www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(3): 778-781

www.thepharmajournal.com
Received: 19-12-2021
Accepted: 22-01-2022

Jyoti Kaushik
Department of Genetics and Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University, Hisar-Haryana, India

Dev Vart
Department of Genetics and Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University, Hisar-Haryana, India

Corresponding Author:
Jyoti Kaushik
Department of Genetics and Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University, Hisar-Haryana, India

Molecular Characterization of pearl millet [*Pennisetum glaucum* (L.) R.Br.] genotypes using Microsatellite markers

Jyoti Kaushik and Dev Vart

Abstract
Bajra also known as bulrush or cattail millet belonging to the family Poaceae is a multipurpose cereal which is grown for both grain and fodder. Among 60 genotypes of pearl millet, DNA was extracted from best selected genotypes of pearl millet by CTAB extraction method. Out of 60 primers used, 30 polymorphic markers have been identified in which only five markers have values above 0.70 and these markers can be considered as highly useful for differentiation of pearl millet germplasm lines. A total of seven major clusters have been identified using UPGMA cluster tree analysis. The present study reveals that SSRs are effective markers for the assessment of genetic diversity in germplasm lines of pearl millet.

Keywords: SSRs, pearl millet, cluster tree, CTAB, PIC

1. Introduction
Pearl millet was originally originated at Africa and was subsequently introduced into India. Since pre-historic times, this crop was grown for grain and fodder in semi-arid and tropical regions of Africa and Asia. It is considered as fourth most important cereal crop after rice, wheat and maize. It is comparable to or even better in some of the nutritional characteristics than major cereals, with respect to its energy value, protein, fat and minerals content (Abdalla *et al.* 1998) [1]. Genetic variability plays a key role in the improvement of target traits as it offers natural and artificial selection to tailor genotypes to better suit diverse agro-ecological conditions. If more genetic variability present in the base material, there will be better chances for improvement. Pearl millet exhibits a tremendous amount of variation at both phenotypic and genotypic levels due to its high out-crossing rate. It is an ideal species for basic and applied research due to low chromosome number, short life cycle, easy selfing and crossing *etc*. The development of DNA based markers such as RFLP and SSR in pearl millet is viewed as a major milestone in providing pearl millet geneticists and breeders with user-friendly and higher efficient molecular markers for use in diversity analysis, marker assisted breeding and varietal characterization. Genetic diversity can be easily studied using PCR-based marker. These markers offer precise means to measure genetic diversity and affinity among germplasm collections than the morphological and biochemical markers due to their environmental insensitivity and abundance in genome. Molecular genetic markers, based on DNA sequence polymorphism (*i.e.* SSR markers), have been applied successfully to accelerate and refine assessment of genetic diversity among genotypes in a wide range of plant species (Chowdari *et al.* 1998; Poland and Rife 2012; Chen *et al.* 2013) [4, 9, 3]. A number of germplasm lines have been developed at CCS Haryana Agricultural University over years. Although these lines have been developed from diverse genetic backgrounds and with diverse morphological traits, these had not been evaluated comprehensively both at phenotypic and genotypic levels. Therefore, the present investigation was carried out with the objective to study genetic diversity of pearl millet lines using PCR-based markers.

Materials and Methods
The present investigation i.e. genotyping was carried out by taking 60 genotypes of pearl millet including hybrids, composites, maintainer lines and white grain lines. All the lines were made available from Bajra Section. The details of DNA extraction and molecular markers used are described as follow:

Molecular markers

A total of 60 Simple Sequence Repeat (SSR) markers widely distributed on different pearl millet linkage groups were used in this study. All these primers were synthesized from Sigma Chemicals Co., India. DNA was extracted from 2-3 weeks old seedlings of pearl millet genotypes and used for genotyping using SSR markers.

Genomic DNA isolation: Genomic DNA was isolated from 2-3 weeks old seedlings of best selected genotypes of pearl millet by CTAB (Cetyltrimethyel ammonium bromide) extraction method as given by Murray & Thompson (1980) and modified by Saghai-Maroof *et al.* (1984) and Xu *et al.* (1994) [11, 13].

Quality and quantity of DNA was estimated by both UV spectroscopy and agarose gel electrophoresis.

