Original paper Open Access

Microsatellite marker dependent genetic divergence assessment within and among heterotic groups of tropical maize inbred lines

Punya, Vinay Kumar Sharma* and Pankaj Kumar

Department of Agricultural Biotechnology & Molecular Biology, Dr. Rajendra Prasad Central Agricultural University, Pusa-848125, Samastipur, Bihar, India
*Corresponding author : V. K. Sharma, Department of Agricultural Biotechnology & Molecular Biology, Dr. Rajendra Prasad Central Agricultural University, Pusa-848125, Bihar, India: E-mail: vksharmarau@yahoo.com

Abstract

Use of microsatellite markers can be helpful in assessing the nature and extent of genetic diversity among inbred lines, assigning inbred lines efficiently to heterotic groups and making the choice of heterotic parents to develop new hybrids. A study was conducted to determine the heterotic groups of 18 inbred lines of maize including three inbred testers and to examine the nature and extent of divergence within and between heterotic groups of inbred lines based on the analysis of targeted microsatellite sites. Using hybrid index and hybrid mean values obtained from the results of experimental trials conducted over the two seasons as the indices, inbred lines were classified into three heterotic groups. Microsatellite profiling with a panel of 28 primer pairs covering all chromosomes revealed ample genetic polymorphism, which allowed unique genotyping and unambiguous classification of inbred lines. Basically, the inbred lines and testers were differentiated into four genotypic groups. Principal coordinate analysis based on similarity indices and spatial ordination of the genetic profiles showed four well defined genotypic groups of 18 inbreds. The distribution pattern of the entries into different heterotic groups formed on the basis of hybrid index value and hybrid mean value corresponded with the microsatellite markers based groups to the extent of 75% and 67% in the first heterotic group, 40% and 29% in the second heterotic group and 33% and 40% in the third heterotic group, respectively. Overall coincidences of inbred lines in heterotic groups based on microsatellite markers with the hybrid index value and hybrid mean value based heterotic groups (47% and 40%, respectively) indicated that microsatellite markers may be effectively and efficiently utilized to assign the inbred lines to heterotic groups for the purpose of reducing the number of single crosses to be generated and evaluated. Average genetic divergence revealed within and between heterotic groups by the microsatellite markers may be utilized as the criterion for parental line selection during development of experimental hybrids

KeyWords *Maize, heterotic group, genetic divergence, cluster analysis, microsatellite marker*

Introduction

India occupies fifth position with respect to area and seventh position with respect to production among the maize growing countries of the world. During the last one decade, maize has registered the highest growth rate among all food grains including wheat and rice because of newly emerging food habits as well as enhanced industrial requirements. Maize occupies an important place in agricultural economy of the country. Traditionally, maize is grown during the summer (monsoon or kharif) season, which is accompanied by high temperatures and rains. Winter (Rabi) cultivation of maize is a relatively new introduction, which started in mid sixties in only some pockets of the country, but now it has spread in the country as a whole. Emerging as a competitive crop, the area under maize has recorded an increasing trend. However, in order to meet the ever growing demand of maize for human food, animal feed, poultry feed, as well as industrial processing to produce value added products, the level of production needs to be essentially and substantially raised further.

Genetically diverse and mutually complementary elite inbred lines are essential requirements for hybrid maize breeding programs or for strategic conservation of germplasm (Adeyemo et al, 2011; Nyaligwa et al, 2015; Smith et al, 2015). The importance of phenetic and genetic divergence among parental inbreds is well established as a significant and most important factor contributing to high yielding hybrids with greater heterotic expression (Dinesh et al, 2016; Nyombayire et al, 2016). Additionally, genetic divergence becomes prerequisite in any crop improvement program as it contributes to the development of superior recombinants (Dutta et al, 2017; Hu et al, 2017; Ghosh et al, 2018). Since greater emphasis is laid on development of single cross hybrids

for commercial exploitation of heterosis manifestation in maize, it becomes obligatory to enhance the yield of inbred parents. Hence, several inbred lines collected from different sources need to be purposefully assessed for their yield performance and divergence.

Assigning the parental lines into different heterotic groups is fundamental for the maximum exploitation of heterosis through hybrid cultivar development in a cross pollinated crop like maize. A heterotic group comprises a set of genotypes that performs well when crossed with genotypes from a different heterotic group. Precise information on heterotic groups of maize inbred lines is, therefore, essential for effective and efficient implementation of hybrid breeding program. Choice of genetically diverse parents for hybridization, as it is amply emphasized, is more likely to generate heterotic hybrids. The high genetic diversity of inbred lines distributed equally among heterotic groups is useful in guiding breeders to select parental candidates for crossing programs (Liu et al, 2003; Legesse et al, 2007; Pabendon et al, 2008). Thus, information on genetic diversity of parental lines is also more or less equally important for hybrid breeding programs. The knowledge regarding genetic diversity pattern and heterotic groups is very useful for proper and effective planning of crossing programs for hybrid cultivar development (Reif et al, 2003).

Phenotypically expressed morphological characters do not reliably portray genetic relationships due to environmental influence. Since the expression of morphological traits is usually influenced by environmental factors, the information generated on the basis of morphological characters is sometimes incomplete and unreliable. Maize breeders have been looking for the possibility of predicting heterosis between inbred lines of maize based on the morphological, pedigree, physiological and biochemical data during the past decades. Recently, molecular markers, which provide reliable and complementary information, have been used by the researchers for the purpose of characterization of inbred lines, assessment of genetic diversity and classification of inbred lines into heterotic groups. Contrary to morphological variation, molecular polymorphism is generally considered to be independent of environment (Gauthier et al, 2002) and therefore more suitable for the evaluation of genetic diversity and as a complementary strategy to traditional approaches in the conservation and utilization of plant genetic resources (Hospital et al, 1997; Gauthier et al, 2002; Ghebru et al, 2002).

Microsatellites are effectively used to measure genetic diversity in many crop plants including maize, because of their high level of polymorphism, repeatability, low cost and amenability to automation. Keeping into consideration that the use of microsatellite markers can help in assessing the nature and extent of genetic diversity among inbred lines, assigning inbred lines efficiently to heterotic groups and making the choice of heterotic parents to develop new hybrids, the present study has been conducted to determine the heterotic groups and examine the nature and extent of divergence between the inbred lines based on the analysis of targeted microsatellite sites

Materials and Methods

Forty-five single cross hybrids were generated from eighteen parental lines including fifteen inbreds and three inbred testers (Table 1) to constitute the experimental materials of the present study. Parental lines of these experimental hybrids were procured from the Department of Plant Breeding & Genetics, Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar, Experimental hybrids along with parental lines were evaluated during the rabi and kharif seasons in randomized block design with three replications. Parental lines and single cross experimental hybrids were randomized independently in contiguous plots. An average value of observations in respect of plant height, ear height, ear length, number of kernels per year and

Table 1. Kernel color and source of inbred lines and testers used in the present study

