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ORIGINAL ARTICLE **OPEN ACCESS**

Utilization of SSR Markers for Studying Genetic Diversity of Popular Maize and their Hybrids

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ABSTRACT
The variability is prerequisite for any breeding programme. SSR markers can be used for analysis of genetic diversity of genotypes. In present study, a total of 75 Simple Sequence Repeat (SSR) primers were used to screen 14 genotypes of maize parents and their hybrids for genetic study. The 14 genotypes were divided into two major clusters at a similarity coefficient of 59%. Major cluster I and II consisted of six genotypes and eight genotypes at a similarity coefficient of 59.5%. Allelic profile for present SSR markers shows that primers amplified a total of eighty nine alleles which varied from 1 (pumc1009) to 3 (pumc1183) with a mean of 1.5 allele per locus. These markers are highly accurate, efficient and reproducible for genetic diversity study and identification at any developmental stage.
Keywords: SSR Markers, pumc1009, pumc1183, Zea mays

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INTRODUCTION

Maize (*Zea mays* L.) is one of the most important food, feed and industrial crop globally. It is predominantly a cross pollinating species, a feature that has contributed to its broad morphological variability and geographical adaptability. Economically, the most important types of maize are grown for grain or fodder and silage production. FAO predicts that an additional 60 Mt 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 Mt to almost 400 Mt in 2030 [13]. As the variability is prerequisite for any breeding programme, improvement of quality and quantity depends on utilization of existing variability [14]. Hybridization is one way to utilize existing genotypic variability. But for better hybridization programme we need to have a clear-cut idea about genetic dissimilarities between parents. Genetic distances determined by molecular markers like simple sequence repeats (SSRs) is a powerful tool to assess the genetic conformity between hybrids and their inbred lines and also for the estimation of genetic diversity. The utilization of molecular markers such as simple sequence repeats (SSRs) for Genetic distances determination is an appropriate as there are few number of morphological markers and biochemical markers available. Detailed knowledge of the diversity and genetic relatedness among the genotypes will be an important aid in the crop improvement strategies. It can be also used for development of varieties that can give optimum agricultural productivity under limited input conditions. Diversity analysis can also be an aid to the gene mining or identifying the regions in genome associated with wider adaptability and agronomically superior traits.

MATERIAL AND METHODS

The experimental plant material comprised of six hybrids of maize along with eight their respective parents and was obtained from Maize Section, Department of Genetics and Plant Breeding, Regional Research Station, Uchani, CCS Haryana Agriculture University, Hisar, Haryana-125004.

Table.1. Parents and their hybrids used for study.

Sr. No.	Hybrids	Parent I	Parent II
1.	HQPM-1	HKI193-1	HKI163
2.	HQPM-4	HKI193-2	HKI161
3.	HQPM-5	HKI163	HKI161
4.	HQPM-7	HKI193-1	HKI161
5.	HM-4	HKI1105	HKI323
6.	HM-2	HKI1352	HKI1344

DNA Isolation

A total of 75 SSR primers were used in the present investigation. Primers were accessed from Imperial Life Sciences, USA. Genomic DNA of maize hybrids and their parents was isolated from the young leaves of all the genotypes using CTAB (Cetyl Trimethyl Ammonium Bromide) extraction method given by Murray and Thompson (1980) and modified by [17]. Followed by treatment with 2 µl of RNAase A solution (5mg/ml) per 50 µl DNA sample to remove RNA contamination. Quality of DNA was checked by submerged horizontal electrophoresis. A 0.8% (w/v) agarose gel was prepared for this [18]. For estimation of quantity by 0.8% agarose gel electrophoresis, a lambda DNA of known concentration (50 ng/ µl) was run along with DNA samples. PCR amplification reaction was carried out in G-Storm and Bio-Rad thermocyclers. PCR amplified products were first checked for amplification on 2.5% agarose gel electrophoresis. Samples were prepared by adding 6X loading dye. PCR amplified products were viewed under UV light fluorescence using Benchtop UV transilluminator.