PCR amplifications were performed using Applied biosystems the rmalcycler. The PCR reaction was carried out in a reaction volume of 20 µl containing 10X PCR buffer, 100 µM each of dNTPs, 0.4 µM of each of forward and reverse primer, 1 unit Taq DNA polymerase and 50 ng template DNA. PCR amplification was performed with initial denaturation at 94^0C for 5 minutes followed by 10 cycles of denaturation at 94˚C for 25sec, annealing at 64˚C - 54˚C (touch-down cycles with -1ºC per min/cycle) for 20sec, and extension at 72˚C for 30sec, followed by 40 cycles of denaturation at 94˚C for 25sec, annealing at 56˚C for 20sec and extention at 72˚C for 30sec, followed by final extension at 72˚C for 20minutes. Amplification products were stored at -20˚C in case these were not separated on agarose gel.

PCR amplified DNA fragments for DNA markers were separated and visualized by submerged horizontal electrophoresis in 1.5% (w/v) agarose gels. Samples were prepared by adding 6X loading dye (Sucrose 4g, bromophenol blue 0.025g, xylene cyanol 0.025g, final volume 10 ml) and pulse centrifuged for proper mixing. 12 µl of PCR products were loaded in the wells and electrophoresis was carried out at constant voltage (3 v/cm of gel) until dye migrated to other end of the gel along with 100 bp ladder. PCR amplified products were visualized under UV light using Gel documentation system.

Molecular data analysis

SSR amplification profiles were scored visually, based on presence (taken as 1) or absence (taken as 0) of bands. The size of the most intensely amplified band for each microsatellite marker was determined based on its migration relative to molecular weight size marker (100 bp DNA ladder from Sigma Chemicals Co. USA). 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 using distance matrix by the unweighted pair group method using arithmetic averages (UPGMA) of JMP 8.0 Software.

Results

SSR markers based polymorphism among pearl millet germplasm lines

In present study, thirty polymorphic markers have been identified (Table 1) which were dispersed throughout the pearl millet genome. A total number of alleles detected which collectively yield unique SSR profiles for all studied germplasm lines were 88. The range of allele was from 1 (*Xpsmp2070, Xpsmp2084, Xpsmp2008, Xpsmp2089, Xipes0216, Xipes0009, Xipes0114, Xpsmp2018)* and 2 (*Xpsmp2088, Xpsmp2066, Xpsmp2019, Xpsmp2040, Xpsmp2063, Xipes0042, Xctm21, Xctm10, Xpsmp2086, Xipes0079, Xpsmp2006, Xpsmp2232, Xpsmp2081*) to 3 (*Xpsmp2237, Xpsmp2027, Xpsmp 2076, Xpsmp2050, Xicmp3017, Xipes0146, Xpsmp2001, Xicmp3080*). Summarized data for the number of alleles detected, band size and polymorphism information content (PIC) values for each of the SSR loci are presented in Table 2. The average number of SSR alleles per locus was 4.1. The range of PIC values for 60 germplasm lines was from 0.20 (*Xctm21)* to 0.79 (*Xpsmp2089*). Out of 30 SSR loci, only five *i.e. Xpsmp2070, Xpsmp2066, Xpsmp2063, Xpsmp2089* and *Xpsmp2001* have values above 0.70 and these five markers can be considered as highly useful for differentiation of pearl millet germplasm lines.

Table 1: Allelic diversity among sixty germplasm lines as assessed by SSR markers

Number of primers used	60
Number of alleles	88
Range of alleles	1-3
Average number of alleles	2.7
Number of polymorphic primers	30
Number of monomorphic primers	6

Table 2. List of SSR primers showing polymorphism

S. No.	Polymorphic primers	Number of Alleles	Band size (bp)	Linkage group	PIC values
1	*Xpsmp2070*	1	220-310	LG3	0.73
2	*Xpsmp2237*	3	220-280	LG2	0.67
3	*Xpsmp2088*	2	130-200	LG2	0.68
4	*Xpsmp2084*	1	210-250	LG4	0.66
5	*Xpsmp2066*	2	150-300	LG2	0.73
6	*Xpsmp2027*	3	230-280	LG7	0.55
7	*Xpsmp 2076*	3	150-200	LG4	0.64
8	*Xpsmp2008*	1	200-280	LG4	0.62
9	*Xpsmp2050*	3	100-150	LG2	0.64
10	*Xpsmp2019*	2	200-300	LG7	0.67
11	*Xpsmp2040*	2	150-180	LG7	0.25
12	*Xpsmp2063*	2	120-500	LG7	0.76
13	*Xicmp3017*	3	200-240	LG1	0.67
14	*Xpsmp2089*	1	130-400	LG2	0.79
15	*Xipes0146*	3	150-200	LG1	0.61
16	*Xipes0216*	1	180-220	LG1	0.60
17	*Xipes0042*	2	410-470	LG1	0.66

