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Identification of polymorphic SSR markers in elite genotypes of pearl millet and diversity analysis

Sushil Kumar [a,b,c,*], Charles T. Hash [d], Govind Singh [b], Ramana Kumari Basava [a], Rakesh K. Srivastava [a,*]

[a] International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Telangana, 502 324, India
[b] Plant Biotechnology Centre, SK Rajasthan Agricultural University, Bikaner, 334 006, India
[c] Centre of Excellence in Biotechnology, Anand Agricultural University, Anand, Gujarat, 388 110, India
[d] International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Niamey, 8001, Niger

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ABSTRACT

Pearl millet is a climate-resilient crop of marginal agro-ecologies and semi-arid tropics of Asia and Africa. With substantial nutritional qualities crop requires low inputs and is capable of giving economic returns. Development of high-yielding hybrids is an important breeding objective for pearl millet worldwide. The knowledge of genetic diversity is a prerequisite for developing superior hybrids. In the current study, attempts were made to evaluate the diversity of 17 important Indian pearl millet inbred genotypes and one popular hybrid 9444 using fluorescent labeled SSR markers. A total of 342 polymorphic alleles with an average of 4.62 alleles per primer were produced from 74 SSR markers. Polymorphic information content (PIC) ranged from 0.10 to 0.89 with an average of 0.55. A very low level of heterozygosity was detected in genotypes. The average genetic dissimilarity detected between pairs of inbred lines was 0.66. Genetic dissimilarity estimates calculated among the inbred lines varied from 0.108 (AIMP-03 and AIMP-08) to a maximum of 0.851(AIMP-03/AIMP-08 and 81B). The results indicated that sufficient genetic variability is available in studied genotypes which can be exploited through heterosis breeding to develop hybrids. The study also presents a suit of SSR markers that could be effectively used for genetic diversity analysis in pearl millet.

1. Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a nutritious staple crop of poor people in the arid and semi-arid tropics of the world [1]. It is a crop of choice in hot and dry climates. Pearl Millet ranks as the sixth most important crop (29 million ha area) globally, sustain one-third of the world's population [2]. India has seven million ha area under pearl millet with a production of 9.25 million tons [3]. In India, pearl millet is the third most widely cultivated food crop after rice and wheat [4]. It is an important and low-cost source of food for the urban poor. Its micronutrients contribution, particularly of iron (Fe) and zinc (Zn), varies from 30 to 50% of the intake of both micronutrients from cereals [5]. The green fodder is rich in protein, calcium, phosphorous and other minerals with low hydrocyanic acid content [6]. It is being experimented as a new forage crop in South America and Korea [7]. Thus, both grain and forage are important for the adoption of improved cultivars. There is a new interest in the USA in growing pearl millet as grain crop because of its drought tolerance and high quality.

Pearl millet can sustain with high temperature and drought, particularly on low-fertility soils with limited water and nutrient holding capacity [8]. In addition, it is a short-duration (65–90 days) crop that allows double cropping in many places. It has a great yield potential and can produce more forage than either sorghum or maize [9] therefore It is an attractive crop for failure agriculture to address environmental concerns like global warming, erratic weather patterns with water scarcity and other abiotic stresses. The major factors that restrict the production potential of pearl millet are low hybrid coverage, slow varietal spread, no fertilizer and various diseases and pests. The major thrust of pearl millet breeding is to improve yield potential in fragile arid regions. The aims of pearl millet breeders are to develop the resistant germplasm against various biotic and abiotic stresses and development of genetically diverse hybrids. These problems, however, can be overcome to some extent by diversification of male-sterile lines, the improvement of restorers, and breeding heterogeneous and heterozygous single, as well

* Corresponding authors.
E-mail addresses: sushil254386@yahoo.com (S. Kumar), r.k.srivastava@cgiar.org (R.K. Srivastava).

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as multicross, hybrids to combat the twin problems of low yield and disease susceptibility [6].

The success of any crop improvement program depends on the availability of genetic diversity conserved in the germplasm [10]. The germplasm of pearl millet is endowed with a wide range of variations for several characters including yield and nutritional traits [11]. The availability, assessment, and exploitation of genetic diversity help to develop new cultivars and heterotic groups [12,13]. The genetic diversity can be easily assessed with DNA markers. The PCR-based DNA markers such as simple sequence repeats (SSRs) are powerful tools for detailed assessment of genetic diversity in crop plants. Molecular markers are already having an impact on pearl millet improvement [14]. Of the various DNA markers used previously to assess the genetic diversity in pearl millet cultivars and land races, SSRs declared more reliable due to their multi-allelism, genome specificity, even distribution, high polymorphism, simply automated and easy detection [15]. In pearl millet, SSRs have been used to develop linkage map and to map quantitative trait loci [16,1]. This study was carried out to assess the utility of fluorescent labeled SSR markers to assess the genetic diversity in elite pearl millet lines consisting of 17 important Indian pearl millet inbred genotypes (and a popular hybrid 9444) being used directly or as founder parents in the hybrid breeding programmes at ICRISAT, state agricultural universities (SAUs) and private sector seed companies.

2. Materials and methods

2.1. Plant material and, DNA isolation

DNA was extracted from young leaves of 15 days old plant following Mace et al. [17] from a set of 18 genotypes (Table 1). Among the 93 SSR markers used, the PSMP series genomic SSR markers were obtained from Qi et al. [18–20]. The CTM and ICMP series SSRs were published by Budak et al. [21] and Senthilvel et al. [22], respectively. All SSRs were screened against 17 inbred and one hybrid (namely 9444).