Sl. No.	Inbred line	Kernel color	Source	Sl. No.	Inbred line	Kernel color	Source
01.	CML467	Yellow	CIMMYT, Mexico	10.	LM13	Yellow	SRI, Coimbatore
02.	CML468	Yellow	CIMMYT, Mexico	11.	Dholi2012	Yellow	TCA, Dholi
03.	CML469	Yellow	CIMMYT, Mexico	12.	HKI162	Yellow	CCS HAU, Hisar
04.	CML470	Yellow	CIMMYT, Mexico	13.	HKI323-B	Yellow	CCS HAU, Hisar
05.	CML471	Yellow	CIMMYT, Mexico	14.	HKI586	Yellow	CCS HAU, Hisar
06.	CML373	Yellow	CIMMYT, Mexico	15.	HKI1105	Yellow	CCSHAU, Hisar
07.	CML115	Yellow	CIMMYT, Mexico	16.	CML161*	Yellow	CIMMYT, Mexico
08.	CML196	Yellow	CIMMYT, Mexico	17.	CML165*	Yellow	CIMMYT, Mexico
09.	CML465	Yellow	CIMMYT, Mexico	18.	CML163*	Yellow	CIMMYT, Mexico

Table 2 Analysis of variance for different characters of parental lines and single cross experimental hybrids of maize across seasons

			Mean sum of squares				
Source of variation		**DF**	**Plant height (cm)**	**Ear height (cm)**	**Ear length (cm)**	**No. of Kernels per ear**	**Grain yield per plant (g)**
Replication	S1	2	0.77	1.86	2.51	1742.82	24.49
	S2	2	0.95	2.06	0.66	40.05	3.20
	S3	2	0.85	1.58	0.37	467.87	11.07
Entries	S1	62	877.36**	541.74**	28.07**	9904.55**	555.51**
	S2	62	572.79**	438.18**	37.88**	2868.25**	719.99**
	S3	62	596.09**	419.61**	31.72**	3564.03**	530.48**
Parents	S1	17	880.54**	485.97**	9.18**	12646.32**	691.17**
	S2	17	457.93**	348.67**	7.75**	1058.60**	277.01**
	S3	17	516.55**	407.55**	7.12**	32.4.57**	289.67**
Hybrids	S1	44	534.67**	271.35**	14.68**	8198.13**	443.73**
	S2	44	560.48**	347.92**	27.41**	2916.25**	430.35**
	S3	44	453.22**	222.79**	19.81**	2991.13**	405.03**
Heterosis	S1	1	15901.86**	13386.71**	938.46**	38376.96**	3167.63**
	S2	1	3067.46**	5931.45**	1010.54**	31520.47**	20994.66**
	S3	1	8234.40**	9284.95**	974.16**	34882.48**	10143.75**
Error	S1	124	1.50	1.85	1.43	946.63	31.92
	S2	124	1.70	1.54	1.15	82.77	31.06
	S3	124	0.87	0.82	0.69	245.20	16.06

S1: Rabi season; S2: Kharif season; S3: Over seasons; *, **: Significant at 5% and 1%, respectively.

grain yield recorded on five randomly chosen plants per entry was used for statistical analysis. Analysis of variance was performed for partitioning of the variance into different sources in order to provide a basis for test of significance. The partitioning of the total variation into different sources was accomplished following cross classification system of the arrangement of various entries.

Heterotic grouping of inbred lines

Heterotic potential of each inbred line was assessed on the basis of grain yield of its experimental hybrid averaged over the two seasons and the average value was considered as the hybrid mean value for the inbred line involved in the cross combination. Hybrid mean value of each inbred line was then compared with the general mean value obtained for grain yield of all hybrid combinations over the seasons. Inbred lines were subsequently classified into high (>GM + ½ Sd), moderate (GM ± ½ Sd) and low (<GM – ½ Sd) heterotic groups. Hybrid index value (Aguiar et al, 2008) of inbred lines was assessed by transforming the grain yield of the hybrids to the index (I) in relation to hybrid means obtained with the same tester as $I=MH/MT$; wherein, I is the hybrid index; MH is the hybrid mean and MT is the mean of all hybrids evaluated with same tester. The value obtained for each of the inbred lines in combination with each of the three testers was averaged to represent the hybrid index value of each inbred line and compared with the mean index (MI) value obtained as average of hybrid index value of all hybrid combinations. Inbred lines were then classified into high (>MI + ½ Sd), moderate (MI ± ½ Sd) and low (<MI – ½ Sd) heterotic groups.

Genotyping of parental lines

Total genomic DNA was isolated from leaf samples collected at four to five leaf stage seedlings of the inbred lines and testers by adopting a standardized maize genomic DNA extraction protocol (Punya et al, 2017). Twenty eight microsatellite primer pairs (Table 3) covering each chromosome existing in the genome were chosen from MaizeDB (http://www.maizegdb.org/ssr.php) and utilized for amplification of targeted genomic regions. Using standard protocol of polymerase chain reaction adjusted to laboratory condition (Punya et al, 2017), targeted amplification of specified genomic regions was selectively and purposely performed by employing selected panel of forward and reverse microsatellite primer pairs in a thermal cycler (Eppendorf). The products generated by primer directed amplification of genomic regions were resolved

Table 3 Allelic diversity of microsatellite markers used for genomic profiling of inbreds

Sl. No.	Marker	Ch. No.	No. of alleles	PIC	Sl. No.	Marker	Ch. No.	No. of alleles	PIC
01.	phi 227562	1	07	0.80	15.	bnlg118	5	13	0.89
02.	bnlg 1429	1	11	0.89	16.	bnlg1136	6	11	0.87
03.	umc 1297	1	13	0.79	17.	umc1083	6	14	0.73
04.	nc 133	2	15	0.71	18.	phi034	7	11	0.87
05.	phi 083	2	14	0.75	19.	phi116	7	08	0.85
06.	phi029	3	11	0.84	20.	umc 1304	8	09	0.34
07.	phi 053	3	16	0.80	21.	umc1161	8	12	0.55
08.	umc1266	3	11	0.88	22.	phi115	8	07	0.81
09.	umc1136	3	06	0.66	23.	phi 014	8	08	0.70
10.	phi072	4	06	0.72	24.	phi065	9	15	0.85
11.	phi093	4	11	0.89	25.	phi 084	10	10	0.83
12.	nc 130	5	09	0.87	26.	umc1367	10	11	0.79
13.	umc1332	5	13	0.79	27.	umc1196	10	07	0.72
14.	umc1152	5	11	0.58	28.	umc1179	10	06	0.93

PIC; Polymorphism information content

with the help of agarose (2%) gel electrophoresis at 110 V for one and half hour in horizontal gel system and then visualized and documented under gel documentation system (Alpha Innotech, USA). Using gel reader (Alpha View Gel Reader), molecular size of amplified products was determined in comparison to the size of markers in the ladder (50 bp).

Allelic diversity and suitability of microsatellite primers based polymorphism for identification of polymorphic and informative markers in order to characterize and differentiate maize inbred lines was assessed on the basis of polymorphism information content (PIC) of the microsatellite primer pairs. Polymorphism information content (Smith et al., 1997) was evaluated manually for each microsatellite locus as $1-\sum fi^2$ where, fi is the frequency of ith allele and summation extends over all alleles generated by a primer pair.

