Indian J. Genet., 78(3): 382-385 (2018)
DOI: doi.org/10.31742/IJGPB.78.3.16

Short Communication

Genetic divergence studies in pearl millet [*Pennisetum glaucum* L. (R.) Br.] inbred lines

Ruchika Bhardwaj*, Tosh Garg, Ejaz A. Malik, Yogesh Vikal, R. S. Sohu and S. K. Gupta[1]

Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana 141 004; [1]ICRISAT, Patancheru , Hyderabad 502 324

(Received: January 2018; Revised: July 2018; Accepted: August 2018)

Abstract

In the present investigation genetic diversity amongst 190 pearl millet accessions *viz*., 95 maintainer (B line) and 95 restorer lines (R line) was analyzed using SSR markers. A total of 40 SSR primers were used for screening, out of which 34 were polymorphic. PIC value ranged from 0.002 (PSMP 2068) to 0.749 (CTM 60) with an average of 0.445. AMOVA showed greater variance represented by individuals within the group (65%) and between group (B and R lines) variance was <35%. The neighbor joining tree based on dissimilarity matrix of germplasm lines differentiated B from R lines into two major clusters with few exceptions of R lines grouping with B lines and *vice versa*. Genetic dissimilarity estimates ranged from 0.12 (RPR 22; IPC 917) to 0.85 (ICMR 10222; ICMB 97333). The maintainers ICMB 97333, RPB 6 and ICMB 92777 and restorers ICMR 10222, RPR 25, RPR 56, RPR 62 and RPR 40 were identified as most distinct and divergent which could be used as parents for heterosis breeding.

Key words: Genetic diversity, maintainers, restorer, pearl millet, AMOVA

Pearl millet, locally known as bajra, is fifth most important coarse-grain cereal after rice, wheat, maize and sorghum. It is cultivated on about 30 m ha in more than 30 countries. In India, it is grown on about 7.13 m ha with an average productivity of 1132 kg/ha in 2015-16 (Directorate of Millet Development 2017). The pearl millet exhibits a tremendous amount of diversity at both phenotypic and genotypic levels (Poncet et al. 1998). Systematic exploitation of this diversity through efficient approach is key to long term improvement programme. Characterization of pearl millet germplasm and estimation of parental polymorphism are the imperative means for categorization of germplasm, identification of desirable genotypes and ultimately introgression of these characteristic genotypes into desired breeding programs. In the present investigation, the genetic diversity within and between maintainer and restorer accessions was analyzed using microsatellite markers and estimates of similarity were used to identify diverse accessions/potential parents for development of CMS based hybrids.

DNA of 190 germplasm lines comprising 95 maintainer lines accession nos., ICMB89111, 91777, 92444, 92666, 92777, 93111, 93222, 93333, 94111, 94333, 94444, 95222, 95333, 96666, 97111, 97333, 97444, 98333, 98444, 98777, 99333, 99444, 95555, 00222, 00555, 00666, 00777, 01444, 01666, 01888, 02222, 05222, 05666, 03222, 03555, 03888, 04222, 04555, 04777, 05888, 06444, 07222, 07333, 07555, 07666, 07777, 07999, 08111, 08222, 08999, 09111, 09222, 09333,09444, 09555, 09888, 10555, 11111, 11666, 11888, 11999, PRB1 to PRB31, 1-6156, 1-6125-1 and 1-6125-2 and 95 restorer lines accession nos., ICMR06111, 07222, 07333, 07666, 07888, 07999, 08111, 08333, 08444, 08999, 09111, 09222, 09333, 09666, 09777, 10222, 10333, 10666, 10777, 11111, 11555, 11666, 11777, 11888, 12222, 12444, 12666, 12777, 12999 and RPR1 to RPR64 germplasm lines was isolated from young leaves following CTAB method (Zidani et al. 2005). DNA was amplified *in vitro* through PCR using Forty SSR primers belonging to PSMP and CTM series (Qi et al. 2004; Budak et al. 2003) following standard protocols as given by Qi et

*Corresponding author's e-mail: ruchipau@gmail.com
Published by the Indian Society of Genetics & Plant Breeding, A-Block, F2, First Floor, NASC Complex, IARI P.O., Pusa Campus, New Delhi 110 012; Online management by indianjournals.com; www.isgpb.org

al. (2004). Amplified DNA fragments were resolved in horizontal electrophoresis system using 2.5% agarose gel containing ethidium bromide (1μg/ml) and 1X TAE buffer. The total number of alleles were recorded for each microsatellite (SSR) marker in all the genotypes under study by giving the number to amplified alleles as 1, 2, 3 and so on. The amplified bands were recorded as 1 (present) and 0 (absent) in a binary matrix.