Table 1
Genotypes and their pedigree.

Genotype	Type of line	Pedigree
ICMB 841-P3	B	Downy mildew resistant selection from 5141B
843-22B	B	Selection from KSU line BKM 2068
863B–P2	B	Togo-13-4-1
AIMP 92901-03	Inbred	Bred by random mating 272 Bold Seeded Early Seeded (BSEC) S1 progenies selected at Aurangabad and BSEC S1s bulk ex Pat/K91.
AIMP 92901-08	Inbred	Bred by random mating 272 Bold Seeded Early Seeded (BSEC) S1 progenies selected at Aurangabad and BSEC S1s bulk ex Pat/K91.
ICMB 92777	B	[843B x (ICMPS 500-4-4-3 x ICMPS 1800-3-1-2-C3-4)]-7-1-3
ICMB 93333	B	(843B × ICMPS 900-9-3-8-2)-21-8-4
ICMB 95444	B	(81–1164 DB/85–1856 LR-16-B x 843DMR1)-14-6-3
ICMB 97111	B	HTBC-48-B-1-1-1-1
ICMB 89111	B	[843B × (GnS × SS-48-40-4)-1-9-8]-30-B-B-1
IPC1518	R	ICRC – F4-146-3
J2340	R	Selection from (F 298 x F4FC −1498)-3-13-2-1-B
ICMR 01004	R	ICMR 01004 was originally selected as a BC4F3 headrow (202) from a single selfed BC4F2 plant derived from the repeated backcrossing of donor parent ICMP 451-P6 with a pure line selection from H 77/833–2: {{[((ICMP 451-P6xH 77/933–2)xH 77/833–2)xH 77/833–2]xH 77/833–2}xH 77/833–2}-F2-202
ICMR 356	R	(B 282 x J 104)-12-B-B-B-B
RIB 3135	R	–
81B	B	Induced downy mildew resistant selection from Tift 23D2B
ICMS 8511	Inbred	Bred from 4 inbreds from DAT 1 in K′84
9444	Hybrid	–

2.2. PCR amplification

PCRs were performed in a 5 μl volume containing 5 ng DNA, 1 pmol of each primer, 2 mM dNTP, 25 mM $MgCl_2$, 0.2U Taq DNA polymerase (Applied Biosystems) and 0.5 μl of 10 × PCR buffer (Applied. Biosystems). A touchdown amplification cycle was adopted for all reactions consisting of a 5 min denaturation at 94 °C, followed by five cycles of 94 °C for 30 s, 62 °C for 30 s (decreasing by 1 °C per cycle) and 72 °C for 30 s, then 30 cycles of 94 °C for 30 s, 52 °C for 30 s and 72 °C for 30 s, and final extension with a 20 min incubation at 72 °C.

To examine the amplification, PCR products together with a 100-base pair ladder were separated electrophoretically on 1.2% agarose gels. To increase the throughput, after checking the amplification, PCR products generated by four different fluorescence dye-labeled primers were pooled in equal volumes and 1.5 μl each of FAM- VIC- NED- and PET-labeled product were mixed with 7 μl of formamide (Applied Biosystems), 0.2 μl of the GeneScan™ LIZ_{500}® Size Standard (Applied Biosystems) and 3 μl of distilled water. Pooled PCR products were denatured and size fractioned using ABI 3730xl DNA Genetic Analyzer capillary electrophoresis (Applied Biosystems, USA).

2.3. Statistical analysis

The raw data files created by the DNA analyzer were processed for allele calling through GeneMapper® software version 4 (Applied Biosystems) for estimating the sizes of the PCR amplicons based on their relative mobility compared to the internal size standard LIZ_{500}. A DNA marker was considered to be valid if it had a peak height of at least 100 fluorescent units. The scoring was also manually checked two times. The basic statistics such as polymorphic information content (PIC), allelic richness as determined by a total number of the detected alleles and the number of alleles per locus, gene diversity, and occurrence of unique alleles and heterozygosity (%) were estimated using the PowerMarker V3.25 [23]. Simple matching allele frequency-based distance matrix was used in DARwin-5.0 program to dissect the genetic structure of genotypes [24]. Support for clusters was estimated by bootstrap analysis using DARwin-5.0. Correlation among number of alleles, PIC and nucleotide repeat was calculated in R.

3. Results and discussion

3.1. Marker polymorphism

Molecular markers are highly useful in precise differentiation of the landraces, hybrids and inbred lines; and thus their planned utilization in hybrid breeding programmes. During the past decades, various molecular markers have been developed for pearl millet [25]. The amplicon fragment analysis of these markers is gel-dependent and has limited capability to rapidly assay large numbers of marker loci. However, the recent improvements in molecular marker technology, such as fluorescent-based automated DNA detection and fragment sizing allow cost-effective genotyping of SSRs to characterize the germplasm for crop improvement.

SSR markers may offer advantages over SNPs in diversity analysis. Due to bi-allelic nature of SNPs, theoretically the PIC values can range between 0 and 0.5. On the other hand, since for SSR markers are mutli-allelic, the PIC values can go well above 0.5 [26]. Many times SSR data could be more in-line with the pedigrees compared to that of with SNP data [27]. Though in the current genomic era, SNP markers are becoming the marker of choice there are many facts that indicate microsatellite markers will continue to be useful and favorable markers [28]. This is due to the fact that (1) in many studies in-depth genotyping as provided by SNP markers are not required there SSRs are a suitable choice, (2) sample size can be largely expanded without significant cost when SSRs are used, which is costly with SNP (3) existed SSR-marker data can be readily incorporated and used with new studies; (4)

Table 2
Amplicon size, polymorphic information content (PIC), gene diversity, and observed heterozygosity of the 74 simple sequence repeat (SSR) loci in 18 pearl millet genotypes.

