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Molecular diversity evaluation in pearl millet parental genotypes using simple sequence repeats

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ABSTRACT

The genetic diversity of of fourteen pearl millet genotypes was investigated using 50 SSR markers. Polymorphism was found in 25 of the 50 markers analyzed. The highest PIC value among these 25 polymorphic SSR markers was reported in SSR marker xpsmp 2251, which was 0.6704. The PIC varied from 0.1239 (3035) to 0.6704 (xpsmp 2251). The power marker UPGMA cluster analysis grouped 14 pearl millet parental genotypes into two major clusters with a 67.5% similarity score. The first cluster has ten parental genotypes: EMRL-14/123, EMRL-14/237, EMRL-14/243, HMS 53 A4, HMS 54 A5, AC 04/13, H77/833-2-202, EMRL-14/111, HMS 30Aegp, EMRL-14/105, while the second cluster contains four parental lines: HMS 58 A1, EMRL-14/103, EMRL-14/127, and EMRL-15/109.

Keywords: *pearl millet, molecular marker, diversity, breeding*

Introduction

Pearl millet is an important grain crop for people living in arid and semi-dry areas of India and Africa. It is a monocot C4 species with a high photosynthetic efficiency. It is a member of the poaceae family and has the chromosome number (2n = 2x = 14). It is an important forage and food crop. In different regions of the world, pearl millet is known as cat millet, bush millet, bajra, and so on. Its grains have a better nutritional value because to their increased protein (9 to 15%), fat (5%), and mineral content (2 to 7%) (Pal *et al*., 1996). It is also strong in vitamins A and B, as well as thiamin and riboflavin, and delivers a lot of energy while being simple to digest.

It is mostly produced as a rainfed crop in dryland areas, however in places such as south India where irrigation facilities are available, it is planted in both seasons, such as *kharif* (July-October) and summer (February-June). It is mostly grown in six states: Rajasthan, Maharashtra, Uttar Pradesh, Haryana, Gujarat, and Andhra Pradesh. After rice, wheat, and maize, it is India's fourth most frequently produced cereal crop, with an area of 7.52 million ha, an output of 10.28 million tonnes, and a productivity of 1374 kg/ha (Anonymous, 2020). It is grown in Haryana over an area of 0.49 million hectares, producing 1.02 million tonnes with a productivity of 1436 kg/ha (Anonymous, 2020).

Biotechnologists and plant breeders use crop diversity as a quick resource to develop new varieties and hybrids in less time and space by searching for desirable genes in a variety of crop species. Crop genetic relationships are an important component of crop improvement programs because they provide information on genetic diversity, compatibility and crop breeding applications.

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Genetic diversity research in Pennisetumgermplasm has opened up possibilities for using it to improve open-pollinated pearl millet varieties and hybrids.

Crop diversity is used by biotechnologists and plant breeders to generate novel varieties and hybrids in less time and space by looking for favorable genes in a variety of crop species. Plant breeders primarily characterize germplasm using morphological features and pedigree data, although these procedures are time-consuming. As a result, a molecular marker-based method for germplasm characterization is thought to be more trustworthy and time-saving. Researchers used a variety of molecular markers for genetic diversity studies, including RFLP, RAPD, ISSRs, AFLP, and microsattelite probes, but SSRs have been shown to be more reliable for genetic diversity studies due to their genome specificity, multiallelic, high polymorphism, and ease of detection (Bharti *et al*., 2018). These are quickly and effectively amplified by PCR and are being found in a variety of crops, including soybean (Akkaya *et al*., 1992), chickpea (Bharadwaj *et al*., 2010), sorghum (Brown *et al*., 1996), and others. Pearl millet has a variety of microsatellites (Allouis *et al*., 2001; Budak *et al*., 2003), and these molecular markers have been employed for diversification studies in this crop.

Materials and Methods

Plant Materials: The experimental material included 14 parental genotypes (4 line and 10 Testers): HMS 58A1, HMS 54A5, HMS 53A4, HMS 30 Aegp, AC 04/13, H77/833-2-202, EMRL-14/243, EMRL-14/127, EMRL-15/109, EMRL-14/111, EMRL-14/237, EMRL-14/123, EMRL-14/103, EMRL-14/105.

