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ASSESMENT OF MOLECULAR DIVERSITY IN MAIZE GENOTYPES (Zea mays L.) THROUGH SSR MARKERS"

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ABSTRACT: The genetic diversity in selected twenty four maize genotypes using 14 SSR primers at molecular level was performed. All the 14 primers yielded amplification and showed 100 % polymorphism. These primers amplified 6 unique loci in 5genotypes. Each primer thus produced on an average 2.5 polymorphic bands. Among the SSR primers, bnlg 1823 produced maximum number of 4 loci. The size of amplification products ranged from 110bp to 750bp. 6 primers showed more than 0.50 PIC value, while 8 others are having less than 0.5 PIC value. Maximum PIC value of 0.75 was observed in SSR primer bnlg 1823 and minimum PIC value of 0.06 was observed in primer umc 1297.The Dice similarity coefficient values ranged from 0.25 to 0.86. The consensus tree constructed by using NTSYSpc 2.02i software revealed four major clusters of maize genotypes. Out of total 14 SSR primers used, 6 primers found to be more informative based on PIC values irrespective of per cent polymorphism. The Dice similarity coefficient values for SSR primers indicated moderate diversity among the maize genotypes. The identical clustering pattern was observed by SSR primers. Unique loci produced by SSR primers may be specific to varieties.

Introduction:

Maize (*Zea mays* L.; 2n = 20) is one of the most important cereal crops in the world's Agricultural economy. It is being used both as food for man and feed for animals. It is also called as "Queen of cereals". The center of origin of maize has been established in the Mesoamerican region, i.e., Mexico and Central America (Watson & Dallwitz, 1992). In India the predominant maize growing states contribute more than 75% of the total maize production. Information regarding the genetic diversity and the relationship among maize inbred lines has had a significant impact on improvement of new cultivars because it is useful for planning crosses for hybrid and inbred line development, assigning lines to heterotic groups, and protecting the plant variety (Hallauer *et al.*, 1988; Pejic *et al.*, 1998).

In conventional breeding, genetic diversity and genetic relationships among maize inbred lines are usually assessed based on the morphological data, the pedigree record of inbred lines and the amount of heterosis expressed by the hybrid. However, these descriptors present several limitations. For example, the morphological characteristics often do not reliably portray the genetic relationships due to environmental interactions.

Molecular markers and their utility have been demonstrated in studies of genetic diversity (Morand *et al.,* 2002; Zeid *et al.,* 2003), mating systems (Durand *et al.,* 2000), pollination biology (White *et al.,* 2002). The purpose of revealing genetic diversity is served by the use of markers. Molecular markers have been utilized for identification of gene(s) of interest on chromosome and are widely used to study the organization of plant genomes. A molecular marker is a DNA sequence that is readily detected and whose inheritance can be easily monitored. Molecular marker does not depend on environmental variables and can be scored at any stage of the plant. During last several years, there has been marked increase in application of molecular markers in breeding crop plants. These markers not only facilitate the development of new varieties by reducing the time required for detection of specific traits, but also fasten the identification of desired genes, thus accelerate breeding for resistance traits.

Simple Sequence Repeats (SSRs) markers are repeats of short nucleotide sequences, usually equal to or less than six bases in length, that vary in number (Rafalski *et al.,* 1996). SSRs are very polymorphic due to the high mutation rate affecting the number of repeat units. These markers have gained considerable co dominant inheritance, reproducibility, relative abundance, extensive genome coverage, chromosome specific location, amenability to automation, high throughput genotyping and their ability to associate with many phenotypes (Parida *et al.,* 2009). SSRs have been widely applied to characterize the genetic diversity in wheat and still continue to be the choice for genetic diversity analysis of trait of interest (Devos *et al.,* 1995; El-Maghraby *et al.,* 2005). SSRs are typically co dominant and multi allelic, with expected heterozygosity frequently greater than 0.7, allowing precise discrimination even of closely related individuals. In natural plant populations, microsatellites have great potential for helping to understand what determines patterns of genetic variation. Du *et al.,*(2001) assayed fifty- eight inbred lines of maize (*Zea mays*) and one teosinte accessions (*Zea Luxuarians*) from significant heterotic group and miscellaneous origins for simple sequence repeats (SSR). They investigated genetic variability and genetic

