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To Assess the Genetic Diversity and Population Structure of Tropical Maize Germplasm using Molecular SSR Markers

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A B S T R A C T

Genetic diversity among 288 Public sector developed tropical maize inbred lines was conducted at the research farm of Plant Breeding Division, (Indian Institute of Maize Research), Delhi and Ludhiana, India during Rabi and Kharif season 2015-17. The genotypes were grouped into six clusters. Cluster I comprised the maximum genotypes (64) which indicated the genetic similarity among them. The minimum genotype (24) was contained in the cluster II. The highest inter-cluster distance was observed between cluster IV and II (0.99) followed by cluster III and IV (0.83) and cluster V and IV (0.33) suggesting wider diversity between them and the genotypes in these cluster could be used as donor parents for new maize hybrid development. The highest intra-cluster distance was observed in cluster IV (0.99) and the cluster VI was had the least intra cluster distance (0.96). The positive absolute values of the two vectors revealed that ear height, ear diameter and yield (t/ha) had the greatest contribution to genetic divergence. The negative values for the two vectors for days to 50% tasseling, ear length and thousand seed weight (TSW) indicated the least responsibility of both the primary and secondary differentiations.

Key words: Genetic diversity, Maize, Inbred, Cluster analysis, Molecular SSR markers

Maize germplasm include landraces, adapted populations, and introduced exotic material. Inbred lines are the prerequisite for hybrid variety development in crop plants. For developing high yielding hybrids in maize, inbred lines need to be developed and evaluated for their diverged gene pool. In addition to its agronomic importance, maize has been a keystone model organism for basic research for nearly a century. It is the most thoroughly researched genetic (Vivodik *et al*. 2017). Yields in maize have increased continually since the 1930s through use of standard plant breeding techniques for selection and hybridization have proposed a framework for improving maize grain yield in tropics maize (Fisher and Palmer 1983).

The genetic diversity between the genotypes is important as the genetically diverged parents are able to produce high heterotic effects. Several studies on maize have shown that inbred lines from diverse stocks tend to be more productive than crosses of inbred lines from the same variety. Manifestation of heterosis usually depends on the genetic divergence of the two parental varieties (Sexna *et al.* 1998). The quantification of genetic diversity through biometrica1 procedure made it possible to choose genetically diverse parents. Genetic diversity in maize is a valuable natural resource and plays a key role in hybrid breeding program. Knowledge of germplasm diversity and the relationship among elite breeding materials has a significant impact on the improvement of crop plants (hallauer *et al.* 1988). In maize, this information is useful in planning crosses for hybrid and line development, in assigning lines to heterotic groups, and in plant variety protection. Evaluation of genetic diversity is important to know the source of genes for a particular trait within the

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available germplasm (Tomooka 1991). The importance of genetically diverse genotypes as a source of obtaining transgressive segregants with desirable combinations has been reported by several workers (Peter and Rai 1978). Genetic resources are, in the sense, the building blocks and also fundamental not only to a crop improvement program, but also for the very survival of the species in time and space (Swaminathan 1983). Moreover, evaluation of genetic diversity is important to know the source of genes for particular trait within the available germplasm. In view of above importance, the present investigation was carried out to identify genetically diverse parents for hybridization. Several previous studies demonstrated the possibilities of molecular markers because they offer a stable and reliable alternative for genetic identification and characterization of germplasm collections.

The most common molecular markers used to assess genetic diversity in tropical maize lines include restriction fragment length polymorphism (RFLP), random amplified polymorphic (RAPD), microsatellite or simple sequence repeats (SSRs), amplified fragment length polymorphism (AFLP) and single nucleotide polymorphism (SNP) (Cheng *et al.* 2017). The SSR markers are known for their dominant inheritance, locus specificity, extensive genome coverage and simple detection of locus using labeled primers (Wu *et al.* 2010, Xu *et al.* 2013, Gideon *et al*. 2017). Molecular markers can be employed to investigate levels of population structure and genetic diversity among maize inbred lines and breeding materials. SSRs, due to its sufficient, exceedingly polymorphic, genome specific, co-dominant in nature, have found application in analyses of population structure, gene mapping, genetic diversity, and assisted selection for maize improvement (Phumichai *et al*. 2012, Wende *et al*. 2013, Semagn *et al*. 2014, Yang *et al*. 2013, Abakemal *et al*. 2015). In the present study, 288 public sector selected lines were analyzed using 55 SSR markers distributed over the maize germplasm. Our objectives were to estimate the levels of genetic diversity and population structure of maize tropical inbred line using molecular markers. The results will be useful to breeders in selecting the best parental combinations for maize breeding program in India.

