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Full Length Research Paper

Determination of genetic polymorphism among indigenous and exotic maize inbreds using microsatellite markers

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Maize (*Zea mays* L.) is an important cereal crop of different countries of world. Undoubtedly, the concerted efforts of plant breeders and their breeding strategies have helped to increase the production and productivity to the tune of two to three folds in many crops including maize. Hybrid cultivars have played a vital role in increasing the acreage and productivity of maize. The success in identifying heterotic hybrid in maize hybrid breeding depends on the availability of genetically diverse maize inbred lines developed from different heterotic gene pool. Hence, generation of information on variability of inbreds at genotypic level become necessary. Molecular markers have proven to be a valuable tool for assessing the genetic diversity in many crop species. Simple sequence repeats (SSR) are currently considered as the molecular markers of choice and are rapidly being adapted by plant researchers for precise estimation of diversity. SSR based molecular diversity analysis of 27 maize inbred lines had produced 23 polymorphic alleles from 10 markers with an average of 2.3 alleles per locus and mean polymorphic information content (PIC) of 0.45. The dendrogram generated with hierarchical unweighted pair group method with arithmetic mean (UPGMA) cluster analysis revealed five major clusters at 0.62 similarity coefficient. The information on diversity of inbred lines generated in this study would be much useful in developing heterotic hybrids.

Key words: Maize, inbreds, marker diversity, simple sequence repeats (SSR).

INTRODUCTION

Maize (*Zea mays* L.) is a widely grown crop with a high rate of photosynthetic activity because of its C_4 pathway, leading to higher grain yield and biomass potential. It is predominantly cross-pollinated species, a feature that has contributed to its broad morphological variability and geographical adaptability. It has assumed greater significance due to its demand for food, feed and industrial utilization. The global production of maize is next to wheat and rice. The Food and Agriculture Organization (FAO) predicts that an additional 60 million tonnes of maize grain will be needed from the annual global harvest by 2030. The demand for maize as an animal feed will continue to grow faster than the demand for its use as a human food, particularly in Asia, where a doubling of production is expected from the present level of 165 million tonnes to almost 400 million tonnes in 2030 (Paliwal et al., 2000). The strength of heterosis breeding programme depends on the availability of diverse superior lines or inbreds. Hybrid cultivars have played a vital role in increasing acreage and productivity especially in maize. The high level of heterosis in a cross indicates that the parents are genetically diverse than those of crosses which show little or low heterosis (Mungoma and Pollack, 1988). Hence, information about genetic diversity of available inbreds is very important to develop heterotic hybrid combinations.

In many studies, genetic diversity among inbred lines or

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Abbreviations: SSR, Simple sequence repeats; **PIC,** polymorphic information content; **UPGMA,** unweighted pair group method with arithmetic mean

Table 1. Details of maize inbred lines taken for molecular diversity analysis.

Parent/inbred line	Kernel		Source
	Colour	Type	
UMI 1023-6, UMI 1044-7, UMI 1051-5, UMI 1053-6, UMI 1054-6, UMI 1690-6, CML 115, CML 357, CML 460, UMI 1093, UMI 1024-5, UMI 61	Yellow	Flint	India/CIM MYT
UMI 1114-10, CML 118, UMI 1025-10, UMI 1029-5, UMI 1131-5, UMI 2244-1, UMI 2128-1, UMI 1137-6	Yellow	Dent	India/CIM MYT
UMI 6143-16, UMI 285, UMI 1119	Orange	Flint	India
UMI 1266-7, UMI 1055-8, UMI 1265-6, UMI 1269-7	Orange	Dent	India

genotypes is being usually assessed based on morphological data, which do not reliably portray genetic relationship, due to environmental influence. Molecular markers have proven to be a valuable tool for genetic diversity analysis of many crop species during the past decade. Their expression, unlike morphological markers, is not influenced by environmental factors; hence, it reflects the actual level of genetic difference existing between genotypes. Genotyping techniques such as restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs) and simple sequence repeats (SSRs) have allowed assessment of the genetic diversity among maize inbred lines to synthesize most heterozygotic hybrid combinations (Trindade et al., 2010; Reif et al., 2006). Thus, attempts have been made to use molecular markers that directly evaluate genetic differences among maize inbred lines. Among the various types of markers, microsatellites or SSRs, which are short sequences containing tandemly repeated copies of one to six nucleotide fragments (Rafalski et al., 1996), are currently considered as the molecular markers of choice and are rapidly being adapted by plant researchers because of their simplicity, high levels of polymorphism (Fufa et al., 2005), high reproducibility and co-dominant inheritance patterns. Therefore, this study was conducted to investigate the genetic polymorphism and genetic relationships among 27 maize inbred lines sourced from India and CIMMYT origin.

