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GENETIC DIVERGENCE AND HETEROSIS AMONG MAIZE GENOTYPES AS INFERRED FROM DNA MICROSATELLITES

NIHARIKA SHUK LA*[1], D. K. MISHRA[1] ARUN CHAVAN[2] AND SANJAY SINGH[1]
[1]Department of Plant Breeding and Genetics, J.N. K.V.V., Jabalpur - 482 004
[2]Department of Genetics and Plant Breeding, Institute of Agricultural Sciences,
Banaras Hindu University, Varanasi - 221 005
e-mail: niharikas86@gmail.com

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***Corresponding author**

ABSTRACT
This study was under taken to investigate the genetic diversity among germplasm of maize and to screen out diversified parent to develop hybrids with high yield potential. Simple sequence repeat (SSR) analysis of 29 markers was performed for 88 germplasm comprising of CIMMYT based QPM lines and *desi*maize collected from tribal areas of Madhya Pradesh.An average of 3.10 alleles per locus was observed with a range from 2 to 5. The polymorphic information content (PIC) of the 29 SSRs ranged from, 0.11 to 0.62 with an average of 0.36. The clustered dendrogram classified the germplasm into two groups that could easily facilitate the selection of diversified parents. Total 11 hybrids, whose parents were diverse, were reported with higher percentage of heterotic effect for yield attributing traits, which may be used as inbreds for obtaining higher yield potential. However results of genotyping of maize inbreds with SSR markers established a benchmark on the genetic diversity existing across the inbreds and hence these DNA marker profiles can be used as criteria for the prediction of heterosis in maize and also for hybrid identification.

INTRODUCTION

Maize (*Zea mays* L.) ismostwidelydistributedand versatile food crop of the world, grown in tropical, sub-tropical and temperate regions. Being high productive among other cereals, it also known as *Queen* of cereals. Since maize is cheap, it becomes the dominant food and main source of dietary energy and protein for poor, particularly in rural and underprivileged segments of the society. Hence there is a need to promote maize breeding on priority basis by adopting various approaches to meet the increasing demand for maize grain and its products.In this context, maize hybrid breeding remains the choice of the methods considering its success over years. For exploiting the potential of hybrid breeding in maize, many maize inbreds have been developed from a limited number of elite lines and elite line synthetics, a practice that heightens the risk of decreased genetic diversity in commercial maize production fields (Hallauer *et al.,* 1988, Soni and Khanorkar 2013). The choice ofthe most efficient breeding scheme for improving maizepopulation is dependent upon the relative amount and typeof genetic variability involved (KuMar *et al.*, 2013). Better understanding on the genetic diversity ensures the breeder in planning crosses for hybrid and line development, in assigning lines to heterotic groups and in plant variety protection (Pejic *et al.,*1998). The developments during the past three decades in the DNA marker technology are enormous and an array of DNA markers is made available as a tool to assess the genetic diversity in plants and animals. Genetic diversity studies using molecular markers reveal patterns of diversity in crops that are obscured by the complexities of pedigree records. Genetic fingerprinting of maize is an efficient method for large scale application to aid breeders in the placement of breeding lines and populations into the correct heterotic group, to aid in the curation of gene bank collections by refining the core subsets formed from field evaluation and to have a better understanding of the evolution of major maize races (Warburton *et al.*, 2002). Molecular genetic markers are clearly a powerful tool to delimit heterotic groups and to assign inbred lines into existing heterotic groups (Melchinger, 1999). The SSR markers offer advantages in reliability, reproducibility, discrimination, standardization, and cost effectiveness over othermarker types (Smith *et al.*, 1997). Therefore this study aimed to investigate the genetic diversity among selected germplasm and to identify potential high yielder hybrids to secure future demand of growing population.