PCR Amplification

Seventy five SSR primer pairs were selected for this study. PCR was performed in a reaction mixture volume of 20 µL containing of 30 ng of template DNA, 1 x PCR buffer with 1.5 mM of MgCl2, 0.2 mM of each dNTPs, 10 pmol of each primers and 1U of Taq DNA polymerase. PCR was carried out in a Thermal Cycler programmed for 35 cycles of 95^0C (5 min), 94^0C (1 min) 56^0C (30 Sec.), 72^0C (1 min) then followed by final-extension at 72^0C for10 min. PCR products (10 µl) were used for electrophoresis and the amplicons were resolved on 2.5 % agarose gel stained with ethidium bromide at 1 µg/ ml, and visualized under UV in a gel documentation system and impurities were identified based on deviations in expected amplification pattern

Allele Scoring

Bands for SSR analysis were scored based on the presence (taken as 1) or absence (taken as 0) of bands. The size (in nucleotides base pairs) of the most intensely amplified bands for each microsatellite marker was determined based on its migration relative to standard DNA marker (100 bp DNA ladder). Multiple alleles were inferred whenever a given marker produced more than one clusters of bands.

Data analysis

The polymorphism information content (PIC) for each SSR marker was calculated according to the formula given by Anderson *et al*. (1993):

$$PIC_i = 1 - \sum_{j=1}^{n} p^2_{ij}$$

Where,

p_{ij} = frequency of the j^{th} allele for marker i

0/1 matrix of allele scoring was used to calculate the similarity genetic distance using 'SIMQUAL' sub-programme of NTSYS-pc (version 2.02e) software (Numerical Taxonomy and Multivariate Analysis System Programme. Dendrogram was constructed by using distance matrix in SAHN sub-programme of NTSYS-pc by the Unweighted Pair-Group Method with Arithmetic Average (UPGMA) algorithm. Principal Component Analysis (PCA) was done to construct 2 and 3 dimensional diagrams.

RESULT AND DISCUSSION

Genetic diversity refers to the variations within the individual gene loci among alleles of a gene, or gene combinations, between individual plants or between plant populations. Analysis of the pattern of genetic diversity in crops is invaluable as it opens up the possibilities for systematic management, conservation and introgression of desirable genes from diverse genetic sources into the elite material, as well as derivation of novel and useful germplasm [23]. The use of DNA markers to obtain genotype specific profiles had distinct advantages over morphological and biochemical methods. The morphological markers may be influenced by the environmental conditions, laborious and time taking. However, the biochemical markers such as isozyme and protein patterns are least influenced by the environment but the polymorphism is again limitation for such markers [10]. SSR had much more polymorphism than most of other DNA markers, and is co-dominant and large in quantity. Therefore, the high polymorphic

information content (PIC) of SSR had promoted the application of microsatellites as molecular markers in fingerprinting [2]. In this study primer pairs of 75 SSRs associated with each hybrid and parental lines were assessed on 2.5 per cent agarose.

Genetic diversity analysis

The SSR diversity data was used for genetic diversity analysis among six maize hybrids and their parents using Numerical Taxonomy and Multivariate Analysis System (NTSYS-PC) software version 2.02e. Seventy five SSR markers were used for analysis in present study. After gel electrophoresis of PCR products, each band was considered as a single allele and alleles were scored as present (1) or absent (0). The matrix was analyzed to construct dendrogram using SAHN sub-program. The SAHN sub-program uses UPGMA algorithm to perform cluster analysis.

SSRs based clustering

Analysis of the pattern of genetic diversity in crops is invaluable as it opens up the possibilities for systematic management, conservation and introgression of desirable genes from diverse genetic sources into the elite material, as well as derivation of novel and useful germplasm [23]. Data obtained from seventy five SSRs were used to construct the dendrogram using Numerical Taxonomy and Multivariate Analysis System (NTSYS-PC) software version 2.02e, and UPGMA algorithm to perform cluster analysis.

The fourteen genotypes were divided into two major clusters at a similarity coefficient of 59%. Major cluster I and II consisted of six genotypes and eight genotypes at a similarity coefficient of 59.5%. These two major clusters I and II were further divided into two sub clusters at similarity coefficient of 60.5% and 59.5% respectively.

