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MOLECULAR ASSESSMENT OF MAIZE INBRED LINES (*Zea Mays* L.) USING MICROSATELLITE MARKERS

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Abstract

Genetic diversity analysis and germplasm characterization are essential steps in plant breeding and molecular markers are proved tool to accomplish. The present study was undertaken at the Molecular Breeding Lab of Plant Breeding Division, Bangladesh Agricultural Research Institute (BARI) to determine the genetic relatedness and molecular characterization of 15 maize inbred lines of BARI. In present study, genetic diversity analysis was performed by using 10 SSR primers to evaluate the polymorphisms, among them six primers showed distinct polymorphism between the maize inbred lines. The maize genotypes E81, E144, E08, E167, E102, E142 and E121 were found more diverged (0.9003) compared to other inbred lines. On the other hand, the lowest genetic distance values (0.1501) were found between the genotype E140 and genotype E80 followed by genotype E126 and genotype E140; genotype E140 and genotype E65; genotype E65 and genotype E80 values were identical (0.4502). The genotypes viz. E81, E144, E08, E167, E102, E142 and E121 were found far away from centroid of the cluster and rest of the genotypes were placed around the centroid. The Principal Coordinate Analysis (PCO) helped to visualize four major clusters and showed that seven maize inbred lines (E81, E58, E08, E167, E102, E142 and E121) were far away from the other genotypes. In conclusion, SSR markers enabled discrimination among accessions and provided valuable information for future use in improvement of these genomic resources.

Keywords: Molecular Diversity, Microsatellite Marker and Inbred Maize

Introduction

Diversity of maize (*Zea mays* L.) inbreds has major importance in the process of maize improvement. The narrow genetic base of the modern highly yielding maize hybrids is problematic in breeding for adaptation to biotic and abiotic stresses, including chilling, drought, heat or salt tolerance. Knowledge on the genetic diversity and relationships among maize inbred lines is helpful in identifying promising combinations for exploitation of heterosis and establishment of heterotic groups for use as source materials in a breeding program. Morphological characteristics are often influenced by the environment

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and therefore, they do not always express genetic relationships. Besides, these traits reveal differences that are not comprehensible in terms of genetic distances. Molecular markers have proven valuable for genetic diversity analysis of many crop species. Their expression, unlike morphological markers, is not influenced by environmental factors; hence reflect the actual level of genetic difference existing between genotypes (Smith and Smith 1992, Westman and Kresovich 1997). In maize, microsatellites have proved to be a valuable tool for genome mapping (Taramino and Tingey 1996). Microsatellites or simple sequence repeats (SSRs) are DNA markers with short stretches of tandemly repeated di-, tri- or tetra-nucleotide motifs (Weber 1990). SSRs are characterized by a great abundance (Matsuoka *et al.* 2002), high variability (Tautz 1989, Schug *et al*. 1998) and even distribution throughout a wide range of genomic regions (Liu *et al.* 1996, Senior *et al.* 1996). They are codominant, highly polymorphic, multi-allelic and have become the marker of choice for genetic analysis in crops (Gupta and Varshney 2000). The objective of the present study was to use microsatellite markers for assessment of genetic diversity among the maize variety and inbred lines.

Materials and Methods

A total of 15 inbred lines of maize were randomly selected from BARI maize inbred lines. Seeds were grown in plastic pots. Then the pots were kept in the net house. After fifteen to twenty days (3 or 4 leaf stage) the fresh leaf was used for DNA isolation. Total DNA was isolated by CTAB method with slight modifications according to Maaß and Klass (1995). After treatment with 10µg/ml RNase A for half an hour at 37ºC, the DNA was purified with propanol. The purified DNA was dissolved in TE buffer and stored at -20ºC and the concentration was determined fluorometrically (Nano drop).

Ten SSR primer pairs were chosen (p-umc1354,p-umc1566,p-umc1292,p-bnlg1124,p-bnlg1179,phi002,phi037,phi038, phi039and bnlg565) to evaluate the polymorphism among the inbred lines. PCR conditions were optimized according to Hoxha *et al.* (2003). Here amplifications were performed in 20µl volumes containing 100ng genomic DNA, 2.5 mMdNTPs, 1.5mM MgCl2 , 10 pmol each forward and reverse primers, 3U TaqDNA polymerase and 10X PCR buffer (Genei). Thermal cycling consisted of initial denaturation at 95ºC for 3min, 30 cycles of 95ºC for 1 min, annealing temperature 55ºC for 1 min and 72ºC for 1 min, followed by a final extension at 72ºC for 5 min. PCR products were stored at 4ºC until use. The PCR products were visualized in Polyacrylamide gel electrophoresis (PAGE).

