Biological Forum – An International Journal **15(3): 27-31(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

DNA Fingerprinting and Molecular Characterization of Newly Developed Maize Inbreds based on Microsatellite Markers

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(Received: 04 January 2023; Revised: 20 February 2023; Accepted: 25 February 2023; Published: 22 March 2023)
(Published by Research Trend)

ABSTRACT: Identification of elite and diverse parents is a critical step in the process of releasing new hybrids. DNA fingerprinting and characterization of germplasm plays a significant role in plant breeding for varietal identification where, molecular markers have proven to be very effective. The current study was performed at the Plant Molecular Biology and Biotechnology Laboratory, RMDCARS, Ambikapur (Chhattisgarh). A total of 27 SSR primers were used to check the polymorphism of eighteen newly developed maize inbreds, eight of which were found to be polymorphic and were subsequently used for DNA fingerprinting and molecular characterization. A total of 25 alleles were obtained using these polymorphic SSR primers, with an average of 3.13 alleles per primer. The PIC value for these primers ranged from 0.10 to 0.82 where, highest value obtained for primer bnlg 1867. The fingerprinting for each inbreds with unique profile identity (ID) were generated using different banding pattern and variation in allele size. These fingerprint data provide distinct allelic profiles for each inbred lines of maize. A dendrogram was also prepared for all these inbreds using the unweighted pair group method with arithmetic mean (UPGMA). It separated them into five major clusters at nearly 84% genetic similarity indicated the existence of genetic variation among the observed inbreds. This enables their further utilization for generating heterotic hybrids in future breeding programmes. Among all the inbreds studied, IAMI-57 and IAMI-43-1 were found to be more genetically diverse. The polymorphic SSR markers facilitated discrimination among genotypes and provided valuable information for future use in improvement of these genomic resources.

Keywords: DNA Fingerprinting, Microsatellite, SSR, Maize, Molecular characterization.

INTRODUCTION

Maize is a monoecious crop grown worldwide due to its wider adaptability and multiple uses. In India, maize is next to wheat and rice and considered as third most important cereal crop. Improved maize germplasm plays a significant role in the advent of diverse and potential parents for making superior hybrids of maize however, for finding the parental components and their crosses genetic identification of maize is very essential step. The PCR-based DNA fingerprinting approaches uses various molecular markers to generate the specific patterns, these patterns are the unique identity generated for an individual. SSR markers are also used in DNA fingerprinting analysis to identify inbred lines and hybrids, as well as to describe the species at the molecular level in order to protect breeders' rights and its protection. Simple Sequence Repeats (SSR) or Microsatellites (Tautz, 1989) markers exhibited high polymorphism, frequent occurrence in genome, high reproducibility and are easily available (Serumaga *et al*., 2014). Therefore, SSRs can be more commonly used to identify and evaluation of varieties of plants genetic resources (Nunome *et al*., 2003). Besides, SSR markers are randomly distributed throughout the genome and revealed significant level of allelic polymorphism and extensively used in maize (Smith *et al.,* 1997).

Diversity among the inbred lines of maize allows the plant breeders to develop new improved hybrids with high yield potential, by efficiently exploiting heterosis by crossing unrelated and distant parents (Dar *et al.,* 2018). There are various methods available for germplasm characterization *viz*., pedigree data, morphological data, isozyme analysis and molecular characterization using DNA fingerprinting. There are several hindrance while using morphological markers for characterization which included late expression, low polymorphism, low heritability and susceptibility to environmental fluctuations which reduces the accuracy of the generated results. All these limitations can possibly be overcome by using molecular markers for diversity analysis (Badu-Apraku *et al.,* 2015).

MATERIALS AND METHODS

In this experiment, DNA fingerprinting and molecular characterisation was performed on a total of eighteen inbred lines, representing collections from IGKV, RMDCARS, Ambikapur, WNC, Hyderabad, Uchani, Haryana and TNAU, Coimbatore (Table 1). The sample leaves were taken from the seedlings of these maize inbred lines, thirty days after sowing. By using CTAB method (Murray and Thompson 1980) with slight modification, the total genomic DNA was extracted and the working stock of DNA (50 ng/ul) was prepared based on quantification results using the mother stock.