Genotypic grouping of inbred lines

Genetic relation among the inbred lines and testers was analyzed by calculating the similarity coefficient (Dice, 1945) for pair-wise combinations of the entries using binary data generated on the basis of presence or absence of the bands in different entries as discrete variables. Based on the proportion of shared bands produced by the primers, similarity coefficient for pair-wise combinations of entries was computed as 2a/(2a+b+c), where, a, b and c represent number of shared bands between J^{th} and K^{th} genotypes, number of bands present in J^{th} genotype but absent in K^{th} genotype and number of bands absent in jth genotype but present in K^{th} genotype, respectively. Cluster analysis was performed on the basis of similarity coefficients by using sequential agglomerative hierarchical non-overlapping (SAHN) clustering as the module for tree building.

Method employed for construction of similarity indices based dendrogram involved un-weighted paired-grouping using mathematical average (UPGMA). Principal coordinate analysis was performed to obtain a two-dimensional ordination of the genetic profiles of the inbred lines and testers. Neighbor joining tree was constructed from similarity matrix. Computational analysis was performed with the help of software (Rohlf, 1997) and the divergence pattern of the inbred lines and testers was examined by identifying the clusters at appropriate phenon levels and comparing the clusters and neighbor joining tree. The groups established on the basis of microsatellite markers were compared with the heterotic groups formed using hybrid index and hybrid mean values by calculating coincidences percentage of lines in the groups (Pinto et al, 2003)

Results

Production potential evaluation of experimental hybrids and molecular characterization of maize inbred lines were carried out in the present study for precise understanding of the nature and extent of molecular level genetic differentiation and divergence and facilitating the use of diverse inbred lines in the hybrid maize breeding programs. Analysis of variance for the experimental design was conducted separately for the two different seasons and then over the seasons based on pooled data (Table 2). Partitioning of variance into

Table 4 Hybrid index and hybrid mean values based on grain yield of hybrids

Sl. No.	Experimental hybrid	HI	HM
01.	CML467×CML161	0.76	53.83
02.	CML468×CML161	1.18	83.30
03.	CML469×CML161	0.81	57.50
04.	CML470×CML161	0.82	57.75
05.	CML471×CML161	1.24	87.55
06.	CML373×CML161	1.02	72.13
07.	CML115×CML161	1.04	73.77
08.	CML196×CML161	1.02	72.33
09.	CML465×CML161	0.81	57.07
10.	LM13×CML161	1.02	71.73
11.	DH2012×CML161	0.95	66.85
12.	HKI162×CML161	1.27	89.95
13.	HKI323B×CML161	1.24	87.58
14.	HKI586×CML161	1.09	77.30
15.	HKI1105×CML161	0.65	45.97
16.	CML467×CML165	0.90	63.42
17.	CML468×CML165	1.03	72.70
18.	CML469×CML165	1.24	87.07
19.	CML470×CML165	1.02	72.22
20.	CML471×CML165	1.21	85.40
21.	CML373×CML165	0.82	57.83
22.	CML115×CML165	1.09	76.98
23.	CML196×CML165	1.05	73.82
24.	CML465×CML165	1.02	72.05
25.	LM13×CML165	0.82	58.22
26.	DH2012×CML165	0.81	57.22
27.	HKI162×CML165	1.06	74.40
28.	HKI323B×CML165	0.78	55.10
29.	HKI586×CML165	1.01	71.08
30.	HKI1105×CML165	1.06	74.83
31.	CML467×CML163	1.10	70.55
32.	CML468×CML163	0.85	54.52
33.	CML469×CML163	1.19	75.90
34.	CML470×CML163	0.84	53.65
35.	CML471×CML163	1.07	68.55
36.	CML373×CML163	0.74	47.22
37.	CML115×CML163	1.27	80.88
38.	CML196×CML163	0.84	54.05
39.	CML465×CML163	1.18	75.47
40.	LM13×CML163	0.88	56.05
41.	DH2012×CML163	0.87	55.75
42.	HKI162×CML163	0.90	57.68
43.	HKI323B×CML163	1.12	71.78
44.	HKI586×CML163	0.93	59.27
45.	HKI1105×CML163	1.16	73.90

HI: Hybrid index; HM: Hybrid mean

various sources revealed the statistical significance of mean sum of squares due to parents, hybrids and parents vs. hybrids (heterosis) under both the seasons and also over the seasons for all the five metric characters.

Phenotyping of parents and hybrids

Among the inbred lines under evaluation, CML467 recorded significantly higher grain yield per plant than all other inbred lines evaluated over the two seasons. The second highest grain yield per plant was observed in the case of tester CML165, which was statistically at par to that recorded for the five inbred lines, namely, LM13, HKI586, CML465, HKI162 and HKI323B. These inbred lines also registered more or less superior mean performance in respect of plant height, ear height, ear length and number of kernels per ear, in comparison to the mean performance of rest of the inbred lines. Sixteen experimental hybrids exhibited significantly higher grain yield per plant in comparison to the general mean value obtained for this character. Among these sixteen hybrid combinations, six combinations, namely, HKI162×CML161, HKI323B×CML161, CML471×CML161, CML471×CML165, CML469×CML165 and CML468×CML161, were observed to be statistically at par in respect of grain yield per plant. Thirteen cross combinations including five amongst sixteen high yielding cross combinations exhibited significantly longer ear length in comparison to the general mean.

Altogether eleven cross combinations including six amongst sixteen high yielding cross combinations had significantly more number of kernels per ear than the general mean. Amongst the high yielding hybrid combinations, seven combinations, namely, CML468×CML161, CML469×CML165, CML469×CML163, CML471×CML161, CML115×CML163, HKI162×CML161 and HKI323B×CML161, recorded significantly higher mean value for either ear length or number of kernels per ear than general mean value. Only two high yielding experimental hybrids, namely, CML196×CML165 and HKI162×CML165, had significantly higher mean value for both ear length and number of kernels per ear than general mean value.

Numerically significant heterosis for grain yield per plant over mid parent ranged from -27.18% in the cross HKI323B×CML165 to 86.96% in the cross CML468 × CML161 during rabi season. Twenty six crosses were found to have positive heterosis. During kharif season, the magnitude of significant heterosis ranged from 22.03% in the cross HKI586×CML163 to 125.09% in the cross CML115×CML163. Thirty nine crosses showed positive heterosis. Seven crosses in rabi season and none of the crosses in kharif season exhibited negative

Table 5. Comparison of heterotic groups of inbred lines formed by using microsatellite markers based genetic similarity, hybrid index value and hybrid mean value

Heterotic groups		Inbred lines
Group 1	GS	CML470,CML471,CML373,CML115,CML196,CML465 ,CML467, CML468, CML469
	HI	CML469, CML471, CML115, HKI162
	HM	CML471, CML115, HKI162
Group 2	GS	HKI323B,HKI586
	HI	CML196, CML465, HKI323B, HKI586,HKI1105
	HM	CML468,CML469,CML196,CML465,HKI323B, HKI586,HKI1105
Group 3	GS	LM13,DH2012,HKI162
	HI	CML467, CML468, CML470, CML373, LM13, DH2012
	HM	CML467, CML470, CML373, LM13, DH2012
Group 4	GS	HKI1105,CML161,CML165,CML163

GS: Genetic similarity; HI: Hybrid Index; HM: Hybrid mean

heterosis. On pooled data basis, the extent of heterosis for grain yield per plant ranged between -14.02% in the cross CML467×CML161 to 89.03% in the cross CML468×CML161. Thirty five crosses exhibited significantly positive heterosis, whereas two crosses were found to exhibit negatively significant heterosis for grain yield per plant.

