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GENOTYPIC DIVERSITY IN PEARL MILLET [*PENNISETUM GLAUCUM* (L.) R. BR.] MAINTAINER LINES USING SSR MARKERS

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SUMMARY

Pearl Millet [*Pennisetum glaucum* (L.) R.Br.] is one of the staple food and fodder crop of the arid and semi-arid regions of Africa and Asia. Pearl millet displays enormous phenotypic and genotypic diversity. Assessment of available genetic diversity in pearl millet through the use of molecular markers will supplement the conventional breeding programme. Keeping this in view, the present study was undertaken to evaluate genetic diversity among forty eight maintainer lines of pearl millet with twenty two SSR primers which produced a total of 85 alleles. Three out of twenty two polymorphic primers showed PIC value of more than 0.70 *i.e. Xpsmp2066, Xpsmp2089* and *Xpsmp2063*. The maximum number of ten amplified products was observed in the profiles of the primer *Xpsmp2001*. The highest PIC value is observed for *Xpsmp2089* with value (0.78). The molecular data grouped the forty eight genotypes of pearl millet into eight main clusters which revealed considerable genetic diversity among the maintainer lines. Among the eight clusters, cluster II was the largest comprising eighteen genotypes. So, SSRs are effective markers for the assessment of genetic diversity in maintainer lines of pearl millet. The diversity assessed can be manipulated to broaden the genetic base of maintainer line for the development of commercial hybrid varieties.

Key words : Pearl millet, genotypic diversity, SSR primers, molecular data, clustering

Genetic diversity is important in driving survival during natural selection and for adaptation and conservation of desired traits. For exploration of plant genetic resources which have potential applications in crop improvement, genetic diversity is considered as an important aspect. Pearl Millet [*Pennisetum glaucum* (L.) R. Br.] known as bulrush or cattail millet is a hardy crop that is the staple cereal crop for the hottest, most arid regions where dry land agriculture is practiced and it is basically cultivated as a rainfed crop, largely under marginal environment and with no or little external inputs where grain yields vary mostly between 400 and 900 kg per ha. It is grown widely in different parts of world for grain, stover, and green fodder on about 27 million hectares of land, primarily in Asia and Africa (Chakauya, 2002). Pearl millet is rich in several nutrients as well as non-nutrients such as phenols. It has 70% starch in dry grain, 8 to 12% protein, 5-7% fat, high fiber (1.2g/ 100g, most of which is insoluble), high iron (30-146 ppm), zinc (25-94 ppm) and calcium (13-52 mg/100g) and is gluten free. Pearl millet contains antioxidants which may be beneficial for the overall health and wellbeing. Pearl millet is a rich source of energy (361 Kcal/100g) which is greater than sorghum and nearly equal to that of brown rice because the lipid content is generally higher (3 to 6%) (Nambiar *et al.* 2011). Pearl millet is an ideal species for genetic studies because of its small diploid genome with large chromosome, abundant phenotypic and genotypic diversity. Genetic variability studies provide basic information which is helpful to know about the nature and extent of variability which is useful in any crop improvement venture depends mainly on the magnitude of genetic variability and heritability present in the source material (Sumathi *et al*. 2010). In heterosis breeding, understanding genetic relationship among parental lines is of paramount importance**.**

In many studies genetic diversity among inbred lines or genotypes is being usually assessed based on morphological characters, which although provides meaningful information but is also affected by environmental factors especially the quantitative traits. Molecular markers allow breeders to dissect complex traits without having to measure the phenotypes, thus reducing the need for extensive field-testing over time and space. Of the various DNA markers, recently simple sequence repeats (SSRs) have

been adjudged as more reliable DNA markers for such studies because of their genome specificity, even distribution, high polymorphism and easy detection. Therefore, the present investigation was carried out with the objective to study genetic diversity of pearl millet lines using SSR markers.

MATERIALS AND METHODS

Genomic DNA was isolated from 2-3 week old seedlings of 48 pearl millet genotypes by CTAB (Cetyltrimethyhel ammonium bromide) extraction method as given by Murray and Thompson (1980) and modified by Saghai-Maroof *et al*. (1984) and Xu *et al*. (1994).