relationship among these inbreds lines used in china, and tested whether it is possible to assign these lines to heterotic groups. Maniruzzaman *et al*., (2018) studied genetic diversity among 15 maize (*Zea mays*L.)inbreed lines using simple sequence repeats (SSR) markers. The analysis was done using 10 SSR primers.

This experiment was planned at the State Level Biotechnology Centre, Mahatma Phule Krishi Vidyapeeth, Rahuri during the year 2017-18 and 2018-19 with a specific objective to study the molecular diversity in maize genotypes using SSR markers.

MATERIALS AND METHODS:

The plant material for the study comprised of twenty four maize genotypes, which were collected from Maize Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri and used for research work. The seeds thus obtained were sown in protrays inside poly house for genomic DNA isolation. The list of maize genotypes along with their pedigree is given in the (Table 1).

Table 1: List of maize genotypes used for analysis

Sr.No.	Name of Genotypes	Pedigree
1.	**52021**	Selection from CM-212
2.	**52065**	Selection from CM-193
3.	**52099**	Selection from 5406-29P24STEC1HC17
4.	**52144**	Selection from CML-189BBB
5.	**52191**	Selection from MAS[MSR/312]-117-2
6.	**52217**	Selection from CML-544W
7.	**52222**	PT963216-B*17
8.	**52285**	HEY POOL-2011-12
9.	**52310**	Selection from IC5859130
10.	**52327**	Selection from 42050-1
11.	**52337**	POOL16BNSEQC3F6*38-1
12.	**52349**	Selection from JCSCH62/8(B)
13.	**52507**	Selection from E57
14.	**IC-470475**	From ICAR-NBPGR, New Delhi
15.	**IC-437070**	From ICAR-NBPGR, New Delhi
16.	**IC/RNG/SW-1-7**	From ICAR-NBPGR, New Delhi
17.	**IC-552819**	From ICAR-NBPGR, New Delhi
18.	**EC-639232**	From ICAR-NBPGR, New Delhi
19.	**EC-639008**	From ICAR-NBPGR, New Delhi
20.	**EC-639290**	From ICAR-NBPGR, New Delhi
21.	**EC-639001**	From ICAR-NBPGR, New Delhi
22.	**DML-1112**	From ICAR-NBPGR, New Delhi
23.	**DML-1285**	From ICAR-NBPGR, New Delhi
24.	**DML-1336**	From ICAR-NBPGR, New Delhi