Statistically significant heterotic effect over better parent for grain yield per plant ranged between -38.40% in the cross CML467×CML161 to 68.46% in the cross CML468×CML161 during rabi season. Similarly, significant heterosis over better parent ranged from 18.04% in CML471×CML163 to 124.28% in CML115×CML163 during kharif season. Across the seasons on pooled data basis, extent of heterosis for grain yield per plant ranged between -28.40% in the cross CML467×CML161 to 73.30% in the cross CML469 × CML163. A perusal of the data on heterosis over better parent further revealed that 15 crosses in rabi season, 34 crosses in kharif season and 31 crosses across the seasons exhibited significant heterosis for grain yield per plant. Eleven crosses in rabi season and three crosses across the seasons showed lesser grain yield per plant than the respective better parents. Sixteen cross combinations, namely, CML469×CML163, HKI162×CML161, CML115×CML163, CML468×CML161, CML468× CML161, CML471×CML161, HKI1105×CML163, CML115×CML161, HKI323B×CML161, CML4×CML161, CML469×CML165, CML471×CML165, CML115×CML165, HKI1105×CML165, CML162×CML165 and CML465×CML163, recorded significantly higher mean performance in conjunction with significantly positive heterosis for grain yield and appeared as most promising amongst the hybrid combinations under evaluation.

Genotyping of parents

Microsatellite sites based molecular profiling was found efficient enough to reveal usable level of polymorphism at molecular level among the maize inbred lines under evaluation in the present investigation. Amplification of genomic template using twenty eight simple sequence repeat primer pairs exhibited different levels of polymorphism among the eighteen maize inbred lines subjected to microsatellite profiling. Molecular level genetic polymorphism was visualized in the form of presence or absence of bands, in addition to the number and position of bands (Fig. 1). Differential ability to determine variability among the inbred lines was clearly exhibited by the panel of primer pairs employed during molecular characterization. Allelic variants generated by some of the primer pairs were higher in number, while some of them yielded lesser number of allelic variants. Altogether 296 allelic variants were

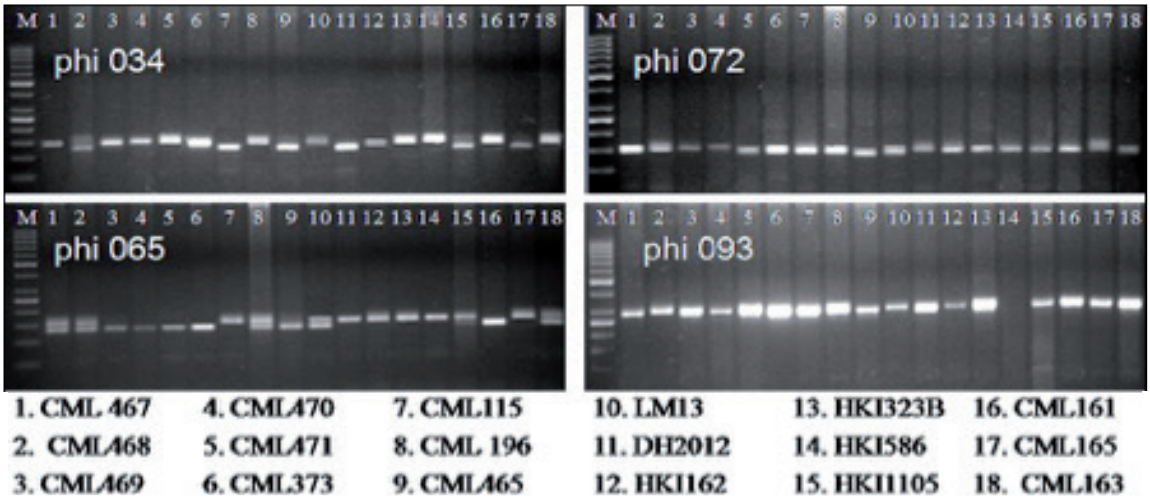

Figure 1 Microsatellite primers dependent amplification patterns of targeted genomic regions of eighteen tropical maize inbred lines.

detected amongst amplified products with a molecular size range between 56 to 352 bp. The number of alleles varied from 6 in the cases of umc1136, phi072 and umc1179 to 16 in the case of phi053. A total of 145 shared alleles ranging from 3 to 10 alleles per primer pair and 151 unique alleles ranging from 2 to 10 alleles per primer pair were detected.

Presence of a microsatellite locus specific amplified product was not recognized in some of the inbred lines under evaluation in the present study. In the absence of amplified product, targeted microsatellite locus specific null allele was assigned to the inbred line. Occurrence of null allele was accordingly inferred for a particular inbred line-marker combination, whenever an amplification product could not be generated in combination with a specific primer pair. Experimental results showed null allele in some of the inbred parental lines subjected to microsatellites based molecular profiling by the primer pairs nc133, bnlg 1429, phi093, phi053, umc1367, phi115 and bnlg118.

Polymorphism information content values ranged from 0.34 (umc1304) to 0.93 (umc1179) with mean value of 0.77 (Table 3), demonstrating sufficiently enough allelic diversity and informativeness of primer pairs along with the potential to discern the genetic differences. Polymorphism per cent, as revealed by the percentage of unique alleles, was recorded to be the maximum in the case of phi084 (70.00%), while the minimum (22.2%) polymorphism per cent was recorded for the primer pair umc1304 with an overall average value of 47.04 percent. Remarkably greater magnitude of polymorphism per cent was registered for the primer pairs umc 1297,phi083, phi029, phi053, umc1266, umc1136, phi093, bnlg118, umc1083, phi034, umc1161, phi115, phi014, phi065, phi084, umc1367, umc1196, umc1179. Microsatellites with di-nucleotide repeat motifs were found to be more polymorphic, generating more number of allelic variants than those with tri-nucleotide repeat motifs. Using the number of alleles generated due to variation in the length of simple sequence repeats flanked by different primer pairs as the criterion in conjunction with the polymorphism information content and polymorphism per cent, the primer pairs umc1297, phi053, umc1266, phi093, bnlg118, phi034, phi115, phi065 and phi084 appeared to be highly polymorphic and informative markers for the purpose of molecular characterization.