A total of 27 SSR markers of maize were initially selected for checking the parental polymorphism and the PCR was carried out in 22 µl reaction mixture containing 2 ul of 50 ng/ul genomic DNA as template, sterile DD H_2O 13.5 µl, PCR buffer (10 X) 2.5 µl, 1 mM dNTPs 1.5 µl, primer pair (forward and reverse) 2 µl and 5 U Taq Polymerase 0.5 µl. The amplification was carried out with initial denaturation of 94°C for 5 min., denaturation at 94°C for 30 s, annealing at 50-62.7°C for 40 s, extension at 72°C for 60 s and the final extension step was carried out at 72°C for 5 min. The resulting PCR amplicons were resolved in 2.0 % agarose gel at 70 V for 50 min. The amplicons were scored as alleles for the loci. The alleles were scored manually and allele sizes (base pairs) were determined comparing with50 bp DNA ladder which is run parallel with the sample DNA of inbred lines. The scored data were then used to obtain similarity matrix according to Jaccard coefficients. At the next step clustering was done and dendrogram generated using unweighted pair-group method using the arithmetic mean (UPGMA) based similarity matrices. The data were analysed using the software NTSYS-pc2.0.

Table 1: List of inbreds used for molecular characterization along with their sources.

Sr. No.	Inbred lines	Source
1.	IAMI-73	IGKV, RMDCARS, Ambikapur
2.	IAMI-83-1	IGKV, RMDCARS, Ambikapur
3.	IAMI-83-2	IGKV, RMDCARS, Ambikapur
4.	IAMI-35	IGKV, RMDCARS, Ambikapur
5.	IAMI-43-2	IGKV, RMDCARS, Ambikapur
6.	IAMI-43-1	IGKV, RMDCARS, Ambikapur
7.	IAMI-57	IGKV, RMDCARS, Ambikapur
8.	VL-18932	CIMMYT, WNC, Hyderabad
9.	VL-18941-2	CIMMYT, WNC, Hyderabad
10.	VL-18941-1	CIMMYT, WNC, Hyderabad
11.	VL-18452	CIMMYT, WNC, Hyderabad
12.	VL-118941	CIMMYT, WNC, Hyderabad
13.	VL-172428	CIMMYT, WNC, Hyderabad
14.	VL-1033	CIMMYT, WNC, Hyderabad
15.	HKI-163	Uchani, Haryana
16.	BML-06	WNC, Hyderabad
17.	UMI-1230	TNAU, Coimbatore
18.	UMI-1201	TNAU, Coimbatore

RESULTS AND DISCUSSION

The genomic DNA of all the eighteen inbreds were used to check the polymorphism using 27 SSR markers, out of which eight SSR primers *viz.,* bnlg 565, bnlg 429, bnlg 1194, bnlg 1867, bnlg 1583, phi-085, phi-402893 and phi-251513 were found to be polymorphic. Using these polymorphic SSR primersa total of 25 alleles were obtained with an average of 3.13 alleles per primer. The number of alleles amplified for each primer ranged from two to eight. Kumar *et al.* (2016) obtained a total of 59 alleles with an average of 2.62 alleles per locus while, Patel *et al.* (2017) identified total 76 alleles with an average of 4.47 alleles per locus however Kumari *et al.* (2018) detected total of 20 alleles with an average of 2.2 alleles per locus. The difference in the number of alleles obtained between studies could be due to sample size, the methodologies used for detecting polymorphic markers that influence allelic differences.

The PIC value for these primers ranged from 0.10 to 0.82 (Table 2). Out of the eight microsatellite markers two markers *viz.,* bnlg-429 and phi-402893 differentiated all the eighteen inbreds at least with a single marker allele difference. The highest PIC value obtained for marker bnlg 1867 (0.82) which separate all the inbreds into amplicons of 260 bp, 300 bp, 320 bp, 420 bp, 460 bp, 560 bp, 700 bp and 1000 bp. The band size of 200 bp were obtained for all polymorphic marker studied except phi-085 and phi-251513 which showed band size of 140 bp. Also, the band size of 400 bp were obtained for bnlg 565 and bnlg 1194. Other band sizes such as 380 bp noticed for bnlg 429, 900 bp for bnlg 1194, 240 bp for bnlg 1583, 140 bp, 280 bp for phi-085, 460 bpfor phi-402893, band sizes of 270 bp, 300 bp, 500 bp and 800 bp for phi-251513 (Table 3). Based onthe allele size (bp) obtained for eight polymorphic SSR primer coding system was generated. These codes were later used to produce SSR fingerprinting map of 18 maize inbreds based on their allele size codes. These fingerprint data provide unique allelic profiles for precisely establishing genotypic identity. San *et al.* (2022) used six SSR primer pairs as a final marker set for variety identification where, the selection of marker varies due to their characterization and discrimination capacity.