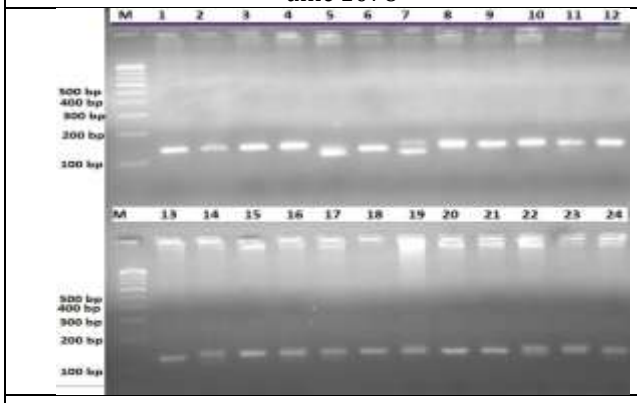
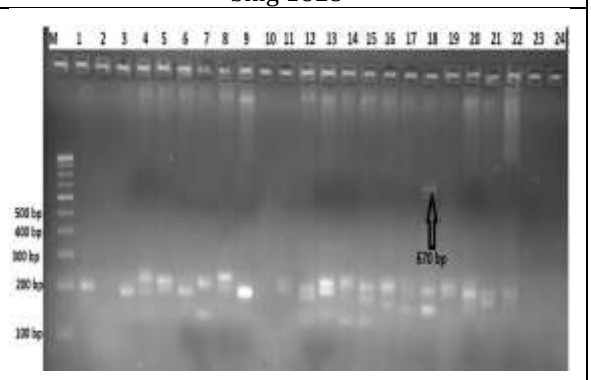
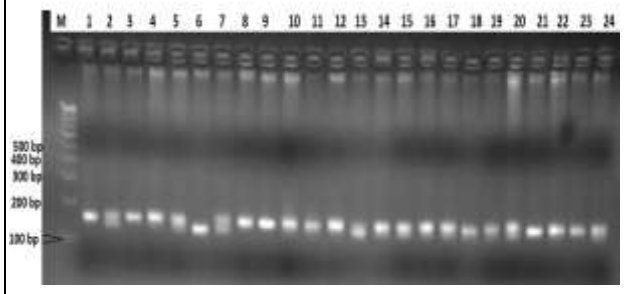
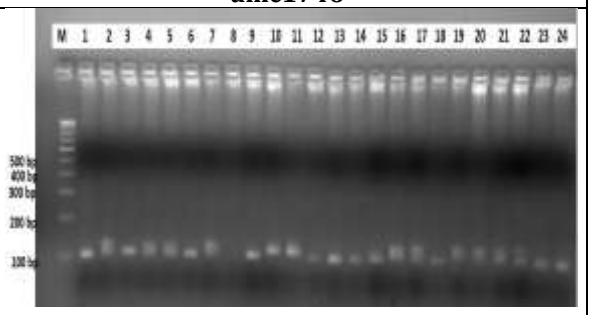
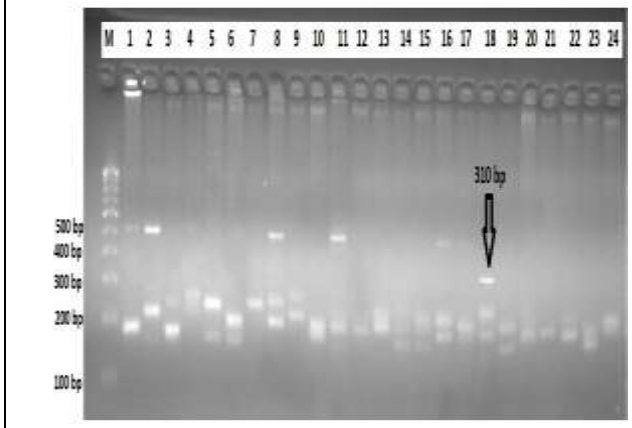
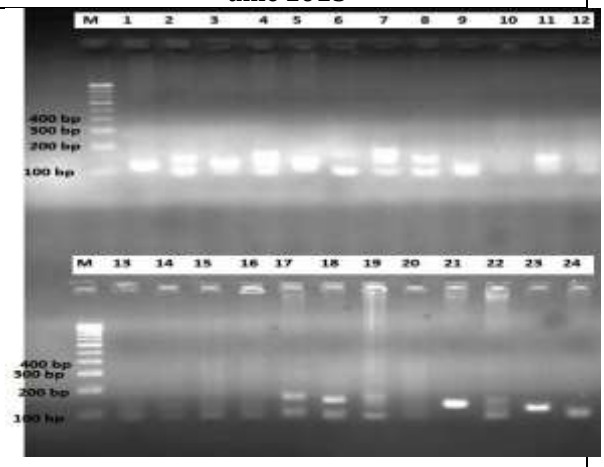
Genomic DNA was isolated from 24 maize genotypes following CTAB (Cetyl Tri methyl Ammonium Bromide) extraction method with some modifications as described by (Helguera *et al.,* 2005).