Chemicals and reagents

All of the chemicals used in DNA extraction and PCR amplification were supplied by Sigma-Chemicals Co USA and Life Technologies (India) Pvt Ltd India. All of the extra chemicals used in this investigation were of molecular/analytical grade and came from Promega, Thermo Fisher Scientific, and Integrated DNA Technologies.

Genomic DNA isolation

The CTAB (CetylTrimethyl Ammonium-Bromide) extraction technique of Saghai-Maroof*et al.* (1984) was used to extract genomic DNA from young leaves of 14 parental genotypes of pearl millet. Young leaves from 4-5 week-old seedlings of 14 parents (4 lines and 10 tester) were obtained for DNA extraction and genotyping during the 2020 *Kharif* season.

Polymerase Chain Reaction (PCR) Amplification

PCR amplification reaction was carried out in BenchtopThemocycler. The optimization of PCR reaction was done by using varying concentrations of template DNA (50, 75, 100 ng), dNTPs mix (100 µM, 150 µM, 200 µM, 250 µM, 300 µM), $MgCl_2$ (2 mM, 2.5 mM), primers (0.3 µM, 0.4 µM, 0.6 µM, 0.8 µM) and *Taq* DNA Polymerase (1 unit, 2 unit, 3 unit) in a reaction volume of 20 µl. The optimized reaction mixture (20 µl) contained50 ng of DNA template, 250 µM of dNTPs mix, 2.5 mM of $MgCl_2$, 0.4 µM of primers, and 2 units of *Taq* DNA Polymerase. A control was also included in each PCR reaction setup, in which sterilized distilled water was used in place of the template DNA, keeping the other reagents' concentration and conditions the same. A total of 50 SSR markers were used for PCR amplification of the template DNA (**Table 1)**.

The PCR reaction (20 µl) was set up in thin-walled 0.2 µl PCR tubes under the following reaction conditions:

i. 94°C for 3:00 minutes (initial denaturation)
ii. 94 °C for 1:00 minute (denaturation)
iii. 49- 53.7°C for 1:50 minute (primer annealing)
iv. 72 °C for 2:00 minutes (primer extension)
v. 72°C for 10 minutes (final primer extension)
vi. Hold at 4ºC for infinity

The amplification reaction was set to repeat the steps (ii) to (iv) for 35 times and the product was kept at 4°C till further use.

Table 1:- A brief description of SSR primers used during the present investigation

