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GENETICS AND PLANT BREEDING

Microsatellite based Molecular Characterization and Genetic Diversity Analysis of Maize (*Zea mays* L.) Inbred Lines

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ABSTRACT

Molecular characterization of the genotypes provides reliable information about the extent of genetic diversity which aids in the development of an appropriate breeding program. In the present study, a total of seventeen polymorphic SSR markers were used across eight maize inbreds for their characterization and discrimination. The number of alleles per locus ranged from 2 alleles (UMC-1657) to 8 alleles (UMC-1097), with an average of 4.47 alleles across 17 loci obtained in the study. The PIC value ranged from 0.46 (UMC-1657) to 0.86 (UMC-1097) with average of 0.68. The dendogram constructed using similarity coefficient values divided 8 genotypes in three divergent clusters.

Highlights

- DNA isolation from eight maize inbreds using modified CTAB method.
- Eight maize inbreds were characterized at molecular level using SSR markers.
- Less genetic resemblance was observed between some of the inbreds indicating better scope for getting more diverse segregants by hybridizing them.
- Some of the SSR primer identified as potential markers to reveal genetic diversity among maize lines at molecular level.

Keywords: Maize, microsatellites, genetic diversity, inbred line, molecular fingerprinting

Maize (*Zea mays* L.) is the third most important cereal crop next to rice and wheat and has the highest production potential among cereals. It belongs to the poaceae family and have somatic chromosome number 2n = 20. It is known as"Queen of Cereals". Many forms of maize are used for food such as popcorn, flint corn, sweet corn, waxy corn, amylose maize, pod corn striped maize and baby corn. The kernel provides feed, food and a resource for many unique industrial and commercial products. By using genetic variation, the composition of the kernel can be altered for both the quantity and quality of starch, protein and oil throughout kernel development. Being a C_4 plant, it is physiologically more efficient and resilient to changing climatic conditions with wider genetic variability and adaptability across wide range of environment.

The area of maize planting is expected to further increase with the increase in the population. An additional corresponding increase in yield in the future will require an efficient breeding program with well-established germplasm in order to exploit potential heterosis. The advancement in use of molecular markers has proven valuable for genetic diversity analysis at the DNA level in plant species (Melchinger and Gumber 1998). Unlike the morphological markers, molecular markers are

not influenced by environmental factors. Thus, they reflect the actual level of genetic difference existing among the genotypes (Westman and Kresovich 1997). Currently several DNA based molecular marker technologies are available for genetic diversity analysis. Simple sequence repeat (SSR) markers were markers of choice as they are polymerase chain reaction (PCR)-based, easy to use, co-dominant, locus-specific, highly reproducible and informative (Powell *et al*. 1996).

SSR markers have been efficiently used to assess the extent of genetic diversity in maize (Shehata *et al.* 2009; Nepolean *et al.* 2013 and Sserumaga *et al.* 2014). Therefore, the present study was undertaken to investigate and quantify the magnitude of genetic diversity at molecular level using set of 17 SSR markers in 8 maize inbred lines.

MATERIALS AND METHODS

Plant material and DNA extraction

Eight inbred lines (Table 1) of maize were characterized using 17 locus specific SSR primers (Table 2). DNA was extracted from the leaves by CetylTrimethyl Ammonium Bromide (CTAB) method (Zidani *et al*. 2005) with some minor modifications.

Table 1: List of maize inbreds

Sl. No.	Inbred	Pedigree	Source
1	CML-307	CIMMYT maize line 0308	CIMMYT, Mexico
2	CM-111	Cuba 342-2-F-#-#	IIMR, New Delhi
3	CLQ-47	CIMMYT QPM line	CIMMYT. India
4	CM-140	I-617-61-1-1-1-1-6-FS5-1-1	IIMR, New Delhi
5	CM-137	IPA-9-7	IIMR, New Delhi
6	HKI-163	CML-163	HAU
7	HKI-193-1	CML-193	HAU
8	CM-500-1	Antiguae Gr.1	IIMR, New Delhi

SSR PCR Amplification and Gel Electrophoresis

SSR sequences were amplified through PCR using locus specific microsatellite primers. PCR amplification (25 ml volume) was carried out in 2.5 µl of 10 × PCR buffer, 0.7 µl of dinucleotide triphosphate (dNTPs) (25mM), 0.5 µl of each of the forward and reverse primer (10 p moles/µl), 0.3 µl of Taq polymerase (5 units/µl), 3.5 µl of DNA (20 ng), and Distilled deionized water (17 µl) using an Eppendroph thermal cycler. The PCR Cycle consist of initial denaturation at 94 °C for 3 min and subsequent cycles each with denaturation at 94 °C for 30 sec, 40 sec at annealing temperature and primer extension at 72 °C for 1 min. The final extension step was carried out at 72 °C for 7 min. The amplified product is then subjected to Gel electrophoresis (2.5 %) along with 100 bp+ DNA ladder (fermentas, USA).