Marker	Repeat Motif	Primer sequence (F/R)	Linkage group (LG)#	Amplicon size (bp)	Major Allele Frequency	Number of alleles	Gene Diversity	Heterozygosity	PIC
CTM12	(CT)12	GTTGCAAGCAGGAGTAGATCGA/ CGCTCTGTAGGTTGAACTCCTT	1	319-339	0.389	7	0.747	0	0.712
ICMP3017	(CAG)7	CACCAAACAGCATCAAGCAG/ AGGTAGCCGAGGAAGGTGAG	1	186-198	0.389	5	0.716	0	0.667
ICMP3032	(GCT)8(ACAT)3	AGGTAGCCGAGGAAGGTGAG/ CAACAGCATCAAGCAGGAGA	1	182-194	0.389	5	0.716	0	0.667
ICMP3080	(AGC)8	CAAACAGCATCAAGCAGGAG/ GCGTAGACGGCGTAGATGAT	1	211-223	0.389	5	0.716	0	0.667
ICMP3088	(TCC)8	TCAGGTGGAGATCGATGTTG/ TTACGGGAGGATGAGGATG	1	141-165	0.25	4	0.75	0	0.703
PSMP2030	(CA)11(GA)10	ACCAGAGCTTGGAAATCAGCAC/ CATAATGCTTCAAATCTGCCACAC	1	110-128	0.5	5	0.659	0.176	0.611
PSMP2069	(CA)26	CCCATCTGAAATCTGGCTGAGAA/ CCGTGTTCGTACATGGTTTTGC	1	202-226	0.5	6	0.694	0	0.664
PSMP2080	(AC)14	CAGAATCCCCACATCTGCAT/ TGCAACTGAGCGAAGATCAA	1	173-183	0.313	6	0.797	0	0.768
PSMP2090	(CT)12)	AGCAGCCCAGTAATACCTCAGCTC/ AGCCCTAGCGCACAACACAAACTC	1	173-183	0.5	4	0.656	0	0.605
PSMP2273	(GA)12	AACCCCACCAGTAAGTTGTGCTGC/ GATGACGACAAGACCTTCTCTCC	1	158-172	0.389	5	0.704	0	0.652
ICMP3063	(GTG)5	TCCGGTAGAGACCGTAATGG/ GGCACTCCCTAGCAAAATGA	2	161-172	0.556	4	0.623	0	0.579
PSMP2013	(CT)19(GT16)	GTAACCCACTAACCCTTACC/ GTCGCACAGAAAAAGAATAG	2	133-257	0.2	11	0.889	0	0.879
PSMP2059	(AC)11	GGGGAGATGAGAAAACACAATCAC/ TCGAGAGAGGAACCTGATCCTAA	2	109-114	0.444	3	0.623	0	0.544
PSMP2077	(CA)15(TA)8	GCCAATATTATTCCCAAGTGAACA/ CTCTTGGTTGCATATCTTTCTTTT	2	136-174	0.5	4	0.656	0.056	0.604
PSMP2088	(CA)24	AAGAAGCCACCAGCACAAAA/ TGCATGAAAGTAGAGGATGGTAAA	2	120-148	0.333	5	0.764	0	0.726
PSMP2089	(AC)15	TTCGCCGCTGCTACATACTT/ TGTGCATGTTGCTGGTCATT	2	108-125	0.4	7	0.773	0	0.749
PSMP2201	(GT)6	CCCGACGTTATGCGTTAAGTT/ TCCATCCATCCATTAATCCACA	2	332-366	0.778	4	0.377	0	0.354
PSMP2225	(GT)12	CCGTACTGATGATACTGATGGTT/ TGGGAGGTAAGCTCAGTAGTGT	2	218-222	0.471	3	0.554	0	0.452
PSMP2231	(TG)12GG(TA)4	TTGCCTGAAGACGTGCAATCGTCC/ CTTAATGCGTCTAGAGAGTTAAGTTG	2	224-244	0.361	7	0.773	0.056	0.742
PSMP2232	(TG)8	TGTTGTTGGGAGAGGGTATGAG/ CTCTCGCCATTCTTCAAGTTCA	2	229-236	0.444	3	0.623	0	0.544
PSMP2237	(GT)8	TGGCCTTGGCCTTTCCACGCTT/ CAATCAGTCCGTAGTCCACACCCCA	2	189-197	0.5	4	0.648	0	0.592
CTM10	(CT)22	GAGGCAAAAGTGGAAGACAG/ TTGATTCCCGGTTCTATCGA	3	170-188	0.438	7	0.742	0	0.714
PSMP2070	(CA)25(TA)6	ACAGAAAAAGAGAGGCACAGGAGA/GCC ACTCGATGGAAATGTGAAA	3	193-265	0.278	8	0.833	0	0.813
PSMP2071	(CA)14	TTGCAGTCCCACGAATTATTTG/ CTATGAATTTATAATCCTGATACT	3	146-181	0.714	4	0.459	0	0.427
PSMP2214	(GT)9	CGCACAGTACGTGTGAGTGAAG/ GATTGAGCAGCAAAAACCAGC	3	241-245	0.444	3	0.642	0	0.568
PSMP2227	(GT)7	ACACCAAACACCAACCATAAAG/ TCGTCAGCAATCACTAATGACC	3	185-181	0.412	4	0.644	0	0.574
PSMP2229	(GT)5	CCACTACCTTCGTCTTCCTCCATTC/GTC CGTTCCGTTAGTTGTTGCC	3	224-252	0.533	5	0.649	0	0.608
PSMP2249	(GT)7 imperfect	CAGTCTCTAACAAACAAACACGGC/ GACAGCAACCAACTCCAAACTCCA	3	126-154	0.444	6	0.704	0	0.661
PSMP2008	(GT)37	GATCATGTTGTCATGAATCACC/ ACACTACACCTACATACGCTCC	4	175-191	0.471	5	0.685	0	0.639
PSMP2081	(AC)15	CTGTGCTGTCATTGTTACCA/ TCAGATCACCTATTACTTTCCCT	4	132-238	0.176	13	0.907	0	0.899
PSMP2084	(AC)42	AATCTAGTGATCTAGTGTGCTTCC/ GGTTAGTTTGTTTGAGGCAAATGC	4	205-255	0.143	10	0.888	0	0.877
PSMP2085	(AC)11	GCACATCATCTCTATAGTATGCAG/ GCATCCGTCATCAGGAAATAA	4	165-177	0.556	5	0.623	0	0.58
PSMP2086	(AC)14	CGCTTGTTTTCCTTTCTTGCTGTT/ CCTTCTCAGATCCTGTGCTTTCTT	4	115-123	0.5	3	0.611	0	0.535
ICMP3027	(GAT)6	ACACCATCACCGACAACAAA/ AGTGACCTGGGGTACAGACG	5	188-197	0.765	3	0.381	0	0.34
PSMP2064	(CA)56	ACCGAATTAAAGTCATGGATCG/ TTGATTCTTCTGACACAAATGAG	5	100-194	0.167	11	0.883	0	0.871
PSMP2078	(CA)42	CATGCCCATGACAGTATCTTAAT/ ACTGTTCGGTTCCAAAATACTT	5	104-171	0.294	8	0.83	0	0.811
PSMP2202	(GT)8	CTGCCTGTTGAGAATAAATGAG/ GTTCCGAATATAGAGCCCAAG	5	145-161	0.75	3	0.387	0.167	0.331

