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Molecular profiling of maize (*Zea mays* L.) inbred lines using SSR markers

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Abstract

Ten maize inbred lines belonging to four source populations were analyzed for polymorphism using a total of 40 microsatellite or Simple Sequence Repeat (SSR) markers. Unique alleles differentiating the inbred lines could be identified. Cluster analysis based on SSR data revealed genetic relationships among inbred lines partially congruent with their pedigree and breeding history. Breeding strategy for handling component lines of different source populations as well as advancement of the available elite lines is proposed based on the present study.

Key words: Maize, inbred lines, molecular characterization, SSR markers

Introduction

The current strategy of maize (*Zea mays* L.) breeding lays greater emphasis on the development of single cross hybrids [1], for which identification of suitable parental lines and their extensive characterization is of utmost importance. The latter would also be helpful in meeting the registration requirements and plant variety protection. As in other crops, morphological traits have long been used to analyze genetic relationships in maize and in the classification of maize races and populations [2]. However, these descriptors are inadequate and suffer from many limitations for precise characterization of the elite breeding lines. Hence, there is a distinct need to utilize molecular markers for unambiguous differentiation of inbred lines [3, 4].

For genotype characterization, various molecular markers (*viz.*, RFLP, RAPD, SSR, AFLP etc.) possess many advantages over the morphological markers. Among these markers, the Simple Sequence Repeat (SSR) markers are of particular importance [5] as these are PCR based, highly polymorphic, reliable and reproducible. The SSR loci comprise of highly variable arrays of 2 to 6 base pair tandem repeats. Nearly 1000 SSR markers are available in maize under public domain facilitating their utilization for diverse purposes in genetics and plant breeding. Several studies have been carried out in recent years in India for molecular profiling of Indian maize inbred lines [6-8], QTL mapping and marker assisted selection [9, 10]. The present study is a further attempt to characterize a set of elite maize inbred lines developed by the Maize Breeding Unit at IARI, New Delhi, so as to utilize them effectively in hybrid maize breeding.

Materials and methods

The ten inbred lines used in the present investigation (designated as DMB101 to DMB110) were developed at Maize Breeding Unit, IARI, Delhi. Pedigree and source population of the inbreds is given in Table 1. Population A-64 was synthesized mainly from the early maturing genotypes, generally exotic germplasm besides material from CIMMYT. MDR-1 and AD-609 were synthesized using several Indian and exotic early maturity germplasm.

DNA was extracted from the leaf samples (from 20-day old seedlings) using the CTAB procedure [11] with minor modifications. Forty SSR primers were selected for this study on the basis of their genomic (bin) locations. Information regarding the map position and repeat type for each of the SSRs is given in Table 2. The PCR profile consisted of the following steps: initial denaturation at 94°C for 4 min, and subsequent 35 cycles, each with denaturation at 94°C for 1 min, primer annealing at 58°C for 1min and primer extension at 72°C for 2 min. The final extension step was performed at 72°C for 7 min. The 12µl reaction mix consisted of 30 ng of template DNA.

The amplified products were resolved on 3.5% SFR (Super Fine Resolution; Amresco) agarose gel that has the capacity to resolve amplified products ranging from 75 to 750 bp. A 100 bp ladder was used for approximate sizing of the products. DNA samples were electrophoresed for 2 h at a constant voltage of 100 V (Bio-Rad sub-cell Model 96) in IX TBE buffer and photographed with a CCD camera attached to a gel documentation system (Vilber Laurmat). SSR alleles were scored sequentially from the largest to the smallest size band based on their position relative to the ladder. Any diffused band or those that were difficult to score were considered as missing data (and designated as '9' in comparison with '1' for the presence of a band and '0' for the absence of a band in the data matrix). The markers which were found to be monomorphic were excluded from the analysis.

Table 1. Geographic origin of the material and pedigree of the maize inbred lines

Source population	Origin of source germplasm	Pedigree	Code No.
A-64	Mexico, USA, Argentina, India and Caribbean region	IPA 3-f-1-2-1#	DMB101
		IPA 3-f-1-#	DMB102
		IPA 3-f-2-#	DMB103
		IPA 1-f-16-2-#-#	DMB104
		TCA 22-f-#-#	DMB109
AD-609	Australia, Yugoslavia, India, Romania and Argentina	IPA 21-f-#-#	DMB105
		IPA 21-f-1-#	DMB106
MDR-1	Philippines, USA, Thailand, Guatemala and Caribbean region	IPA 34-5-f-#	DMB107
		IPA 34-62-f-#	DMB108
MDR-1 × A-64	Population developed from MDR-1 × A-64	SC 7-2-f-#	DMB110

Polymorphism information content (PIC) for each SSR marker was determined as per the procedure outlined by Senior *et al.*, [12]. Jaccard's coefficient was used to calculate the genetic similarities (GS) among pair-wise comparison of genotypes based on SSR data. The similarity matrix was analysed using NTSYS-pc version 2.02 to produce on agglomerative hierarchical classification by employing Unweighted Pair Group Method using Arithmetic Averages (UPGMA). "Cophenetic correlation coefficient' was estimated using the COPH and MXCOMP options in NTSYS-pc 2.02 to test the goodness of fit of clustering to the SSR data matrix. Canonical discriminant analysis was carried out using SPSS 9.0 software for determining the optimal number of clusters.