The genetic diversity was computed using computer software programme – DARwin 5.0 (Perrier and Jacquemond-Collet 2006). Dissimilarity matrix for SSR primers was constructed using Jaccard's coefficient of associations to find out genetic relationships. The data were subjected to unwieghted pair group's method with arithmetic mean (UPGMA) analysis to generate dendrogram. The grouping of B and R lines into clusters and subclusters was done at 5% dissimilarity level. Polymorphic information content (PIC) value was estimated using the equation as given by Botstein et al. (1980). Hetrozygosity and gene diversity were calculated with the Power marker 3.25 software (Liu 2005). GenALEX integrated Excel software package (Excoffier et al. 1992) was used to estimate the variance components among and within B and R line groups.

Out of 40 SSR markers only 34 showed polymorphism and a total of 103 polymorphic patterns revealed at least one difference among germplasm lines. The alleles ranged from two to six per locus with an average of 3.029 alleles per locus. Similarly, number of alleles per primer ranged from 2 to 6 with an average of 2.76 per primer as reported by Sumanth et al. (2013). Primer CTM-60 amplified highest number of alleles followed by PSMP 2001 whereas, 11 primers amplified two alleles only (Table 1). The lower number of alleles per SSR locus detected was in comparison with the findings of Stich et al. (2010). This probably due to the inbreds used in the present investigation originated from ICRISAT, Patancheru breeding program which might narrow down the diversity in comparison to the available pearl millet global diversity. Thus the levels of polymorphism is attributed to how diverse are the lines from each other and the type of primers used in the study.

The PIC value ranged from 0.002 (PSMP 2068) to 0.749 (CTM60) with the average of 0.445 values across pearl millet germplasm lines (Table 1). Markers with PIC more than 0.5 are efficient in discriminating genotypes and extremely useful in detecting the polymorphism rate at a particular locus (DeWoody et al. 1995). Fourteen SSR primers recorded PIC values $\geq$0.5 suggesting the high discriminating nature of these markers. PIC value was highest for the primer (CTM 60) with maximum number of alleles. However, there was no association between PIC value and numbers of alleles at a locus. Budak et al. (2003) also could not correlate number of alleles with degree of polymorphism as observed in the present study. Recently evolved microsatellites would have fewer polymorphisms because of fewer occasions for mutation, even if they have long repeats (Xu et al. 2000). The relative gene diversity averaged 0.40 within B lines, 0.40 within R lines, and 0.43 between the two groups of lines, suggesting no significant divergence at molecular level between the parental groups. The level of heterozygosity in SSRs across B and R lines ranged from 0.15 to 0.81 and averaging 0.44. Heterozygosity was not detected at four SSR loci (*psmp3043*, *psmp2090*, *psmp2232*, and *psmp2251*). The R-lines had greater average heterozygosity (0.44) than B-lines (0.43).

All the germplasm lines were also investigated via analysis of molecular variation (AMOVA) (Excoffier et al. 1992), as implemented in GenAlEx, which was used to hierarchically partition genetic variation and revealed significant differences between B and R line groups. Greater variance (65%) was represented by individuals within the group and between group (B and R lines) variance was <35%. The findings that greater variance was represented by individuals within groups, while between groups variance was <33.9% has been supported by Ganapathy et al. (2012) and Nepolean et al. (2012). Parents involved in respective B and R line development are morphologically quite diverse from each other. Maintainer lines are generally bred for short height (<100 cm) and larger seed size while R lines are generally bred for taller height (150-180) cm), more tillers, relatively small seed size, and profuse pollen production (Rai et al. 2006). This distinctness in parents and plant type in the study was also reflected in the marker based clustering pattern, which clearly separated B lines from R lines in two clusters. This distinct grouping of maintainers and restorers is largely due to the fact that separate breeding programmes are being followed for seed parents and their restorers, and more importantly separate gene pool are being maintained to maximize the level of heterosis (Rooney and Smith 2000). Differential selection for certain characters like plant height and flowering time contributed to the distinctive nature of the parental lines.

