LG6	Xicmp3086	ACCAAACGTCCAAACCAGAG	ATATCTCTTCGCTGCGGTGT
55	LG6	Xpsmp2018	CGCAAGACATTTTAGTATCACC	ACAGTCATCCTCAGTCGTCC
56	LG6	Xpsmp2048	TGAATTGGGAATAAAGGAGACC	ACGTGTGCCTGCTTTTAGTAAC
57	LG6	Xpsmp2270	AACCAGAGAAGTACATGGCCCG	CGACGAACAAATTAAGGCTCTC
58	LG7	Xctm08	GCTGCATCGGAGATAGGGAA	CTCAGC AAGCACGCTGCTCT
59	LG7	Xpsmp2013	GTAACCCACTAACCCTTACC	GTCGCACAGAAAAAGAATAG
60	LG7	Xpsmp2019	TGTGCCACAGCTTGTTCCTC	CAAGCAGCCAGTTCCTCATC
61	LG7	Xpsmp2027	AGCAATCCGATAACAAGGAC	AGCTTTGGAAAAGGTGATCC
62	LG7	Xpsmp2033	CTATACCATTGAATTGAAAGGTC	CAATCTTTAGCTTTTTCAAGAGAC
63	LG7	Xpsmp2040	CATTACACGTTTCTTCAAACGC	TCTTCGGCCTAATAGCTCTAAC
64	LG7	Xpsmp2043	TCATATTCTCCTGTCTAAAACGTC	ACAAATCGTACAAGTTCCACTC
65	LG7	Xpsmp2063	GAGCACATGAAATAGGAAGCAG	AAGGTAGTTATAGTTAGCTTGATC
66	LG7	Xpsmp2074	AGGACTGTAGGAGTGTGGACAACACA	CCAGACCTACCAGTGAATGAGA
67	LG7	Xpsmp2079	AGCCGAAGGCTAATCAACAA	GTGGTCAGCAGCAGATGTAA
68	LG7	Xpsmp2087	GGAACAGACTCCATACCTGAAA	TACCTGCCTGTGCTGTTAGT
69	LG7	Xpsmp2203	GAACTTGATGAGTGCCACTAGC	TTGTGTAGGGAGCAACCTTGAT
70	LG7	Xpsmp2224	GGCGAATTGGAATTCAGATTG	CGTAATCGTAGCGTCTCGTCTAA

sion at 72^0C for 30s, followed by final extension at 72^0C for 20 min. Amplification products were separated on 2.5% agarose gel containing ethidium bromide and visualized under UV light using gel documentation system.

Molecular data analysis: To estimate genetic similarity/dissimilarities between different inbreds the data of banding pattern was used as input. The presence of band run on agarose gel was taken as one and absence of band was read as zero. The binary data was used to calculate similarity genetic distance using JMP 8.0 software, SAS Institute Inc., Carry, NC, 1989-2007. Dendrogram was constructed by using distance matrix by the unweighted pair group method using arithmetic averages (UPGMA) of JMP 8.0 Software. The genetic diversity of each microsatellite locus was assessed by calculating the frequency of the microsatellite alleles based on polymorphic information content (PIC). PIC is a measure of allele diversity at a locus and formula is given by

$PIC = 1 - \sum_{j=1}^{n} P^2_{ij}$

Where, pij is the frequency of the jth allele for ith marker (Anderson *et al.,* 1993). PIC is synonymous with the term "gene diversity" as described by Weir (1996). The PIC value ranging from '0' (Monomorphic) to '1' (highly discriminative with many alleles in equal frequency) is an indication of discriminative power of marker, not only for number of alleles at a locus but also for relative frequencies of those allele in the inbreds under study.