Scoring and Analysis of Data

Clear and distinct bands amplified by SSR primers were scored for the presence and absence of the corresponding band among the 8 maize inbreds. To evaluate the characterizing potential of each marker, the polymorphism information content (PIC) for each SSR locus was calculated according to formula suggested by (Weir 1996). Coefficients of similarity were calculated by using Jaccard's similarity coefficient by SIMQUAL function and cluster analysis was performed by agglomerative technique using the UPGMA (Un-weighted Pair Group Method with Arithmetic Mean) method by SAHN clustering function of NTSYS-pc version 2.02 (Rohlf, 1994).

RESULTS AND DISCUSSION

Analysis of all the 17 locus specific SSR primer revealed polymorphism across the 8 inbred lines. A total number of 76 alleles were amplified with an average of 4.47 alleles. Nepolean *et al.* (2013) generated a total of 111 polymorphic alleles with an average of 3.17 alleles per locus. While, Sserumaga *et al.* (2014) detected total of 184 alleles were identified at all the loci with an average of 7.36 and a range between two and 19 alleles per locus. Differences in the numbers of alleles between studies could be explained mainly due to the size of the samples under study, the methodologies employed for detection of polymorphic markers which influence allelic differences, expected diversity or uniformity based on pedigrees. The PIC value ranged from 0.46 (UMC-1657) to 0.86 (UMC-1097) with an average of 0.68 which is comparatively low as compared to polymorphism information content observed by Adeyemo*et al.* (2011) (PIC = 0.17 to 0.84) and Sserumaga *et al.* (2014) (PIC = 0.16 to 0.91).

Table 2: Results of SSR analysis

Sl. No.	Primer	Molecular band size (bp)	Total no. of bands	No. of polymorphic band	Per cent polymorphism	PIC value
1	UMC-1035	69.67 to 84.94	9	4	44.44	0.70
2	UMC-1933	56.81 to 63.23	10	4	40	0.58
3	UMC-1944	56.14 to 75.23	10	6	60	0.78
4	UMC-1657	52.43 to 63.59	8	2	25	0.46
5	PHI-027	71.94 to 88.57	11	5	45.45	0.74
6	PHI-115	122.40 to 149.16	8	4	50	0.65
7	UMC-1754	68.31 to 103.97	11	6	54.54	0.80
8	UMC-1122	78.43 to 94.43	9	4	44.4	0.69
9	UMC-1097	51.21 to 65.04	15	8	53.3	0.86
10	UMC-1492	72.11 to 91.20	14	6	42.85	0.77
11	UMC-1433	96.63 to 116.27	8	3	37.5	0.62
12	UMC-1507	61.40 to 78.02	14	5	35.71	0.77
13	UMC-1620	62.23 to 74.69	9	3	35.17	0.59
14	UMC-1636	54.92 to 66.26	8	3	37.5	0.59
15	UMC-1282	126.81 to 156.73	10	5	50	0.74
16	Y1	58.61 to 69.31	8	3	37.5	0.59
17	UMC-1366	61.0 to 80.55	14	5	35.71	0.77
	Total	—	**176**	**76**	—	—
	Average	—	—	—	**42.88**	**0.68**

Table 3: Similarity matrix for Jaccard's coefficient for 8 maize genotypes based on SSR analysis

	CML-307	CM-111	CQL-47	CM-140	CM-137	HKI-163	HKI-193-1	CM-500-1
CML-307	1.00							
CM-111	0.74	1.00						
CQL-47	0.62	0.57	1.00					
CM-140	0.50	0.61	0.65	1.00				
CM-137	0.42	0.47	0.54	0.76	1.00			
HKI-163	0.47	0.45	0.57	0.66	0.74	1.00		
HKI-193-1	0.53	0.53	0.62	0.58	0.55	0.71	1.00	
CM-500-1	0.53	0.53	0.59	0.61	0.58	0.63	0.66	1.00

The similarity coefficient (Table 3) for 8 inbred based on SSR analysis showed maximum genetic similarity between CM-140 and CM-137 (0.76) because these two lines are from same source followed by CML-307 and CM-111 (0.74) which was also obtained from same source. While, minimum genetic resemblance (0.42) was observed between parents CML-307 and CM-137 this may be due to the different sources of origin. So, crossing between these two lines will be more rewarding in heterosis breeding.