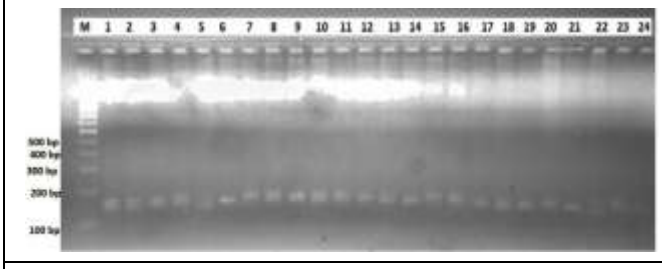
Table 3: Sequences and fixed optimum annealing temperature for SSR primers.

Sr. No.	Name of the primer	Primer direction	Sequence	No. of bases	Annealing Temp (℃)
1	umc 1078	F	AGGCACTAGCAGGCGAGAGG	20	54
		R	GCGTAGTAACATCCATCCAACCAA	24	
2	bnlg 1823	F	TGTGACTCCATACCGCACAT	20	51
		R	CTCATCATGTTGTACATGGCG	21	
3	umc 1136	F	CTGCATACAGACATCCAACCAAAG	24	53
		R	CTCTCGTCTCATCACCTTTCCCT	23	
4	umc 1746	F	ACCTTGCCTGTCCTTCTTTCTCTT	24	55
		R	ACACGAGCATCCTACATCCTCCTA	24	
5	umc 1015	F	CAGACACAAGCAGCAAAGCAAG	22	54
		R	TCCGACTCCAAGAAGAGGAGAA	22	
6	bnlg 2235	F	ATCCGGAGACACATTCTTGG	20	52
		R	CTGCAAGCAACTCTCATCGA	20	
7	Phi 109275	F	CGGTTCATGCTAGCTCTGC	19	54
		R	GTTGTGGCTGTGGTGGTG	18	
8	bnlg 2204	F	AGGCGACTTAGCTGCAGAAG	20	49
		R	CGACTTTCGGTTTGGAAAAG	20	
9	umc 2383	F	CATAGACGTGCCCCTTGTCATC	22	55
		R	CTCGCAACTGCGCTTCTAGATACT	24	
10	bnlg 1043	F	TTTGCTCTAAGGTCCCCATG	20	51
		R	CATACCCACATCCCGGATAA	20	
11	bnlg 1346	F	CATCATGAAGCAATGAAGCC	20	49
		R	CCGCGCCATTATCTAGTTGT	20	
12	bnlg 1070	F	TTCCAGTAAGGGAGGTGCTG	20	52
		R	TAAGCAACATATAGCCGGGC	20	
13	umc 1297	F	ATCGCCTCAACACACCTTCATATT	24	53
		R	TGGTCACTGACTGTTTCGACTAGC	24	
14	bnlg 1006	F	GACCAGCGTGTTGATCCC	18	54

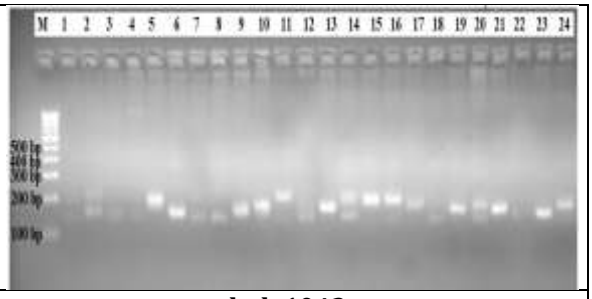
RESULTS AND DISCUSSION

Out of 14 SSR primers used all 14 primers amplified and showed the polymorphism in maize genotype.