MATERIALS AND METHODS

A total of 288 tropical maize genotype including indigenous, exotic accession breeding lines along with 55 SSR marker and inbred lines from the public sectors were used in the present study. These genotypes were taken from the germplasm collection maintained at IIMR, New Delhi, CIMMYT, AICRP and NBPGR.

SSR markers and genotyping

Genomic DNA was extracted from approximately 200 mg fresh leaf tissue using the cetyltrimethylammonium bromide (CTAB) method (Saghai-Maroof *et al*. 1984). A total of 55 SSR primers, which were distributed evenly over the 6 maize chromosomes, were selected and synthesized according to the information available in the Maize-GDB database (http://archive.maizegdb.org/). PCR amplifications were carried out in 15 µl reaction volumes containing 20-30ng of 2µL template DNA, 1µM each of 2.0µL primer, 5 × 2.25 Taq-buffer, 0.05µL of 5 units μL^{-1} Taq DNA polymerase, 2.5µl dNTPs of 0.80µL, 25mM $mgcl_2$ of 0.60 and dH_2O 7.30 µL. PCR protocols consisted of 32 cycles of 94 for 45s, an annealing temperature at either 45, 50, 55 or 60°C depending on the individual SSR primers for 45s, and 72°C for 60s, and a final extension step of 72°C for 10 min. PCR products were analyzed by 3.5% Metaphor gel electrophoresis and visualized by blue dye staining with gel-doc.

Genetic diversity analysis

For each SSR locus, polymorphic bands were scored as 1 or 0 for presence or absence of the bands at the same mobility, respectively. Gene diversity (PIC) was calculated for each marker according to the formula: $PIC=1-\sum fi2$, where fi is the allele frequency for the i^{-th} locus summed across all alleles for that locus. The program Power-Marker v3.25 and Micro Soft Excel was used to calculate allele number, allele frequency, and gene diversity of each locus (Liu *et al*. 2005).

Population structure analysis

The STRUCTURE v2.3.3 was employed to assess the population structure of the 288 maize inbred lines using the Bayesian model-based approach (Pritchard *et al*. 2000). The number of subgroups (K), with each K repeated five times, was ranged from 1 to 12, with burn-in of 100,000 and run length of 100,000. We used the ad hoc criterion △K related to the second order rate of change in the log probability of data (LnP(D)) to determine the most probable K value (Evanno *et al*. 2005). To examine genetic diversity among the 288 maize inbred lines, the data matrices of the genetic similarity were used to create the dendrogram using UPGMA clustering with the computer structure softwareand NTSYS-pc v2.2 (Rohlf 2009). Principal coordinate analysis (PCoA) was also employed to reveal relationships among the 288 inbred lines using the software.

RESULTS AND DISCUSSION

A subset of 55 randomly selected polymorphic SSRs marker was amplified with 288 accessions of Maize which include tropical inbred line collection (collections from different states of India). The PIC value for these 55 polymorphic SSRs markers with respect to 288 accessions varied from 0.33 to 0.99. The average of polymorphism information content for the SSR markers was 0.57±0.19. Maximum PIC value was noticed for dupssr14 (0.99) followed by Phi 015 (0.96) and umc1232 (0.83), while the lowest PIC value (0.01) was noticed Bnlg1092 (0.33), umc2208, and Phi 92. Majority of the SSRs (60%) showed PIC value (>0.50) and there were only four SSRs which showed PIC value in between 0.83 and 0.99 (Table 1). The highest PIC value was observed in individual markers dupssr14 (0.99) indicated that this marker had shown more allelic variation among all. However, Lowest PIC value indicated the low allelic variation. The Groups wise highest PIC value was observed in Bnlg252, 0.57 whereas; lowest

PIC value was observed in group Phi033 markers with value of 0.54.

Table 1 Genetic diversity at genome level of 288 tropical maize inbred lines revealed by 55 SSR markers with chromosomes number and PIC values

Chromosome	Markers	No. of alleles (range)	PIC values
8	dupssr14	53	0.99
4	umc1232	52	0.69
5	umc1332	64	0.83
5	Umc2303	38	0.83
4	Bnlg252	57	0.63

Based on the maximum membership probability, 288 inbred lines were assigned into 6 subpopulations (Red, Green, Blue, yellow, Purple and Sky blue) (Fig 1). Red included 64 tropical maize inbred lines closed to yellow color 57 maize lines in genetic backgrounds. Green had 24 inbred lines related to green color in genetic background. Blue color had included 22 inbred lines. Purple color had included 52 tropical maize inbred lines. Or sky-blue color had 38 included inbred lines at 6 genetic backgrounds, which named as population structure subgroup described by (Xie *et al*. (2008).

