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Research Article

Molecular diversity analysis of maize (*Zea mays*L.) inbreds using SSR markers

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Abstract
Maize is the third most important cereals. Inbreds are the widely used parental material in the hybridization program. Many of these accessions have specific features, but only a few of them have been utilized in maize improvement programs.There is an important role of understanding the genetic diversity among and within inbred lines at the molecular level for maize improvement. The present investigation consists of 25 maize inbreds collected from the All India Co-ordinated Maize Improvement Project, Department of Genetics and Plant Breeding, IASc, BHU, Varanasi, were analysed for diversity using a total of 40 SSR markers during rabi 2015-16. Dendrogram was constructed based on UPGMA from the Jaccard's similarity coefficient and the inbreds were subjected to cluster analysis. Among the 40 SSR markers used only 20 found informative (polymorphic) with total 70 alleles, provide a reference for determining the Simple Sequence Repeats (SSR) alleles number in genetic relationship analysis of maize inbred line and other crop germplasm. The PIC value ranged from 0.286 (umc226) to 0.966 (csu308) with an average PIC of 0.736. The first three PCs contributed 23.70, 7.63 and 6.73% respectively, with a cumulative variation of first 3PCs was 38.07%. The cluster analysis indicates inbred HUZM 252 and HUZM 265 are highly diverse. The present study has indicated the need for evaluating the component lines derived from each cluster. Cluster analysis indicates there is an enough diversity found between the inbreds tested and the information on diversity of inbred lines generated in this study would be much useful in developing heterotic hybrids.

Key words
Maize, SSR markers, molecular diversity, UPGMA

Introduction

Maize (*Zea mays* L.) is an important cereal crop in the world. It is the most versatile emerging crops having wider adaptability under varied agro-climatic conditions and is being cultivated in tropic, sub-tropic and temperate regions worldwide under all irrigated to semi-arid conditions. It is grown from latitude 58^0 N to 40^0 S, from sea level to higher than 3000 m altitude and in areas receiving yearly rainfall of 250 to 5000 mm (Shaw, 1988; Downsell *et al*., 1996) and with a growing cycle ranging from 3 to 13 months (CIMMYT, 2000).

Globally, maize is known as queen of cereals because of its highest genetic yield potential among the cereals. It has nearly doubled its production in the early 2000s to today mainly due to adoption of single cross hybrids and integration of molecular techniques in conventional breeding approaches (Prasanna and Sharma, 2005). Despite this, worldwide spectacular increase during the last decade, Indian corn yields are still significantly low when compared with other maize growing countries.

Achievements in hybrid development should be adequately backed up by germplasm enhancement, synthesizing of new gene pools and heterotic populations representing variability for diverse requirements. Assessment of genetic variability in the existing population assumes prime importance in this context. Germplasm, which is a prerequisite for any breeding program, serves as a valuable source material as it provides scope for building of genetic variability. Molecular profiling studies of inbred lines provides a real estimate of variability among the genotypes at the molecular level and hence will serve as a reliable tool for selecting elite germplasm. SSR markers are very useful in molecular profiling as these are PCR based, highly polymorphic and reproducible. The SSR loci are having variable arrays of 2 to 6 base pair tandem repeats. A very large amount of SSR markers have been designed in maize and there is enough information available for the genetic studies. The present study is to characterise 25 inbred lines based on their marker analysis.

Material and Methods

Twenty five inbred lines of maize were used in the present investigation were obtained from the All India Co-ordinated Maize Improvement Project,

Department of Genetics and Plant Breeding, IASc, BHU, Varanasi. (Table. 1)

The DNA was extracted from the leaf samples following CTAB extraction method (Doyle and Doyle, 1987) with minor modifications. Out of 40 primers 20 were found polymorphic. Information regarding the 20 SSR primers is given in table 3. PCR was carried for the reaction mixture and the polymerase chain reaction (PCR) was carried out as per the standard procedure. The amplified products of PCR were resolved on 2.5% agarose gel. A 50 bp ladder was used for sizing of the products. Electrophoresis was carried for 3.5 hours at a constant voltage of 65V in TAE buffer and gel were visualised under a UV light source in gel documentation system (**Gel** Doc™ **XR+**, BIO-RAD, USA). SSR markers, generated clear and unambiguous bands of various molecular sizes, were scored with the number of alleles and the polymorphism based on the base pair differences.