MATERIALS AND METHODS

The genotypes used in this study consisted of 27 maize inbred lines including 24 University Maize Inbred lines (Tamil Nadu, India) and four CIMMYT lines (Mexico). The details on kernel colour and shape of inbreds used in the study was presented in Table 1.

DNA extraction

Genomic deoxyribonucleic acid (gDNA) was extracted using the cetyl trimethyl ammonium bromide (CTAB) method described by Dellaporta et al. *(*1983) with some modifications. The concentration of genomic DNA was determined on the basis of optical density readings and agarose gel electrophoresis. The final concentration of all the samples was adjusted to 25 ng/µl for amplify in polymerase chain reaction (PCR).

Primers and PCR amplification

10 SSR primers, based on their high polymorphism information content from previous studies (Smith et al., 1997; Ambika, 2010) and listed in the website maize genomic database (MaizeGD: http://www.agron.missouri.edu) were selected to determine the genetic variability in maize inbred lines (Table 2). PCR was performed in 15 µl reaction mixes consisting of 25 ng template DNA, 2.5 mM dNTP mix, 10 µM SSR primers (forward and reverse), 0.2 µl Taq polymerase and 10× PCR reaction buffer in a 1.5 ml microcentrifuge tube on ice. In a 15 µl PCR reaction volume, 13 µl of master mix was mixed with 2 µl of 25 ng DNA. The PCR profile was programmed with initial denaturation at 95°C for 5 min, followed by 35 cycles of 94°C for 1 min, 56°C for 1 min and 72°C for 1 min. This was followed by one final extension cycle at 72°C for 5 min, and an indefinite hold at 4°C.

Electrophoresis and fragment detection

Briefly, 5 µl of the final reaction product was mixed with 10 µl of electrophoresis loading buffer. After denaturation and immediate cooling, 2 µl of the sample was loaded onto a 6% polyacrylamide gel and electrophoresed at 400 V and 40 W till bromophenol blue reaches the bottom of the gel. The separated fragments were then visualized using a silver staining.

SSR statistical analysis

Gel photographs were scored manually and repeated twice to limit errors in scoring. The bands were binary coded by 1 or 0 for their presence or absence for each genotype, respectively. The binary matrix based on marker scores was subjected to cluster analysis. Cluster analysis was conducted with the unweighted pair group method based on arithmetic averages (UPGMA) to generate a dendrogram. The entire analysis was performed using NTSYS pc version 2.02 software (Rohlf, 1998).

RESULTS AND DISCUSSION

Genetic diversity is of prime importance for the survival, successful adaptation to certain agro-climatic conditions and improvement of any crop species. Without enough genetic diversity in the germplasm, it is practically

Table 2. SSR primer pairs used in the study and their sequences.

Primer	Sequence 5' to 3'
umc1166	Forward: CGATCAGATCATACACAACCTTGC Reverse: GAGGATCGATTCTTGGCGAGT
bnlg615	Forward: CTTCCCTCTCCCCATCTCCTTTCCAA Reverse: GCAACCTGTCCATTCTCACCAGAGGATT
phi299852	Forward: GATGTGGGTGCTACGAGCC Reverse: AGATCTCGGAGCTCGGCTA
bnlg1160	Forward: AATACTGGACCACCAGGCAC Reverse: CGTGGGTCACCAGGAGTC
phi037	Forward: CCCAGCTCCTGTTGTCGGCTCAGAC Reverse: TCCAGATCCGCCGCACCTCACGTCA
umc1484	Forward: GCGTACAGAACAGAGCAGTTACGA Reverse: ACTGAAGCTGCCTGCCTTCTATTT
phi079	Forward: TGGTGCTCGTTGCCAAATCTACGA Reverse: GATGTGGGTGCTACGAGCC
phi113	Forward: GCTCCAGGTCGGAGATGTGA Reverse: CACAACACATCCAGTGACCAGAGT
phi114	Forward: CCGAGACCGTCAAGACCATCAA Reverse: AGCTCCAAACGATTCTGAACTCGC
phi126	Forward: TCCTGCTTATTGCTTTCGTCAT Reverse: GAGCTTGCATATTTCTTGTGGACA