PSMP2208	(GT)10	GAAAGAGCAAACTGAACAATCCC/ ACTTTGCCCTGGATGATCCTC	5	246-252	0.765	3	0.388	0	0.355
PSMP2219	(GT)7	ACTGATGGAATCTGCTGTGGAA/ GCCCGAAGAAAAGAGAACATAGAA	5	282-288	0.889	3	0.204	0	0.194
PSMP2233	(TG)9	TGTTTTCTCCTCTTAGGCTTCGTTC/ ACCTTCTCCGCCACTAAACAACT	5	256-262	0.5	4	0.642	0	0.583
PSMP2261	(GA)16	AATGAAAATCCATCCCATTTCGCC/ CGAGGACGAGGAGGGCGATT	5	176-192	0.333	7	0.79	0	0.762
PSMP2274	(GA13)	CACCTAGACTCTACACAATGCAAC/ AATATCAAGTGATCCACCTCCCAA	5	229-263	0.353	5	0.74	0	0.697
PSMP2276	(CA)16	TGTGGCAATTACGGTCGAGC/ CTACCTCTATCTTACTTCACC	5	271-279	0.444	5	0.716	0	0.677
ICMP3002	(AAG)7	CGAGCCGCCATAGTTGAC/ TACACACACATTGCCACACG	6	195-203	0.556	2	0.494	0	0.372
ICMP3038	(TGT)6	CTCTCGGTTTGACGGTTTGT/ GGGGAAAACAAAGTTGCTCA	6	197-200	0.588	2	0.484	0	0.367
ICMP3050	(TA)8	ATGTCCAGTGTTGACGGTGA/ CGGGGAAGAGACAGGCTACT	6	207-219	0.5	4	0.648	0	0.592
ICMP3058	(GA)9	TGTCAGCTTGGATGTTTGGA/ GCAAGCCACAAGCCTATCTC	6	178-192	0.353	5	0.754	0	0.715
PSMP2213	(GT)10	CCCAAAAGAACCACACCCAC/ GTTGATGCTACTGCTCGTTTG	6	193-195	0.941	2	0.111	0	0.105
PSMP2248	(TG)10	TCTGTTTGTTTGGGTCAGGTCCTTC/ CGAATACGTATGGAGAACTGCGCATC	6	162-166	0.722	2	0.401	0	0.321
PSMP2270	(GA)26 imperfect	AACCAGAGAAGTACATGGCCCG/ CGACGAACAAATTAAGGCTCTC	6	151-175	0.722	4	0.444	0	0.409
PSMP2275	(GTT)10	CCAGTGCCTGCATTCTTGGC/ GCATCGAATACTTCATCTCA	6	265-273	0.722	3	0.438	0	0.396
PSMP2027	(GT)31	AGCAATCCGATAACAAGGAC/ AGCTTTGGAAAAGGTGATCC	7	188-194	0.563	3	0.539	0	0.447
PSMP2033	(CA)9	CTATACCATTGAATTGAAAGGTC/ CAATCTTTAGCTTTTTCAAGAGAC	7	199-203	0.722	3	0.438	0	0.396
PSMP2040	(CA)nd	CATTACACGTTTCTTCAAACGC/ TCTTCGGCCTAATAGCTCTAAC	7	228-264	0.75	2	0.375	0	0.305
PSMP2063	(AC)22(AT)5	GAGCACATGAAATAGGAAGCAG/ AAGGTAGTTATAGTTAGCTTGATC	7	112-174	0.231	8	0.849	0.077	0.831
PSMP2074	(AC)11	AGGACTGTAGGAGTGTGGACAA/ CCAGACCTACCAGTGAATGAGA	7	103-227	0.333	6	0.773	0	0.74
PSMP2087	(AC)10	GGAACAGACTCCATACCTGAAA/ TACCTGCCTGTGCTGTTAGT	7	114-124	0.639	5	0.554	0.056	0.522
PSMP2203	(GT)18 imperfect	GAACTTGATGAGTGCCACTAGC/ TTGTGTAGGGAGCAACCTTGAT	7	336-356	0.389	5	0.702	0.056	0.647
PSMP2210	(GT)12	CAATGATGACCGTAATCTGGGTG/ GGGCAAGATATGTGAAATCAAG	7	145-193	0.882	2	0.208	0	0.186
PSMP2224	(TG)10	GGCGAAATTGGAATTCAGATTG/ CGTAATCGTAGCGTCTCGTCTAA	7	148-157	0.583	5	0.583	0.111	0.53
PSMP2236	(TG)4(GT)4	ATAAGTGGGACCCACATGCAGCAC/ CGAAAGACTAGCAAAATTGCGCCTTC	7	260-264	0.882	2	0.208	0	0.186
PSMP2263	(AG)33	AAAGTGAATACGATACAGGAGCTGAG/CA TTTCAGCCGTTAAGTGAGACAA	7	204-252	0.333	8	0.802	0	0.779
PSMP2266	(GA)17	CAAGGATGGCTGAAGGGCTATG/ TTTCCAGCCCACACCAGTAATC	7	169-183	0.375	6	0.75	0	0.712
PSMP2271	(GA)11	CCTTATATTGGACCGACTGCTGAC/ CTCCCCCATACACGAGCGAGAA	7	180-184	0.556	3	0.586	0	0.517
ICMP3001	(AAAC)4	GCCGTCGACATTAACCAACT/ AAACATAGGGCTTGGCACTG	-	149-153	0.722	2	0.401	0	0.321
ICMP3048	(GTGCG)3	CGGAACTGCTGGAGTGAAAT/ GCGACTTCGACCGACTTTT	-	244-249	0.889	2	0.198	0	0.178
PSMP2045	(CA)11(GA)5	TCATCTTCCCCTATCCGAAAC/ ACTTGCCAATGCTATCTTCAC	-	189-195	0.5	3	0.555	0	0.456
PSMP2082	(AC)12	GCAGGTCATATCTTTCGTGTC/ AAAAGCATCCTCAAATACCCAT	-	106-108	0.688	2	0.43	0	0.337
PSMP2204	(GT)7	TGCTTCTTGACTATGTTTTCC/ AGATATGGCGAACGTGAGGAG	-	264-266	0.824	2	0.291	0	0.248
PSMP2209	(GT)6(CT)7	TTGGACGATTTGGAAGCATAG/ GAGGAAAAGAGCCATACAGAGAC	-	337-355	0.5	4	0.663	0	0.615
PSMP2212	(GT)6	GATTGGATGGCAGTGCTTGG/ CAAACCAGCCATCAACAACCAG	-	189-227	0.667	5	0.525	0	0.497
PSMP2223	(GT)7	CATGCTTCTTCTTCTTTTGTAACC/ CAGCTCTTTGATCTCACTACAC	-	178-187	0.438	3	0.602	0	0.516
PSMP2242	(TG)9 imperfect	TAGCCTAATTGAACATTTTACGGAG/ GCTTATCCAGGACATGCAATAC	-	189-191	0.938	2	0.117	0	0.11
PSMP2246	(TG)7 imperfect	CGGATGCTAAATTAACCGAAGC/ CCAGCTTGCTTCTGTTGCGTTC	-	258-262	0.556	3	0.537	0	0.441
Mean				100-366	0.519	4.622	0.598	0.01	0.551