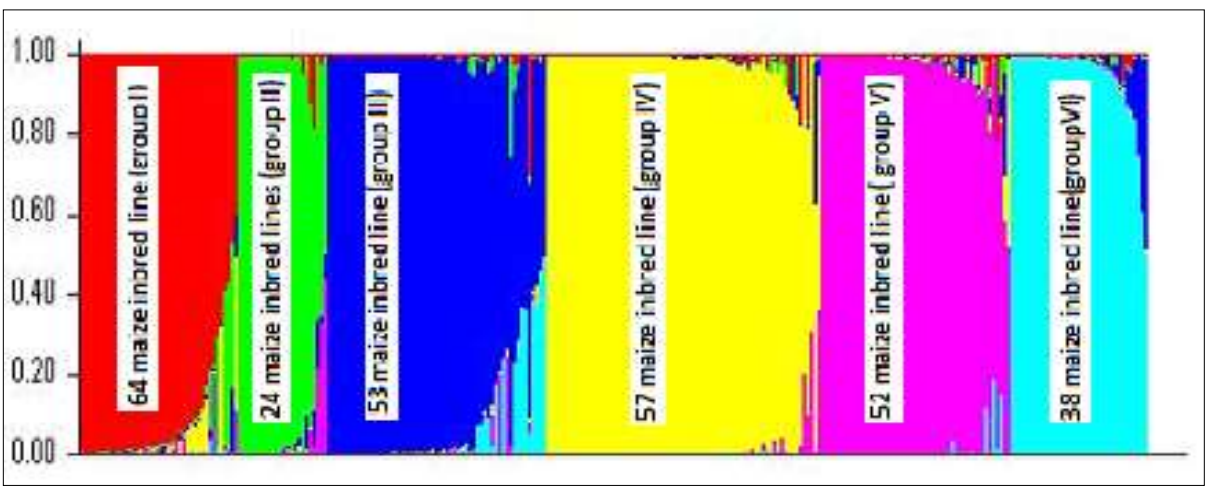

Fig 1 Population structure of the 288 tropical maize inbred lines (K=6)
Note: the vertical coordinate of represents the288 inbred line. The vertical coordinate of each subgroup indicates the coefficients for each individual along with colors

The similarity coefficient among 288 maize inbred lines ranged from 0.33 to 0.99 and the average was 0.60. When the similarity coefficient was 0.67, cluster analysis also clearly grouped 288 inbred lines into six subpopulations than clustering were consistent with their assignments using STRUCTURE software.

The statically analysis based on 55 SSR markers also separated the 228 maize inbred lines into six major groups. As inferred by STRUCTURE analysis, the inbred lines in Red were mainly distributed in between 2 to 6 cluster group, green distributed in between 1 to 5 group, blue distributed in between 4 to 5 group, yellow distributed in between 3 to 6 group, purple distributed in between 2 to 3 group, sky blue distributed in between 1 to 4 group. Most individuals within Red and sky-blue subpopulations were grouped more closely. The green color or II group indicating that higher genetic diversity subpopulations.

SSR markers, due to their abundance, co-dominance, and locus specificity, have been extensively used to assess genetic diversity in maize genotypes (Sarcevic *et al*. 2008). In the present study, all the 288 tested maize inbred lines were derived from public sector inbred selected lines. The average polymorphic information content (PIC) value was 0.33, which was lower than that in important inbred lines with PIC over 0.97 (Wang *et al*. 2008, Xie *et al*. 2008). The number of alleles found in this study is also in agreement with other studies (Wang *et al*. 2008, Park *et al*. 2015). Wang *et al*. (2008) reported a total of 1,365 alleles with an average of 9.4 alleles per locus by screening 95 inbred lines using SSR markers. Park *et al*. (2015) genotyped 174 maize inbred lines by 150 SSR markers and detected a total of 1082 alleles with an average of 7.21 alleles per locus. In our study, alleles were obtained at the genome level Chromosome 1 showed the lowest allele number (24 alleles) and chromosome 8 the highest (64 alleles). Therefore, we have thus determined that there is a higher-level genetic diversity in the 288 tropical maize-selected lines, which has the potential to enhance the genetic diversity of Indian and foreign maize breeding materials.