Molecular weight for each amplified allele was measured in base pair using Alpha-Ease FC 5.0 software. The allele frequency data from Power Marker

version 3.25 (Liu and Muse, 2005) was used to export the data in binary format (allele presence="I" and allele absence = "0") for analysis with NTSYS-pc version 2.2 (Rohlf, 2002).

The summary statistics including the number of alleles per locus, major allele frequency, gene diversity, polymorphism information content (PIC) values were determined using Power Marker version 3.25 (Liu and Muse, 2005). A similarity matrix was calculated with the Simqual subprogram using the Dice coefficient, followed by cluster analysis with the SAHN subprogram using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering method as implemented in NTSYS-pc was used to construct a dendrogram showing relationship among the genotypes. The similarity matrix was also used for principal coordinate analysis (PCA) with the D Center, Output, and MXPlot subprograms in computer program Numerical Taxonomy and Multivariate Analysis System (NTSYS-pc).

Results and Discussions

Microstallites displayed a high level of polymorphism.Out of 10 SSR markers employed to investigate the polymorphism, six markers (p-umc1354, p-umc1566, p-umc1292, phi037, phi039 and bnlg565) revealed clear and consistent amplification profiles. Among these, four markers (phi038, bnlg1179, bnlg1124, phi002) showed monomorphic pattern and hence were not included in further analysis.

A total of 48 alleles were detected at 10 SSR markers among 15 maize inbred lines with an average of 8 alleles per-microsatellite/genotypes locus (Table 1).The highest number of alleles per locus/genotype was detected using SSR primer set bnlg565, showing 12 alleles with an average of 0.8 alleles per genotype (Table 1). The lowest allele number per locus among the homologous chromosomes was observed using SSR primer set p-umc1566, showing a total of 4 with an average of 0.27 alleles per genotype (Table 1). The average number of alleles obtained in the present study was higher than those reported in previous maize diversity studies (Lu and Bernardo, 2001, Enoki *et al.* 2002, VazPatto *et al.* 2004). Lu and Bernardo (2001) reported 4.9 alleles per SSR locus for a sample of 40 US inbreds analyzed by 83 SSR markers; Warburton *et al.* (2002) investigating 57 CML lines with 85 SSR markers reported 4.9 alleles per marker; Senior *et al.* (1998) found 5.0 alleles/locus in a study of 94 elite US maize inbreds with 70 SSR markers, and Vaz Patto *et al.* (2004) reported 5.33 alleles per locus in 104 Portuguese and inbreds using 15 SSRs. In addition, Pejic *et al.* (1998) reported 6.8alleles/locus in 33 inbreds from US corn belt using 27 SSRs; Enoki *et al.* (2002), studying 65 inbred lines adapted to cold regions of Japan and imported American materials with 60 SSRs reported 7.3 alleles per locus and Xia

et al. (2004) reported 7.4 alleles per locus in 155 tropical lowland inbreds using 79 SSRs. On the other hand, Liu *et al.* (2003) reported average 21.7 alleles per locus in a study including 260 US inbreds analyzed at 94 SSR loci. It is important to note that the total number of alleles reported in diversity studies is usually proportional to sample size, and some differences seen here may be attributable to sampling differences. However, another factor influencing the number of alleles is the use of di-nucleotide repeat SSRs, which can produce large number of alleles. However the more diverse set of inbreds from the gene bank collections included in the study may also contribute to the observed higher allelic richness. Range of allele size (bp) was from 4 (p-umc1566) to 12 (bngl565) (Table 1). The highest allele size difference in phi039 followed by bnlg565 and lowest in p-umc1566. According to Nei's (1973) the highest level of gene diversity value (0.8991) was observed in loci bnlg565 and the lowest of gene diversity value (0.6234) was observed in loci p-umc1566 with a mean diversity of 0.7773 (Table 2). It was observed that marker detecting the lower number of alleles showed lower gene diversity than those detected the higher number of alleles showed higher gene diversity. This result is consistent with previous work done by Herrera *et al.* (2008).

Table 1. Number of alleles, range of allele (bp) and gene diversity (GD) found in 15 maize inbred lines for 6 SSR markers.

Sl. No.	Markers	Chro[a] No.	Repeat type	Allele number	Range of allele size (bp)	Diff[b] (bp)	Gene diversity
1	p-umc1354	1.01	(CCG)5	6	36-62	26	0.7556
2	p-umc1566	1.01	(GCC)6	4	65-68	3	0.6756
3	p-umc1292	1.01	(TGG)6	8	68-100	32	0.8356
4	phi037	1.08	(AG)	8	131-164	33	0.8356
5	phi039	1.08	(ATT)	10	78-129	51	0.8000
6	bnlg565	3	(CT)21	12	47-92	45	0.9067
	Mean			8			0.8015

The frequency of the most common allele at each locus ranged from 13.33 % (bnlg565) to 46.66% (p-umc1566). On an average, 32.22% of the 15 maize inbred lines shared common major allele at any given locus (Table 2).