Primer name	Forward primer (5' to 3')	Reverse primer (5' to 3')
Xpsmp 2085 F	GCACATCATCTCTATAGTATGCAG	GCATCCGTCATCAGGAAATAA
Xpsmp 2203 F	GAACTTGATGAGTGCCACTAGC	TTGTGTAGGGAGCAACCTTGA
Xpsmp 2027 F	AGCAATCCGATAACAAGGAC	AGCTTTGGAAAAGGTGATCC
Xpsmp 2225 F	CCGTACTGATGATACTGATGGTT	TGGGAGGTAAGCTCAGTAGTGT
Xpsmp 2070 F	ACAGAAAAAGAGAGGCACAGGAGA	GCCACTCGATGGAAATGTGAAA
Xpsmp 2204 F	GAACTTGATGAGTGCCACTAGC	TTGTGTAGGGAGCAACCTTGAT
Xpsmp 2206 F	AGAAGAAGAGGGGGTAAGAAGGAG	AGCAACATCCGTAGAGGTAGAAG
Xpsmp 2219 F	ACTGATGGAATCTGCTGTGGAA	GCCCGAAGAAAAGAGAACATAGAA
Xpsmp 2231 F	TTGCCTGAAGACGTGCAATCGTCC	CTTAATGCGTCTAGAGAGTTAAGTTG
Xpsmp 2074 F	AGGACTGTAGGAGTGTGGACAA	CCAGACCTACCAGTGAATGAGA
Xipes0004 F	GTGCGTTCTTCCTTGCCTAC	TCATCACACAGGGCTAGCTG
Xipes0105 F	GGGGGCTCACAGAACAAGTA	CCGAAGTTCCCACAGAATGT
Xctm08 F	GCTGCATCGGAGATAGGGAA	CTCAGCAAGCACGCTGCTCT
Xctm10 F	GAGGCAAAAGTGGAAGACAG	TTGATTCCCGGTTCTATCGA
Xctm12 F	GTTGCAAGCAGGAGTAGATCGA	CGCTCTGTAGGTTGAACTCCTT
Xicmp3002 F	AAGATGGATGATGGATTGATGA	TACACACACATTGCCACACG
Xicmp3032 F	GCGTAGACGGCGTAGATGAT	CAACAGCATCAAGCAGGAGA
Xicmp3088 F	TCAGGTGGAGATCGATGTTG	TTACGGGAGGATGAGGATG
Xpsmp2273 F	AACCCCACCAGTAAGTTGTGCTGC	GATGACGACAAGACCTTCTCTCC
Xpsmp2267 F	GGAAGGCGTAGGGATCAATCTCAC	ATCCACCCGACGAAGGAAACGA
Xpsmp2251 F	TCAAACATAGATATGCCGTGCCTCC	CAGCAAGTCGTGAGGTTCGGATA
Xpsmp2249 F	CAGTCTCTAACAAACAAACACGGC	GACAGCAACCAACTCCAAACTCCA
Xpsmp2248 F	TCTGTTTGTTTGGGTCAGGTCCTTC	CGAATACGTATGGAGAACTGCGCATC
Xpsmp2237 F	TGGCCTTGGCCTTTCCACGCTT	CAATCAGTCCGTAGTCCACACCCCA
Xpsmp2232 F	TGTTGTTGGGAGAGGGTATGAG	CTCTCGCCATTCTTCAAGTTCA
3005 F	CGCGGTGTTCTCACACAC	TGTGAATTCCGCGGGTATAG
3016 F	TTGTGGCTGAAGAAGAGATCC	AATGTGGGGAGAGACACACG
3020 F	GTTCCATGGAGCTGGAAGTC	GCTAGAACAGGGCCGTTACA
3035 F	GCCAAGGAGGTCAAGATCG	ACACGACTCGACTCAGACCA
3039 F	GGCACGAGGGGCTAAGTAA	GGAACGCCGAGTACACAGAT
CTM-1 F	TCTGGGGATTGGCTGGAATTACA	AAGTTGGGTAACGCCAGGGTTTTC
CTM-2 F	GGTGATTAAAATCGAGGGTT	AGCAACTTGAGCAGCGG

CTM-3 F	GTCCATCGTCGCCGACGAA	GGATTTGCTAGTTGTGGGCT
CTM-8 F	GCTGCATCGGAGATAGGGAA	CTCAGCAAGCACGCTGCTCT
CTM-9 F	GCCTCCTCTTGATACCATATT	TAGCCTTGGCTGCTATATTC
CTM-12 F	GTTGCAAGCAGGAGTAGATCGA	CGCTCTGTAGGTTGAACTCCTT
CTM-26 F	GCAAGTGATCCATGACATTACGA	ACTTGCTAGCTGCTGCTCTTG
CTM-55 F	CGTCTTCTACCACGTCCT	CATAATCCCACTCAACAATCC
CTM-58 F	TACGTGCTACAAGAATGG	GCTGGCTAGGACACAA
CTM-60 F	AAGCCCCGATCACATCAA	AGCCGAGCCTCATCCC
ICMP3050 F	ATGTCCAGTGTTGACGGTGA	CGGGGAAGAGACAGGCTACT
ICMP3024 F	ATCGAGGCCAAGTACGTGAT	CGAGCTTCTAGCTCCAATCC
ICMP 3025 F	GTTGCAGATGAGCGATCGTA	CGCCGACCAAGAACTTCATA
ICMP3063 F	TCCGGTAGAGACCGTAATGG	GGCACTCCCTAGCAAAATGA
ICMP3032 F	GCGTAGACGGCGTAGATGAT	CAACAGCATCAAGCAGGAGA
ICMP3042 F	TAGTTAATGGGGGTGCGTGT	AAGCACCATCAGCATACCC
ICMP3043 F	TCCTGTACAAGGACGTGCAG	TATCGACGCCAACGATACTG
ICMP3045 F	ACAAGGACGACAAGGACCAC	CCTCTCCAAGCACATGTTTC
ICMP3080 F	CAAACAGCATCAAGCAGGAG	GCGTAGACGGCGTAGATGAT
ICMP3081 F	ACGCCGTTTTCGTGTAGTCT	TCCACAAGGTGACCTCACTG