Genotypic grouping of inbred lines

Ample genetic differentiation was discerned amongst the inbred lines subjected to microsatellite profiling for the assessment of their genetic relationships. Amongst pair-wise combinations of entries under evaluation, the magnitude of similarity

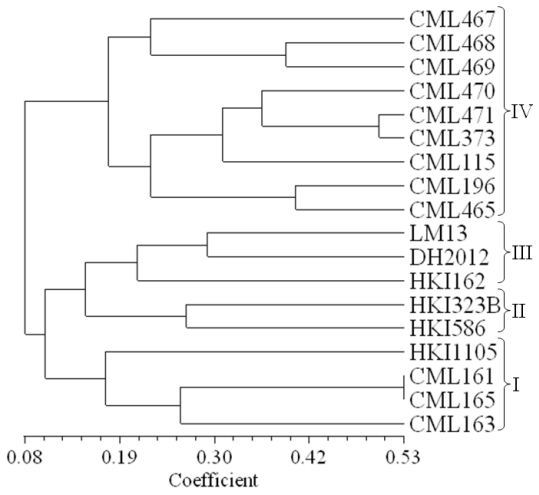

Figure 2 - Hierarchical classification pattern of maize inbred lines and testers based on similarity coefficients for twenty eight microsatellite primer pairs

coefficient between CML 165 and CML 161 was found to be the maximum, reflecting close similarity of these two testers with respect to the regions of the genome targeted by the primer pairs used for molecular profiling in the present study. Basically, the inbred lines and testers were differentiated into four genotypic groups (Fig. 2). Cluster analysis clearly indicated that the microsatellite markers utilized in the present study revealed a remarkably higher level of genetic polymorphism, which allowed unique genotyping and unambiguous classification of inbred lines. Since the markers were chosen from all the chromosomes existing in the genome of maize, the molecular level genetic diversity exhibited by them seemed to be unbiased and not due to chance. Neighbor joining tree (Fig. 3) and principal coordinate analysis based spatial distribution pattern of the microsatellites primers dependent genetic profiles (Fig. 4) exhibited more or less similar type of genetic associations amongst the inbred lines and testers.

Table 6. Average genetic similarity within and between hybrid index (above diagonal) and hybrid mean value (below diagonal) based heterotic groups of inbred lines

Heterotic group	Group 1	Group 2	Group 3	Hybrid index
Group 1	0.153	0.127	0.195	0.132
Group 2	0.135	0.113	0.109	0.125
Group 3	0.203	0.135	0.132	0.151

GS: Genetic similarity; HI: Hybrid Index; HM: Hybrid mean

Heterotic grouping of inbred lines

Considering the hybrid index value (Table 4) as the basis, the inbred lines were divided into three groups. Highly heterotic group (Group 1) included four inbred lines (Table 5). Similarly, moderately heterotic group (Group 2) contained five inbred lines, whereas poor heterotic group (Group 3) accommodated six inbred lines. On the basis of hybrid mean value, the inbred lines were also classified into three groups. Highly heterotic group (Group 1) consisted of three inbred lines, whereas moderately heterotic group (Group 2) and poor heterotic group (Group 3) had seven and

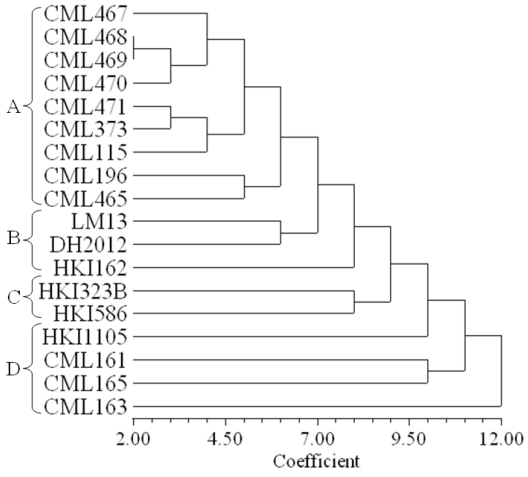

Figure 3 - Neighbor joining tree of maize inbred lines and testers based on similarity coefficients for twenty eight microsatellite primer pairs

five inbred lines, respectively. Microsatellite profiling based cluster analysis discriminated the inbred lines and testers into four broad groups. All the CML lines, with the exception of three testers, namely, CML161, CML165 and CML163, were accommodated in one multi-genotypic group. Similarly, second and third groups consisted of two and three inbred lines, respectively. In the fourth group, one inbred line was included along with the three testers. The distribution pattern of entries into different heterotic groups formed on the basis of hybrid index value and hybrid mean value corresponded with the microsatellite markers based genotypic groups to the extent of 75% and 67% in the first heterotic group, 40% and 29% in the second heterotic group and 33% and 40% in the third heterotic group, respectively. Since, heterotic grouping based on hybrid index value and hybrid mean value directly reflected the heterotic effects, the first, second and third heterotic groups were regarded as highly, moderately and low heterotic groups, respectively.

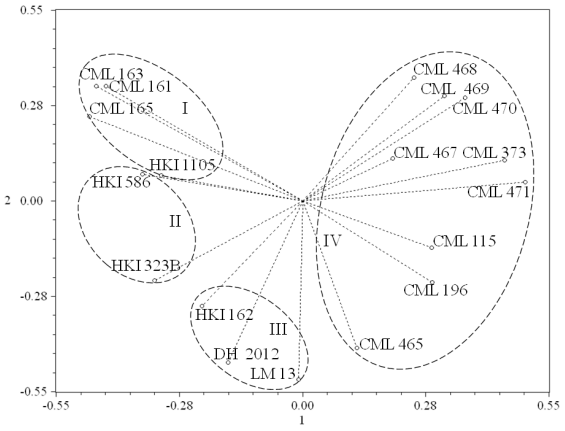

Figure 4 - Spatial distribution pattern of twenty eight microsatellite primer pairs based genetic profiles of maize inbred lines and testers

Overall coincidences of inbred lines accommodated in different genotypic groups on the basis of microsatellite markers assisted molecular characterization were considerably higher with the inbred lines included in hybrid index value and hybrid mean value based heterotic groups (47% and 40%, respectively), indicating thereby that microsatellite markers were effectively and efficiently utilized to establish heterotic groups of maize inbred lines and to assign the inbred lines to heterotic groups for grain yield heterosis. Microsatellite markers based average genetic divergence revealed within the hybrid index value and hybrid mean value based heterotic groups (Table 6) was extremely comparable, indicating the practical usefulness of the separation of maize inbred lines into heterotic groups by employing microsatellite markers as the criterion during parental selection for the development of single cross experimental hybrids. The inbred lines with same heterotic groups seemed to be suitable for the development of synthetic varieties while those in different heterotic groups appeared to be desirable for the development of hybrid varieties to maximize the manifestation of heterosis.

Discussion

During the course of present investigation, maize inbred lines collected from four different sources or geographical origins were subjected to molecular characterization using a panel of microsatellite markers covering all the chromosomes existing in the genome. The purpose of this study was basically to investigate the nature and extent of divergence between the inbred lines and to separate the inbred lines into heterotic groups based on the comparative analysis of targeted microsatellite sites. The efficiency and adequacy of used panel of microsatellite markers in heterotic grouping of

inbred lines was tested according to the affiliation to heterotic groups on the basis of performance of single cross experimental hybrids generated from inbred lines of different heterotic groups.