MATERIALS AND METHODS

The experiment was conducted for three years at two,field and molecular level both. During firstyear (*Kharif*-2012), selection of inbred as parents was conducted in which 88

germplasm was evaluated at phenotypic level, andthose found promising for various yield attributing traits were subjected to molecular diversity analysis through SSR during second year (*Rabi*-2012-13).Based on the electrophoretic banding pattern of SSR markers (Table 1), pair wise genetic distances among genotypes were estimated. Out of which, 10 better and most diverse parents based on the genetic distance were selected to be used as parents for conducting crossing programs as per 10 x 10 diallel mating design duringsecond year (*Rabi*-2012-13). Hybridization of selected inbred was varied out planting them in paired parent's arrangement for diallel mating (excluding reciprocal) in paired method for coinciding the flowering. Seed obtained from parents were harvested separately.During third year (*Rabi*- 2013-14), the 10 parents along with 45 single cross hybrids (SCH) and a standard check (HQPM-1) were evaluated (Table 2) in Randomized complete Block Design with three replications. Standard package of practices were followed to raise the good crop. The observations were recorded for different yield and yield related traits.

SSR diversity analysis

Collection of samples

Green young and healthy leaves were used from each of the selected germplas mone month after sowing (before flowering) in the morning hours from the fieldfor extraction of DNA. The collected samples were placed in cooling pads to transfer and then stored at -80ºC.The genomic DNA was isolated as per protocol proposed by Sangai-Maroof *et al*. (1984) with some modifications. After isolation the genomic DNA, it was checked under 0.8 % agarose gel and as per requirement was then subjected to dilution and quantification. Genomic DNA was dissolved in nuclease free water and final concentration was made 25 mg/μL.

PCR reaction

Total 29 polymorphic SSR primers were used to identify genetic divergence among germplasm (Table 1). The PCR was run using genomic DNA under varying temperature range. With standardized conditions (Shukla *et al.*, 2014); each amplified PCR product was checked on 4% agarose gel and then theses PCR products were photographed in 'Syngene' Multigenious Bio-imaging System.

Data Analysis

Phlylogenetic reconstruction was based on the neighbor-joining method implemented in Power Marker version 3.25 (Liu and Muse, 2005). Power Marker was also used to calculate the average number of alleles, gene diversity, and polymorphic information content (PIC) values.Based on the electrophoretic banding pattern of 29 SSR markers, pair wise genetic distance amongst genotypes were estimated and a dendrogram was generated using UPGMA clustering by neighbor-joining method.

RESULTS AND DISCUSSION

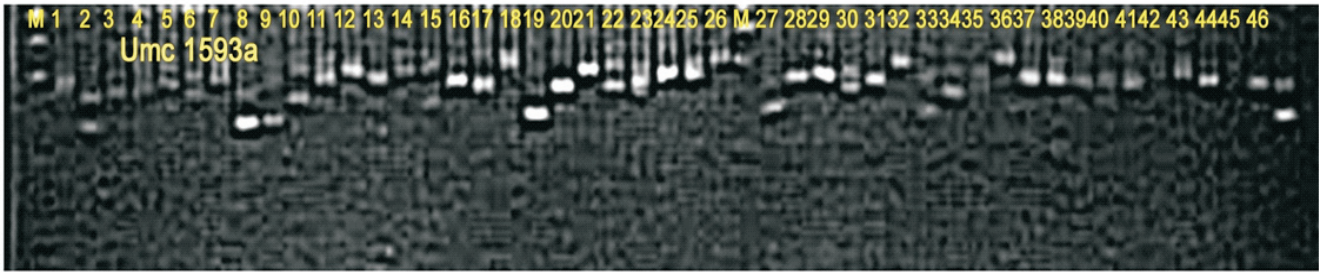
A set of 29 SSR markers (Table 1) used for genotyping, chosen on the basis of their properties of detecting single loci, their