Results and discussion

Out of 40 SSR markers employed to investigate the polymorphism (ensuring coverage of all the ten chromosomes), 32 revealed clear and consistent amplification profiles. Among these, five markers (*phi014, bnlg400, phi023, phi062 and umc1996*) showed monomorphic pattern and hence were not included in further analysis. The pattern of polymorphism for two SSR markers is indicated in Fig. 1. Of the 27 polymorphic SSR loci, eight showed four alleles each, twelve revealed three alleles each, and for the remaining seven loci, two alleles were amplified.

Polymorphism information content (PIC), which is a measure of allelic diversity at a locus, ranged from 0.38 to 0.70. PIC is estimated using the formula '$1-\Sigma (P_{ij}^2)$ where P_{ij} is the frequency of j^{th} allele for i^{th} locus summed across all alleles in the locus. The PIC values ranging from '0' (monomorphic) to '1' (highly discriminative with many alleles in equal frequencies) indicate discriminative power of the marker by taking into account not only the number of alleles at a locus, but also the relative frequencies of those alleles in the genotypes under study.

Some SSR markers were found to have higher discriminating power for differentiation of inbred lines as they uncovered several unique or rare alleles (frequency less than 0.1) in the genotypes analyzed.

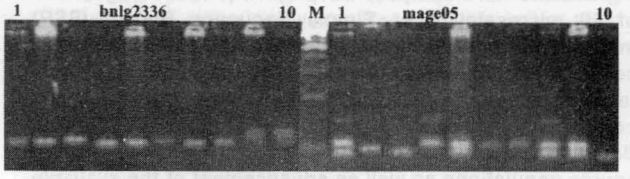

Fig. 1. SSR allele patterns among the maize inbred lines revealed by primers *bnlg2336* and *mage05*. M = Molecular weight marker, 1-10 = Ten inbred lines as per information provided in table 1.

Eight unique alleles were detected in four of the genotypes *viz.*, DMB101, DMB105, DMB106 and DMB109. Of these, three unique alleles were specific to DMB105 (amplified by *umc1225, bnlg1796, bnlg127*), two each were uncovered in DMB106 (*umc1014, bnlg572*) and DMB109 (*bnlg1796, bnlg162*), while one allele (*bnlg1452*) was specific to DMB101. These unique or rare SSR alleles provide an opportunity for unambiguous differentiation of the respective inbred lines. For instance, in the present study DMB105 has at least one rare allele each for *bnlg1796* and *umc1225*. Also, primers of different size range can be used in a single PCR reaction (multiplexing) allowing distinction of DMB105 from other inbred lines studied. It is also possible to effectively utilize a group of SSR markers,

Table 2. Microsatellite markers used in the present study

Primer	SSR Repeats	Bin location	Primer	SSR Repeats	Bin location
bnlg147	GT	1.02	*Phi075*	CT	6.00
bnlg615	CT	1.07	*umc1006*	GCCAGA	6.02
bnlg400	AG	1.09	*umc1014*	GA	6.04
umc1331	GGT	1.11-1.12	*phi089*	ATGC	6.08
bnlg371	CA	2.05	*bnlg572*	AG	7.03
mage05	AG	2.05	*bnlg339*	CT	7.03
phi029	CCCTCT	3.04	*phi045*	AAC	7.06
bnlg1452	AG	3.04	*phi119*	AG	8.02
Bnlg1796	AG	3.06	*phi014*	GGC	8.04
phi079	AGATG	4.05	*phi121*	CCG	8.04
dupssr34	TTG	4.07	*bnlg162*	CT	8.05
phi093	AGCT	4.08	*phi033*	AAG	9.01
bnlg143	CA	5.01	*bnlg127*	CT	9.04
bnlg105	AG	5.02	*phi108411*	AGCT	9.05-9.08
umc1225	AG	5.08	phi062	ACG	10.04
bnlg389	CT	5.09	*bnlg2336*	AG	10.04

where alleles in combination could provide unique genotyping of several inbreds included in the present study.

The profiles of the inbred lines as revealed by SSR markers also gave information regarding their status of homogygosity at the genomic level. While DMB102 and DMB104 were found to be homozygous (with no double bonds detected among the SSR alleles amplified), in other lines some amount of heterozygosity was implied by the appearance of double bands. The reasons for double bands in some inbreds may be due to residual heterozygosity, contamination by stray pollen during inbred development or due to mutation caused spontaneously or by transposable elements during inbred development. Specifically, two of the lines (DMB107 and DMB109) might need special attention of the breeders in view of high frequency (10/26) of double bands. The need for increasing the level of homogygosity of the inbred lines especially for identifying essentially derived varieties was also emphasized by Heeknberger *et al.*, [13], who analyzed SSR profiles of many different accessions of maize inbreds.