For molecular characterization, eighteen inbred lines were taken for the experiment to study the similarity

index based on the polymorphism shown by the eight markers. Mainly five clusters were formed at nearly 84% genetic similarity, among inbreds. Cluster I contains three inbreds *viz.* IAMI-73, IAMI-83-1 and IAMI-83-2 having 88% similarity whereas, Cluster II consisted of IAMI-35, IAMI-43-2, VL-18941-2, VL-18941-1, VL-172428, VL-18932, VL-18452 and VL-1033 having 87% similarity. Cluster III contains five inbreds with 91 % similarity which included VL-118941, BML-06, UMI-1230, UMI-1201 and HKI-163. Among the lines, in accordance with the markers taken for the study IAMI-57 and IAMI-43-1 were found to be genetically diverse inbreds. Inbred line IAMI-83-2 which comes in Cluster I along with IAMI-73 and IAMI-83-1 showed similarity with both lines as obtained from cluster analysis. The genotypes of five major groups can be used as parents to get better heterosis as they exhibited genetic diversity. Inferring a higher level of efficacy for establishing the association between closely related genotypes, microsatellite markers thus enabled more precise separation of clusters.

Table 2. Frequency of alleles and PIC value of eight microsatellite loci in eighteen newly developed maize inbreds.

Marker name	Annealing temperature (°C)	Forward Primer	Reverse Primer	No. of alleles	PIC = 1-ΣX_i^2)	Allele frequency
bnlg 565	51	TAAGAACGACGAACGGTAACTG	GCTCACTGCACGCCAACAC	2	0.10	0.90
bnlg 429	54	CTCCTCGCAAGGATCTTCAC	AGCACCGTTTCTCGTGAGAT	2	0.50	0.50
bnlg 1194	52	GCGTTATTAAGGCAAGCTGC	ACGTGAAGCAGACGATCCAT	3	0.19	0.82
bnlg 1867	54	CCACCACCATCGTAGGAGTT	CAGTACACAGCAGGCAGCTC	8	0.82	0.18
bnlg 1583	51	ATCAAGCTTATCGAGAGAGAGAGAG	CGACGGTTTAAAGACTGC	2	0.48	0.52
phi-085	50	AGCAGAACGGCAAGGGCTA	TTTGGCACACCACGCCGA	2	0.32	0.68
phi-402893	50	GCCAAGCTCAGGGTCAAG	CACGACGTTATTCGCTGT	2	0.50	0.50
phi-251513	54	CCAGTCCAATTGGAGAGGG	GAGATTCCCCTGCAGGACT	4	0.45	0.55

Table 3: Coding system of selected eight SSR primer set based on allele size (bp).

Code	bnlg 565	bnlg 429	bnlg 1194	bnlg 1867	bnlg 1583	phi-085	phi-402893	phi-251513
01	200	200	200	260	200	140	200	270
02	400	380	400	300	240	280	460	300
03			900	320				500
04				420				800
05				460				
06				560				
07				700				
08				1000				

Table 4: SSR fingerprinting map of 14 maize inbreds based on their allele size codes.

Genotype name	bnlg 565	bnlg 429	bnlg 1194	bnlg 1867	bnlg 1583	phi-085	phi-402893	phi-251513
IAMI-73	01	0102	01	030508	01	01	0102	01
IAMI-83-1	01	0102	01	02050708	01	0102	0102	01
IAMI-83-2	01	0102	01	01030506	01	0102	0102	01
IAMI-35	01	0102	01	01	02	0102	0102	01
IAMI-43-2	01	0102	01	02	02	0102	0102	01
IAMI-43-1	0102	0102	0102	0204050708	02	01	0102	0102
IAMI-57	01	0102	0103	01050708	02	01		01
VL-18932	01	0102	01	0305	02	01		01
VL-18941-2	01	0102	01	0207	02	01	0102	010203
VL-18941-1	01	0102	01	0207	02	01	0102	010203
VL-18452	01	0102	01	020305	02	01	0102	01
VL-118941	01	0102	01	02	02			01
VL-172428	01	0102	01	02	02	01	0102	010304
VL-1033	01	01	01	030507	02	01	0102	01
HKI-163	01	01	01	020305	01			01
BML-06	01	01	01	02	01	01		01
UMI-1230	01	0102	01	02	01	01		01
UMI-1201	01	0102	01	02	01	01		01

Table 5: Distribution of eighteen newly developed maize inbreds in different clusters.