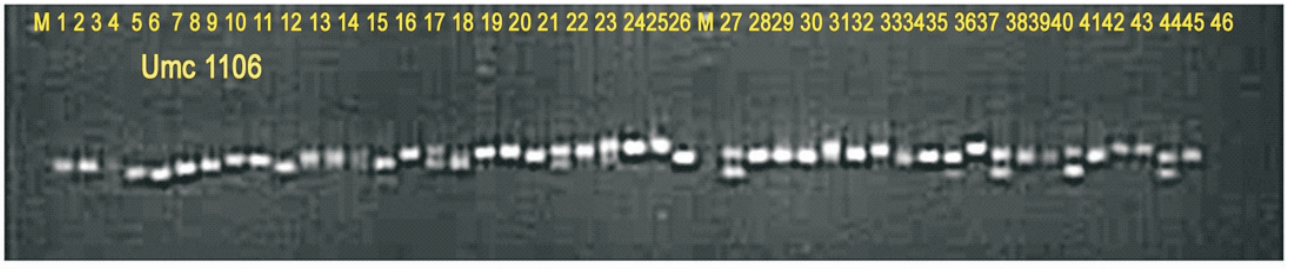
Table 1: Major Allele Frequency, Gene Diversity, Heterozygosity and PIC Values Obtained using SSR markers

Sr. No.	Marker	Genotype No.	Major Allele Frequency	Allele No.	Gene Diversity	Heterozygosity	PIC Value
1	Bnlg490	2.0000	0.6739	2.0000	0.4395	0.0000	0.3429
2	Bnlg1014	3.0000	0.6630	2.0000	0.4468	0.1087	0.3470
3	Bnlg1185	5.0000	0.8804	5.0000	0.2202	0.2174	0.2131
4	Bnlg1520	6.0000	0.6196	5.0000	0.5586	0.1304	0.5131
5	Bnlg2086	3.0000	0.4565	3.0000	0.6248	0.0000	0.5470
6	Dupssr12	4.0000	0.6522	4.0000	0.5293	0.0000	0.4897
7	Dupssr17	8.0000	0.4565	4.0000	0.6103	0.2391	0.5312
8	Dupssr34	2.0000	0.6196	2.0000	0.4714	0.7609	0.3603
9	Phi001	4.0000	0.7826	4.0000	0.3684	0.3478	0.3448
10	Phi057	3.0000	0.8696	3.0000	0.2353	0.0000	0.2224
11	Phi112	2.0000	0.8261	2.0000	0.2873	0.0000	0.2461
12	Phi 961100	3.0000	0.7391	3.0000	0.4045	0.0870	0.3507
13	Phi037	4.0000	0.4565	3.0000	0.6040	0.0435	0.5200
14	Umc1035	4.0000	0.6630	3.0000	0.4724	0.0652	0.3946
15	Umc1042	4.0000	0.7283	3.0000	0.4067	0.2826	0.3398
16	Umc1066	5.0000	0.5000	3.0000	0.5910	0.2391	0.5073
17	Umc1106	2.0000	0.9348	2.0000	0.1219	0.0000	0.1145
18	Umc1231	6.0000	0.5000	4.0000	0.5952	0.1522	0.5149
19	Umc1288	2.0000	0.9348	2.0000	0.1219	0.1304	0.1145
20	Umc1395	3.0000	0.6957	3.0000	0.4612	0.0000	0.4075
21	Umc1432	2.0000	0.5652	2.0000	0.4915	0.0000	0.3707
22	Umc1446	2.0000	0.8913	2.0000	0.1938	0.2174	0.1750
23	Umc1519	3.0000	0.9130	3.0000	0.1616	0.0000	0.1537
24	Umc1593a	8.0000	0.4783	5.0000	0.6708	0.2826	0.6206
25	Umc1859	6.0000	0.4348	4.0000	0.6704	0.4565	0.6088
26	Umc1866	3.0000	0.9130	3.0000	0.1626	0.0000	0.1563
27	Umc2382	2.0000	0.7826	2.0000	0.3403	0.0000	0.2824
28	Zag125	5.0000	0.5761	3.0000	0.5352	0.5435	0.4459
29	Zct439	4.0000	0.6522	4.0000	0.5168	0.0652	0.4665
	Mean	3.7931	0.6848	3.1034	0.4246	0.1507	0.3690

Table 2: crosses showing genetic diversity and % standard heterosisfor yield attributing traits in maize genotypes

