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Molecular Genetic Diversity Analysis of Quality Protein Maize lines using SSR markers

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ABSTRACT

Genetic diversity and relationships among QPM (quality protein maize) lines would help and broaden the genetic base of crops in national improvement programmes. In this study, we report the analysis of 18 QPM germplasm lines were arranged using microsatellite markers or Simple Sequence Repeats (SSR). Genetic variability among eighteen QPM genotypes were assessed employing 34 primers spread over the whole genome. The polymorphism information content (PIC) values for SSR markers were ranged from 0.50-0.95 and the genotypes were grouped into different clusters using NTSYSpc2.1 programme. The PIC value was found to be highest for the primer *bnlg 1401*(0.95) followed by *bnlg1043, bnlg1194* and *Umc1005* (0.94) while the lowest value was recorded for the primer *bnlg1506* (0.50) with the mean value of 0.83.

Key words : Dendrogram, Quality Protein Maize, Simple Sequence Repeats.

Maize is a cereal crop for human consumption and livestock feed as well as raw material for several industrial uses. However, conventional maize contains low protein quality due to its deficiency in two essential amino acids lysine and tryptophan (FAO, 1992). The opaque-2 (*o2)* mutation was found to be the most suitable for genetic manipulation in breeding programmes aimed at developing maize lines with high lysine and tryptophan. Maize homozygous for the *o2* (recessive) mutation was shown to have substantially higher lysine and tryptophan content than maize that was either homozygous dominant (*O2O2*) or heterozygous (*O2o2*) for the *opaque-2* locus.

Genetic diversity is the basic for any crop improvement programme. The hybrids involving the parents with more diversity among them are expected to exhibit higher amount of heterotic expression and broad spectrum of variability in segregating generations. Information on germplasm diversity and relationships among elite materials is fundamentally important in crop improvement (Hallauer and Miranda, 1988). Assignment of inbred lines to heterotic groups using molecular markers allows the characterization of a greater number of lines, thus potentially increasing the efficiency of maize breeding programs (Reif *et al*., 2003 and Melchinger and Gumber, 1998). Diversity analysis of germplasm collections can be carried out using morphological, geographical, molecular (DNA, sequence, gene) and functional levels (Buckler *et al*., 2006). Microsatellites or simple sequence repeats (SSRs) are stretches of tandemly arranged short sequence motifs (ranging from two to six nucleotides), which are abundant and highly polymorphic in several eukaryotic genomes, including maize (Powell *et al*., 1996 and Gupta *et al.,* 1996). The objective of the present study was to determine the levels of genetic diversity and relationships among the selected QPM lines and to evaluate the useful-ness of microsatellites in diversity analysis of Quality Protein Maize germplasm.

MATERIAL AND METHODS

The QPM germplasm lines (Table. 1) for the present study were procured from Directorate of Maize Research, New Delhi and these lines were sown at Maize Research Centre, Hyderabad during *rabi*-2009.

The primer sequences used for PCR amplifica-tion were selected from the MaizeGDB data-base (http://www.agron.missouri.edu). The mi-crosatellite loci selected were chosen based on the size of the repetitions and their location to obtain a representative sampling of the whole genome (Table 2).

About 2 g of young and healthy leaf tissue was collected from 30 days old plants and ground into a fine powder in liquid nitrogen using a pre-chilled

Table1. Maize inbred lines used in the present study.

S. No	Name of QPM line	S. No	Name of QPM line
1	CML142(w)	10	CML 158
2	CML172.1	11	CML 154
3	CML165	12	CLQRCYQ-30
4	CML142(P)	13	HKI17-2
5	CML 163	14	HKI164-4(1-3)-2
6	CML 181	15	CLQRCY51
7	CML 186	16	CML142
8	CML 150	17	HKI193-2-2-1
9	CML 157	18	CML165X G26SEQC3

Table 2. SSR primers shown polymorphism with their repeat motif and PIC value.