[a]Linkage group adopted-in black font from Ref. [32]; in red [22]; Blue font [19].

multi-allelic nature of SSRs makes them highly suitable for studying small subpopulations; and (5) microsatellites are the best markers of choice for small-to-medium-throughput laboratories with limited budget. Unlike maize and rice, in case of pearl millet, SNP chips are still not available.

A very few studies have been carried out on elite pearl millet germplasm using fluorescent-labeled SSRs as compared to other major cereal crops [29]. In the present study, a total of 74 (79%) SSRs were polymorphic in a panel of 18 pearl millet genotypes (Table 2). Allele size of the markers ranged from 100bp to 366bp. The number of alleles per

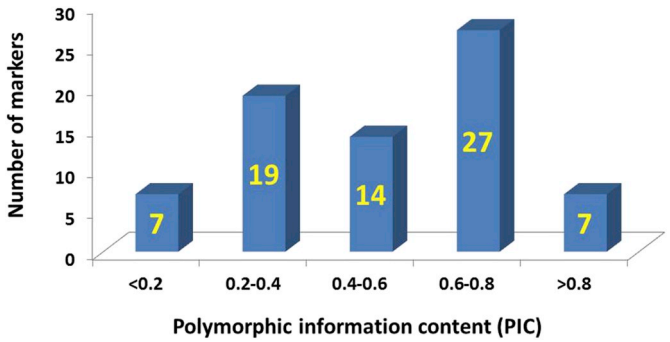

Fig. 1. Bar diagram of Polymorphic information content (PIC).