18	*Xctm21*	2	300-380	LG2	0.20
19	*Xpsmp2001*	3	200-440	LG5	0.72
20	*Xctm10*	2	180-200	LG3	0.25
21	*Xpsmp2086*	2	110-130	LG4	0.48
22	*Xipes0079*	2	210-230	LG1	0.48
23	*Xicmp3080*	3	180-220	LG1	0.43
24	*Xipes0009*	1	130-150	LG1	0.52
25	*Xppsmp2006*	2	200-220	LG1	0.64
26	*Xpsmp2232*	2	150-170	LG1	0.55
27	*Xipes0114*	1	130-150	LG4	0.62
28	*Xpsmp2081*	2	160-190	LG4	0.25
29	*Xpsmp2018*	1	210-240	LG6	0.61
30	*Xpsmp2270*	3	100-140	LG6	0.30

Cluster analysis

The UPGMA cluster tree analysis led to the grouping of sixty germplasm lines into two major groups which were further subdivided into seven major clusters. Further, seven clusters grouped all the sixty germplasm lines in such a way that lines within each cluster had high similarity than those in other clusters (Table 3). Cluster pattern revealed that, cluster1 was the largest consisting of 26 germplasm lines. This was followed by cluster2 (23 germplasm lines), cluster4, cluster5 and cluster6 (3 germplasm lines each) and cluster3 and cluster7 (1 germplasm line each).

Table 3. Distribution of sixty germplasm lines in different clusters based on SSR markers

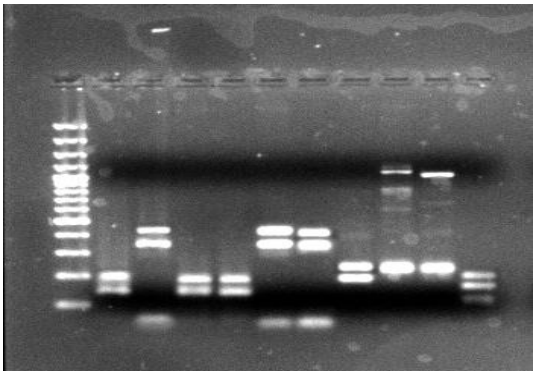
Cluster	Germplasm lines	Number of germplasm lines
Cluster1	HHB 272, HMS 70B, HMS 58B, HMS 33B, HMS 34B, HMS 39B, HMS 38B, HMS 50B, HMS 68B, WHC 802, HMS 55B, HMS 20B, HMS 66B, HMS 40B, HMS 44B, HMS 46B, HMS 42B, HMS 69B, HMS 43B, HMS 45B, HMS 48B, WG 33-4, WG 35-4Y, WG 35-4, HMS 41B, HMS 21B	26
Cluster2	HHB 234, HHB 226, HHB 223, HMS 13B, HMS 14B, HMS 16B, HMS 26B, HMS 32B, HHB 216, HMS 7B, ICMB 94555, ICMB 843-22, HMS 37B, ICMB 89111, HMS 6B, HHB 67, HHB 117, HHB 94, HC 20, HMS 47B, HHB 146, ICMB 97111, HC 10	23
Cluster3	HHB 197	1
Cluster4	ICMB 94222, 36B×9422B, 04999B	3
Cluster5	78/711, HBL 34, HBL 11	3
Cluster6	EBL 12/237, SGP 10-107, ISK 51	3
Cluster7	HB 15/085	1

Discussion

Molecular markers are very helpful to breeders in understanding the complex traits without measuring the phenotypes and reduce the need of extensive field testing over time and space. Recently, SSRs are considered as more reliable DNA marker because of their multi-allelism, genome specificity, even distribution, easy detection and high polymorphism. Out of 60 primers, 30 polymorphic primers (Table 2) were found which produced a total of 88 alleles. The number of alleles detected per primer pair ranged from 2 to 3 with an average of 4.1 alleles per primer. It was observed that PIC value was highest for the SSR primer *Xpsmp2089* (0.79) followed by *Xpsmp2063* (0.76) and lowest for the primer *Xctm21* (0.20). It is considered that higher the PIC value, the more informative is the SSR marker and hence, primer *Xpsmp2089* was found to be highly informative. Based on the dendrogram, the sixty genotypes of pearl millet were grouped into seven main clusters (Table 3). Among the seven clusters, cluster I was the largest comprising of twenty six genotypes. The second cluster comprised of twenty three germplasm lines followed by three genotypes each in cluster IV, V and VI, one genotypes each in cluster III and VII. Clustering using SSR markers was also done by Kapila *et al.* (2007); Nepolean *et al.* (2012); Sumanthi *et al.* (2013) and Kannan *et al.* (2014). Ramya *et al.* (2018) [6, 8, 12, 5, 10] genotyped 343 hybrid parental (maintainer (B-) lines and restorer (R-) lines using 88 polymorphic SSR markers and found that the range of alleles was from 2 (*Xipes0142*, *Xipes0079*, *Xipes0026*, *Xipes0205*, *Xpsmp2235*, *Xpsmp2253* and *Xipes0147*) to 28 (*Xpsmp2070*) alleles per locus, followed by *Xipes0233* (21), *Xipes0027* (17) and *Xipes0098* (16). The PIC values ranged from 0.02 (*Xipes0147*) to 0.90 (*Xpsmp2070*). Ten and eleven clusters were created for B- and R-lines, respectively. Adeoti *et al.* (2017) [2] used 14 polymorphic SSR markers to screen 114 accessions and found a total of 57 alleles with an average of 4.07 alleles per locus.