Genetic dissimilarity estimates as per Jaccard's index of dissimilarity between pairs of lines ranged from 0.12 (RPR 22; IPC 917) to 0.85 (ICMR 10222; ICMB 97333), which indicated the genetic relatedness among these lines and moreover B lines were found more similar based on the dismiliarity matrix. The maximum dissimilarity was observed between ICMB 97333; RPR 27 and ICMB 03888; RPR 25. Earlier in a study by Kapila et al. (2008) reported genetic similarity estimates ranged from 0.05 to 0.73 with an average value of 0.29 in pearl millet. All the 190 germplasm lines were grouped into 2 major clusters and one minor group (Fig. 1). One major group consisted majority of B lines and another group included majority of R lines while minor group consisted of only four lines. Thus, the neighbor joining tree based on simple matching dissimilarity matrix of germplasm lines clearly differentiated B lines from R lines with exceptions of few maintainer lines (ICMR 07888, RPR 40, ICMR 12999 and ICMR 12777) clustered into restorer (R lines) group and *vice versa*. Such results were not unexpected because by considering that these lines might have shared a common pedigree. Similar variation in clustering of B and R lines was reported by Nepolean et al. (2012). Minor group consisted of both B (ICMB05888, ICMB04777) and R lines (ICMR1299, ICMR 12777).

Further clustering of B and R groups, the lines were grouped into 5 and 4 sub clusters, respectively

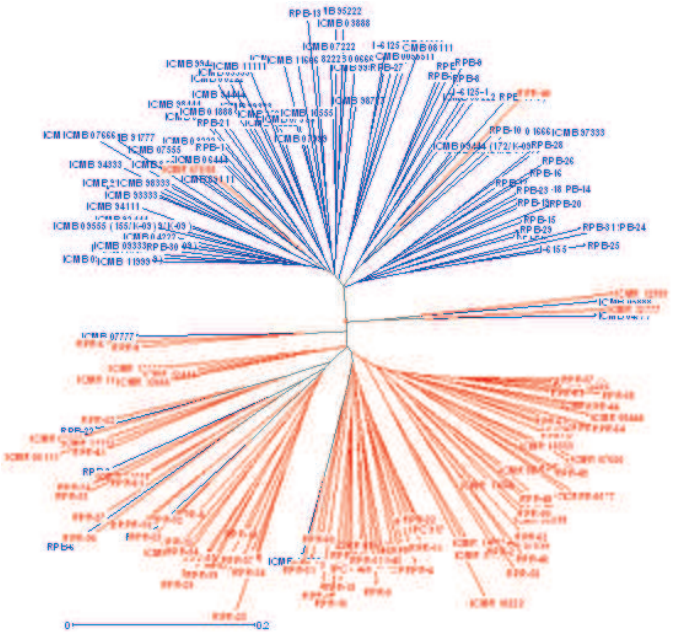

Fig. 1. Unweighted neighbor joining tree based on a simple dissimilarity matrix across 95 B and 95 R lines

which indicate the diversity within these groups. Majority of the RPB lines were clustered in sub cluster I, II and majority of ICMB lines were sub clustered into III, IV and V sub cluster. This again agreed with the differentiation of the lines according to their source of origin. In case of R lines, further grouping clustered the lines into four sub clusters with maximum number

Table 2. Level of polymorphism and PIC values for 34 SSR primers in pearl millet germplasm lines

S.No.	Primer name	Total No. of alleles	PIC value	S. No.	Primer name	Total No. of alleles	PIC value
1	PSMP2001	5	0.730	19	PSMP2050	4	0.391
2	CTM-10	2	0.500	20	PSMP2059	3	0.223
3	CTM-25	2	0.365	21	PSMP2063	2	0.668
4	CTM-26	4	0.576	22	PSMP2064	2	0.497
5	CTM-57	2	0.500	23	PSMP2066	4	0.577
6	CTM-59	3	0.263	24	PSMP2068	4	0.002
7	CTM-60	6	0.749	25	PSMP2069	2	0.342
8	PSMP2006	2	0.500	26	PSMP2070	4	0.338
9	PSMP2008	5	0.216	27	PSMP2072	2	0.612
10	PSMP2013	2	0.553	28	PSMP2076	3	0.569
11	PSMP2019	4	0.645	29	PSMP2077	2	0.402
12	PSMP2024	3	0.446	30	PSMP2078	4	0.527
13	PSMP2027	2	0.323	31	PSMP2080	3	0.273
14	PSMP2033	2	0.279	32	PSMP2088	3	0.365
15	PSMP2040	3	0.490	33	PSMP2089	4	0.607
16	PSMP2043	2	0.476	34	PSMP2090	2	0.255
17	PSMP2045	2	0.443		**Total**	103	
18	PSMP2048	4	0.432		**Mean**	3.029	0.445

of lines in sub cluster IV (35). The most divergent sub clusters was cluster I from maintainer group and cluster IV from the restorer group. Lines from these sub clusters could be identified and used for potential parental development in different breeding strategies for better heterotic responses. The maintainers ICMB 97333, RPB 6 and ICMB 92777; and restorer ICMR 10222, RPR 25, RPR 56, RPR 62 and RPR 40 lines were identified as most distinct and divergent which could be used as candidate parents for varital development programme.