locus ranged from 2 to 13 (PSMP2081) with an average of 4.6 alleles per locus (Table 2). All sample analyses were conducted in two replications to test the reproducibility and only the reproducible and unambiguous peaks or alleles were used for the analysis. The SSRs with dinucleotide repeat motifs showed higher allele numbers than trinucleotide. This high polymorphism has also been detected in SSRs with dinucleotide repeat motifs in pearl millet Budak et al. [22]. The range in allele number and the average number of alleles detected were higher than those reported [21] (2–9 alleles across 15 inbred lines) and Senthilvel et al [22] (3.1 alleles per locus; 22 genotypes). However, the number of alleles per locus was comparatively lower than Mariac et al. [30] with 2–18 alleles (6.8 alleles per locus) and Oumar et al. [31] with (12.5 alleles per locus) which may be due to the study of the diverse world collection of germplasm. The reason for these differences presumably relates to the quantity and/or type of germplasm studied and the nature of the markers used.

The polymorphic information content (PIC) values ranged from 0.105 (PSMP2213) to 0.899 (PSMP2081), with an average of 0.551. More than 36% (27 SSR) markers had high PIC value and were between 0.6 and 0.8 (Fig. 1). The mean PIC value across all the SSR loci was in line with previously reported [30,31,33,34] but little lower than that recorded by Gupta et al. [29] which could be due to the difference in the sampled germplasm. A significantly positive correlation was recorded between repeat motif and the number of alleles (0.60***); and PIC and number of alleles (0.51***). PIC values also showed a strong positive linear correlation with the number of alleles (r = 0.85) implying that alleles amplified can be indirectly used to assess PIC in pearl millet (Fig. 2).Kapila et al. [34]. also reported similar results based on 72 inbred lines of pearl millet using 34 SSR loci.

Gene diversity is defined as the probability that two randomly

Table 3
Estimates of mean number of alleles and polymorphic information content (PIC) across seven linkage groups and repeat number groups of nucleotide motifs in pearl millet.

Linkage group[a]	Number of alleles	PIC
LG1 (10)	5.20	0.67
LG2 (11)	5.00	0.61
LG3 (7)	5.28	0.62
LG4 (5)	7.20	0.71
LG5 (10)	5.20	0.56
LG6 (8)	3.00	0.41
LG7 (13)	4.46	0.52
Unlinked (10)	2.80	0.43
Repeat number groups		
10–20	3.38	0.43
21–30	4.6	0.58
32–40	5.5	0.67
42–50	6.5	0.72
>50	7.11	0.71

[a] Value in parenthesis is number of marker in each linkage group (LG).

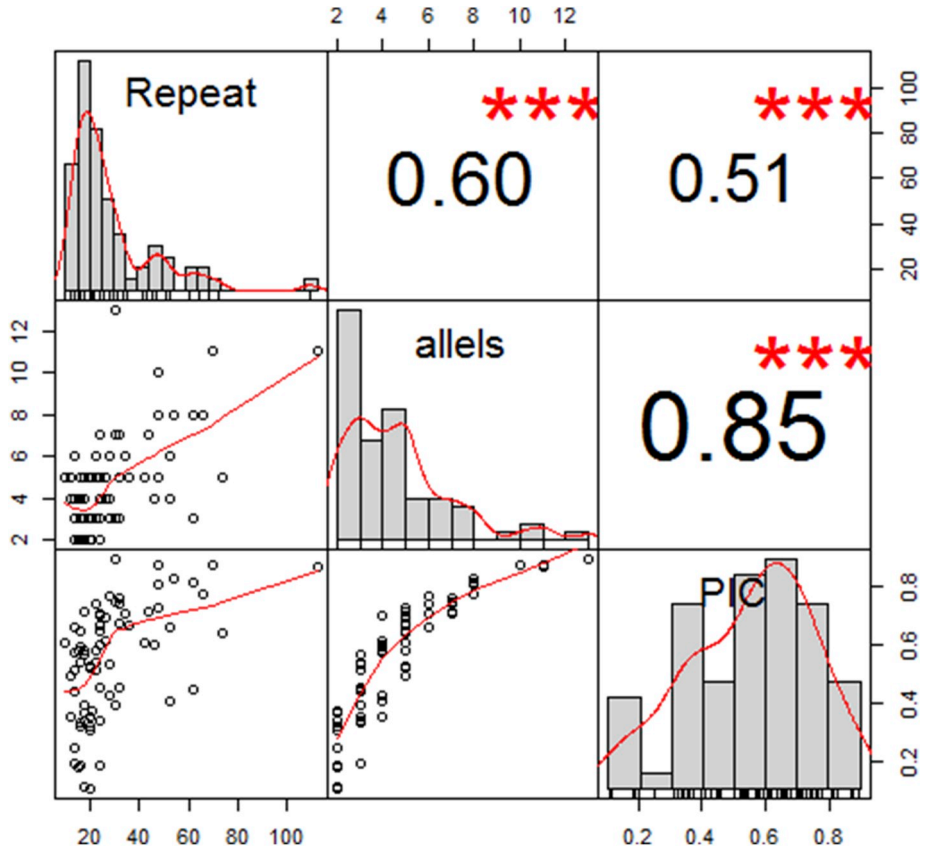

Fig. 2. Correlogram among SSR repeat motif, number of alleles and Polymorphic information content (PIC); *** = Significant at 0.001 level of probability.

Table 4
Pairwise dissimilarity indices of 18 pearl millet genotypes based 74 SSR markers.

