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Full Length Research Paper

Assessment of genetic diversity in quality protein maize (QPM) lines using simple sequence repeat (SSR) markers

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Maize is a primary source of energy supplement and can contribute up to 30% protein, 60% energy and 90% starch in an animal's diet. In the present investigation, 48 microsatellite markers, spread across the maize genome were used for analyzing genetic diversity among the sixty three quality protein maize (QPM) lines, including lines developed in India and CIMMYT, Mexico. Polymorphic profiles for 37 simple sequence repeat (SSR) loci aided in differentiating the QPM inbred lines. Using SSR procedures, the number of alleles per locus ranged from two to six, giving a total of 151 alleles for the 37 SSR loci. The genotypes were grouped into different clusters using NTSYSpc2.11 programme. The polymorphic information content (PIC) value was found to be highest for the primers *bnlg*1401*, bnlg*2136*, bnlg1633* and *umc* 1357 (0.96) while the lowest value was for the primer *umc 1656* (0.75) with the mean value of 0.48. From this study, the inbred line CML 142(w) is to give better combinations with CML 172, CM 161, CML 163, CLQRCYQ 281, CML 121, HQPM 5, HQPM 7, CML165 and CML 161 × CML 165 for the development of hybrids suitable for India.

Key words: QPM, SSR, cluster analysis.

INTRODUCTION

Maize is an important cereal food crop in the world after wheat and rice. It is a staple food for millions of people in poor countries around the world. Worldwide, maize is cultivated in an area of 165 million ha with a total production of 820 million metric tonnes giving an average yield of 3.5 metric tonnes /ha (Anonymous, 2012). During 2011 to 2012, the crop was grown on 8.11 million ha in India with total production of 16.8 million metric tonnes. Andhra Pradesh occupies first place in the production (21%) and productivity (3248 kg/ha) in the country. A typical mature maize kernel contains a small embryo and much larger endosperm, with 90% starch and 10% protein. Approximately 70% of this protein is composed of

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several types of prolamines known as zeins that are alcohol soluble. Four types of zeins namely, α, β, γ and δ are found in maize and they are distributed in a distinctive pattern, but it is deficient in two essential amino acids, lysine and tryptophan. Babies and adults consuming normal maize suffer from nutritious disorders such as marasmus and kwashiorkor (Ortega et al., 1986).

However, using the maize mutant *opaque-2* (o2) discovered in the early 1960s (Mertz et al., 1964) scientists developed high lysine and tryptophan maize with normal kernels of vitreous appearance (Ortega and Bates, 1983). The conventional breeding procedures were successful in releasing several quality protein maize (QPM) hybrids both in Africa and Latin America. India has imported the Mexican QPM lines and developed several QPM hybrids in recent past. However, it is very much essential to develop the QPM hybrids which can yield on par with normal hybrids. SSR markers could be used for the detection of polymorphism among QPM inbred lines (Krishna et al., 2012). The objective of present research was to identify the genetic relationships among the 63 QPM inbred line.

MATERIALS AND METHODS

Plant material

QPM germplasm lines for the present investigation were procured from Directorate of Maize Research (DMR), New Delhi, Maize Research Centre (MRC), Rajendranagar, Hyderabad and Vivekananda Parvatiya Krishi Anusandhan Sansthan (VPKAS), Alomora. Marker information about Simple Sequence Repeats (SSRs) was collected from maize database (www. maizegdb.org). Sixty three QPM lines developed in India and Mexico (Table 1) were sown at Maize Research Centre, Hyderabad during *rabi*-2009. The genotypes were diverse in view of geographical distribution.