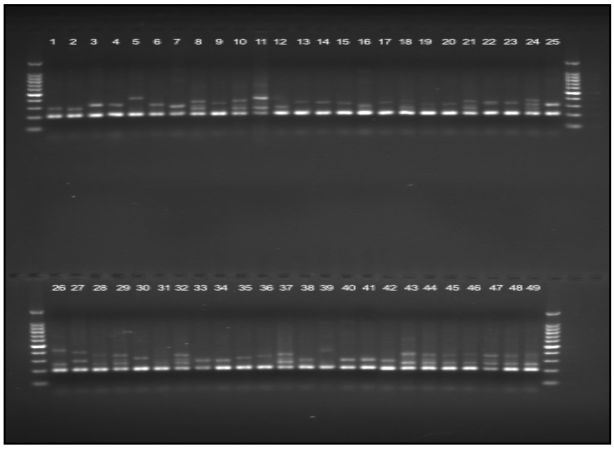

Fig. 1. *Polymorphism in different forty nine inbreds of pearl millet by using primer Xpsmp2001.*

RESULTS AND DISCUSSION

Molecular marker analysis: The DNA was extracted from 49 inbreds of pearl millet and checked for quality and quantity. The spectrophotometer revealed the 260/280 ratio of about 1.7-1.9 which indicated the presence of good quality of genomic DNA. The electrophoretic separation of genomic DNA on 0.8% agarose gel revealed the presence of single intact band.

Table 3. List of SSR primers showing polymorphism.

S.N.	SSR Locus	SSR Motif	Number of alleles	Range of band sizes (bp)	Linkage group	PIC values
1	Xpsmp2001	$(TC)_8(AC)_{47}$	6	200-450	LG5	0.72
2	Xpsmp2008	$(TG)_{38}(CT)_1$	8	180-1000	LG4	0.85
3	Xpsmp2013	$(GT)_{10}$	4	150-300	LG7	0.34
4	Xpsmp2019	$(CA)_{38}$	3	200-300	LG7	0.61
5	Xpsmp2027	$(GT)_{31}$	3	250-600	LG7	0.14
6	Xpsmp2030	$(CA)_{11}(GA)_{10}$	2	100-200	LG1	0.48
7	Xpsmp2043	$(CA)_{13}(GA)_6$	2	200-700	LG7	0.45
8	Xpsmp2048	$(AC)_{33}$	2	200-400	LG6	0.49
9	Xpsmp2063	$(AC)_{22}(AT)_5$	3	100-500	LG7	0.59
10	Xpsmp2066	$(CA)_{54}$	4	200-500	LG2	0.70
11	Xpsmp2069	$(CA)_{19}CTCG(CA)_7$	3	200-600	LG1	0.57
12	Xpsmp2070	$(CA)_{25}(TA)_6$	13	200-1200	LG3	0.87
13	Xpsmp2072	$(CA)_{24}TC(TA)_5$	12	100-1000	LG2	0.82
14	Xpsmp2077	$(CA)_{15}(TA)_8$	2	150-250	LG2	0.42
15	Xpsmp2078	$(CA)_{42}$	3	100-250	LG5	0.61
16	Xpsmp2084	$(CA)_{44}$	3	200-300	LG4	0.64
17	Xpsmp2085	$(AC)_{11}$	2	150-200	LG4	0.35
18	Xpsmp2086	$(CA)_{13}$	2	80-150	LG4	0.46
19	Xpsmp2088	$(CA)_{24}$	4	100-700	LG2	0.61
20	Xpsmp2089	$(AC)_{14}$	4	100-400	LG2	0.54
21	Xpsmp2203	$(GT)_{18}$	2	300-400	LG7	0.46
22	Xpsmp2237	$(GT)_8$	2	200-300	LG2	0.30
23	Xpsmp2273	$(GA)_{12}$	5	150-600	LG1	0.61
24	Xctm10	(CT)22	2	180-230	LG3	0.48
25	Xctm21	$(CT)_{24}$	4	150-500	LG2	0.51
26	Xipes0009	$(CGTA)_6$	2	150-200	LG1	0.40
27	Xipes0042	$(TGA)_7$	2	350-450	LG1	0.14
28	Xipes0098	$(TGA)_{12}$	2	200-300	LG1	0.40
29	Xipes0146	$(ATCTTC)_6$	2	100-200	LG1	0.49