S.No	Primer	Repeat motif	PIC	S.No	Primer	Repeat motif	PIC
1	Bnlg1633	AG	0.90	18	Umc1656	CGGT	0.65
2	Phi072	AAAC	0.82	19	Bnlg1136	AG	0.93
3	Phi075	CT	0.59	20	Bnlg1297	AG	0.85
4	Bnlg1179	AG	0.93	21	Bnlg339	AG	0.89
5	Bnlg1643	AG	0.93	22	Umc1708	GGA	0.75
6	Umc1111	CAAAA	0.70	23	Umc1407	GGC	0.85
7	Umc1147	CA	0.92	24	Bnlg1194	AG	0.94
8	Bnlg1297	AG	0.90	25	Bnlg2037	AG	0.77
9	Bnlg108	AG	0.92	26	Umc1005	GT	0.94
10	Mmc0401	GGA AG	0.86	27	Umc1141	GA	0.77
11	Umc2369	GCAC	0.79	28	Bnlg1401	AG	0.95
12	Bmc 2136	AG	0.91	29	Bnlg1506	AG	0.50
13	Bnlg1754	AG	0.84	30	Bnlg1810	AG	0.89
14	Nc004	AG	0.83	31	Umc1357	CTG	0.84
15	Bnlg1937	AG	0.60	32	Bnlg210	AG	0.90
16	Umc1574	GCC	0.80	33	Umc1038	CT	0.89
17	Bnlg1043	AG	0.94	34	Umc1152	ATAG	0.89

mortar-pestle. 50–100 mg of ground material was transferred into a 2 ml microcentrifuge tube containing 800 µl extraction buffer (Tris –HCL 75 mM, pH 8.0, EDTA 20 mM, NaCl 500 mM, SDS 1.2% and PVP 1% to be added after autoclaving in case of mature leaf tissue) and the contents were mixed gently by swirling action. After suspending the tissue in the buffer for 5–10 min, 500 µl of ice-cold chloroform: iso-amyl alcohol (24:1) mixture was added, mixed well with the suspended tissue and spun at 10,000 rpm for 1 min preferably at 4^0 C. Without disturbing the interface, the supernatant was transferred to another 2 ml microcentrifuge tube and 1 ml absolute alcohol was added to the supernatant and mixed gently. The tube was spun for 3 min in a microcentrifuge with maximum speed and the supernatant was decanted. DNA was suspended in 200 µl of 1XTE (Tris-HCl 10 mM, pH 8.0, EDTA 1 mM) buffer containing 10 mg/ml of RNase A and then stored at -20^0C for long term storage or used directly for PCR amplification reactions. PCR cycling consisted of initial denaturation at 94^0C for 4 min, followed by 35 cycles of amplification at 94^0C for 1 min, 55–65^0C for 1 min, and 72^0C for 2 min. A final extension step at 72^0C for 7 min was followed by termination of the cycle at 4^0C. The amplified products (10 µl) were resolved on a 3 % agarose (Merc) gel.