DNA extraction and SSR marker analysis

About 2 g of young and healthy leaf tissue was collected from 30 days old plants and into a fine powder in liquid nitrogen using a pre-chilled mortar-pestle. Fifty to hundred milligram (50 to 100 mg) of ground material was transferred into a 2 ml microcentrifuge tube containing 800 µl extraction buffer [2% cetyltrimethylammonium bromide (CTAB)] and the contents were mixed gently by swirling action. After suspending the tissue in the buffer for 5 to 10 min, 500 µl of chloroform: iso-amyl alcohol (24:1) mixture was added, mixed well with the suspended tissue and spun at 10,000 rpm for 1 min. Without disturbing the interface, the supernatant was transferred to another tube and 1 ml absolute alcohol was added to the supernatant and mixed gently. DNA was suspended in 200 µl of 1X TE (Tris-HCl 10 mM, pH 8.0, EDTA 1 mM) buffer containing 10 mg/ml of RNase A and then stored at -20°C. Polymerase chain reaction (PCR) cycling consisted of initial denaturation at 94°C for 4 min, followed by 35 cycles of amplification at 94°C for 1 min, 55 to 65°C for 1 min, and 72°C for 2 min. A final extension step at 72°C for 7 min was followed by termination of the cycle at 4°C. The amplified products (10 µl) were resolved on a 3% agarose (Merc) gel and detected by Gel Document. A 100 bp DNA ladder (New England Biolabs Ltd) was used as a molecular weight marker. For the same primer, the products of different sizes were considered different alleles. The information obtained was coded in an excel spreadsheet for further analysis.

Data analysis

The polymorphism information content (PIC) value, described by Botstein et al. (1980), was used to refer to the relative value of each marker with respect to the amount of polymorphism exhibited. PIC value was estimated by:

$$PIC=1-\Sigma Pi^2 - \Sigma\Sigma Pi^2\, Pj^2$$

Where, 'i' is the total number of alleles detected for SSR marker and 'Pi' is the frequency of the i^{th} allele in the set of 63 genotypes investigated and j = i+1. This formula gives us an indicator of how many alleles a certain marker has, and how much these alleles divide evenly. Average linkage (unweighted pair group with arithmetic mean, UPGMA) cluster analysis was performed with the matrix of genetic similarity (GS) estimates. All computations were carried out with appropriate procedures of the software package NTSYSpc vers.2.11.

RESULTS

In the present study, 48 hyper variable microsatellite markers were used to assess the genetic diversity of 63 popular cultivars of QPM. Thirty seven (37) of the 48 SSR markers showed polymorphisms, while eleven markers were monomorphic. A total of 151 alleles were identified from the 37 polymorphic SSR loci among 63 QPM inbreds. The average number of alleles per locus was 4.08, with a range of 2 (*umc* 1656) to 6 (*umc*1357 and *bmc* 2136) (Table 2). The banding pattern of 63 QPM genotypes with SSR loci *bmc* 2136 is shown in Figure 1.

The PIC value ranged from a minimum of 0.75 (Umc1656) to maximum of 0.96 (*bnlg*1633, *bmc* 2136 and *bnlg*1401), with an average of 0.91. The average PIC values, in the present study (0.91). The UPGMA cluster analysis based dendrogram grouped the 63 genotypes into two major clusters with a genetic similarity range of 66 to 97% (Figure 2). Cluster 'A' represents four

Table 1. List of QPM germplasm used for genetic diversity.

S/N	QPM inbredLINE	S/N	QPM inbredLINE
1	CML142(W)	33	S5367
2	CML172.1	34	S5163
3	CML165	35	S5121-1
4	CML142(P)	36	S5555
5	CML163	37	HKI163
6	CML181	38	HKI193
7	CML186	39	HKI5072-2-BT
8	CML150	40	HKI193-2-2
9	CML157	41	HKI193-1
10	CML158	42	CML194
11	CML154	43	(CML161XCML451)28-2-16-1-2-1
12	CLQRCYQ-30	44	(CML150XCL02839)26-1-2
13	HKI17-2	45	CLQ6601XCL02843
14	HKI164-4(1-3)-2	46	HKI5072-2-BT
15	CLQRCYQ51	47	CML181-B-B
16	CML142	48	CML154
17	HKI193-2-2-1	49	HKI193-2-2-4
18	CML165XG26SEQC3	50	HKI164-7-4
19	S5340	51	HQPM5
20	S5180	52	HQPM7
21	S5347	53	CML165
22	S5170	54	CML161XCML165
23	S5330	55	CML169
24	S5175	56	HKI139
25	S5258	57	CML384X176
26	S5207	58	CML165
27	S5342	59	CML172
28	S76-13-3-3	60	CML161
29	S5204	61	CML163
30	S771	62	CML121
31	S5291	63	CLQ281
32	S5238		

genotypes namely, CML 161× CML 165, CML 165, HQPM 5 and HQPM 7. Cluster ‘B’ primarily composed of 59 QPM inbred lines.