The polymorphic information content (PIC) values ranged from 0.6234 to 0.8991 with an average *of* 0.7733. The highest PIC value (0.8991) was obtained for bnlg565 followed by p-umc1292 (0.8159), phi37 (0.8159) respectively. The lowest PIC value (0.6234) was obtained for p-umc1566 (Table 2). PIC value revealed that bnlg565 was considered as the best marker for 15 maize inbred

lines followed by p-umc1292 and phi37. P-umc1566 could be considered as least powerful marker.

The average PIC value determined in our investigation agreed well with the earlier findings reported based on SSR marker in maize inbred lines (Senior *et al.* 1998, Heckenberger *et al.* 2002 and VazPatto *et al.* 2004). Dinucleotide SSR loci (phi 037, nc003, bnlg619, phi054) identified the largest mean number of alleles (4.8) and mean PIC (0.67) as compared to tri, tetra and penta nucleotide repeats in this study, which is also in close agreement with previous observations for maize (Smith *et al.* 1997, Senior *et al.* 1998 and Enoki *et al.* 2002).

Table 2. Data on sample size, No. of observation, major alleles frequencies and Polymorphism information content (PIC) found among 15 maize inbred lines.

Sl. No.	Locus	No. of observation	Major allele frequencies (%)	PIC	Mean PIC
1	p-umc1354	15	0.40	0.7237	0.7773
2	p-umc1566	15	0.47	0.6234	
3	p-umc1292	15	0.27	0.8159	
4	phi037	15	0.27	0.8159	
5	phi039	15	0.40	0.7858	
6	bnlg565	15	0.13	0.8991	

The UPGMA clustering system also generated four genetic clusters with similarity coefficient of 15%. Maize inbred line E58 alone formed a single cluster named cluster III which showed 88% dissimilarity with the cluster IV (E121, E144, E142, E132, E139, and E89) and 79% dissimilarity with cluster II (E102, E126, E140, E80, E65 and E81).

Two inbred lines form a single cluster I where E08 and E167 are closed to each other. In cluster II inbred lines E140 and E80 showed maximum similarity 83% followed by E132, E139; E121, E144 (34% similar and 66% dissimilar). Cluster IV and Cluster II had maximum six inbred lines. In cluster IV all inbred were closed to each other by about 26%.

The pair-wise genetic dissimilarity coefficients based on 6 SSR marker indicated that the maximum genetic distance values (0.9003) was recognized between genotype E08 and genotype E121, genotype E08 and genotype E102, genotype E102 and genotype E121, genotype 139 and genotype E65, genotype E140 and genotype E132, genotype E58 and genotype E126, genotype E80 and genotype E144, genotype E81 and genotype E142, genotype E89 and genotype E81 and so on (Table 3). These results were in agreement with findings of Principal

Coordinate Analysis and suggested that these genotypes were diversed. The lowest genetic distance values (0.1501) were found between the genotype E140 and genotype E80 followed by genotype E126 and genotype E140; genotype E140 and genotype E65; genotype E65 and genotype E80 values were identical (0.4502).

Each lower and higher intergenotypic distances between pairs of maize inbred lines based on 6 SSR markers were given in the (Table 3) on the basis of Nei distance (Nei, 1973). Future breeding program for crop improvement could choose these genetically diversed parents for crossing program to create genetic variability and transgressive segregants.

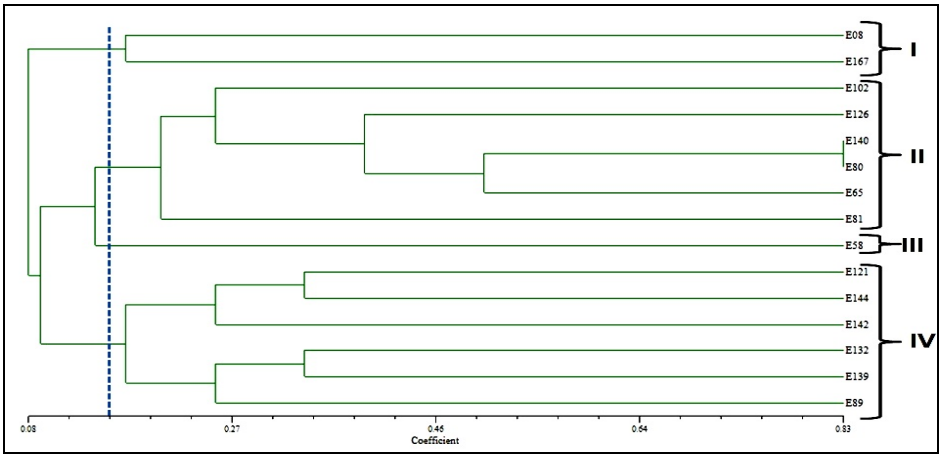

Fig. 1. Associations among maize inbred lines revealed by cluster analysis of SSR distance databased on the alleles detected by 6 SSR markers.