impossible to increase the yield and other desirable traits of any crop, since selection depends on the availability of genetic variability within the breeding material. Hence, characterization of the germplasm available would be the essential first step especially in case of heterosis breeding. Morphological characterization is easier and cheap but it does not reveal the genetic relationship reliably due to environmental influences. Therefore, the molecular markers are being employed now-a-days for precise assessment of variability at genome level. Of the 10 polymorphic SSR primer pairs used to determine the genetic diversity in 27 inbred lines, the number of alleles per locus in the lines ranged from two to four, for a total of 23 alleles. SSR marker profile of all the 27 genotypes with primer umc1166 is shown in Figure 1. The highest number of alleles was observed in loci phi126 (four alleles). Genetic diversity of the studied materials is the most important factor limiting average number of alleles identified per microsatellite locus during screening.

However, other factors such as (1) number of SSR loci and repeat types and, (2) the methodologies employed for detection of polymorphic markers have been reported to influence allelic differences. Wu et al. (2004) determined 5.4 alleles in pop corn landraces using 61 SSR loci; Liu et al. (2005) with 50 SSR loci found 4.1 alleles; Choukan et al. (2006) found 2 to 11 alleles with 4.9 alleles per locus with 46 SSR loci; Legesse et al. (2006) reported 3.85 alleles per locus using 27 SSR loci and Qi-Lun et al. (2008) found 6.4 alleles using 45 SSR loci. However, our value (2.3) closely agreed with the findings reported by Shah et al. (2009) and Aguiar et al. (2008) who reported 1.56 alleles using 10 SSR loci and 3.4 alleles with 28 polymorphic SSR loci. It is important to note that the total number of alleles reported in diversity studies is actually proportional to sample size. Therefore, the differences observed in the study may be attributable to sampling differences and lesser number of loci used for genetic diversity analysis.

Polymorphic information content

The polymorphism information content (PIC) demonstrates the informativeness of the SSR loci and their

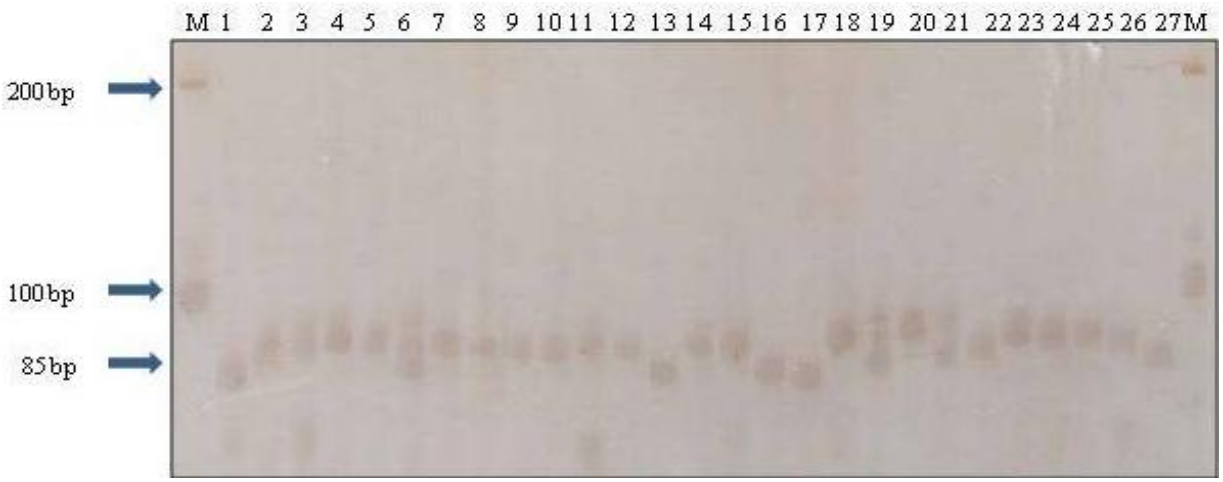

Figure 1. SSR marker profile of 27 maize inbreds produced by primer umc1166.

