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Molecular diversity studies in maize germplasm of eastern Uttar Pradesh

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ABSTRACT

Maize *(Zea mays L.)* is an important crop next to wheat and rice due to its high yield efficiency. It is one of the prominent widely grown C4 crop with a high rate of photosynthetic activity leading to high grain and biomass yield potential. Genetic diversity is very obligatory for Upgrading of maize crop**.** This study was conducted to determine genetic and molecular diversity among eight maize genotypes using ten simple sequence repeat (SSR) primers. The number of alleles per marker varied from one to three with average of 0.7 alleles. Bnlg1885 and Bnlg1600 detected maximum of three alleles while phi101 detected lowest of one allele. Seven primer out of ten viz. bnlg1018, bnlg1396, phi049, phi029, phi088, umc1008, phi072 studied failed to amplify any band and showed monomorphic pattern. The PIC values of the SSR loci varied from 0.60 (bnlg1600) to 0.70 (phi101) with the mean of 0.19. SSR markers being co-dominant in nature identified heterozygotes among the twenty inbreds lines. Out of ten markers, seven markers bnlg1018, bnlg1396, phi049, phi029, phi088, umc1008, phi072 did not show any heterozygotes, while others detected as 6 heterozygotes out of 8 inbred lines investigated with an average of 0.75 heterozygotes per marker. The pair wise genetic similarity was calculated by Jaccard's similarity coefficients, which ranged from 0.08 to 1.55 with an average similarity of 0.63. The minimum genetic similarity (0.08) was found between five pairs, HUZM-356 with HUZM-47 and HUZM-53 with HUZM-147. The maximum similarity (0.73) was found between HUZM-47 with HUZM-36.

Keywords: C4 Crop**,** Heterozygotes, Molecular diversity, PCR reaction, SSR marker

Introduction

Maize is an annual, prominent widely grown C4 crop and cross-pollinated plant. It is an angiosperm and monocot. It is native to America and is the only species of genus Zea (2n=20) having two close relatives, gamma grass (Tripsacum, 2n=36, 72) and Teosinte (Euchlaena 2n=20) in annual forms. Molecular analysis identified one form of teosinte (Zea mays ssp. parviglumis) as the progenitor of maize (Doebley, 2004). Maize has 10 chromosomes (2 n=2 x=20). The total genetic length of these chromosomes is roughly 1500cm. To improve genetic diversity of local germplasm, it is important to know the extent of already existing genetic variability in the material. To estimate genetic diversity in maize genotype, different kind of markers can be used. Before, 1970, genetic diversity in crop plant was generally determined from pedigree data (Lubberstedt et al., 2000) and morphological traits (Yee et al., 1999).

Goodman (1972) used phenotypic markers and quantitative traits for selection. Further, isozymes and storage proteins were used for analysing diversity (Melchinger et al., 1990; Cox et al., 1988). The advent of DNA marker technology in the 1980s begins to study the inheritance of important agronomic traits (Peleman and Van der Voort, 2003) by different molecular markers.

Amplified Fragment Length Polymorphism (Vos et al., 1995) and Simple Sequence Repeats (Weber and May, 1989) have been most widely used for the analysis of plant genetic diversity (Morgante and Olivieri, 1993). Simple Sequence Repeats (SSRs) are DNA markers with short stretches of tandemly

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repeated di-, tri- or tetra nucleotide motifs (Weber, 1990). Microsatellite markers being co-dominant, highly polymorphic and multi-allelic become the most suitable choice for genetic analysis in various crops (Gupta and Varshney, 2000). Microsatelites (SSRs) occur frequently in most eukaryotic genome and can be very informative, multiallelic and reproducible (Vos et al., 1995; Senior and Heun, 1993) and were suggested in order to overcome the limitations associated with RFLP and RAPD.

At present, SSRs are the most promising molecular markers which are able to identify or differentiate genotypes within a species (shah et al. 2009). SSRs are ubiquitously interspersed in eukaryotic genomes and can find applications as highly variable and multiallelic PCR based genetic markers (Brown et al., 1996). The application of SSR techniques to finger print plant species was first reported by Akkaya et al. (1992). The present study was carried out in order to study genetic and molecular diversity in maize.

Material and Method

Plant material

In the present investigation the experimental material used comprised of twenty advance inbred lines of maize (Zea mays L.) obtained from the Demonstration of hybrid Rice and Hybrid Maize and Their Seed Production Through Farmers Participatory Approach in Eastern Uttar Pradesh, Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221 005. The genotypes used are listed in the Table-1.

DNA isolation

DNA was isolated from young leaves by Kit method. A protocol from Chromous Biotech (Cat#:rkt 08A) India Limited is followed.

Take 100mg young leaves and wash them in distilled water. Crush in pestle and mortar with 750

Table 1 List of inbred lines used in the experiment.