Genotype	9444	ICMB841-P3	843-22B	863B_P2	AIMP92901-03	AIMP92901-08	ICMB92777	ICMB93333	ICMB95444	ICMB 97111	ICMB89111	IPC1518	J2340	ICMR01004	R356	RIB3135	81B
ICMB 841-P3	0.770																
843-22B	0.709	0.574															
863B–P2	0.750	0.709	0.662														
AIMP 92901-03	0.689	0.716	0.655	0.642													
AIMP 92901-08	0.689	0.743	0.655	0.642	0.108												
ICMB 92777	0.662	0.622	0.466	0.682	0.730	0.703											
ICMB 93333	0.716	0.595	0.493	0.723	0.676	0.635	0.365										
ICMB 95444	0.649	0.622	0.277	0.723	0.689	0.676	0.527	0.473									
ICMB 97111	0.635	0.676	0.439	0.588	0.703	0.703	0.473	0.554	0.500								
ICMB 89111	0.682	0.615	0.351	0.689	0.736	0.723	0.412	0.480	0.358	0.453							
IPC1518	0.615	0.770	0.703	0.709	0.703	0.689	0.676	0.622	0.676	0.649	0.676						
J2340	0.615	0.824	0.743	0.757	0.757	0.716	0.757	0.703	0.676	0.757	0.797	0.703					
ICMR 01004	0.655	0.730	0.757	0.716	0.716	0.703	0.743	0.689	0.689	0.703	0.743	0.797	0.689				
R 356	0.601	0.696	0.716	0.682	0.628	0.628	0.696	0.723	0.709	0.696	0.649	0.588	0.723	0.642			
RIB 3135	0.595	0.703	0.642	0.723	0.581	0.608	0.730	0.743	0.608	0.676	0.709	0.608	0.703	0.595	0.534		
81B	0.777	0.642	0.649	0.804	0.851	0.851	0.703	0.682	0.635	0.730	0.676	0.750	0.764	0.743	0.743	0.709	
ICMS 8511	0.730	0.709	0.703	0.770	0.743	0.770	0.669	0.669	0.757	0.716	0.716	0.770	0.797	0.770	0.777	0.676	0.473

chosen alleles from the population are different and it varied from 0.11 (PSMP2213) to 0.89 (PSMP2081), with an average of 0.59. Oumar et al. [31]. found the same level of gene diversity in cultivated germplasm of pearl millet. This value is lower than the gene diversity (0.74) estimates observed in landraces by Stich et al. [35]. This difference might be explained by the fact that in the latter study genotypes were derived from landraces from West and Central Africa (WCA).

Due to the codominant inheritance of SSRs, they facilitate analysis of genetic purity as well as homozygosity of the inbred lines [33]. A very low level of heterozygosity (%) was detected in the germplasm ranged from 0.00 to 0.17, with an average of 0.01 indicating that each SSR detected a single locus and that each of the genotypes used was inbred and homogeneous. However, detected heterozygosity for a few SSRs could be due to residual heterozygosity or occurrence of mutation at a specific SSR locus.

Of the 342 alleles detected, 120 alleles were rare as exclusively present in one genotype. Rare alleles were detected in 52 (55.9%) SSR loci and ranged from 1 to 10 (PSMP2081) with an average of 2.3 rare alleles per locus. In general, markers detecting a greater number of alleles per locus detected more rare alleles. Limited information is available across the linkage groups wise genetic diversity in pearl millet. Average PIC (0.41–0.71) varied across the linkage groups in pearl millet (Table 3). Similar to Kapila et al. [34] PIC for LG 6 (0.41) was lesser than the remaining linkage groups indicating the evolutionary less dynamic nature of this linkage group. Moreover, the presence of important domestication genes might be responsible for low PIC of LG 6 which indicating the conserved nature of LG 6 in pearl millet [34,36].

3.2. Genetic dissimilarity and cluster analysis

A dendrogram was constructed using the NJ method. This approach successfully categorized all the genotypes used. The bootstrap analysis revealed moderate to high statistical support for major nodes in the dendrogram. On the basis of polymorphism data, the average genetic dissimilarity detected between pairs of lines was 0.66, indicated considerable diversity among them (Table 4). Genetic dissimilarity estimates calculated among the genotypes varied from 0.108 (AIMP-03 and AIMP-08) to a maximum of 0.851(AIMP-03/AIMP-08 and 81B). RIB3135 and ICMB841 were found to span the extremes of the dendrogram, with all other germplasm distributed in-between. Studied genotypes were grouped into three clusters, I, II and III with nine, six and three lines, respectively. R-lines were dispersed in cluster I indicate the significant genetic divergence among them. High genetic dissimilarity (>0.5) among R lines and (>0.6) between R lines and most of the maintainer lines indicates the promising performance in hybrid development.