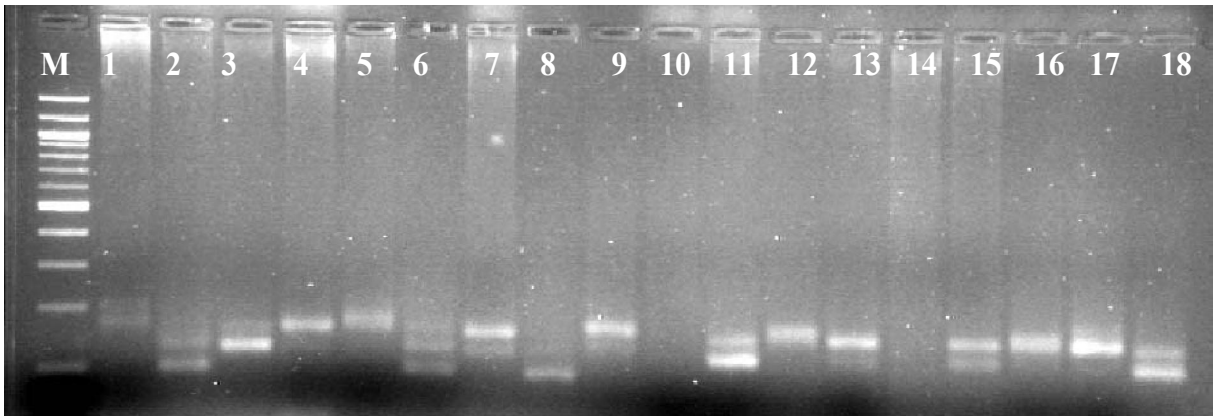


Fig: 1 SSR Profile of QPM germplasm generated by Bmc2136

M: 100 bp ladder, 1.CML142(W), 2. CML172.1, 3.CML165, 4. CML142(P) 5, CML163 6, CML181, 7, CML186, 8, CML 150, 9. CML 157, 10, CML 158, CML 154, 11. CM154,12.CLQRCYQ-30, 13, HKI17-2, 14, HKI164-4(1-3)-2, 15, CLQRCY51, 16. CML 142, 17.HKI 193-2-2-1, 18. CML165X G26SEQC3

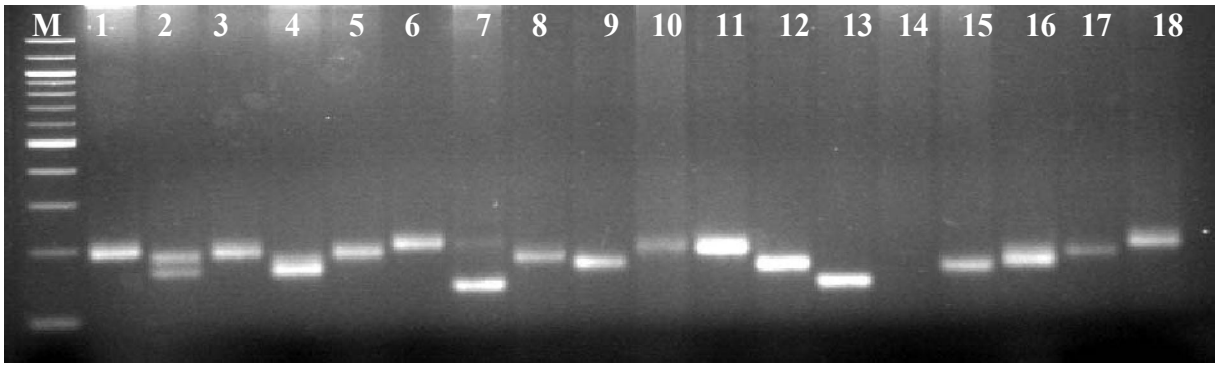


Fig: 2 SSR Prifile of QPM germplasm generated by Bnlg1194

M: 100 bp ladder, 1.CML142(W), 2. CML172.1, 3.CML165, 4. CML142(P) 5, CML163 6, CML181, 7, CML186, 8, CML 150, 9. CML 157, 10, CML 158, CML 154, 11. CM154, 12. CLQRCYQ-30, 13, HKI17-2, 14, HKI164-4(1-3)-2, 15, CLQRCY51, 16. CML 142, 17.HKI 193-2-2-1, 18. CML165X G26SEQC3