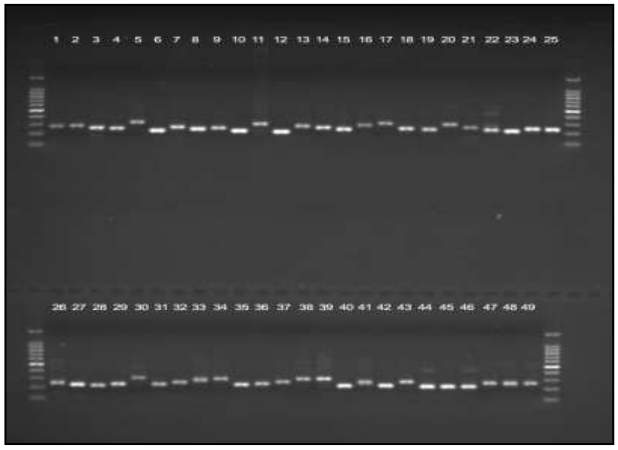

Fig. 2. *Polymorphism in different forty nine inbreds of pearl millet by using primer Xpsmp2084.*

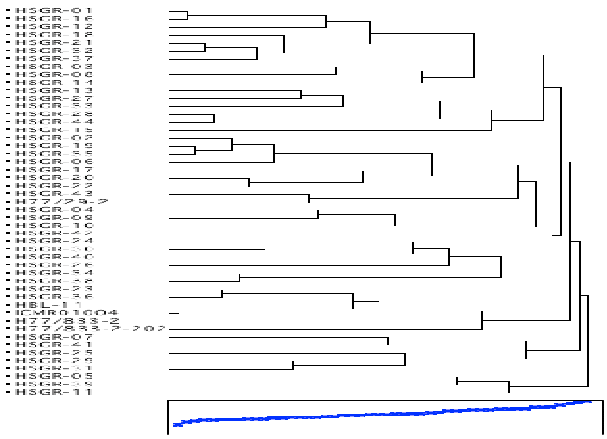

Fig. 3. *Dendrogram showing the clustering pattern of forty nine inbreds of pearl millet on the basis of SSR marker consequences.*

The present study comprised of detailed assessment of the utility of SSR markers in effectively discriminating forty nine inbred lines. Total 29 pair polymorphic SSR primers were identified on the basis of initial screening of 70 primers, used for assessing genetic diversity among pearl millet inbreds. A total of 108 alleles were detected, collectively yielding unique SSR profiles for all the 49 inbreds. Results given in Table 3 revealed that the average number of SSR alleles per locus was 3.72, with a range from 2 (Xpsmp2077, Xpsmp2203, Xpsmp2237, Xpsmp2086, Xpsmp2030, Xpsmp2085, Xpsmp2043, Xpsmp2048, Xctm10, Xipes0009, Xipes0098, Xipes0042, Xipes0146) to 13 (Xpsmp2070). Singh *et al*. (2013) identified total of 21 pair polymorphic SSR primers on the basis of initial screening of 60, were used for assessing genetic diversity among the pearl millet cultivars. These primers amplified a total of 64 alleles, which varied from 2 to 6 per locus, with a mean of 3.0 per locus. The overall polymorphism among the cultivars was 92%.

Based on the allele frequencies, PIC (Polymorphism Information Content) values for different SSR loci were estimated and found that value ranged from 0.14 (Xpsmp2027, Xipes0042) to 0.87 (Xpsmp2070) with an average of 0.51 per locus. Figure 1 and 2 shows a sample of polymorphic bands generated by primers Xpsmp2001 and Xpsmp2084 respectively. It is significant to note that 5 out of 29 SSR loci, namely Xpsmp2070, Xpsmp2001, Xpsmp2008, Xpsmp2066, Xpsmp2072 revealed PIC values above 0.70, can be considered highly useful for differentiation of pearl millet inbred lines. Chakauya and Tongoona (2008) found that out of the ten primers screened for the ability to amplify and detect polymorphism among pearl millet landraces, four did not produce amplification products, four were monomorphic (PSMP2019, PSMP2059, PSMP2056, PSMP2069), while two primers (PSMP2008, PSMP2013) were polymorphic with 2 -4 bands ranging between 150-220 base pairs. The two polymorphic primers were used for cluster analysis.

In previous studies, Stich *et al*. (2010) observed more number of alleles per locus, while analyzing diversity in germplasm lines. Similarly, Kapila *et al*. (2008) found an average of 6.26 alleles per locus, in a set of maintainer lines. In this study, lower number of alleles per locus observed, could be explained that we have used agarose gel, which has lower resolution power in comparison to polyacrylamide gel used in these studies. The level of polymorphism displayed by the SSR markers was considerably high in the present analysis, as showed by the high mean PIC value (0.51). The PIC values of SSR loci as genetic markers are also usually directly correlated to the type and number of repeats present. Kapila *et al*. (2008) also observed similar degree of SSR polymorphism, who recorded mean PIC values of 0.58. Mean PIC varied across the linkage groups in pearl millet *i.e.* 0.44, 0.55, 0.67, 0.57, 0.66,

Table 4. Distribution of forty nine pearl millet inbreds in different clusters based on SSR markers.