Out of nine inbreds grouped in Cluster I, three downy mildew resistant lines with a large grain (AIMP-03, AIMP-08 and 863B–P2) were derived from the *Iniadi* landrace from Togo and Ghana and four (R3135, R356, J2340 and ICMR01004) were restorer lines (Fig. 3). Some of the lines of this cluster derived from the *Iniadi* landrace known to have inherent drought tolerance mechanisms and having high grain Fe and Zn contents have been used as a parent in a mapping population to tag QTLs in pearl millet [5,16,37]. Grouping of *Iniadi* genotypes was expected hence results served as an internal control for our analysis Cluster II grouped B lines and most of the lines involving 843B as one of the parents in pedigree. The four B lines (ICMB 89111, ICMB 95444, ICMB 92777 and ICMB 92777) are expected to cluster together as they share 843B as a common ancestor. Inbred line ICMB 97444 and 843B clustered together at 28% dissimilarity had downy mildew resistance. These results are in line with previously reported by Kapila et al. [34]. B- Line ICMB 97111 had split at 48% dissimilarity from other lines of cluster II. This could be due to a derivative of HTBC (High Tillering *Bajra* Composite) and 843B. Maintainer lines, ICMB 97111 and ICMB 93333, clustered together at 55% dissimilarity. These results indicate that B-lines were developed from a narrow gene pool. Cluster III included three

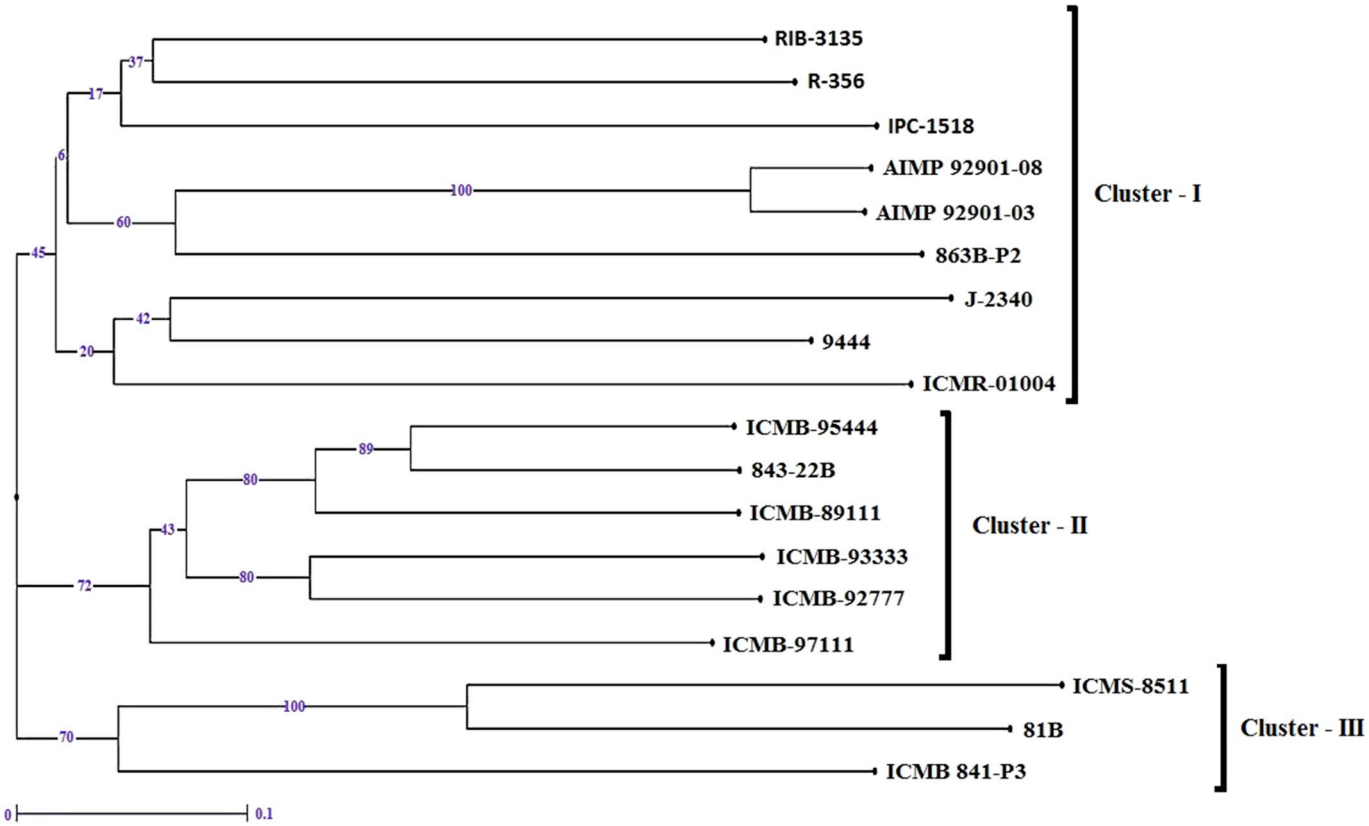

Fig. 3. SSR marker based dendrogram showing clusters of 18 pearl millet genotypes.

low grain Fe and Zn containing small grain lines (81B, ICMS 8511 and ICMB 841-P3). Chowdari et al. [6]. and Kapila et al. [34] also reported grouping of 81A and 841A in same the cluster.

4. Conclusion and implications

The present study resulted in the development of a suite of SSR markers that can be efficiently used for the assessment of genetic diversity in inbred lines of pearl millet. The genetic relationships identified among the panel of inbred lines may be useful in designing strategies to improve the use of available genetic variation in the context of pearl millet breeding globally. This may include heterotic gene pool studies using SSRs [12], and developing a decision support system for generating crosses based on SSR-based genetic distance matrices. The highly polymorphic SSR loci will enable the population structure study in pearl millet and SSR based association mapping. The marker can also be used to confirm the hybridity of F_1 plants generated through different pairs of inbred used in the current study.

Author contributions

Conceived and designed the study: CTH and RKS. Performed the study: SK. Analyzed the data: SK and RKB. Interpretation of results: SK, RKS and GS. Wrote first draft of the paper: SK and RKS. Revised the manuscript: SK, RKS, GS and RKB.

Declaration of competing interest

The authors have no conflict of interest.

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