Group No.	Number of genotypes	Genotypes
1	3	IAMI-73, IAMI-83-1, IAMI-83-2
2	8	IAMI-35, IAMI-43-2, VL-18941-2, VL-18941-1, VL-172428, VL-18932, VL-18452, VL-1033.
3	5	VL-118941, BML-06, UMI-1230, UMI-1201, HKI-163
4	1	IAMI-57
5	1	IAMI-43-1

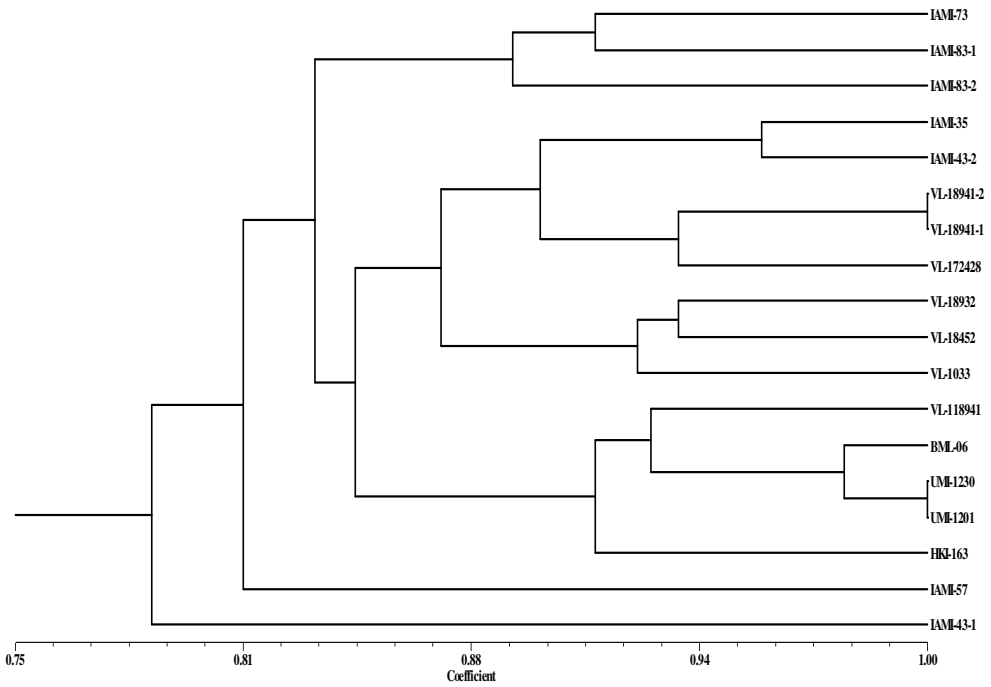

Fig. 1. Dendrogram of maize inbreds based on microsatellite markers.

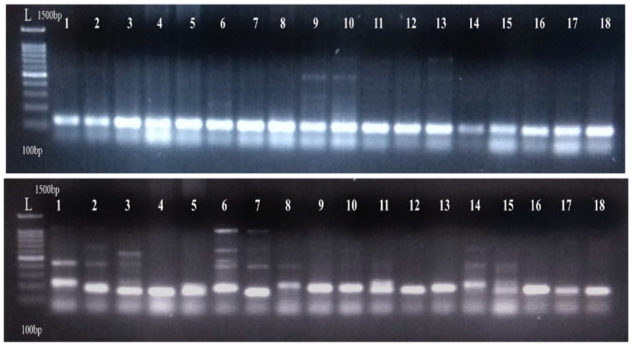

Fig. 2. DNA profile of eighteen maize inbreds obtained through microsatellite marker.

CONCLUSIONS

The DNA fingerprinting catalogue of maize inbreds were generated using different banding pattern and variation in allele size. These fingerprint data provide distinct allelic profiles that can be used to precisely determine genotypic at any stage of crop growing cycle. Furthermore, the SSR markers based characterization facilitated discrimination among all the eighteen maize inbreds where, inbreds *viz.,* IAMI-57 and IAMI-43-1 were found to be more genetically diverse. Though, the data were generated using a limited number of SSR primers, inclusion of more number of primers would lead to an unambiguous result.

FUTURE SCOPE

Fingerprint data generated for all the inbreds studied in this experiment may be used for future study. The diverse parents found in this study may be employed in future breeding programmes of maize to generate the heterotic hybrids.

Acknowledgements. The authors would like to thank Department of genetics and Plant Breeding and Plant Molecular Biology and Biotechnology Laboratory, RMDCARS, Ambikapur (C.G.) for providing all the facilities to conduct this research work.
Conflict of Interest. None.

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How to cite this article: Jyoti Sahu, Deepika Parte, Priya Gupta, Naresh Kumar Sahu, J.K. Tiwari, S.K. Sinha and Prabharani Chaudhari (2023). DNA Finger Printing and Molecular Characterization of Newly Developed Maize Inbreds based on Microsatellite Markers. *Biological Forum – An International Journal, 15*(3): 27-31.