Using the dataset generated by SSR profiles, genetic relationships among the ten inbred lines was analyzed. The dendrogram presented in Fig. 2 shows the genetic relationships as reaveleded by cluster analysis using Jaccard's coefficient and UPGMA algorithm. The cophenetic value was estimated to be 0.75, indicating a good correspondence between the dendrogram and the genetic similarity matrix derived using the Jaccard's coefficient. In order to determine optimal number of clusters, canonical discriminant analysis was carried out, which revealed three distinct clusters (Fig. 3). While eight of the inbred lines clustered in one group, DMB106 (IPA21) and DMB102 (IPA3) separated out into distinct groups.

Grouping of the ten genotypes on the basis of SSR profiling was found to be partially congruent with their pedigree and breeding history. This could be due to various reasons including small number of SSR loci analyzed in this study as well as the broad genetic base of the source populations. In general, cluster analysis revealed high genetic divergence among the inbred lines. Even among the eight lines forming the first cluster, the genetic diversity was considerably high. DMB102 and DMB106, which formed distinct clusters, originated from different source populations. Genotypes (DMB105 and DMB106) belonging to AD-609 source population were found to be relatively different from other lines. Genotypes derived from A-64 and MDR-1 appeared to be more closely related, including DMB110, which shared these two source populations. DMB107 and DMB108 which are MDR-1 derivatives, clustered

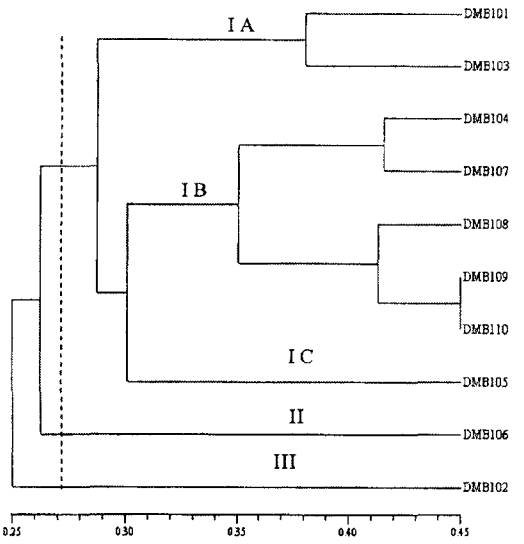

Fig. 2. Dendrogram depicting genetic relationships among selected inbreds based on SSR data, using Jaccard's similarity coefficient and UPGMA clustering algorithm.

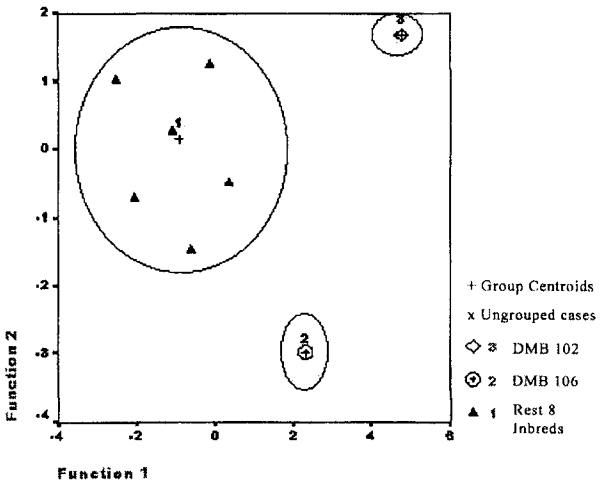

Fig. 3. Canonical discriminant analysis of SSR data for determining optimal number of clusters.

together along with DMB103, DMB104 and DMB109. However, the prominent exception was DMB102, which was found to be different from other lines involving or sharing A-64 source population. It is important to note here that the ten genotypes profiled in the present study were chosen mainly on the basis of their desirable features relating to productivity, maturity, etc. with an eye on their eventual use in generating cross combinations. Consequently there was unequal representation of the genotypes from each source population (Table 1). Further, pedigree and molecular marker data may not always be congruent in highly cross-pollinated crops like maize due to selection drift during the development of inbreds [14]. Yu *et al.*, [15] also reported some inconsistencies in cluster patterns using SSR data.

Smith *et al.*, [3, 16] reported the utility of SSRs as genetic markers to discriminate 58 inbred lines of maize using 131 SSR. They concluded that SSR profiling represent the optimum approach for identification and pedigree validation of maize genotypes compared to other currently available methods. While SSR polymorphism might very well represent genetically divergent and heterotic patterns, predicting F_1 grain yield performance based on SSR divergence is not always possible [16, 17]. SSR, on the other hand, can be effectively employed in a breeding programme to identify parental lines for hybrid development as well as for diverse purposes in molecular breeding [9, 18]. In order to utilize SSR markers for identification of inbred lines and essentially derived varieties, it is essential to employ suitable number of SSR markers that cover the entire genome [19].

The present study, while implying the broad base of the source populations, has indicated the need for reorganizing the component lines derived from each of them. It might be desirable to systematically assess the heterotic relationships among all the component lines derived from the four source populations using suitable testers besides SSR data. Such efforts should facilitate better and more meaningful utilization of the available germplasm, with better conformity between pedigree, source population, heterosis and molecular marker data. The present study is a step towards this direction.

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