The UPGMA based dendogram of 8 inbred lines shown in Fig. 1 separated them into 3 well-defined groups. Group C has maximum number of lines (5) followed by group A (2). While, group B has only line CQL-47 which derived from QPM line which is most diverged from all. Lines with greater similarityCM-140 and CM-137, CML-307 and CM-111 included in the same cluster *i.e.* cluster C and A, respectively. Das *et al.* (2012) grouped 25 maize genotypes into 4 different clusters. Adeyemo*et al.* (2011) also observed genetic difference among 38 maize genotypes detected by SSR emphasizing the efficiency of SSR markers in diversity analysis and found two distinct Groups A and Group B with 23 and 15 genotypes, respectively.

The genotypes with greater dissimilarity were included in different clusters. In general, the clustering pattern generated was according to

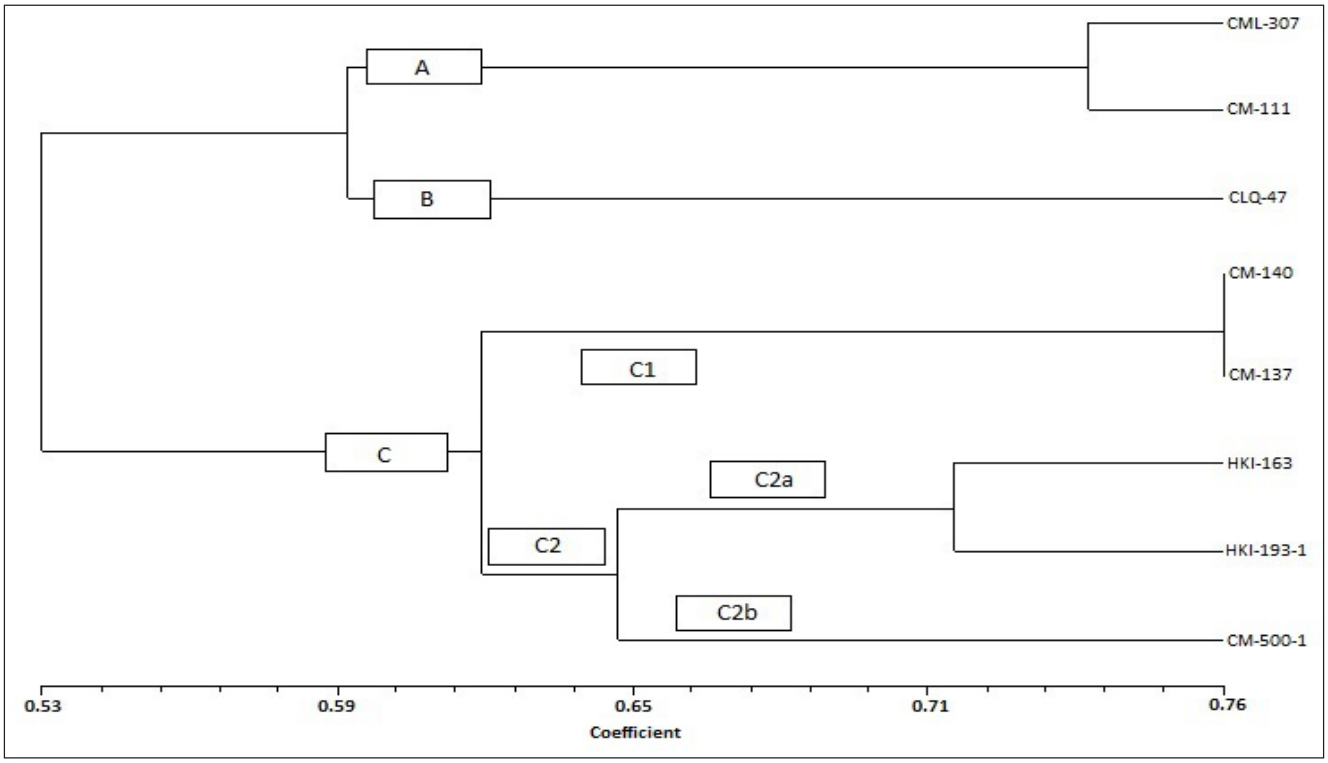

Fig. 1: Dendrogram showing clustering of 8 maize genotypes constructed using UPGMA based on Jaccard's coefficient obtained from SSR analysis

geographical diversity of studied inbreds which is not in agreement with findings reported by Yu *et al*. (2001). The variability detected using SSR markers could potentially contribute towards effective utilization of the inbred lines for the exploitation of heterosis and formation of genetically diverse source populations.

CONCLUSION

The ability of the SSR markers to amplify specific allele is very important. Molecular analysis revealed that SSR primer UMC-1097, UMC-1754 and UMC-1944 as potential markers to disclose genetic diversity among maize lines at molecular level. The available information regarding diversity can be efficiently utilized in selection of parents for maize improvement programme.

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