Sr.No.	Crosses	Molecular Genetic diversity by UPGMA cluster analysis	(%) Standard heterosis			
			(GYPP)	1000 kernel weight	Harvest index%	Number of rows/ear
1.	JLM 2 X CML 470	76.55 %	48.59	47.94	49.64	42.54
2.	JLM 2 X VL 101123	58.62 %	37.09	41.80	63.95	21.66
3.	JLM 2 X CML 429	56.90 %	40.04	29.89	35.17	27.05
4.	JLM 2 X CML 472	68.28 %	45.17	40.40	48.30	56.17
5.	JLM 2 X VL 1031	51.26 %	39.58	39.03	34.02	22.54
6.	JLM 3 X CML 470	50.00 %	25.20	24.08	28.53	46.70
7.	JLM 3 X VL 101123	50.00 %	38.02	38.86	23.70	35.38
8.	JLM 3 X CML 429	58.28 %	30.59	26.33	25.03	30.60
9.	JLM 3 X CML 472	66.55 %	48.17	41.01	53.68	45.20
10.	JLM 3 X VL 1031	54.44 %	42.25	47.15	44.02	50.24
11.	JLM 7 X CML 470	50.00 %	29.92	25.75	38.11	25.20
12.	JLM 7 X VL 101123	51.72 %	36.25	37.64	44.90	27.05
13.	JLM 7 X CML 429	68.07 %	43.08	46.33	65.49	49.44
14.	JLM 7 X CML 472	66.00 %	44.58	47.09	43.34	46.17
15.	JLM 7 X VL 1031	49.67 %	40.57	26.02	67.00	25.28
16.	JLM 22 X CML 470	58.28 %	41.31	42.13	43.21	48.65
17.	JLM 22 X VL 101123	60.37 %	38.52	39.86	86.91	58.62
18.	JLM 22 X CML 429	55.17 %	15.44	20.00	29.00	26.17
19.	JLM 22 X CML 472	60.00 %	16.16	21.22	23.69	25.20
20.	JLM 22 X VL 1031	50.00 %	36.58	31.83	75.19	25.38
21.	JLM 50 X CML 470	62.88 %	45.08	35.97	36.79	37.23
22.	JLM 50 X VL 101123	51.72 %	18.08	27.97	33.70	27.94
23.	JLM 50 X CML 429	51.72 %	31.85	38.48	33.54	21.66
24.	JLM 50 X CML 472	59.23 %	32.43	44.87	34.45	28.82
25.	JLM 50 X VL 1031	55.38 %	26.57	27.97	33.63	27.05





Picture 1 and 2: Electrophoretic banding pattern of SSR amplification product resolved on 4% agrose gel for genetic diversity analysis and parental polymorphism study.

Lane 1	Bulk Line 22	Lane 9	VL 1087	Lane 17	HKI 13441	Lane 25	CML 472	Lane 33	JLM 50	Lane 41	JLM 42
Lane 2	BML 7	Lane 10	VL 109183	Lane 18	BML 6	Lane 26	VL 1031	Lane 34	JLM 51	Lane 42	JLM 48
Lane 3	CML 474	Lane 11	VL 1016196	Lane 19	HKI 161	Lane 27	JLM 1	Lane 35	JLM 8	Lane 43	JLM 29
Lane 4	CML 470	Lane 12	VL 1018798	Lane 20	HKI 163	Lane 28	JLM 4	Lane 36	JLM 32	Lane 44	JLM 13
Lane 5	VL 1030	Lane 13	VL 1029	Lane 21	CML 286	Lane 29	JLM 2	Lane 37	JLM 49	Lane 45	JLM 14
Lane 6	VL 1047	Lane 14	VL 108729	Lane 22	CML 407	Lane 30	JLM 3	Lane 38	JLM 20	Lane 46	JLM28
Lane 7	VL 1046	Lane 15	L No. 21	Lane 23	VL 101123	Lane 31	JLM 7	Lane 39	JLM 27		
Lane 8	VL 1033	Lane 16	L No. 27	Lane 24	CML 429	Lane 32	JLM 22	Lane 40	JLM 23		