Conclusion

The present study demonstrates that SSRs are effective markers for the assessment of genetic diversity in germplasm lines of pearl millet. The study reveals that the number of alleles detected for a SSR marker can be a good indicator to assess PIC. And it can also be said that selection of the markers based on higher repeat number will be more efficient for genetic diversity studies.



Polymorphism in different ten hybrid lines of pearl millet using primer *Xipes0079*

[HHB272, HHB234, HHB226, HHB223, HHB216, HHB67

(Improved), HHB197, HHB117HHB146, HHB94]

References

1. Abdalla AA, Tinay AH, Mohamed BE, Abdalla AH. Proximate composition, starch, phytate and mineral contents of ten pearl millet genotypes. Food Chemistry. 1998;63(2):243-246.
2. Adeoti K, Gustave D, Eben-Ezer E, Thierry B, Sylvain S, Alain R, Estelle J. Assessment of genetic diversity among cultivated Pearl millet (Pennisetum glaucum, Poaceae) accessions from Benin, West Africa. African Journal of Biotechnology. 2017;16(15):782-790.
3. Chen F, Gao M, Zhang J, Zuo A, Shang X, Cui D. Molecular characterization of vernalization and response genes in bread wheat from the Yellow and Huai Valley of China. BMC Plant Biology. 2013;13:199.
4. Chowdari K, Venkatachalam S, Davierwala A, Gupta V, Ranjekar P, Govila O. Hybrid performance and genetic distance as revealed by the (GATA) 4 microsatellite and RAPD markers in pearl millet. Theoretical & Applied Genetics. 1998;97:163-169.
5. Kannan B, Senapathy S, Raj AGB, Chandra S, Hash CT. Association analysis of SSR markers with phenology, grain, and stover-yield related traits in Pearl Millet [Pennisetum glaucum (L.) R. Br.]. The Scientific World Journal. 2014;1:1-14.
6. Kapila RK, Yadav RS, Plaha P, Rai KN, Yadav OP, Hash CT, Howarth CJ. Genetic diversity among pearl millet maintainers using microsatellite markers. Plant Breeding. 2007;127:33-37.
7. Murray MG, Thompson WF. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Research. 1980;8:4321-4325.
8. Nepolean T, Gupta SK, Dwivedi SL, Bhattachajee R, Rai KN, Hash CT. Genetic Diversity in Maintainer and Restorer Lines of Pearl Millet. Crop Science. 2012;52:2555-2563.
9. Poland JA, Rife TW. Genotyping-by-Sequencing for Plant Breeding and Genetics. Plant Genome. 2012;5:92-102.
10. Ramya RA, Lal Ahamed, M Tara, Satyavathi C, Rathore A, Katiyar P, *et al*. Towards Defining Heterotic Gene Pools in Pearl Millet [Pennisetum glaucum (L.) R. Br.]. Frontiers in Plant Science. 2018;8:1-11.
11. Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allad RW. Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, Chromosomal location and Population dynamics. Proceedings of National Academy of Sciences, U.S.A. 1984;81:8014-8019.
12. Sumanthi M, Sumathi P, Vinodhana NK, Sathyam. Assessment of genetic distance among the inbred lines of Pearl Millet [Pennisetum glaucum (L.) R. Br.] using SSR markers. International Journal of Biotechnology & allied Fields. 2013;1(3):153-162.
13. Xu Y, Shimoro X, Hofstra H. Plant DNA isolation protocol. Nucleic Acids Research. 1994;22:2399-2403.