The data matrix was subjected to analysis using NTSYS-pc version 2.11 (Rohlf, 1997). The SIMQUAL program was used to calculate the Jaccard's similarity coefficients. The resulting similarity matrix was used to construct UPGMA (**U**nweighted **P**air **G**roup **M**ethod with **A**rithmetic Mean) based dendrogram. Polymorphic information content (PIC) for each SSR marker was calculated as per the standard PIC formula. PIC=1- $\sum_{i=1}^{k} P_i^2$. Where, P_i is the frequency of the i^{th} allele and k is the total number of different alleles at the specific locus.

Result and Discussion

In the present investigation 40 SSR primers were used out of which 20 primers recorded polymorphic. These 20 SSR markers yielded a total of 70 alleles. The polymorphic pattern for 2 SSR markers is indicated in fig 1. The number of polymorphic bands per primer ranged from 2 to 7 with an average of 3.5. The PIC value ranged from 0.286 (umc226) to 0.966 (csu308) with an average PIC of 0.736. The Jaccard's similarity coefficient ranged from 0.00 to 0.64 due to diversification in morphology and pedigree among the genotypes (fig. 2). The maximum similarity was observed between HUZM-91-1 and HUZM-71 which shows similar origin. Minimum similarity was found between HUZM-252 and HUZM-59, It describes greater degree of diversity. These could be used for hybrid breeding program. High yielding and superior cultivar development relies on efficient and effective utilization of present variability in the inbred lines. Cultivar with specific characteristic could be developed to meet the specific requirements by the use of modern molecular markers efficiently. Suitable number of SSR markers covering the entire genome should be deployed. The selected SSR primers showed a high level of polymorphism depicted by their corresponding PIC value ranged from 0.286 (umc226) to 0.966 (csu308) with an average PIC of 0.736 (Table 2). This result was comparative to the findings of Ying *et al.* (2011) and Hongbo *et al.* (2011).

The coefficient of genetic similarity ranged from 0 to 0.64 which exerted presence of higher diversity in the 25 maize inbred lines studied. Gupta and Singh (2010) reported higher range of diversity (0.12 to 0.73) with an average similarity of 0.42. Narrow range of diversity (0.39-0.61) was found by Kumar *et al.* (2008) among 16 inbreed lines. Roy *et al.* (2015) reported relatively higher range of similarity (0.22-0.87). Higher estimated genetic distance could be due to diverse pedigree of the genotypes.

SSR data, using Jaccard's similarity coefficient and UPGMA clustering algorithm.25 genotypes were broadly grouped into 4 clusters namely, Cluster I, II, III and IV. Cluster I consisted of 8 genotypes whereas Cluster II consisted of 9 genotypes (Fig. 2). The Cluster I is subdivided into two sub-groups, namely IA (4 genotypes) and IB (4 genotypes). The Cluster III, being a smallest cluster, consisted of 3 genotypes namely HUZM-69, HUZM-88, HUZM-47. These are found to be of diverse origin with parentage DMR WN-3, HUM 242 (local) X Suwan 1 F_2. Cluster IV consisted five genotypes in which HUZM 59 and HUZM 265 are highly polymorphic. The present study has indicated the need for evaluating the component lines derived from each of them. The similar diversity pattern in different sets of inbreds was reported by Sharma *et al.* (2017), Synrem *et al.* (2017), Thakur *et al.* (2017), and Samanthi *et al.* (2012).