Picrure 1 and 2: Elecrophoretic banding of SSR amplification product resolved on 4% agrose gel for genetic diversity analysis and parental polymorphism study

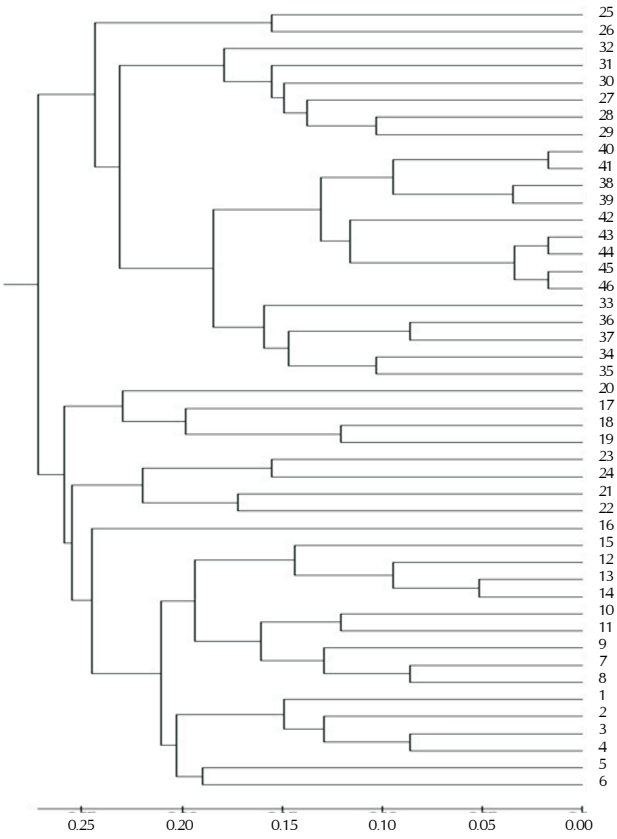


Figure 1: Dendrogram generated using UPGMA analysis showing relationship among maizegermplasmusing SSR marker

broad coverage of the genome and high levels of polymorphism when applied to a broad range of maize germplasm, which are reported earlier. The sequence of the primer pairs were chosen from the maize Genome Database (www. gramineae. org). High level of polymorphism as depicted by all selected SSR primers was observed.The corresponding PIC value ranging from 0.1145 (Umc 1106) to 0.62 (Umc 1593a) with an average of 3.10 alleleper locus (Picture 1 and 2, Table 1) and was found greater than that in previous studies by Barcaccia *et al.* (2003), Hoxha *et al.* (2004), Babu *et al.* (2012), Kumar *et al.* (2012), Mishra and Singh (2012) and Wasal and Prasanna (2013); which indicates that selected primers are highly polymorphic and the degree of diversity among genotypes was also very high.

Genetic diversity is defined as the probability that two randomly chosen alleles from the population are different among the maize genotypes (Liu and Muse, 2005). The maximum amount of gene diversity was exhibited by the marker UMC 1593a (0.67) whereas, the minimum gene diversity of (0.12) was recorded for UMC 1106 and Umc 1288. The mean value of the gene diversity was 0.42. Similar findings were also reported by Choukan and Warburton (2005) where the average gene diversity was found to be 0.42 in 36 early maturing Iranian maize inbred lines. Since the genetic diversity and PIC values recorded in these studies were within those recorded for genetic diversity studies in maize, these SSR markers are considered useful for genetic purity analysis of maize varieties.