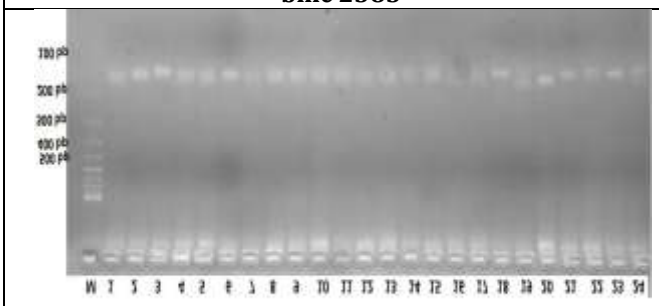
umc 1078	bnlg 1823
	
umc 1136	**umc1746**
	
bnlg 2235	**umc 1015**
	
Phi 109275	**bnlg 2204**



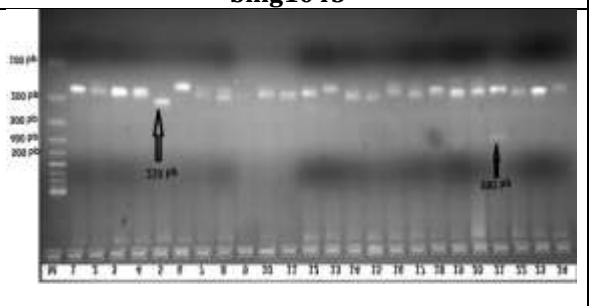
bmc 2383



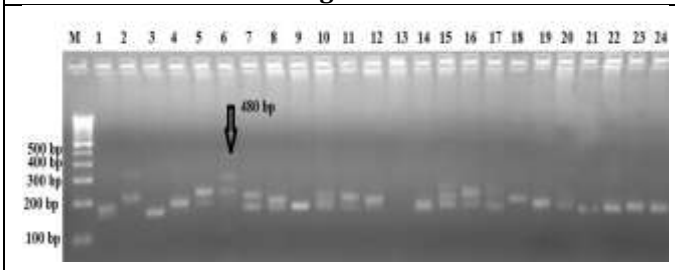
bnlg1043



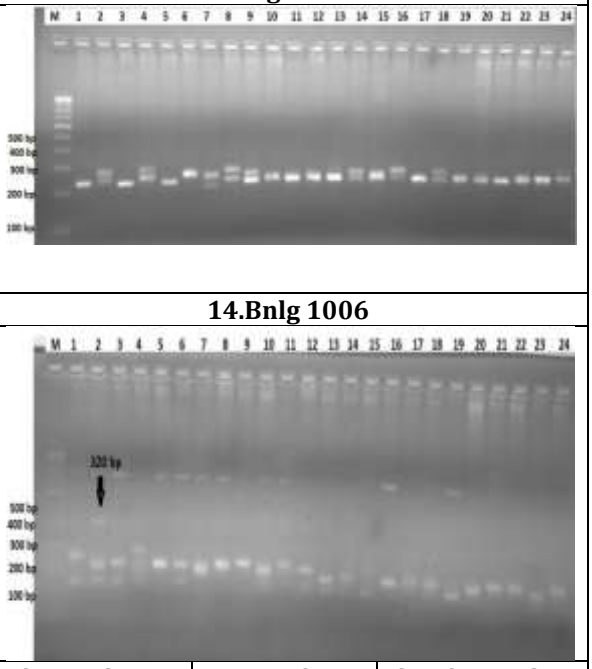
bnlg 1346



bnlg 1070



13. Umc 1297



14.Bnlg 1006

LANE NO.	GENOTYPES	LANE NO.	GENOTYPES	LANE NO.	GENOTYPES
1	52021	9	52310	17	IC-552819
2	52065	10	52327	18	EC-639232
3	52099	11	52337	19	EC-639008
4	52144	12	52349	20	EC-639290
5	52191	13	52507	21	EC-639001
6	52217	14	IC-470475	22	DML-1112
7	52222	15	IC-437070	23	DML-1285
8	52285	16	IC/RNG/SW-1-7	24	DML-1336

From the SSR analysis it was observed that a total of 35 bands were generated by amplification with 14 polymorphic primers. 35 of them were polymorphic, from which 6 were unique. The size of amplification product ranged from 110 bp to 750 bp. Each primer produced on an average 2.5 bands and each polymorphic primer produced on an average 2.5 polymorphic bands. The results can be summarized as following.