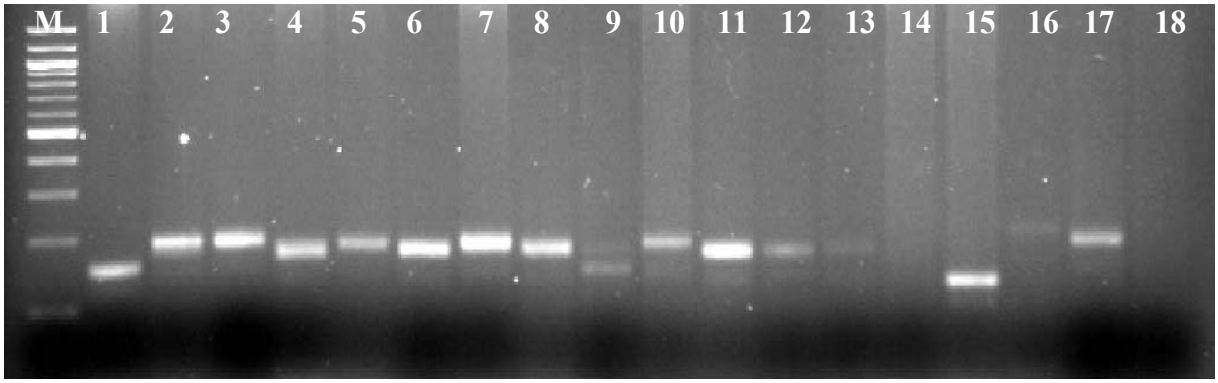


Fig: 3 SSR Profile of QPM germplasm generated by Bnlg1401

M: 100 bp ladder, 1.CML142(W), 2. CML172.1, 3.CML165, 4. CML142(P) 5, CML163 6, CML181, 7, CML186, 8, CML 150, 9. CML 157, 10, CML 158, CML 154, 11. CM154, 12. CLQRCYQ-30, 13, HKI17-2, 14, HKI164-4(1-3)-2, 15, CLQRCY51, 16. CML 142, 17.HKI 193-2-2-1, 18. CML165X G26SEQC3

The amplification products were resolved on agarose gel (3%) and detected by Gel Document. The different bands obtained were evalu-ated by visual inspection, a 100 bp DNA ladder (New England Biolabs Ltd.) was used as a molecular weight marker. For the same primer, the products of different size were considered different alleles. The information obtained was coded in a worksheet for further analysis.

RESULTS AND DISCUSSION

The current investigations were carried out to detect the diversity among the QPM lines. Information regarding diversity and relationships among breeding material is necessary for hybrid maize breeding (Enoki *et al.*, 2002)

Out of 48 SSR markers used for this study, 34 markers were found to be polymorphic among the eighteen QPM lines. Variations in the DNA sequence have been used as molecular markers in plants and animals dur-ing the last two decades. Simple sequence repeats (SSRs) have been used to assess genetic relationships among maize inbred lines (Smith *et al.*, 1997; Yuan *et al.*, 2001and Enoki *et al.*, 2002).

Out of the 34 primers tested, the number of alleles generated was higher (5) for the primers Bmc2136 (Fig. 1), Bnlg1194 (Fig. 2) and Bnlg1401(Fig. 3). The number of alleles ranged from 2 to 5 across the primers.