	Inbreds	Number of inbreds
Cluster 1	HSGR-01, HSGR-16, HSGR-12, HSGR-18, HSGR-21, HSGR-32, HSGR-37, HSGR-03, HSGR-08, HSGR-14	10
Cluster 2	HSGR-13, HSGR-27, HSGR-33, HSGR-28, HSGR-44, HSGR-15	6
Cluster 3	HSGR-02, HSGR-19, HSGR-35, HSGR-06, HSGR-17, HSGR-20, HSGR-22, HSGR-43, H77/29-2	9
Cluster 4	HSGR-04, HSGR-09, HSGR-10, HSGR-42	4
Cluster 5	HSGR-24, HSGR-30, HSGR-40, HSGR-26,HSGR-34, HSGR-38	6
Cluster 6	HSGR-23, HSGR-36, H77/833-2-202,H77/833-2, HBL-11, ICMR-01004	6
Cluster 7	HSGR-07, HSGR-41, HSGR-25, HSGR-29, HSGR-31	5
Cluster 8	HSGR-05, HSGR-39, HSGR-11	3

0.49 and 0.43 for LG1, LG2, LG3, LG4, LG5, LG6 and LG7, respectively. A number of reports are available in cereals that show different contributions of chromosomes/linkage groups to molecular diversity (Kleinhofs *et al*., 1993, Boyko *et al*., 1999, Huang *et al*., 2002, Ni *et al*., 2002). In pearl millet, LG 6 having the lowest number (1) of polymorphic SSR markers and low PIC (0.49) were observed in present study. Thus, the presence of some important genes involved in domestication might be responsible for conserved nature of LG 6 in pearl millet (Poncet *et al*., 2000).

Genetic diversity analysis: To understand the genetic relationships among the 49 inbreds, we clustered them into different groups by using WARD's minimum variance method (Fig. 3). On the basis of this method, 49 inbreds were separated into 8 major groups. Further, eight clusters grouped all the forty nine inbreds in such a way that inbreds within each cluster had high similarity than those in other clusters (Table 4). Cluster pattern revealed that, cluster1 was the largest consisting of 10 inbreds. This way followed by cluster3 (9 inbreds), cluster2 (6 inbreds), cluster5 (6 inbreds), cluster6 (6 inbreds), cluster7 (5 inbreds) and cluster4 (4 inbreds) and cluster8 (3 inbreds). Four inbreds, H77/833-2-202, H77/833-2, HBL-11 and ICMR-01004 were used as check lines in present study, grouped under same cluster (cluster6). This indicates that these genotypes might have an ancestral relationship.

The clustering pattern was not completely based on definite character. The set of primers used were not able to group all the genotypes into exactly similar phenotypically intended categories. This may be due to the fact that the numbers of SSR primers under study were not sufficient to represent all morpho-agronomical characters.

Since pearl millet is highly cross-pollinated crop, hybrid development for commercial exploitation is a viable option. Depending upon the diversity available among inbred lines for trait of interest, these inbreds could effectively be utilized as parents through their conversion into male sterile and fertility restorer lines for developing commercial productive hybrids in pearl millet. Another possibility could be to develop open-pollinated population using inbreds from different clusters and their subsequent genetic improvement through recurrent selection to concentrate desirable genes in the population which can further be used for developing improved inbred lines for targeted traits.

Conclusion

The study concludes that in case of pearl millet, the value of Polymorphic information content (PIC) ranged from 0.14 to 0.87 with an average of 0.518 specified the substantial efficiency of markers. Out of 70 primers, 29 primers showed higher polymorphism among the inbred lines. The study has provided an imminent of the inter-relationship among the genotypes and places of interest the genetic distance by SSR markers to competently reveal the genetic inter-relationship among the genotypes. The study provides the basis for future pearl millet crop variety identification, maintenance, and management.

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