Nearly half of the total numbers of allelic variants in the present study was detected by only nine (39%) of the 28 microsatellite markers employed during molecular characterization, suggesting the existence of significant polymorphism among the markers. Similarly, more than half (64%) of the microsatellite primers recorded polymorphic information content value greater than the overall mean, indicating that most of the markers used had sufficiently high discriminatory power and utility for genetic diversity studies. More or less similar efficiency and significant polymorphism among microsatellites markers was reported by earlier researchers (Nyaligwa et al, 2015). Remarkably greater number of primer pairs (64%) generated more than one amplified product due to amplification of more than one primer binding genomic region, thereby reflecting most probably the existence of residual heterozygosity in the genetic background of the inbred lines and the co-dominant nature of the microsatellite markers.

Potential of the genomic markers to detect the genetic differences among the genotypes based on the number of alleles per locus and distribution of allele frequencies is reflected by their polymorphic information content. Demonstrating the informativeness of the primer pairs used in the present study, the range of its numerical values (0.34 to 0.93) clearly indicated the presence of appreciably greater level of allelic richness among the inbred lines. Average value calculated for this parameter in the present study was very close to the value obtained by several earlier research workers (Hoxha et al, 2004; Reid et al, 2011). However, the average number of allelic variants per primer detected in the present study was lower than that reported in the literature (Wasala and Prasanna, 2013), but higher than that documented in the reports of some other earlier researchers (Adeyemo et al, 2011; Nyaligwa et al, 2015). Such noticeable discrepancies in respect of the number of detected alleles might be due to differences in the diversity of the lines used, the number of lines examined and the genetic profiling method adopted (Adeyemo et al, 2011). Among the primers which had higher PIC values, umc1297, phi053, umc1266, phi093, nc130, umc1332, bnlg118, phi034, phi116, phi115, phi065 and phi084 generated considerably greater number of allelic variants as a consequence of sequence length variation revealed by the amplification of simple sequence repeats flanked by these primer pairs. Furthermore, umc1297, phi053, umc1266, phi093, bnlg118, phi034, phi115, phi065 and phi084 generated considerably greater percentage of unique alleles amongst the twelve primers which had higher PIC values along with greater number of alleles. Taking into consideration the number of alleles generated by different primer pairs in conjunction with the level of polymorphism detected in the present study, the primers umc1297, phi053, umc1266, phi093, bnlg118, phi034, phi115, phi065 and phi084 appeared to be highly effective, polymorphic and informative primers, which contributed most to the differentiation between the genotypic groups

Similarity coefficient based dendrogram discriminated and clustered the inbred lines and testers into four major clusters with somewhat non-homogeneous distribution of entries within the cluster (Fig. 2). In general, the inbred lines originating from the same center showed remarkably greater tendency to be clustered together. All the CML inbred lines, with the exception of the three inbred testers, were accommodated into a single cluster. Similarly, the three CML inbred testers occupied the same cluster along with one HKI inbred line and two of the remaining three HKI inbred lines were included in another cluster. In spite of showing reasonable level of genetic similarity, the entries were found to be well separated in all the clusters, thereby indicating that they were genetically divergent also. Remarkably greater differentiation and divergence of inbred testers from other inbred lines could be attributed to high inbreeding and lesser number of effective alleles (Saavedra et al, 2013). Neighbor joining tree (Fig. 3) and principal coordinate analysis based two-dimensional ordinations of the microsatellites primers dependent genetic profiles (Fig. 4) exhibited more or less similar type of genetic associations amongst the inbred lines and testers, consistent with the relationships revealed by the sequential agglomerative hierarchical non-overlapping cluster analysis based dendrogram.

Using different approaches to examine the nature of differentiation and divergence among the inbred parental lines, it was clearly established that the markers utilized in this study revealed a remarkably higher level of genetic polymorphism, which enabled unique genotyping and unambiguous classification of inbred lines. Similar inference was derived from microsatellite markers based molecular profiling of inbred lines carried out by earlier researchers (Beyen et al, 2005; Pabendon et al, 2008; Shah et al, 2010; Shiri et al, 2014). Ample molecular level divergence exhibited within sources of collection signified that substantial variation existed among the inbred lines within sources of collection, in addition to the contribution of the sources of inbred lines to the total molecular variance (Kashiani et al, 2012; Nyaligwa et al, 2015; Richard et

al, 2016). Further, the separation of most of the lines derived from different source germplasm into well defined groups suggested that these inbred lines can be effectively utilized as parental lines to develop inbred lines belonging to different heterotic groups with the usefulness to produce hybrids that may optimize expression of heterosis in maize breeding programs. Therefore, the present study has revealed appreciable level of genetic diversity among the inbred lines with the genetic potential to facilitate the selection of parents with diverse alleles.

Practically reliable and effective discrimination of inbred lines efficiently promotes the utilization of genetic materials in breeding programs. Parental genetic divergence in this context defines the manifestation of heterosis and the heterotic pattern is largely determined by the genetic divergence of the parental lines. Therefore, crossing programs involving distant inbred lines of maize might ensure greater success in the production of desirable genetic variability (Abera et al, 2012; Xu et al, 2013; Kanagarasu et al, 2013; Kage et al, 2013; Mikic et al, 2017) and thus might maximize the exploitation of heterosis and segregation (Molin et al, 2013l; Saavedra et al, 2013; Nyombayire et al, 2016). Consequently, the observed relationships in this study could be exploited accordingly in order to design a strong hybrid maize breeding program. The information acquired from this study regarding the extent of genetic diversity and relationships revealed amongst some maize inbred lines would be explored to pin point suitable heterotic patterns and assign the inbred lines into specific heterotic groups.

Parental line selection and breeding strategies for the successful and efficient hybrid development program are greatly facilitated by heterotic grouping of parental lines. Heterotic grouping is grouping of related or unrelated genotypes from the same or different populations that indicate similar combining ability and heterotic response when crossed with testers from other genetically diverse germplasm groups (Hundera, 2017). Being an important activity in hybrid maize breeding programs, it enables efficient parental lines selection. Assigning lines to heterotic groups avoids the tedious and time consuming efforts usually required for the development and evaluation of crosses that should be discarded, allowing maximum heterosis to be exploited by crossing inbred lines belonging to different heterotic groups. Heterotic pattern refers to a specific pair of two heterotic groups that express high heterosis and high hybrid performance in their cross (Rajendran et al, 2014). Several approaches have been suggested and adopted for the classification of inbred lines into heterotic groups (Fan et al, 2003; Pinto et al, 2003; Aguiar et al, 2008; Delucchi et al, 2012; Bidhendi et al, 2012; Kanyamasoro et al, 2012; Rajendran et al, 2014; Richard et al, 2016; Singode et al, 2016; Suni et al, 2016; Ejigu et al, 2017; Meena et al, 2017). Recently, microsatellite markers have been developed and used as a tool to assess the genetic diversity among inbred lines of maize and to assign them to different heterotic groups (Smith et al, 2000; Bantte and Prasanna, 2004; Tian et al, 2004; Reif et al, 2005; Aguiar et al, 2008; Balestre et al, 2008; Pabendon et al, 2008; Shah et al, 2010; Shiri et al, 2014; Suni et al, 2016; Hu et al, 2017). Molecular markers offer the possibility of evaluating only the more promising crosses between the most divergent lines.