RESULTS

Twenty two polymorphic SSR markers identified in the present study (Table 1) were dispersed throughout the pearl millet genome. A total of 85 alleles were detected, collectively yielding unique SSR profiles for all the 48 maintainer lines. Summarized data for the number of alleles detected per SSR locus analyzed as well as 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 3.04, with a range from 1 (*Xpsmp2086*, *Xpsmp2040*, *Xctm10*, *Xipes0079*, *Xctm21*) to 3 (*Xpsmp2001*). PIC values of various SSR loci across all the 48 maintainer lines ranged from 0.12 (*Xpsmp2040*) to 0.78 (*Xpsmp2089*) with an average of 0.56 per locus. It is significant to note that 3 out of 22 SSR loci, namely *Xpsmp2066*, *Xpsmp2089*, *Xpsmp2063* revealed PIC values above 0.70, can be considered highly useful for differentiation of pearl millet maintainer lines. Figure 1 showing polymorphism of primer *Xpsmp2008* in twenty six out of forty eight maintainer lines of pearl millet.

TABLE 1
Allelic diversity among forty eight maintainer lines as assessed by SSR markers

Number of primers used	70
Number of alleles	85
Range of alleles	1-3
Average number of alleles	3.04
Number of polymorphic primers	22
Number of monomorphic primers	6

The UPGMA cluster tree analysis led to the grouping of forty eight maintainer lines in eight major groups. Further, eight clusters grouped all the forty eight maintainer lines in such a way that maintainer lines within each cluster had high similarity than those in other clusters (Table 3). Cluster pattern revealed that, cluster2 was the largest consisting of 18 maintainer lines. This way followed by cluster5 (10 maintainer lines), cluster8 (6 maintainer lines), cluster6 (5 maintainer lines), cluster4 (4 maintainer lines), cluster1 (3 maintainer lines) and cluster3 and cluster7 (3 maintainer lines).

DISCUSSION

Molecular markers allow breeders to dissect complex traits without having to measure the phenotype, thus reducing the need for extensive field-testing over time and space. Information provided by the genetic markers is used to design optimal procedures to manage extensive germplasm collections, in particular to highlight priorities in further sampling missions, to design germplasm regeneration programs and to construct core collections. Of the various DNA markers, recently SSRs have been adjudged as more reliable DNA markers for such studies because of their multi-allelism, genome specificity, even distribution, high polymorphism and easy detection.

Out of 70 primers, 22 polymorphic primers were found which produced a total of 85 alleles. The number of alleles detected per primer pair ranged from 1 to 3 with an average of 3.04 per primer. In the study done by Gupta *et al.* (2015), the combined clustering analysis of 379 hybrid parents (213 previously designated parents of set-I and 166 newly developed parents of set-II) based on Polymerase Chain Reaction (PCR) products detected by 28 SSR primer pairs, showed 12.68 alleles per locus while Bashir *et al.* (2015) observed 13.3 alleles per locus. For instance, Sumathi *et al.* (2010) reported 2.76 alleles per primer pair among 42 inbred lines bred primarily at Tamil Nadu Agricultural University, Coimbatore, India. Chandra-Shekara (2007) found the PIC values ranged from 0.24 to 0.60 for the SSR markers.

PIC value was highest for the SSR primer *Xpsmp2089* (0.78) followed by *Xpsmp2063* (0.75) and lowest for the primer *Xctm21* (0.04). The 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 forty eight genotypes of pearl millet were grouped into eight main

TABLE 2
List of SSR primers showing polymorphism

S. No.	Polymorphic primers	Number of alleles	Band size (bp)	Linkage group	PIC values
1.	*Xpsmp2070*	2	220-310	LG3	0.69
2.	*Xpsmp2001*	3	200-440	LG5	0.69
3.	*Xpsmp2008*	1	200-280	LG4	0.54
4.	*Xctm10*	1	150-200	LG3	0.25
5.	*Xpsmp2237*	2	220-280	LG2	0.59
6.	*Xpsmp2086*	1	110-150	LG4	0.48
7.	*Xpsmp2027*	2	230-280	LG7	0.50
8.	*Xpsmp2066*	2	150-300	LG2	0.72
9.	*Xpsmp2084*	2	210-250	LG4	0.59
10.	*Xpsmp2019*	2	200-300	LG7	0.64
11.	*Xpsmp2088*	2	130-200	LG2	0.68
12.	*Xctm21*	1	300-380	LG2	0.04
13.	*Xpsmp2089*	2	130-400	LG2	0.78
14.	*Xipes0042*	2	410-470	LG1	0.65
15.	*Xipes0146*	2	150-200	LG1	0.59
16.	*Xpsmp2063*	2	120-500	LG7	0.75
17.	*Xpsmp 2076*	2	150-200	LG4	0.62
18.	*Xpsmp 2050*	2	100-150	LG2	0.62
19.	*Xpsmp 2040*	1	150-180	LG7	0.12
20.	*Xipes 0216*	2	180-220	LG1	0.57
21.	*Xipes 0079*	1	210-230	LG1	0.48
22.	*Xicmp 3017*	2	200-240	LG1	0.67