Authors' contribution

Conceptualization of research (RB, YV); Designing of the experiments (RB, YV); Contribution of experimental materials (SKG); Execution of field/lab experiments and data collection (RB, EAM, RSS, YV); Analysis of data and interpretation (RB, TG, EAM); Preparation of manuscript (RG, TG, EAM, SKG).

Declaration

The authors declare no conflict of interest.

References

Botstein D., White R. L., Skolnick M. H. and David R. 1980. Construction of genetic linkage map in man using restriction fragment length polymorphisms. American J. Human Genet., **32:** 314-31.

Brinkmann B., Klintschsar M., Neuhuber F., Huhne J. and Rolf B. 1998. Mutation rate in human microsatellites and influence of the structure and length of the tandem repeat. American J. Human Genet., **62**: 1408-15.

Budak H., Pedraza F., Cregan P. B., Baenziger P. S. and Dweikat I. 2003. Development and utilization of SSRs to estimate the degree of genetic relationships in a collection of pearl millet germplasm. Crop Sci., **43**: 2284-90.

DeWoody J. A., Honeycutt R. L. and Skow L. C. 1995. Microsatellite markers in white-tailed deer. Heredity, **86**: 317-19.

Directorate of Millet Development. 2017. http://www.dacnet.nic.in/millets.

Excoffier L., Smouse P. and Quattro J. 1992. Analysis of molecular variance inferred for metric distances among DNA haplophytes: Applications to human mitochondrial DNA restriction data. Genet., **131**: 479-91.

Ganapathy K. N., Gomashe S. S., Rakshit S. et al. 2012. Genetic diversity revealed utility of SSR markers in classifying parental lines and elite genotypes of sorghum [*Sorghum bicolor* (L.) Moench]. African J. Crop Sci., **6**: 1486-93.

Kapila R. K., Yadav R. S., Plaha P., Rai K. N., Yadav O. P., Hash C. T. and Howarth C. J. 2008. Genetic diversity among pearl millet maintainers using microsatellite markers. Plant Breed., **127**: 33-37.

Liu J. 2005. Power Marker V. 3.25. Available at http://www.powermarker.net.

Nepolean T., Gupta S. K., Dwivedi S. L., Bhattacharjee R., Rai K. N. and Hash C. T. 2012. Genetic diversity in maintainer and restorer lines of pearl millet. Crop Sci., **52**: 2555-63.

Poncet V., Lamy F., Enjalbert J., Joly H., Sarr A. and Robert T. 1998. Genetic analysis of the domestication syndrome in pearl millet [*Pennisetum glaucum* (L.) R. Br.], Poaceae) inheritance of the major characters. Heredity, **81**: 648-58.

Perrier X. and Jacquemoud-Collet J. P. 2006. DARwin software http://darwin.cirad.frl.

Qi X., Pittaway., Undup T. S., Iiu S., Waterman H. et al. 2004. An integrated genetic map and a new set of simple sequence repeat markers for pearl millet [*Pennisetum glaucum* (L.) R. Br.] Theor. Appl. Genet., **109**: 1485-93.

Rai K. N., Kulkarni V. N., Thakur R. P., Haussmann B. I. G. and Mgonja M. A. 2006. Pearl millet hybrid parents research approaches and achievements. (Eds. C. L. L. Gowda, K. N. Rai, B. V. S. Reddy and K. B. Saxena. Hybrid Parents Research at ICRISAT Patancheru, Andhra Pradesh, India. pp: 11-74.

Rooney W. L. and Smith C. A. 2000. Techniques for developing new cultivars. (Eds. Smith C. W. and Frederiken). Sorghum origin, history, technology and production Wiley, New York pp. 329-47.

Stich B., Haussmann B. I. G., Pasam R., Bhosale S., Hash C. T., Melchinger A. E. and Parzies H. K. 2010. Patterns of molecular and phenotypic diversity in pearl millet [*Pennisetum glaucum* (L.) R. Br.] from West and Central Africa and their relation to geographical and environmental parameters. BMC Plant Biol., **10**: 216-26.

Sumanth M., Sumathi P., Vinodhana N. K. and Sathya M. 2013. Assessment of genetic distance among the inbred lines of pearl millet [*Pennisetum glaucum* (L.) R. Br.] using SSR markers. Int. J. Biotechnol. Allied Fields, **1**: 153-62.

Xu X., Peng M. and Fang Z. 2000. The direction of microsatellite mutations is dependent upon allele length. Nat. Genet., **24**: 396-99.

Zidani S., Ali F. and Mohamed C. 2005. Genomic DNA extraction method from pearl millet (*Pennisetum glaucum*) leaves. African J. Biotech., **4**: 862-66.