Among the three approaches adopted to separate the inbred lines into heterotic groups in the present study, two were based on crosses with the same set of testers. Since a systematic comparison was attempted by classification based on the group of lines evaluated by the same set of testers, the concordance index of the two test crossing based grouping methods was obtained by counting the method-wise total coincidences of all evaluated lines. A more or less similar approach was adopted by earlier researchers (Aguiar et al, 2008). Microsatellite markers based classification was found highly effective in heterotic grouping of inbred lines consistent with their source or geographical origin and remarkably greater number of inbred lines procured from the same source were placed in the same heterotic group. Similarly, the three testers procured from the same source were placed in the same heterotic group. A comparative assessment of heterotic grouping by using three different criteria, namely, microsatellite markers based genetic similarities; hybrid index values and hybrid mean values, further indicated that CML471 and CML115 or HKI323B and HKI586 or DH2012 and LM13 belonged to same heterotic group in all the cases.

Grouping methods showed inconsistency of the procedures involved in heterotic group formation, particularly in respect of the lines included in the groups, which were not the similar using three different methods. Differential classification of inbred lines into heterotic groups by the hybrid index and hybrid mean was also evident. However, heterotic grouping based on hybrid index value appeared to be discriminatory (Aguiar et al, 2008), since the lines were included in the same group on the basis of their relative performance in combinations with three distinct testers. Inconsistency in classification based on the hybrid index can be explained by taking into consideration the fact that the hybrid index value was based on the performance recorded over two seasons. Therefore, heterotic grouping based

on the mean of all hybrids with the testers represented an attempt of establishing a method of unbiased heterotic group formation. Contrarily, the constitution of heterotic groups based on the direct evaluation of each hybrid was influenced by the combining ability of the tester. Consequently, the test crosses with testers that probably had high combining ability recorded a higher mean and the lines involved in the tests crosses tended not to be classified in this group. Obviously, the consistency in the formation of heterotic groups using microsatellite markers was not absolute for grouping based on the hybrid mean and hybrid index values, though the proportion of concordance of the grouping based on the microsatellite markers on the one hand and the grouping based on the hybrid index and the hybrid mean on the other hand was highly comparable (47% and 40%, respectively). While the formation of heterotic groups on the basis of test crosses is tester-dependent, labor and cost-intensive and influenced by genotype-environment interaction, microsatellite markers based discrimination of heterotic groups does not suffer from all these limitations. Microsatellite markers are, therefore, able to more efficiently classify closely related maize inbred lines than morphological characters (Beyen et al, 2005; Pabendon et al, 2008; Shah et al, 2010; Shiri et al, 2014). Thus, the results of the present study provided the evidence to support the view point that the use of microsatellite markers for separation of maize inbred lines into heterotic groups would effectively and efficiently lower the number of single crosses to be evaluated, thus, increasing the efficacy of hybrid maize breeding programs.

Acknowledgements

The authors gratefully acknowledge the Department of Plant Breeding & Genetics, Dr. Rajendra Prasad Central Agricultural University Pusa, Bihar, India for providing the seeds of maize inbred lines used in the study.

References

Abera W, Shimelis H, Derera J, Mosisa W, Danson J and Laing MD, 2012. Genetic interrelationships among medium to late maturing tropical maize inbred lines using selected SSR markers. Euphytica 10: 1-9.

Aguiar CG, Schuster I, Junior ATA, Scapim CA and Vieira ESN, 2008. Heterotic groups in tropical maize germplasm by test crosses and simple sequence repeat markers. Genet Mol Res 7: 1233-1244.

Adeyemo O, Menkir A, Melaku G and Omidiji O, 2011. Genetic diversity assessment and relationship among tropical yellow endosperm maize inbred lines using SSR markers. Maydica 56: 1-7.

Bantte K and Prasanna BM, 2004. Simple sequences repeat polymorphism in quality protein maize (QTL) lines. Euphytica 129: 337-344.

Balestre M, Pinho RGV, Souza JC and Lima JL, 2008. Comparison of maize similarity and dissimilarity genetic coefficients based on microsatellite markers. Genet Mol Res 7: 695–705.

Beyene Y, Botha AM and Myburg AA, 2005. A comparative study of molecular and morphological methods of describing genetic relationships in traditional Ethiopian highland maize. Afr J Biotechnol 4: 586-595.

Bidhendi MZ, Choukan R, Darvish F, Mostafavi K and Mjidi E, 2012. Classifying of maize inbred lines into heterotic groups using diallel analysis. World Acad Sci Eng Technol 67: 1368-1371.

Delucchi C, Eyherabide GH, Lorea RD, Daniel AP, Otegui ME and Lopez CG, 2012. Classification of argentine maize landraces in heterotic groups. Maydica 57: 26-33.

Dice LR, 1945. Measures of the amount of ecologic association between species. Ecology 26: 297-302.

Dinesh A, Patil A, Zaidi PH, Kuchanur PH, Vinayan MT and Seetharam K, 2016. Genetic diversity, linkage disequilibrium and population structure among CIMMYT maize inbred lines, selected for heat tolerance study. Maydica 61(M29): 1-7.

Dutta R, Kumar A, Chandra S and Ngachan SV, 2017. Genetic divergence, path coefficient, principal component and cluster analyses of maize genotypes in the mid-altitudes of Meghalaya. Maydica 62 (M8): 1-5.

Ejigu YG, Tongoona PB and Ifie BE, 2017. Classification of selected white tropical maize inbred lines into heterotic groups using yield combining ability effects. Afr. J. Agric. Res 12: 1674-1677. DOI: 10.5897/AJAR2016.11872.

Fan XM, Tan J, Chen HM and Yang JY, 2003. Heterotic grouping for tropical and temperate maize inbreds by analysing combining ability and SSR markers. Maydica 48: 251-257.

Gauthier O, Gouesnard B, Dallard J, Redaelli R, Rebourg C, Charcosset A and Boyat A, 2002. RFLP diversity and relationships among traditional European maize populations. Theor Appl Genet 105: 91-99.

Ghebru B, Schmidt RJ and Bennetzen JL, 2002. Genetic diversity of Eritrean sorghum landraces assessed with simple sequence repeat (SSR) markers. Theor Appl Genet 105: 229-236.

Ghosh A, Das PK, Ghosh A and Kundagrami S, 2018. Heterosis, potence ratio and genetic distance for yield and yield contributing traits in single cross maize hybrids. Maydica 63(M9): 1-9.

Hospital F, Moreau L, Lacoudre F, Charcosset A and Gallais A, 1997. More on the efficiency of marker assisted selection. Theor Appl Genet 95: 1181-1189.

Hoxa S, Shariflou MR and Sharp P. 2004. Evaluation of genetic diversity in Albanian maize using SSR markers. Maydica 49: 97-103.

Hu C, Zhang L, Dong Y, Zhou Q, Li X, Ren Y, Zhu S, Zhang L, Liu Z and Li Y, 2017. Revealing core heterotic germplasm and characterizing of maize inbred lines using SSRs. Maydica 62 (M18): 1-10.

Hundera NB, 2017. Combining ability and heterotic grouping in maize (Zea mays L.) inbred lines for yield and yield related traits. World J Agric Sci 13: 212-219.