S. No.	Inbred lines
1.	KHI 536
2.	HUZM 97-1-2
3.	HUZM 509
4.	HUZM 121
5.	V-323
6.	KHI 164-4(1-3)-2
7.	HUZM 88
8.	KHI PC-8
9.	HUZM 47
10.	HUZM 60
11.	V 25
12.	CM 126
13.	V 348
14.	V 386
15.	V 388
16.	V 351
17.	CML 395
18.	KHI 209
19.	KHI 1352-5-8-9
20.	KHI 586

micro litre suspension buffer. Mix 250 micro litre more suspension buffer. Take 750 micro litre final volume in the vial. Mix 1 ml lysis buffer and store at 65^0 for 15 min. Cool it at room temperature. Now spin it at 10000g for 3 min.

Transfer supernatant in fresh vial and discard the pellet. Now 600 microlitre supernatant is transferred into the spin tube. Then spin at 10000g for 3 min and discard the supernatent from collection tube. Repeat this step. Now spin the empty column with the collection tube at 10000g for 3 min. Place the spin column in a fresh vial. Add 50 microlitreelution buffer (already stored at 65^0) into the spin column. Keep the vial along with the spin column at 65^0 for 1 min. After that spin at 10000 g for 3min. Repeat previous two steps. So that the final volume of DNA was 100 microlitre. Now DNA sample were stored at 4^0C.

Table 2 Bin location, primer sequence, allele number and PIC values of SSR loci.

SSR Locus	Bin Location	Sequence (5'-3')	No. of Alle	PIC	T_a (^{0}C)
hi101	5.06	Forward TGTTCGCCGTCTAGCCTGGATT Reverse TCATCAGCAACGACGACTACTCC	1	0.70	58
bnlg1885	5.07	Forward GACAGACGCAACTACCGAAA Reverse TGTTCAATTTGATGTTCATTGC	3	0.68	58
bnlg1600	6.00	Forward CGATCAGTGCGTGGAGAGTA Reverse TAGGCATGCATTGTCCATTG	3	0.60	58
bnlg1018	2.04	Forward CGAGGTTAGCACCGACAAT Reverse CGAGTAAATGCTCTGTGCCA	1	0.00	58
bnlg1396	2.06	Forward CGCATTTCTCCTGCAGTACA Reverse TGCTTGAGTCGTCGAATCTG	1	0.00	58
phi049	3.01	Forward CTTCTGTTCCGCCATCCAGTATGTT Reverse GATTGCGATAACATTGCGGCAAGTTGT	1	0.00	58
phi029	3.04	Forward TTGTCTTTCTTCCTCCACAAGCAGCGAA Revrese ATTTCCAGTTGCCACCGACGAAGAACTT	1	0.00	58
phi088	3.08	Forward GATTGCGATAAGCATTGCGGCAGTT Reverse CTTCTGTTCCGCCATCCAGTATGT	0	0.00	58
umc1008	4.01	Forward TCTAGCTTGTGGTGGTGGTTA Reverse ACATGAGCACAAAGACTGACC	0	0.00	58
phi072	4.01	Forward ACCGTGCATGATTAATTTCTCCAGCCTT Reverse GACAGCGCGCAAATGGATTGAACT	0	0.00	58

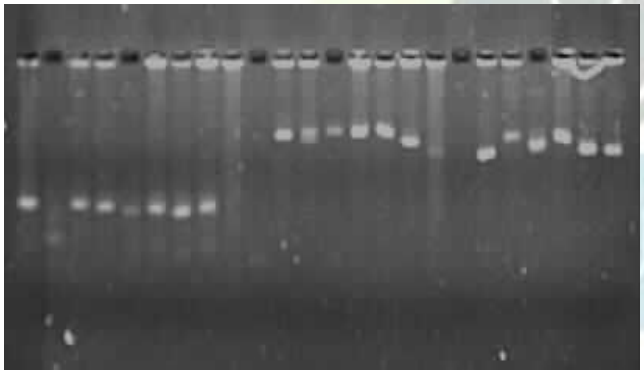

Fig 4(a) phi101, bnlg1885, bnlg1600

PIC= Polymorphism information content,
*= not used in the calculation,
Ta: annealing temperature.

Figure 1 Bin location, primer sequence, No. of allele and PIC Values of SSR Loci.

Polymerase chain reaction (PCR)

All PCR reactions were carried out in 20 µl reaction containing 2 µl template DNA, 5.0 µl of each primer, 1.2µl of each dATP, dGTP, dCTP, dTTP, 2.0 µl of 10 X PCR buffer, 0.2 µl of Taq DNA polymerase and 9.6 µl of ddH_2O. Amplification conditions were: an initial denaturation step of 5 min at 94°C, followed by 25-35 cycles each consisting of a denaturation step of 1 min at 94°C, annealing temperature was variable according to primer. It is ~5^0C below T_m of primers and an extension step of 2 min at 72°C. The last cycle was followed by 10 min extension at 72°C. (Shah et al. 2009).All amplification reactions were performed using PCR system programmable thermocycler. The amplification products were electrophoresed on 1.5% agarose gel, and visualized by staining with ethidium bromide under ultraviolet (UV) light and photographed using gel documentation system.