The two dimensional graphical view of Principal Coordinate Analysis (PCO) showed the spatial distribution of the 15 maize inbred lines along the two principal axes. The genotypes viz. E81, E144, E08, E167, E102, E142 and E121 were found far away from centroid of the cluster and rest of the genotypes were placed around the centroid (Fig.2). The genotypes were placed far away from the centriod were more genetically diverged compared to the genotypes placed near the centriod were likely to be genetically more similar. However, centriod may be defined as the vector representing the middle point of the cluster which contained at least one number for each variable. The connecting line between the each genotype and the centriod represented eigen vectors for the respective genotypes.

Table 3. Lower and higher inter genotypic distances (Nei, 1973) between pairs of Maize inbred lines based on 6 SSR markers

	E08	E102	E121	E126	E132	E139	E140	E142	E144	E167	E58	E65	E80	E81	E89
E08															
E102	0.9003														
E121	0.9003	0.9003													
E126	0.7503	0.7503	0.6002												
E132	0.7503	0.7503	0.7503	0.6002											
E139	0.9003	0.9003	0.7503	0.7503	0.6002										
E140	0.9003	0.6002	0.7503	0.4502	0.9003	0.7503									
E142	0.7503	0.9003	0.7503	0.7503	0.7503	0.7503	0.9003								
E144	0.9003	0.9003	0.6002	0.7503	0.7503	0.7503	0.9003	0.6002							
E167	0.7503	0.7503	0.9003	0.7503	0.9003	0.7503	0.7503	0.7503	0.9003						
E58	0.9003	0.9003	0.7503	0.9003	0.7503	0.7503	0.7503	0.9003	0.9003	0.9003					
E65	0.7503	0.7503	0.7503	0.6002	0.9003	0.9003	0.4502	0.7503	0.9003	0.7503	0.6002				
E80	0.9003	0.6002	0.6002	0.6002	0.9003	0.7503	0.1501	0.9003	0.9003	0.7503	0.7503	0.4502			
E81	0.9003	0.7503	0.7503	0.9003	0.9003	0.9003	0.6002	0.9003	0.9003	0.9003	0.7503	0.7503	0.6002		
E89	0.7503	0.9003	0.7503	0.7503	0.7503	0.6002	0.7503	0.7503	0.7503	0.9003	0.9003	0.9003	0.7503	0.9003	

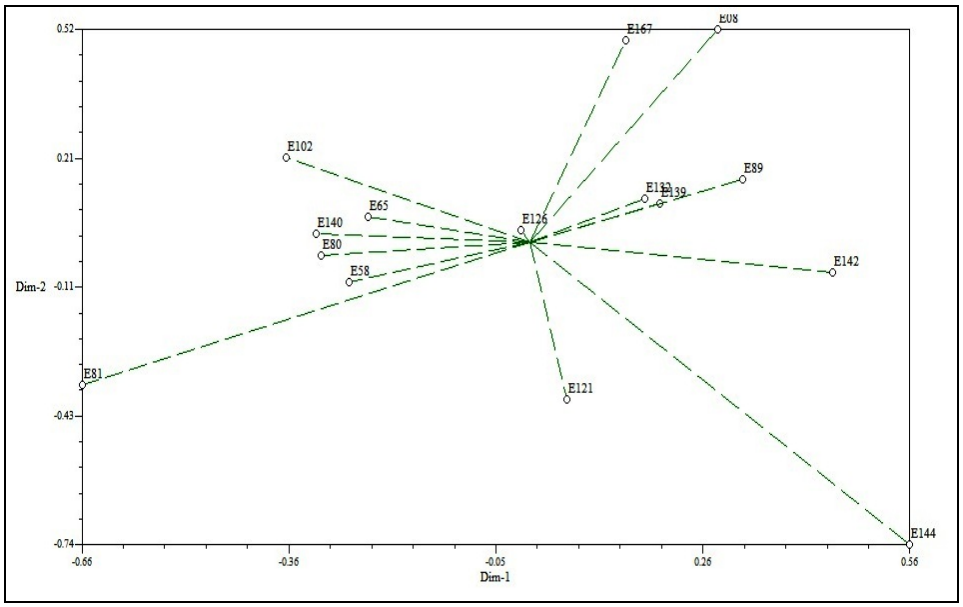

Fig. 2. Two-dimensional view of principal coordinate analysis (PCO) with 6 SSR markers over 15 maize inbred lines

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