Table 3. Repeat types, bin number, number of alleles and PIC values of SSR markers used in the study.

SSR primer's code	Repeat type	Bin number	Number of alleles	PIC value
umc1166	CT	1.02	2	0.35
bnlg615	-	1.07	3	0.56
phi299852	AGC	6.07	2	0.47
bnlg1160	AG	3.06	2	0.48
phi037	AGC	1.08	2	0.34
umc1484	AGC	1.01	2	0.42
phi079	AGATG	4.05	2	0.25
phi113	GTCT	5.03	2	0.48
phi114	GCCT	7.03	2	0.49
phi126	AG	6.00	4	0.66
Mean	-	-	2.3	0.45

potential to detect differences among the inbred lines based on their genetic differences. In the present study, PIC values of the SSR loci ranged from 0.25 to 0.66 with mean of 0.45 (Table 3). The average PIC value determined in our investigation agrees with the earlier findings reported based on SSR marker in maize inbred lines. Choukan et al. (2006) found the PIC value of 0.54; Legesse et al. (2006) reported the PIC of 0.58 and Aguiar et al. (2008) with PIC value of 0.51.

Clustering of the inbred lines

The dendrogram constructed using the UPGMA clustering algorithm grouped the inbred lines into five clusters (Figure 2). The three yellow kernel type genotypes of Indian origin *viz.,* UMI 1023-6, UMI 1114-10 and UMI 1093 were grouped in cluster I, only two genotypes UMI 1025-10 and UMI 1119 were grouped into cluster II. Among the genotypes analysed, six and eight genotypes were grouped in cluster III and IV, respectively with one or two inbreds of CIMMYT origin. Fifth cluster contains eight genotypes which includes both yellow and orange type of grains with one line of CIMMYT origin (Table 4). It could be noted that the four genotypes from CIMMYT grouped into different cluster indicates the existence of good variability within the indigenous lines too. Even though the genotypes were grouped in different clusters, some of the flint and dent genotypes, yellow and orange kernel genotypes are grouped in same cluster.

This may be due to the reason that inbreds are developed from the same ancestral cross combining both flint and dent kernel genotypes. Further, the effects of selection drift and mutation or human error might be the cause of the aforementioned discrepancies (Warburton et al., 2002; Legesse et al., 2006). By utilizing the aforementioned genetic diversity information generated, 72 hybrid combinations were synthesised using these 27 genotypes by including genotypes viz., UMI 285, UMI 61 and UMI 1119 as testers and remaining 24 inbreds as lines, in a Line x Tester mating design. The resultant hybrids were found to be promising since testers selected in such a way that they occupied the different clusters. The parents of the hybrids which