Population structure in the present study was also investigated using three complementary analysis methods NTSYS and STRUCTURE, statistical analysis based on

SSR data. We selected maximum membership probability as the group criterion, 288 maize inbred lines were assigned into six subpopulations which was in agreement with the assignments obtained by structure software clustering. Nevertheless, for the 288 tested maize inbred selected lines, the pedigree information was not in accordance with their clustering. This finding can be partially explained by complex genetic background in tropical maize inbred line. Therefore, it is of significant importance to understand population structure and genetic diversity among inbred lines is for maize improvement. The allele frequencies, gene diversity and population structure obtained in the present study lead us to conclude that the 288 inbred lines derived from genetically diverse inbred maize lines contain extensive genetic variation and are a valuable resource for Indian maize breeding. It means that highest frequencies of polymorphism shown the susceptibility and survival in adverse condition.

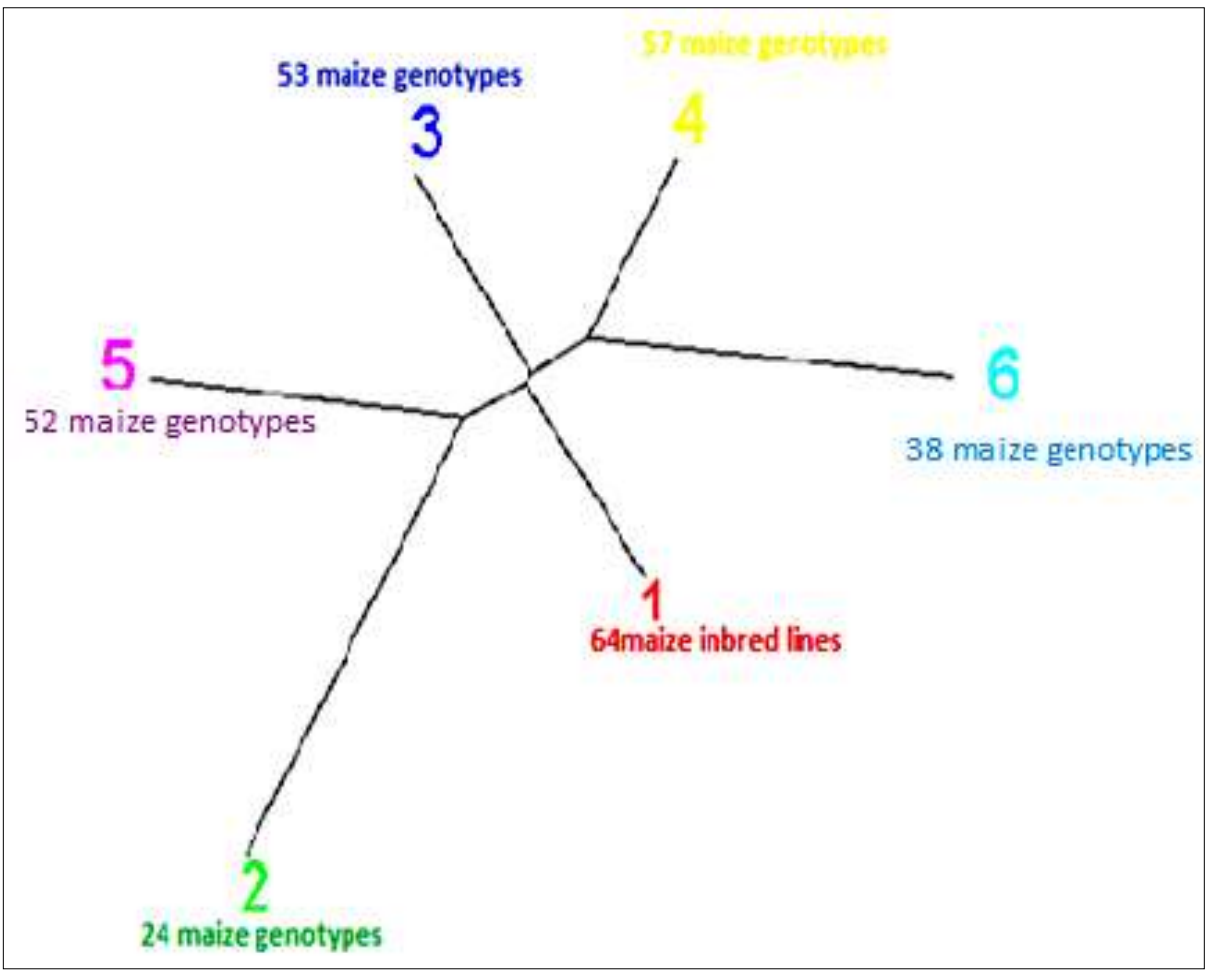

Fig 2 Dendrogram of 288 maize inbred lines by structure cluster analysis

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