Principal component (PC) analysis revealed the first three PCs contributed 23.70%, 7.63% and 6.73% of total variation respectively, with a cumulative variation of first 3PCs was 38.07%. Two dimensional plot were prepared (Fig.3 and Table 3) for which upto 50 % variability can be accounted. In 2-D plot genotypes were grouped into 3 clusters. Most of the UPGMA cluster IA, II and III genotypes were grouped into one cluster with an exception the genotype HUZM-69 and HUZM-88 of cluster III were included in cluster II in PCA 2-D. The clustering pattern generated by UPGMA and PCA were mostly similar with some exceptions. In the present study the first 3 PC accounted for 38.07% of total variation. Several studies have been performed for diversity

estimation in maize using UPGMA and PCA for molecular analysis; in general, the results obtained from PCA agreed with UPGMA clustering (Kumar *et al*., 2008, Gupta and Singh, 2010).

Conclusion

The 20 informative (polymorphic) markers with 70 alleles provided a way to determe the Simple Sequence Repeats (SSR) alleles number in genetic relationship analysis. The PIC value ranged from 0.286 (umc226) to 0.966 (csu308) with an average PIC of 0.736. The cluster analysis indicates inbred HUZM 252 and HUZM 265 are highly diverse. This investigation indicated the need for evaluating the component lines derived from each cluster. Cluster analysis showed there is an enough diversity found between the inbreds tested and the information on diversity of inbred lines generated in this study would be much useful in developing heterotic hybrids. Further studies by increasing the number of markers and correlating the current data with morpho-physiological traits may help in better selection strategies to come out with the genuine genotypes with durable promising outcome.

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Table 1. List of 25 inbred lines of maize

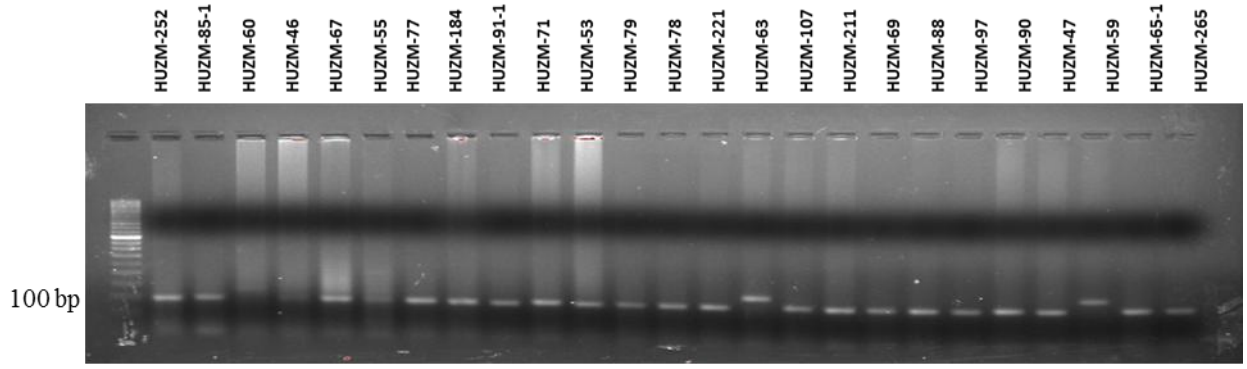
Sl. No.	Inbred lines	Source/Inbred lines derived from
1	HUZM-252	HKH 1211 (Y)
2	HUZM-85-1	-
3	HUZM-60	X1321AWXVC2
4	HUZM-46	MBR-ET (W) CIF139-2-*1-B-2*9 Bulk # Bulk
5	HUZM-67	DMR WN-2
6	HUZM-55	ISO2 X1381 WA-4K
7	HUZM-77	DMR WN-8 x Local
8	HUZM-184	X-2006
9	HUZM-91-1	Dewaki X VC2
10	HUZM-71	DMR WN-5 (Tuxpeno pool c7)
11	HUZM-53	ISO2 X 1381 WA
12	HUZM-79	DMR WN-8 X Local
13	HUZM-78	DMR WN-8 X Local
14	HUZM-221	JKMH-168
15	HUZM-63	X1381HWXVC2
16	HUZM-107	SEEDTECH-701
17	HUZM-211	R-9702
18	HUZM-69	DMR WN-3
19	HUZM-88	HUM 242 (local) X Suwan 1 F2
20	HUZM-97	X Jaunpur Local
21	HUZM-90	HUM 145 (local) X Suwan 1 F2
22	HUZM-47	P502c2-185-3-4-1-3-B-B-B
23	HUZM-59	X1321AWXVC2
24	HUZM-65-1	X1381HW
25	HUZM-265	SWS013 Y-6 Normal