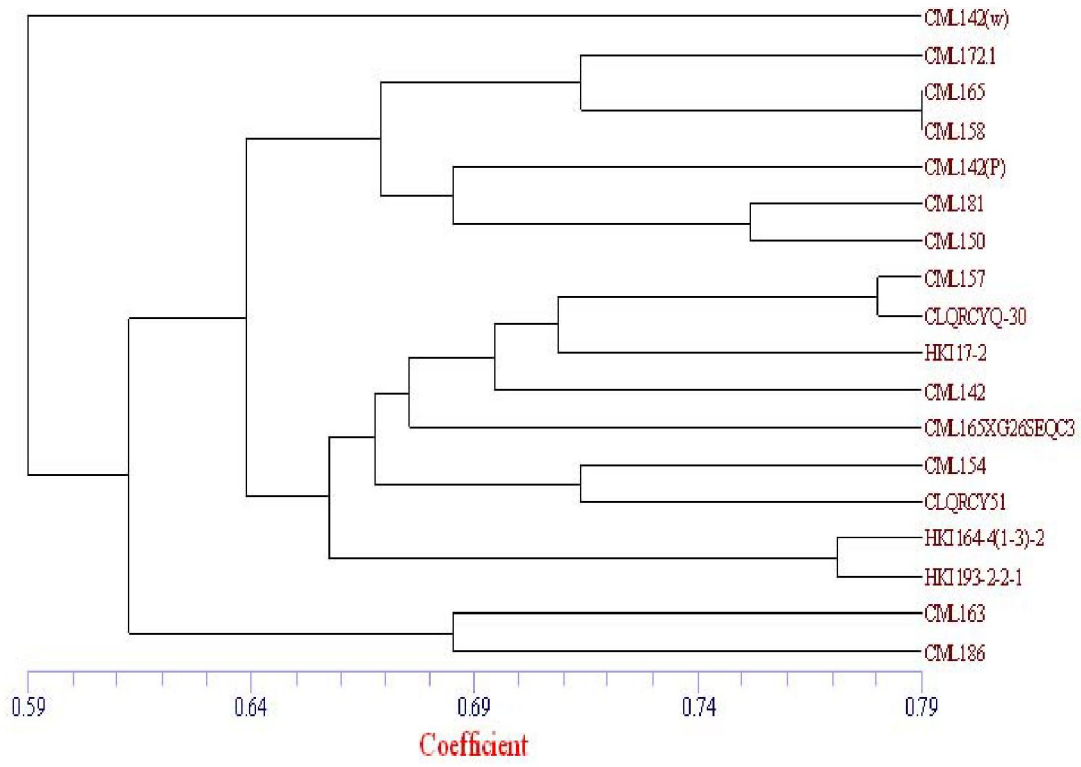
The Jacard's value estimated across all the polymorphic SSR loci was 0.83. The PIC values in the present study ranged from 0.50 (Bnlg1506) to 0.95 (Bnlg1401).The polymorphism information content (Botstein *et al.*,1980) is a parameter indicative of the degree of informative ness of a marker. The expected heterozygosis (*He*), sometimes known as PIC or polymorphic information content (Smith *et al.*, 1997) was estimated according to Nei (1978).

Twenty three SSR loci revealed PIC values more than the average. Among them seventeen primers Bnlg1401 is having highest PIC value 0.95. However the selected primers with high PIC value will be useful for further studies.

The similarity coefficients during the present investigation were used as input data for cluster analysis using NTSYSpc2.1a program and the resulting dendrogram is shown (Fig. 4).

According to the data obtained from the dendrogram all the eighteen QPM lines were divided into two major clusters I and II. Cluster I consists of CML 142(w) which is highly diversified from other

Fig. 4. Dendrogram for 18 QPM lines based on similarity matrix from 34 SSR Markers



QPM lines. CML 142(w) line can be used for the development of single cross hybrids. Cluster II is the biggest cluster consisting of 17 lines. Cluster II was segmented into two groups IIA and IIB. Cluster IIB consists of CML 163 and CML 186. These two QPM lines may be derived from common ancestor. Cluster IIA sub divided into IIA1 and IIA2. Cluster IIA1 consists of six lines *viz.,* CML172.1, CML165, CML158, CML142 (P), CML181 AND CML150. Cluster IIa2 again partitioned into IIA2x and IIA2y. Cluster IIA2y consists of HKI 164-4(1-3)-2 and HKI 193 -2 -2 -1. CML157, CLQRCYQ-30, HKI 17-2, CML142, CML165X G26SEQC3, CML154 and CLQRCY51 were grouped under Cluster IIA2x. .

CML 142(w) is highly diversified from CML 163 and CML 186.these lines can be used as parents for the development of maize hybrids. Crosses between genetically divergent lines generally produce better-performed hybrids than crosses between closely related parents (Tracy and Chandler, 2004 and Kamalesh *et al.,* 2009).

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