Results and Discussion

Qualitative and quantitative estimation of DNA

Genomic DNA was isolated and its quality and quantity were determined using 0.8% agarose gel electrophoresis with -DNA as a control.On agarose gel electrophoresis, a single high molecular weight band demonstrated that genomic DNA was intact and free of mechanical and enzymatic degradation and it was then used for the PCR amplification step.

PCR Amplification using SSR markers

A total of 50 SSR markers were used for genetic diversity analysis in this investigation. Out of 50 markers 25 markers showed polymorphism **(Fig 1-2)** and the results of amplification of these 25 markers are presented in the **Table 2.** Among these 25 polymorphic SSR markers, the maximum PIC value was observed in SSR marker xpsmp 2251, which was 0.6704. The range of PIC values varied from 0.1239 (3035) to 0.6704 (xpsmp 2251) **(Table 3).**These results are comparable with some of the previous studies on pearl millet by Stich *et al.* (2010), Bashir *et al.* (2015), Hemender *et al.* (2018) and Kumar *et al.* (2020).

Table 2:- Results of SSR markers

Markers	Number of markers
Number of markers used	**50**
Number of markers that show amplification	**48**
Number of markers that did not show amplification	**2**
Number of polymorphic markers	**25**
Number of monomorphic markers	**23**

Cluster analysis using SSR markers

The presence or absence of the band was used to assess the data supplied by 25 polymorphic markers. The presence of a band was assigned a value of 1 and the lack of a band was assigned a value of 0, which was then utilized to cluster the dendrogram with the help of power marker software **(Fig 3).**

The UPGMA cluster analysis led to the grouping of 14 pearl millet parental genotypes into two major clusters at 67.5 % similarity index. The major cluster consists of 10 parental genotypes *viz*., EMRL-14/123, EMRL-14/237, EMRL-14/243, HMS 53 A_4, HMS 54 A_5, AC 04/13, H77/833-2-202, EMRL-14/111, HMS 30A_{egp}, EMRL-14/105 and the second cluster consists of four parental lines *viz*., HMS 58 A_1, EMRL-14/103, EMRL-14/127, EMRL-15/109. The current molecular clustering results revealed that considerable genetic diversity exists among the selected parents, which can be used in breeding programs. Similar results have been reported by Govindaraj *et al.* (2009), Nepoleon *et al.* (2012), Singh *et al.* (2013), Adeoti *et al.* (2017), Ambawat *et al.* (2020) and Bougma *et al.* (2021). The diversity studied through molecular marker analysis is a time-saving and feasible technique for selecting the genotypes to be used in the breeding program.

Table 3:- List 25 SSR primers showingpolymorphism

Sr. No.	MARKER	Amplification Range (bp)	PIC
1	**xpsmp 2074**	110-190	0.3249
2	**xctm10**	185-400	**0.5680**
3	**xicmp 3002**	200-750	**0.6122**
4	**xpsmp 2232**	230-250	0.3698
5	**xipes 0105**	170-185	0.3750
6	**xpsmp 2251**	130-210	**0.6704**
7	**xpsmp 2249**	120-150	0.2800
8	**icmp 3081**	210-230	0.3538
9	**ctm3**	250-450	0.3750
10	**ctm12**	330-350	0.2149
11	**ctm9**	180-200	0.3457
12	**ctm 58**	260-480	0.3698
13	**ctm 26**	260-280	0.2800
14	**xpsmp 2273**	260-290	0.3698
15	**xpsmp 2070**	180-220	**0.5291**
16	**xicmp 3088**	180-200	0.3698
17	**xcmp 3032**	210-220	0.2149
18	**xpsmp 2231**	220-250	0.3538
19	**xpsmp 2204**	320-350	0.3249
20	**xpsmp 2203**	330-250	0.3538
21	**xipes0004**	120-280	0.3737
22	**xpsmp 2267**	170-210	0.2503
23	**3035**	190-210	0.1239
24	**icmp 3042**	210-220	0.3538
25	**icmp 3045**	300-320	0.3698