Kage U, Madalageri D, Malakannavar L and Ganagashetty P, 2013. Genetic diversity studies in newly derived inbred lines of maize (Zea mays L.). Mol Plant Breed 4: 77-83.

Kanagarasu S, Nallathambi G, Ganesan KN, Kannan S, Shobhana VG and Senthil N, 2013. Determination of genetic polymorphism among indigenous and exotic maize inbreds using microsatellite markers. Afr J Biotech 12: 5723-5728.

Kanyamasoro MG, Karungi J, Asea G and Gibson P, 2012. Determination of the heterotic groups of maize inbred lines and the inheritance of their resistance to the maize weevil. Afr Crop Sci J 20: 99 – 104.

Kashiani P, Saleh G, Jothi MP, Abdullah NAP and Selamat A, 2012. Molecular characterization of tropical sweet corn inbred lines using microsatellite markers. Maydica 57: 154-163.

Legesse BW, Myburg AA, Pixley KV and Botha AM, 2007. Genetic diversity of African maize inbred lines revealed by SSR markers. Hereditas 144: 10-17.

Liu K, Goodman M, Muse S, Smith JS, Buckler E and Doebley J, 2003. Genetic structure and diversity among maize inbred lines as inferred from DNA microsatellites. Genetics 165: 2117–2128.

Meena AK, Gurjar D, Patil SS and Kumhar BL, 2017. Concept of heterotic group and its exploitation in hybrid breeding. Int J Curr Microbiol App Sci 6: 61-73.

Mikic S, Kondic-Spika A, Brbaklic L, Stanisavljevi D, Ceran M, Trkulja D and Mitrovic B. 2017. Molecular and phenotypic characterisation of diverse temperate maize inbred lines in Southeast Europe. Zemdirbyste-Agriculture 104: 31–40. DOI 10.13080/z-a.2017.104.005

Molin D, Coelho C, Máximo D, Ferreira F, Gardingo J and Matiello R, 2013. Genetic diversity in the germplasm of tropical maize landraces determined using molecular markers. Genet Mol Res 12: 99-114.

Nyaligwa L, Hussein S, Amelework B and Ghebrehiwot H, 2015. Genetic diversity analysis of elite maize inbred lines of diverse sources using SSR markers. Maydica 60(M29): 1-8.

Nyombayire A, Derera J, Sibiya J, Gasura E and Ngaboyisonga C, 2016. Genetic diversity among maize inbred lines selected for the mid-altitudes and highlands of Rwanda. Maydica 61(M17): 1-7.

Pabendon MB, Azrai M, Mejaya MJ and Sutrisno, 2008. Genetic diversity of QPM and normal maize inbreds as revealed by SSR markers and its relationship with the hybrid performance. J. Agro Biogen 4: 77-82.

Pinto RMC, Garcia AAF, Souza JCL and Souza AP, 2003. Comparison between molecular markers and diallel crosses in the assignment of maize lines to heterotic groups. Maydica 48: 63-73.

Punya, Sharma VK and Kumar P, 2017. A modified method for maize genomic DNA extraction without liquid nitrogen and phenol. Indian Res J Genet & Biotech 9: 45-51.

Rajendran A, Muthiah A, Joel J, Shanmugasundaram P and Raju D, 2014. Heterotic grouping and patterning of quality protein maize inbreds based on genetic and molecular marker studies. Turk J Biol 38: 10-20.

Reid LM, Xiang K, Zhu X, Baum BR and Molnar SJ. 2011. Genetic diversity analysis of 119 Canadian maize inbred lines based on pedigree and simple sequence repeat markers. Can. J. Plant Sci 91: 651- 661.

Reif JC, Melchinger AE, and FrischM, 2005. Genetical and mathematical properties of similarity and dissimilarity coefficients applied in plant breeding and seed bank management. Crop Sci 45: 1-7.

Reif JC, Melchinger AE, Xia XC, Warburton ML, Hoisington DA, Vasal SK, Beck D, Bohn M and Frisch M, 2003. Use of SSRs for establishing heterotic groups in subtropical maize. Theor Appl Genet 107: 947-957.

Richard C, Osiru DS, Mwala MS and Lubberstedt T, 2016. Genetic diversity and heterotic grouping of the core set of southern African and temperate maize (Zea mays L) inbred lines using SNP markers. Maydica 61(M3): 1-9.

Rohlf F, 1998. NTSYS-pc Numerical, Multivariate Analysis System. Version 2.02 g, Exeter Software, Applied Biostatistics Inc., Taxonomy. New York, USA.

Saavedra J, Silva TA, Mora F and Scapim CA, 2013. Bayesian analysis of the genetic structure of a Brazilian popcorn germplasm using data from simple sequenec repeates (SSR). Chil J Agric Res 73: 99-107

Shah MM, Hassan SW, Maqbool K, Shahzadi I and Pervez A, 2010. Comparisons of DNA marker-based genetic diversity with phenotypic estimates in maize grown in Pakistan. Genet Mol Res 9: 1936-1945.

Shiri MR, Choukan R and Aliyev RT, 2014. Study of genetic diversity among maize hybrids using SSR markers and morphological traits under two different irrigation conditions. Crop Breeding J 4: 65-72.

Singode A, Manivannan A, Ahmad B, Srivastava E and MahajanV, 2017. Heterotic grouping in early maturing Indian maize lines. IJAIR 6: 2319-1473.

Smith JSC, Chin ECI, Shu H, Smith OS, Wall SJ, Senior ML, Mitchel SE, Kresovitch S and Ziegle J, 1997. An evaluation of utility of SSR loci as molecular markers in maize (Zea mays L.): comparisons with data from RFLPs and pedigree. Theor Appl Genet 95: 163-173.

Smith OS, Sullivan H, Hobart B and Wall SJ, 2000. Evaluation of a divergent set of SSR markers to predict F1 grain yield performance and grain yield heterosis in maize. Maydica 45: 235-241.

Smith SD, Murray SC and Heffner E, 2015. Molecular analysis of genetic diversity in a Texas maize (Zea mays L) breeding program. Maydica 60(M20): 1-8.

Suni FM, Farkhari M, Taheri H, Mohammadi PP and Barzegari M, 2016. DNA molecular marker depicted variability and heterotic pattern among maize inbred lines. J Appl Biotechnol Reports 3: 465-472.

Terron AE, Cordova PH, Mickelson H and Lopez R, 1997. Heterotic pattern of 30 maize lines derived from CIMMYT's population 43 SR. Agron. Mesoamericana 8: 26-34.

Tian QZ, Li XH, Li MS, Jiang W and Zhang SH, 2004. Molecular marker assisted selection of quality protein maize. J Maize Sci 12: 108–110.

Wasala SK and Prasanna BM. 2013. Microsatellite markers based diversity and population genetic analysis of selected lowland and mid-altitude maize landrace accessions of India. J. Plant Biochem. Biotechnol 22: 392-400.

Xu J, Liu L, Xu Y, Chen C, Rong T, Alli F, Zhou S, Wu F, wang LYJ and Cao M, 2013. Development and characterization of simple sequence repeat markers providing genome wide coverage and high resolution in maize. DNA Res 20: 497-509