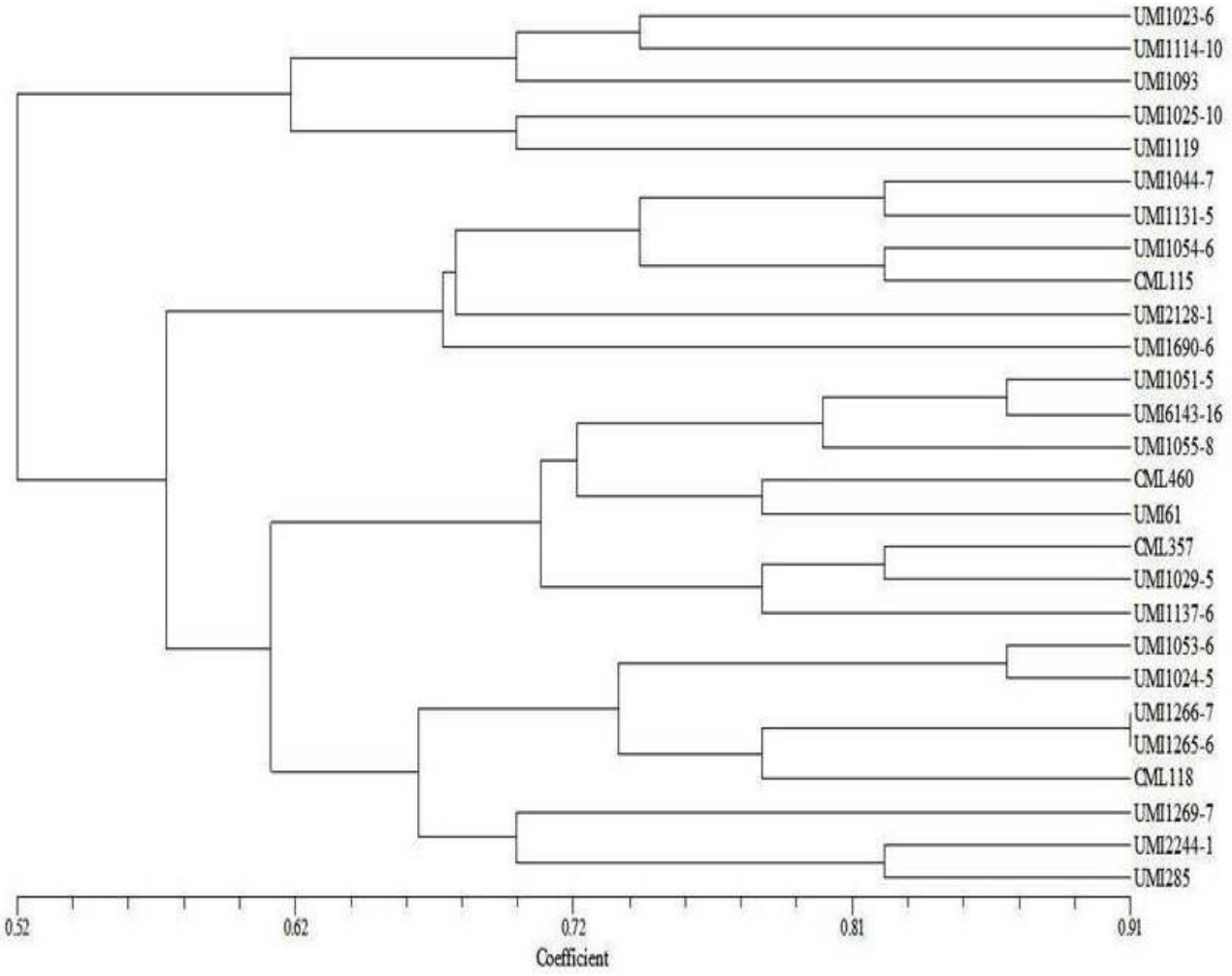

Figure 2. Dendrogram showing the relationship among 27 maize inbreds based on 10 SSR primer pairs.

Table 4. Clustering of maize inbred lines by SSR markers.

Cluster	Genotype/inbred
I	UMI1023-6, UMI1114-10, UMI1093
II	UMI1025-10, UMI1119
III	UMI1044-7, UMI1131-5, UMI1054-6, UMI2128-1, UMI1690-6, CML115
IV	UMI1051-5, UMI6143-16, UMI1055-8, UMI61, UMI1029-5, UMI1137-6, CML460, CML357
V	UMI1053-6, UMI1024-5, UMI1266-7, UMI1265-6, UMI1269-7, UMI2244-1, UMI285, CML118

show higher yielding ability and heterosis were also from different clusters (Table 5). Hence, these results further support that combining the diverse inbreds in heterosis would yield heterotic hybrids.

Conclusion

Present study indicates that SSR markers clearly separated the inbred lines into different clusters based on their genetic divergence and facilitated for the development of heterotic hybrid combination. Hence, whenever the inbred from indigenous and exotic origin is included in the development of hybrids, it is advisable to assess the genetic divergence in order to avoid narrow genetic base in resultant hybrids.

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Table 5. Hybrids combinations showing their parents cluster origin.

Hybrid	Grain yield per plant (G)	Heterosis over check variety (%)	Parent cluster
UMI 1044-7 × UMI 61	182.63	18.70	III × IV
UMI 1093 × UMI 61	182.35	18.51	I × IV
UMI 2244-1 × UMI 1119	177.87	15.61	V × II
UMI 1093 × UMI 1119	177.70	15.49	I × II
CML 460 × UMI 1119	172.49	12.11	IV × II
UMI 1044-7 × UMI 1119	170.78	11.00	III × II
UMI 1024-5 × UMI 1119	168.77	9.69	V × II
UMI 1266-7 × UMI 1119	165.59	7.62	V × II
UMI 1093 × UMI 285	162.29	5.48	I × V

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