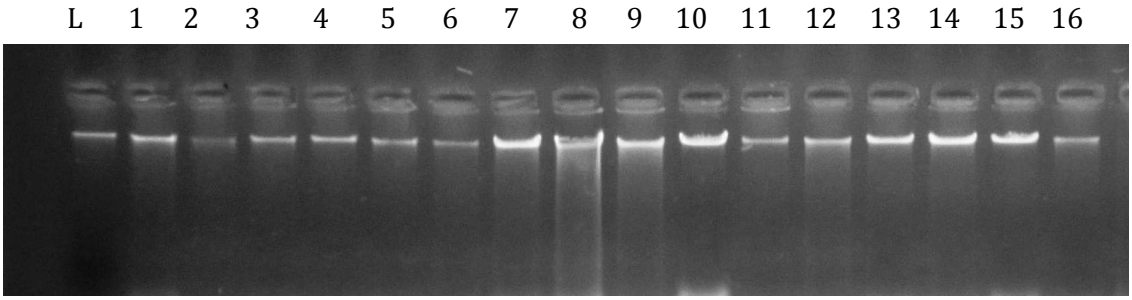

Plate.1: Genomic DNA L: 50ng Lambda DNA, lane 1: HKI 193-1, lane 2: HKI193-2, lane 3: HKI163, lane 4: HKI163, lane 5: HKI161, lane 6: HKI161, lane 7: HKI1352, lane 8: HKI1105, lane 9: HKI1344, lane 10: HKI323, lane 11: HQPM-1, lane 12: HQPM-4, lane 13: HQPM-5, lane 14: HQPM-7, lane15: HM2, lane 16: HM4

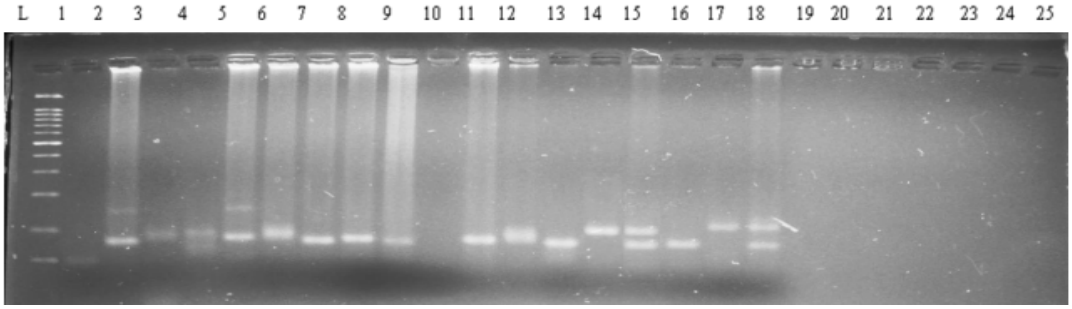

Plate.2: Polymorphic SSR marker profile obtained with pumc1040; L: 100bp ladder, lane13: HKI 1105, lane14: HKI 323, lane15: HM4, lane16: HKI 1352, lane17: HKI 1344, lane18: HM2

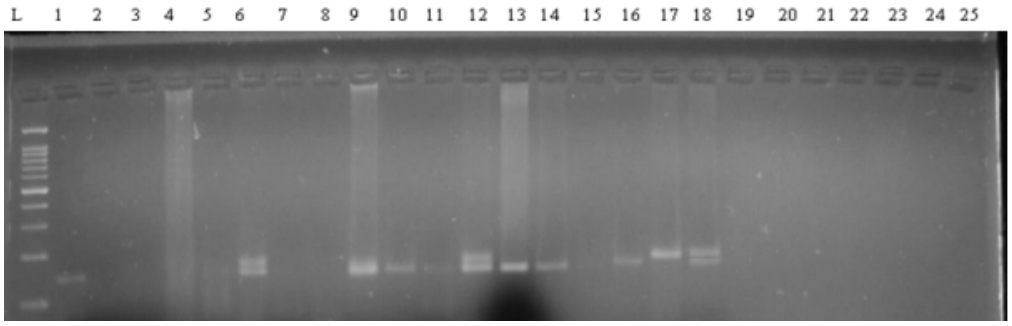

Plate.3: Polymorphic SSR marker profile profile obtained with HM2 with pumc1064; L: 100bp ladder, lane16: HKI 1352, lane17: HKI 1344, lane=18: HM2