Table 2. Total number of alleles found in each primer and their respective PIC value.

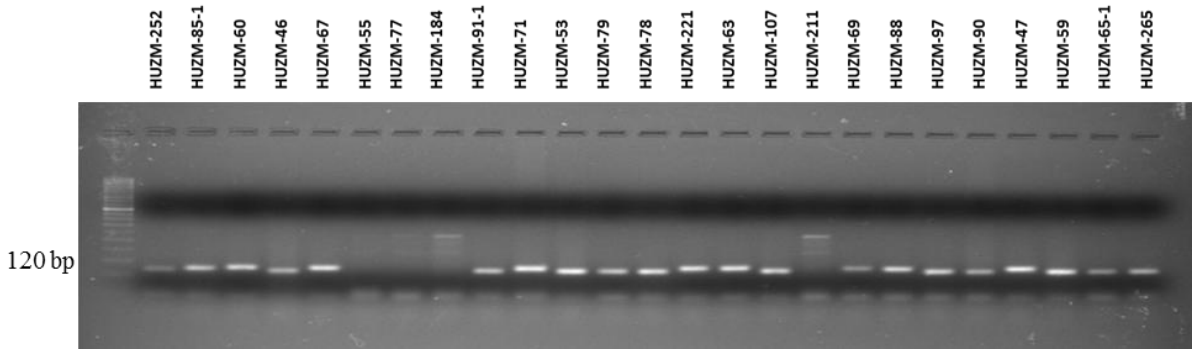
Sl. Number	Primer	Linkage group	Number of alleles	PIC
1	phi328189	2	5	0.512
2	bnlg1287	5	4	0.778
3	umc226	3	3	0.286
4	umc1603	1	2	0.557
5	csu308	5	3	0.966
6	umc1482	5	2	0.582
7	umc1867	9	3	0.85
8	umc2088	2	4	0.781
9	umc1735	8	3	0.952
10	bnlg1893	2	4	0.779
11	umc1085	1	3	0.870
12	umc2258	3	3	0.702
13	bnlg339	7	6	0.885
14	umc1657	9	4	0.763
15	umc1015	7	7	0.859
16	umc2190	7	3	0.675
17	phi059	10	2	0.646
18	umc1662	4	3	0.690
19	umc2284	4	3	0.882
20	phi113	5	3	0.714

Table 3. Grouping of the genotypes based on PCA values.

Group 1	Group 2	Group 3
HUZM-91-1, HUZM-252, HUZM-53, HUZM-53	HUZM-85-1, HUZM-60, HUZM-46, HUZM-67, HUZM-55, HUZM-77, HUZM-184, HUZM-79, HUZM-78, HUZM-221, HUZM-63, HUZM-107, HUZM-211, HUZM-69, HUZM-88, HUZM-97, HUZM-90, HUZM-47, HUZM-65-1	HUZM-59, HUZM-265



Size of ladder: 100bp



Size of ladder: 100bp

Fig. 1. SSR allele pattern among the 25 maize inbred lines revealed by primer 1.bnlg1893, 2. phi113.