Fig 1:- Polymorphism in Fourteen different genotypes of pearl millet by using SSR primer icmp 3081

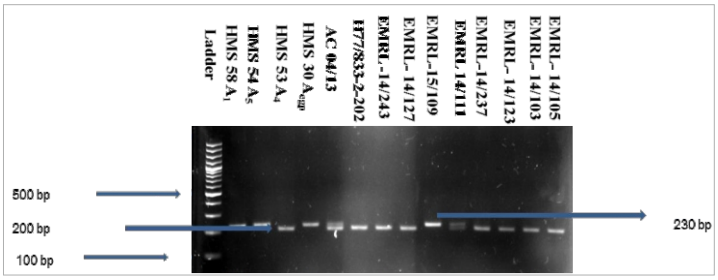


Fig 2:- Polymorphism in Fourteen different genotypes of pearl millet by using SSR primer xpsmp 2074

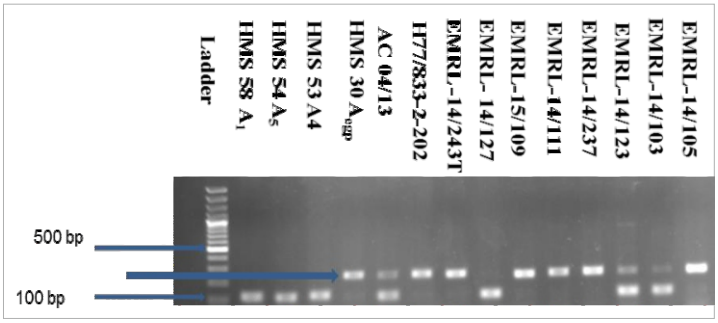
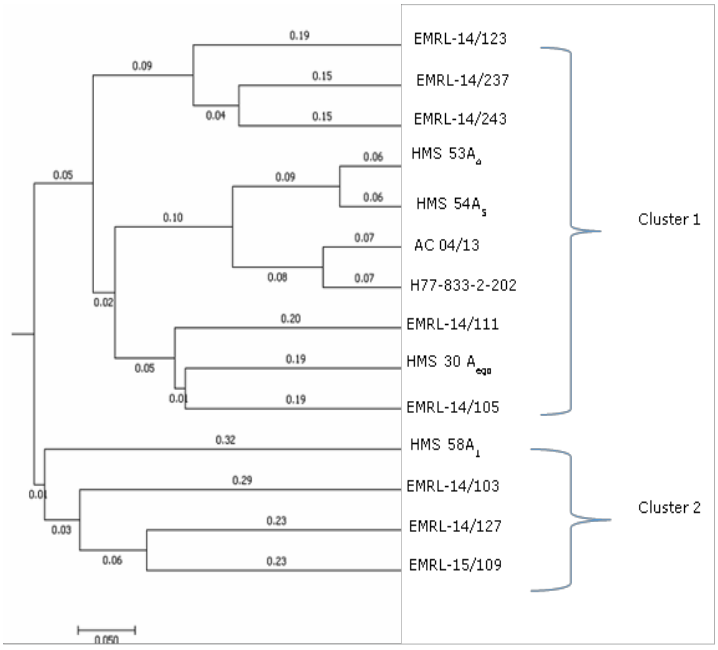


Fig 3:- UPGMA dendrogram showing relationship among fourteen pearl millet genotypes based on similarity matrix data using 25 SSR markers



Conclusion

The power marker UPGMA cluster analysis led to the grouping of 14 pearl millet parental genotypes into two major clusters at a 67.5% similarity index. The major cluster consists of 10 parental genotypes *viz*., EMRL-14/123, EMRL-14/237, EMRL-14/243, HMS 53 A_4, HMS 54 A_5, AC 04/13, H77/833-2-202, EMRL-14/111, HMS 30A_{egp}, EMRL-14/105 and the second cluster consists of four parental lines *viz*., HMS 58 A_1, EMRL-14/103, EMRL-14/127, EMRL-15/109. The current molecular clustering results revealed that considerable genetic diversity exists among the selected parents, which can be used in breeding programmes.

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