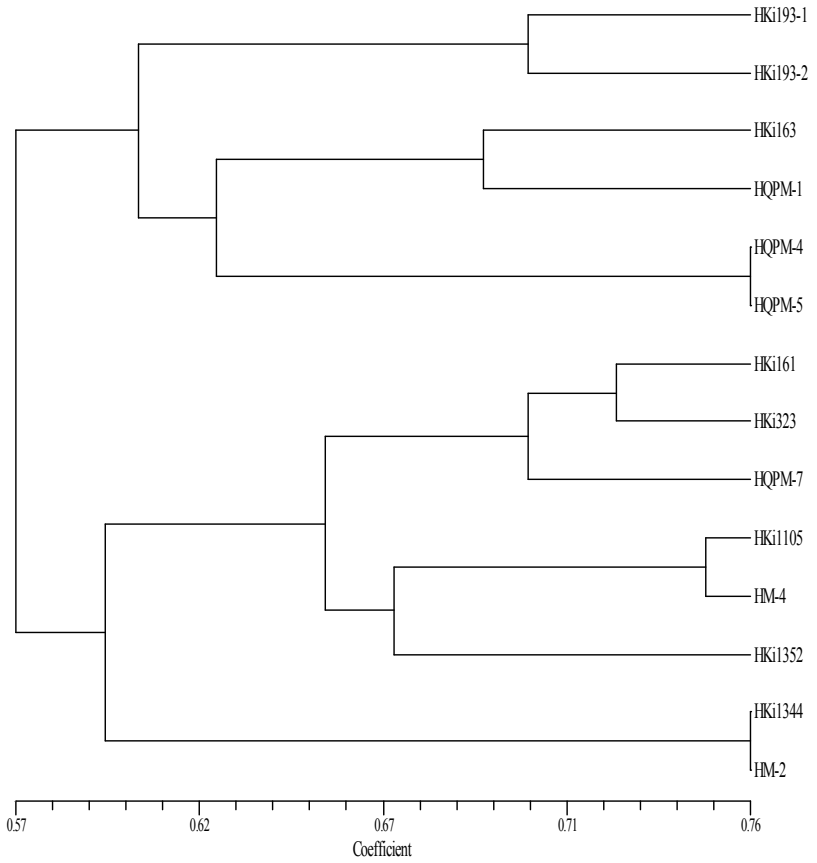

Fig.1 Dendrogram (NTSYS-pc) showing genetic relationships among six hybrids and their eight parental genotypes using allelic data at seventy five SSR loci

HQPM1 similar to one of its parent HKI163 at a similarity coefficient of 70%. In the second sub cluster hybrid HM2 is closer to its parent HKI1344 at similarity coefficient of 76%. HKI161 and HKI323 are closer to each other at similarity coefficient of 72% and these were found to be diverse from HKI1105 at 67.2% of similarity coefficient. HM-4 is more similar to one of its parent HKI 1105 at a similarity between 65-75%. Choudhary *et al.* [4] used sixty five SSR markers to make cluster of 24 genotypes which divided these genotypes in four major cluster genotypes HKI161, HKI323 and HKI1105 fall in same sub-cluster here also as in our study HKIO161 is more close to HKI323 at a similarity coefficient of 70% and these two genotypes are diverse from HKI1105 at a similarity coefficient 62%. Genetic diversity value obtained by [6] 59.2% was comparable to the value in the present study.

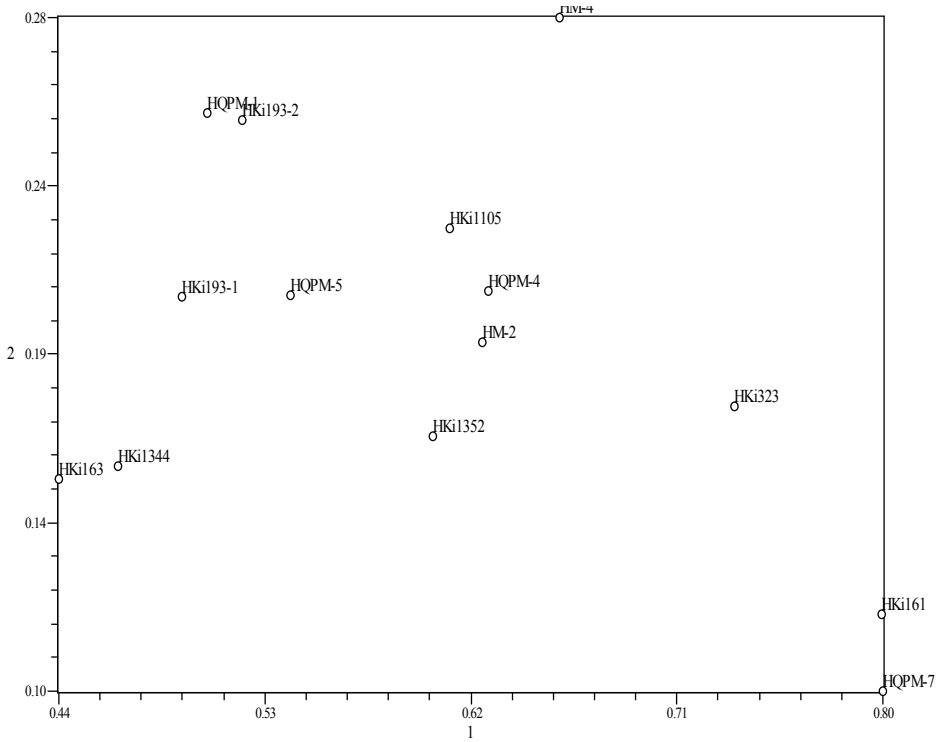

Fig. 2 Two-dimensional PCA scaling of six hybrids and their eight parental genotypes using genetic distance matrix data at seventy five SSR loci