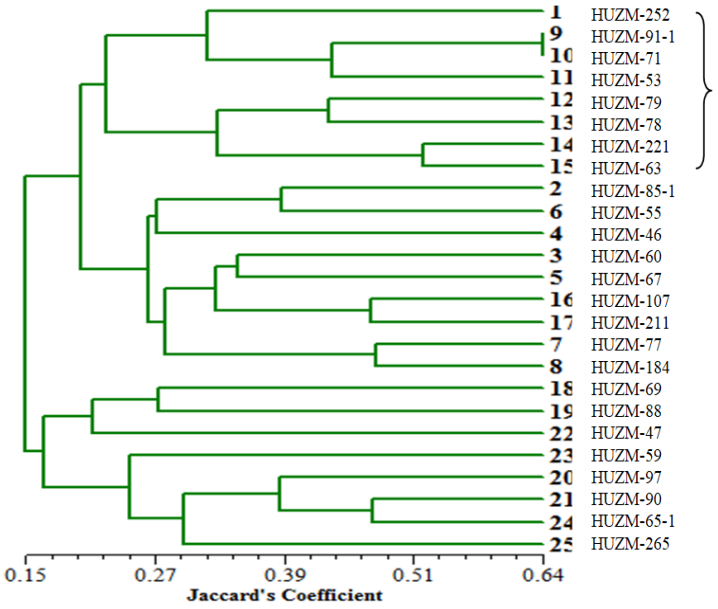


Fig. 2 Dendrogram showing genetic relationship among the maize inbreed lines based on the

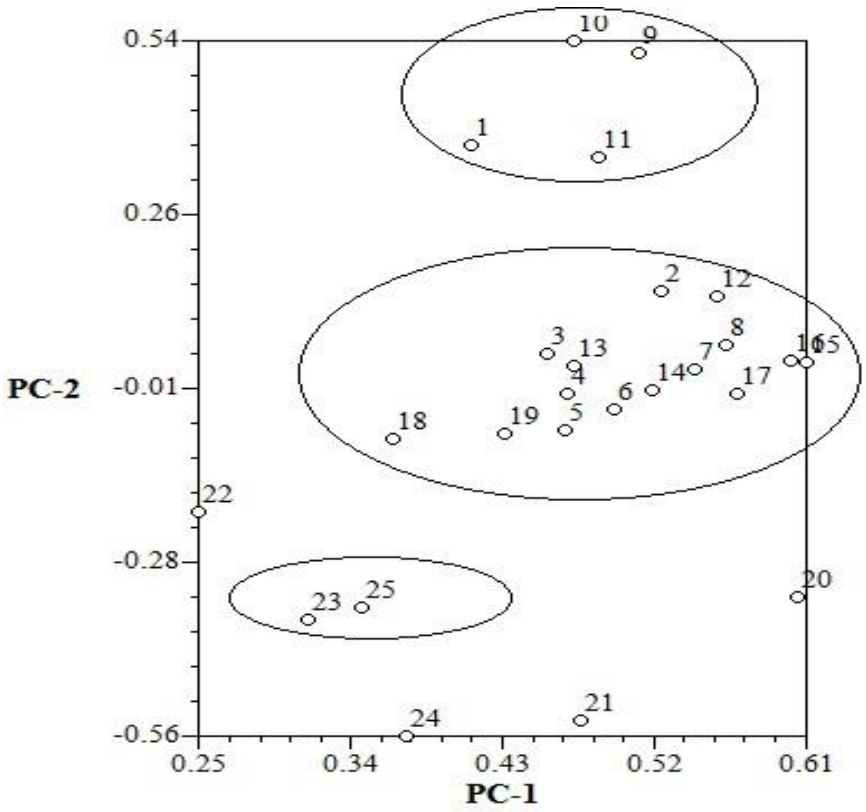


Fig. 3. 2-D PCA analysis of maize inbreed lines based on SSR data