- Maximum 4 loci were amplified by the primer bnlg 1823 followed by bnlg 2235 (3 bands), bnlg 2204 (3 bands), bnlg 1043 (3 bands), bnlg 1346 (3 bands) and bnlg 1006(3 bands). Remaining all primers showed 2 bands. All 14 primers showed 100 % polymorphism
- Among the SSR primers used, six primers have PIC value more than 0.50 and other eight have the PIC value less than 0.50.
- PIC values of primers ranged from 0.06 for SSR primer umc 1297 to 0.75 for SSR primer bnlg 1823.
- The maximum number of bands were observed in 52222 (20 bands) while minimum bands were observed inDML-1336 (11 bands).
- All 14 polymorphic primers together amplified 6 unique loci specific to 5 particular genotypes.
- The similarity coefficient values based on SSR markers data ranged from 0.25 to 0.86. Maximum similarity coefficient value of 0.86 was noticed two times in the genotypes between DML-1285 and 52099, 52337 and 52327 while minimum similarity value of 0.25 was observed in between IC-552819 and 52217.
- The size of amplification product ranged from 110 bp to 750 bp.
- The UPGMA based dendrogram revealed 24 genotypes were divided into four Major clusters of maize genotypes. Major cluster I largest cluster, comprised of 20 genotypes. Major cluster II comprised of one genotype, major cluster III comprised of one genotype and major cluster IV comprised of two genotypes.
- Major cluster I has been divided into five sub clusters.Sub cluster IA comprised of three genotypes 52021, EC-639232, EC-639001. Sub cluster IB comprised of three genotypes 52065, EC-639008, EC-639290. Sub cluster IC comprised of four genotypes 52099, DML-1285, 52310 and 52144. Sub cluster ID comprised of five genotypes 52222, 52285, 52327, 52337, and DML-1112. Sub cluster IE comprised of five genotypes IC-437070, IC-470475, IC-552819, DML-1336 and IC/RNG/SW/1-7.
- The major cluster II comprised of single genotype 52349. The major cluster III comprised of single genotype 52191. The major cluster IV comprised of two genotypes *viz*., 52217 and 52507.
- Grouping results observed in dendrogram analysis were reflected in 2-D PCO scatter plot analysis (fig.2). In 2D PCO scatter plot analysis, 8 different groups were formed namely IA, IB, IC, ID, IE, II, III and IV. There is very narrow differences as per 2D scatter plot (PCO analysis) with most of the genotypes placed closely.

5.2 Conclusions

Molecular analysis of maize in relation to assessment of diversity using SSR analysis revealed that SSR markers have a higher discrimination capacity. All the 14 SSR primers showed 100% polymorphism, representing capability of these primers to amplify the less conserved regions of the DNA.

- Out of total 14 SSR primers used, 6 primers *viz*., bnlg 1823, umc 1015, bnlg 2235, phi 109275, bnlg 2204 and bnlg 1043 found to be informative based on PIC values and these primers can be used for the molecular characterization of maize genotypes under evaluation.
- The Dice similarity coefficient values for SSR primers indicated moderate diversity among maize genotypes. Unique bands produced by SSR primers may be variety specific.
- Based on the Dice similarity coefficient values, minimum similarity value of 0.25 was observed between the genotypes IC-552819 and 52217 followed by 0.31 between the genotypes EC-639290 and 52191, IC-437070 and 52217; 0.32 between the genotypes 52349 and 52217, DML-1336 and 52222, DML-1336 and 52349 indicating they are more diverse to each other.

- These genotypes can be used for hybridization programme for exploitation of heterosis and crop improvement.
- The genotypes having more diversity between them can be used for association mapping to identify major and minor quantitative trait loci (QTL) and also in convergent crop improvement.

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