Total 29 different SSR markers, selected for yield and yield attributing traits found to be polymorphic for 46 selected germplasm and out of which, majority of primers showed clear amplification and sharp resolution. The study revealed that the total number of alleles amplified were 90 with a mean value of 3.10 $\pm$ 0.23 (Table 1). Maximum number of allele *i.e.* 5 was amplified by marker Umc 1593a. Average number of bands per primer was found to be 2.89. The size of the amplified markers ranged from 100 bp (Bnlg 490) to 360 bp (ZAG 125). The Major allele frequency for 46 Maize genotypes ranged from 0.45 in (Bnlg 2086, Dupssr 17) to 0.93 (Umc 1106) with an average frequency of 0.68. Based on the electrophoretic banding pattern, pair wise GD amongst the genotypes of maize for genetic diversity were estimated and a dendrogram was generated with UPGMA cluster by neighbor-joining method implemented in Power Maker. The dendrogram generated could clear cut differentiate the *Desi* maize germplasm from the CIMMYT based QPM lines that comprises of the total of the germplasm under study.With the Analysis of genetic structure and diversity among maize inbred lines as inferred from DNA microsatellites could be a timely tool for identifying right inbred for maize hybrid breeding LIU *et al.* 2005. The level of diversity reported ranged from 0.0 to 68.28%.

Through dendrogram,cluster 1 comprises of 2 groups, in first group 2 line of QPM (CML 472 and VL1031) are present (Fig. 1 and Table 2). However, in second group 20 *Desi* germplasm were present. In the earlier studies the QPM and *D*esi lines had also been grouped separately using SSR markersshowing genetic diversity in maize (Pabendon *et al.,* 2009). The Genetic distance that was reported among the parents are presented in the table (Table 1) was carried out using various operational taxonomic units (OTUs). Therefore it was easy to screen 10 most diverse parents occupying different clusters and based on genetic distance near about or more than 50% of genetic distance between them. These parents were CML 470, VL 101123, CML 429, CML 472, VL 1031 (QPM lines) and JLM 2, JLM 3, JLM 7, JLM 22, JLM 50 (*Desi* lines). These most promising (for yield and related traits) and diverse genotypes including the *Desi* maize and QPM lines used to conduct 10 x 10 diallel mating design (during *Rabi* 2012-13) for crossing programme.

These parental combinations or hybrids exhibiting higher percentage of genetic diversity between them also revealed greater percentage of mid parent, better parent and economic heterosis over the commercial check HQPM-1 when evaluated along with their parents during *Rabi* 2013-2014 for all yield attributing traits (Table 2). Most interestingly the grain yield per plant which was recorded high level of heterosis over all the three levels, which was also supported by Dubey *et al.* (2009), who observed heterosis over mid parent (MP), better parent (BP) and standard check (SC) against the non-conventional hybrid L10 x T1 - VC in which the heterosis at all the three levels was highly significant and positive for grain yield per plant. The percent standard heterosis for grain yield per plant ranged from -7.23 to 48.82 %, with an average of 27.16% (Table 2). The maximum molecular genetic diversity was reported by the cross JLM 2 X CML 470 (76.55%)and which was also showed higher percentage of economic

heterosis(48.59%).Rest of the crosses*viz.*JLM 2 X CML 472, JLM 3 X CML 472, JLM 7 X CML 472, JLM 7 X CML 429, JLM 7 X CML 472, JLM 22 X CML 470, JLM 22 X VL 101123, JLM 22 X CML 472, JLM 50 X CML 470 and JLM 50 X CML 472 also reported to be higher in terms of standard heterosis for yield attributing traits *i.e.* grain yield per plant, 1000 kernel weight, harvest index and number of rows/ear.

The parents with higher gene diversity in the all the performed crosses observed to be shown higher percent heterosis, which exhibited genetic potential of them to develop new desirable hybrids or may be develop as potential inbreed for hybrid breeding programme. In maize, genetic diversity analysis to determine the genetic distance (GD) using various operational taxonomic units (OTUs) remains routine practice. From the genetic diversity analysis results of present study, maize inbreds lacking their pedigree data could be identified based on their GD to make hybridization between them. The results of genotyping 45 maize inbreds with SSR markers established a benchmark on the genetic diversity existing across the inbreds. Supplementing the existing morphological descriptors with reliable and repeatable DNA based marker profiles is a must considering the ramifications in the future maize breeding in India.

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