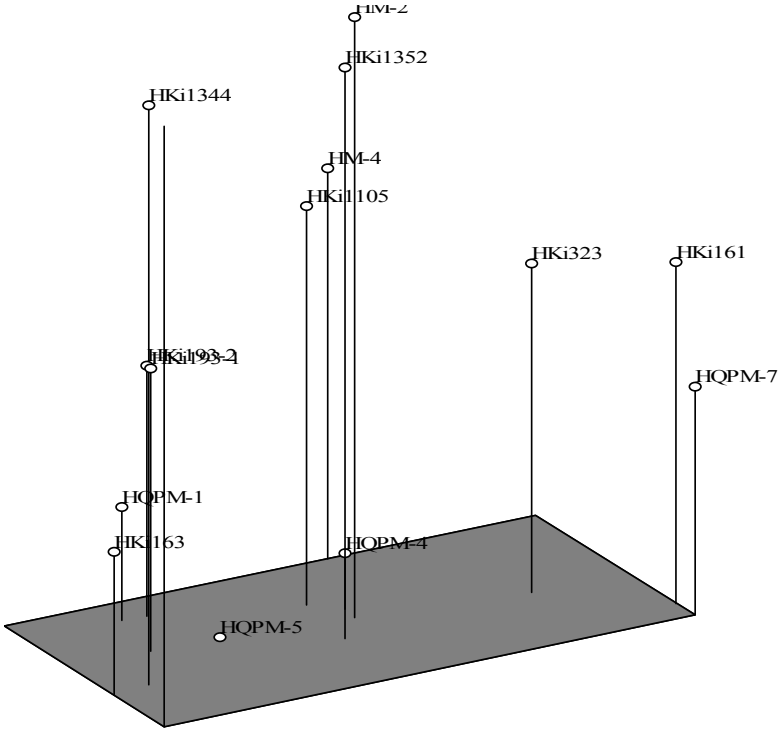

Fig.3 Three-dimensional PCA scaling of six hybrids and their eight parental genotypes using SSR similarity matrix data at seventy five SSR loci

The minimum similarity of 59.5% was observed between (HM-2 hybrid and HKI193-1) and maximum was 76% between the hybrids (HQPM-4 and HQPM-5) and (HM-2 and HKI1344). In the first cluster Furthermore, simple matching matrix was subjected to principal component analysis (PCA). The grouping

of fourteen genotypes using PCA analysis in 2-D (Fig 2) and 3-D scaling (Fig 3)followed the same pattern as depicted in dendrogram with minor differences is also supported by [4].

Allelic profile for SSR markers

In the present investigation, Primers amplified a total of eighty nine alleles which varied from 1 (pumc1009) to 3 (pumc1183) with a mean of 1.5 allele per locus. Similar results were also found by Shah *et al.* [19] as they reported 1.56 alleles per locus and the value was comparable to value 2.07 per locus obtained by Nguyen *et al.* [12]. This value is much lower than the value of mean allele per locus reported earlier by [21] they found an average of 4.6 alleles per primer. Similarly high values (4.9 alleles per locus) were also reported by [11], [22] (4.9 alleles) with 85 SSR loci, [15] (6.4 SSR alleles per locus), [16] (4 alleles per locus), [8] (4.9 alleles per locus), [9] (5.8 alleles per locus) and [4] (4.1 alleles per locus).

Polymorphic information content (PIC) value obtained in the present study was 0.402 for 2 alleles and 0.523 for 3 alleles which is near to average value of 0.59 by (Senior *et al.* 1998). Similar PIC values (an average PIC of 0.58 on 56 inbred lines of maize) were also recorded by [5] (0.61) and by [6] (0.512) while [3] reported average PIC value of 0.49 on 22 maize genotypes. The values are much lower than value obtained by [1] (0.75 to 0.94 and by [7] (0.70). These minor differences in the results are due to variation in amplification pattern obtained by different set of primers.

CONCLUSION

In this study, appearance of novel bands evidencing polymorphism may be because of chromosome variation or gene rearrangements in hybrid populations. The results of the present study suggested that there are still chances to incorporate genetic variations to the hybrids so, to increase the quality and yield of maize. New variation may be due to hybridization crossing breeding. SSR markers are very efficient and reliable for estimation of existing genetic variability.

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