TABLE 3
Distribution of forty eight pearl millet maintainer lines in different clusters based on SSR markers

Clusters	Maintainer lines	Number of maintainer lines
Cluster1	HMS 6B, HMS 23B, HMS 29B	3
Cluster2	HMS 49B, HMS 56B, HMS 53B, HMS 58B, Tift 23 D2B, HMS 61B, HMS 55B, HMS 59B, HMS 63B, ICMB 97111, HMS 60B, HMS 62B, 81B, ICMB 843-22, ICMB 94555, HMS 52B, HMS 54B, HMS 64B	18
Cluster3	HMS 51B	1
Cluster4	HMS 45B, HMS 46B, HMS 48B, HMS 47B	4
Cluster5	HMS 7B, HMS 13B, HMS 16B, HMS 41B, HMS 42B, HMS 38B, HMS 39B, HMS 40B, HMS 43B, HMS 44B	10
Cluster6	HMS 18B, HMS 32B, HMS 33B, HMS 36B, HMS 34B	5
Cluster7	HMS 20B	1
Cluster8	HMS 21B, HMS 30B, HMS 26B, HMS 28B, HMS 22B, HMS 37B	6

clusters (Table 3). Among the three clusters, cluster II was the largest comprising of eighteen genotypes. The first cluster comprised of three maintainer lines followed by eighteen genotypes, one genotype, four genotypes, ten genotypes, five genotypes, one genotype and six genotypes in the subsequent clusters. Bashir *et al.* (2015) identified a total of seven phylogenic groups with variable sizes and the average PIC values obtained across the seven linkage groups varied signi?cantly from 0.40 to 0.91. Clustering using SSR markers was also done by Gupta *et al.* (2015), Kannan *et al.* (2014), Sumanthi *et al.* (2013), Nepolean *et al.* (2012).

CONCLUSION

The present study demonstrates that SSRs are effective markers for the assessment of genetic diversity in maintainer 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 that selection of the markers based on higher repeat number will be more efficient for genetic diversity studies.

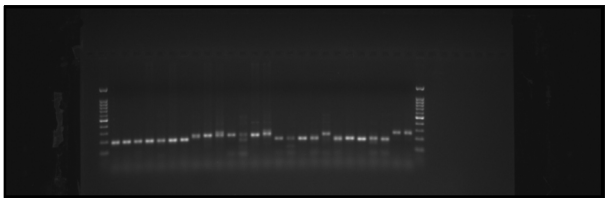

Fig. 1. Polymorphism in twenty six maintainer lines of pearl millet using primer *Xpsmp2008.*
(1-HMS 6B, 2-HMS 7B, 3-HMS 13B, 4-HMS 16B, 5-HMS 18B, 6-HMS 20B, 7-HMS 21B, 8-HMS 22B, 9-HMS 23B, 10-HMS 26B, 11-HMS 28B, 12-HMS 29B, 13-HMS 30B, 14-HMS 32B, 15-HMS 33B, 16-HMS 34B, 17-HMS 36B, 18-HMS 37B, 19-HMS 38B, 20-HMS 39B, 21-HMS 40B, 22-HMS 41B, 23-HMS 42B, 24-HMS 43B, 25-HMS 44B, 26-HMS 45B).

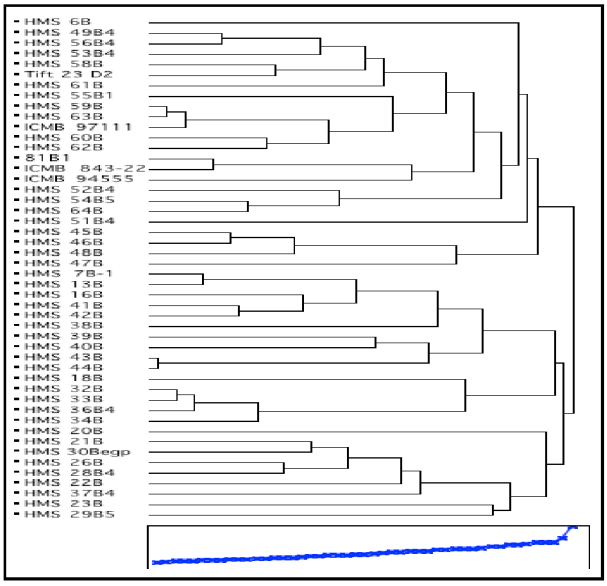

Fig. 2. Dendrogram showing the clustering pattern of forty eight maintainer lines of pearl millet on the basis of genotyping by SSR markers

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