Statistical analysis

For statistical analysis, every band was considered as a single locus/allele. The loci were scored as present (1) or absent (0). Bi-variate 1-0 data matrix was generated. Mean PIC value was calculated based on PCR values obtained across various SSR loci, The PIC values, ranging from '0' (monomorphic) to '1' (very highly discriminative, with many alleles in equal frequencies) provide an estimate of the

discriminatory power of a 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. There are several agglomerative hierarchical methods that are commonly used in clustering technique. Among them the UPGMA (Unweighted Paired Group Method using Arithmetic averages) method and the wards' method are the most commonly used methods suited for various applications.

Result

Ten SSR primers identified a total of seven alleles among eight maize inbred lines. Seven primers out of ten bnlg1018, bnlg1396, phi049, phi029, phi088, umc1008, phi072 studied failed to amplify any band and showed monomorphic pattern. The number of alleles per marker varied from one to three with average of 0.7 alleles. Bnlg1885 and Bnlg1600 detected maximum of three alleles while phi101 detected lowest of one allele. The PIC values of the SSR loci varied from 0.60 (bnlg1600) to 0.70 (phi101) with mean of 0.19. (Table-2).

SSR markers being co-dominant in nature identified heterozygotes among the lines. Out of ten markers investigated, seven markers bnlg1018, bnlg1396, phi049, phi029, phi088, umc1008, phi072 did not show any heterozygotes, while others detected a tatal of six heterozygotes out of eight inbred lines. The pair wise genetic similarity was calculated by Jaccard's similarity coefficients, which ranged from 0.08 to 1.55 with an average similarity of 0.63, which shows a higher level of genetic diversity. Agglomerative hierarchical classification based on Jaccard's similarity coefficient put the eight inbred lines in four main clusters. Cluster I and II represents the biggest cluster with 3 genotypes each, followed by cluster III and IV, represents one genotype each are monogenotypic with genotype CM 145 and CM 118 respectively. Cluster I had three genotypes CM 104, CM 126 and V 25 respectively. Similarly cluster II also shows CM 105, CM 119 and CM 212 genotypes respectively.

Discussion

Genetic diversity plays a key role in crop improvement. Present study was aimed at identifying genetic diversity in eight maize genotypes using ten chromosome specific Simple Sequence Repeat (SSR) primer sets. SSRs are more potent than any other marker system and are a robust tool to detect polymorphism at molecular level.

Ten SSR markers used in the present study to detect molecular polymorphism among eight inbred lines. The average number of alleles (0.7) detected per locus in the study was lower than the earlier reports of Warburton et al. (2002) and Patto et al. (2004) who reported an average of 4.9 and 5.3 alleles using 85 and 80 SSR loci, respectively. However, the estimated value of 2.5 is close to the findings of Bantte and Prasanna (2003) and Legesse et al. (2007) who recorded 3.25 and 3.85 alleles using 36 and 27 polymorphic SSR loci, respectively.

Estimation of Jaccard's similarity coefficient among eight Maize genotypes

Detecting the lower number of alleles per locus may be attributed towards the use of screening technique followed in different studies. Di-nucleotide SSR loci, bnlg1885 and bnlg1600 identified the largest number of allele three with the PIC values of 0.68 and 0.60 respectively. Smith et al. (1997) have reported that di-repeat SSR loci reveal higher number of alleles, but sometime they also produce additional stutter bands. This probably may be one of the causes for the high allele numbers and PIC values. This is in contrary with the observations made by Enoki et al. (2002) and Legesse et al. (2007). The mean PIC value (0.19) determined in the study is lower than findings of Xu et al. (2004) and Legesse et al. (2007) who reported average PIC values of 0.62 and 0.58, respectively. Molecular analysis revealed a very high level of diversity among the lines. This clearly indicates high genetic variability among the lines. High variability in maize inbred lines has also reported by Legesse et al., (2007). The dendogram constructed using the UPGMA clustering algorithm fit well with the similarity matrix with high

cophenetic correlation. The pair wise genetic similarity was calculated by Jaccard's coefficients, which ranged from 0.08 to 1.55. Cluster I and II shows the biggest cluster both with 3 genotypes each, followed by Cluster III, IV with 1 genotype each are monogenotypic.

Conclusion

Molecular analysis shows diversity among eight inbreds and grouped them in same cluster or different clusters. Some genotypes out of eight were not showing polymorphism which may be due to less number of primers used in the study.

Molecular analysis revealed a very high level of diversity among the lines, which ranged between 0.08 and 1.55 with an average of 0.19. This clearly indicates high genetic variability among the lines. The eight inbred lines were grouped into four clusters in which cluster I and cluster II are the large cluster both contain three genotypes each respectively, followed by Cluster III and IV both contain 1 genotype each are monogenotypic. The present study indicates that CM 104, CM 126 and V 25 are grouped in same cluster while CM 145 and CM 118 are monogenotypic at molecular level as well as genetic level.

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