It can be again divided into sub clusters B1 and B2. Sub cluster B1 comprised of five inbred lines namely, CML 121, CLQRCYQ 281, CML 163, CML 161 and CML 172. B2 sub cluster again sub divided into B2a and B2b. B2a comprised of 15 lines namely, CML 165 × G26SEOC3, CLQRCY51, CML154, CML142, HKI17-2, CLQRCYQ-30, CML 157, CML 186, CML 163, CML 150, CML 181, CML158, CML 165, CML142 (P) and CML 172. B2b again sub divided into B2b1 and B2b2. The case of sub cluster B2b1 consists of eight QPM inbred lines namely, CML 154, HKI164-7-4, HKI193-2-2-4, CML181-B-B, CML194, HKI193-1, HKI193-2-2 and HKI 5072-2-BT. B2b2 sub cluster again subdivided into B2b2x and

Table 2. SSR locus, Number of alleles, PIC and bin location and SSR repeat motif.

SSR locus	No. of alleles	PIC	Bin	SSR repeat motif
Bnlg1179	4	0.91	1.01	AG(16)
Bnlg1643	5	0.95	1.08	AG(24)
Umc1111	3	0.84	1.11	(CAAAA)4
Umc1147	4	0.91	1.07	(CA)7
Bnlg1297	5	0.94	2.02	AG(32)
Bnlg108	4	0.93	2.04	AG(12)
Mmc0401	5	0.95	2.05	(GGA)2(AG)27
Bnlg1633	5	0.96	2.07	AG(16)
Bmc2136	6	0.96	3.04	CA(31)
Bnlg1754	5	0.95	3.09	AG(20)
Umc2369	4	0.92	3.02-3.03	(GCAC)4
Phi 072	3	0.88	4.01	AAAC
Nc004	5	0.95	4.03	AG
Bnlg1937	3	0.87	4.05-06	AG(21)
Umc1574	3	0.85	4.09	(GCC)5
Phi096	4	0.91	5.03	AGGTG
Phi 075	5	0.92	6.0	CT
Bnlg1043	4	0.93	6.00	AG(20)
Umc1656	2	0.75	6.03	(CGGT)7
Umc1490	3	0.83	6.07	(AC)6
Bnlg1136	4	0.91	6.07-08	AG(14)
Bnlg1292	4	0.91	7.01	AG(14)
Bmc339	3	0.87	7.03	AG(12)
Umc1708	3	0.84	7.04	(GGA)4
Umc1407	4	0.93	7.05	(GGC)6
Phi116	3	0.87	7.06	ACTG/ACG
Bnlg1194	5	0.95	8.01-02	AG(33)
Bnlg2037	4	0.94	8.01	AG(16)
Umc1005	4	0.94	8.08	(GT)15
Umc1141	4	0.92	8.06	(GA)7
Bnlg1401	6	0.96	9.02	AG(22)
Bnlg1506	4	0.92	9.07-08	AG(19)
Bnlg1810	4	0.92	9.01	AG(14)
Umc1357	6	0.96	9.05	(CTG)8
Bnlg210	3	0.88	10.03	AG(14)
Umc1038	4	0.92	10.07	(CT)15
Umc1152	4	0.93	10.01	(ATAG)6
Mean	4.08	0.91		

B2b2y. B2b2x comprised of two inbred lines CML 142(w) and CML 384 × CML 176. CML 142(w) was used as a potential donor for conversion breeding programme. B2b2y consists of 29 inbred lines. Among these 29 inbred lines 28 lines were developed in India and one QPM line (CML161 × CML165) in Mexico.

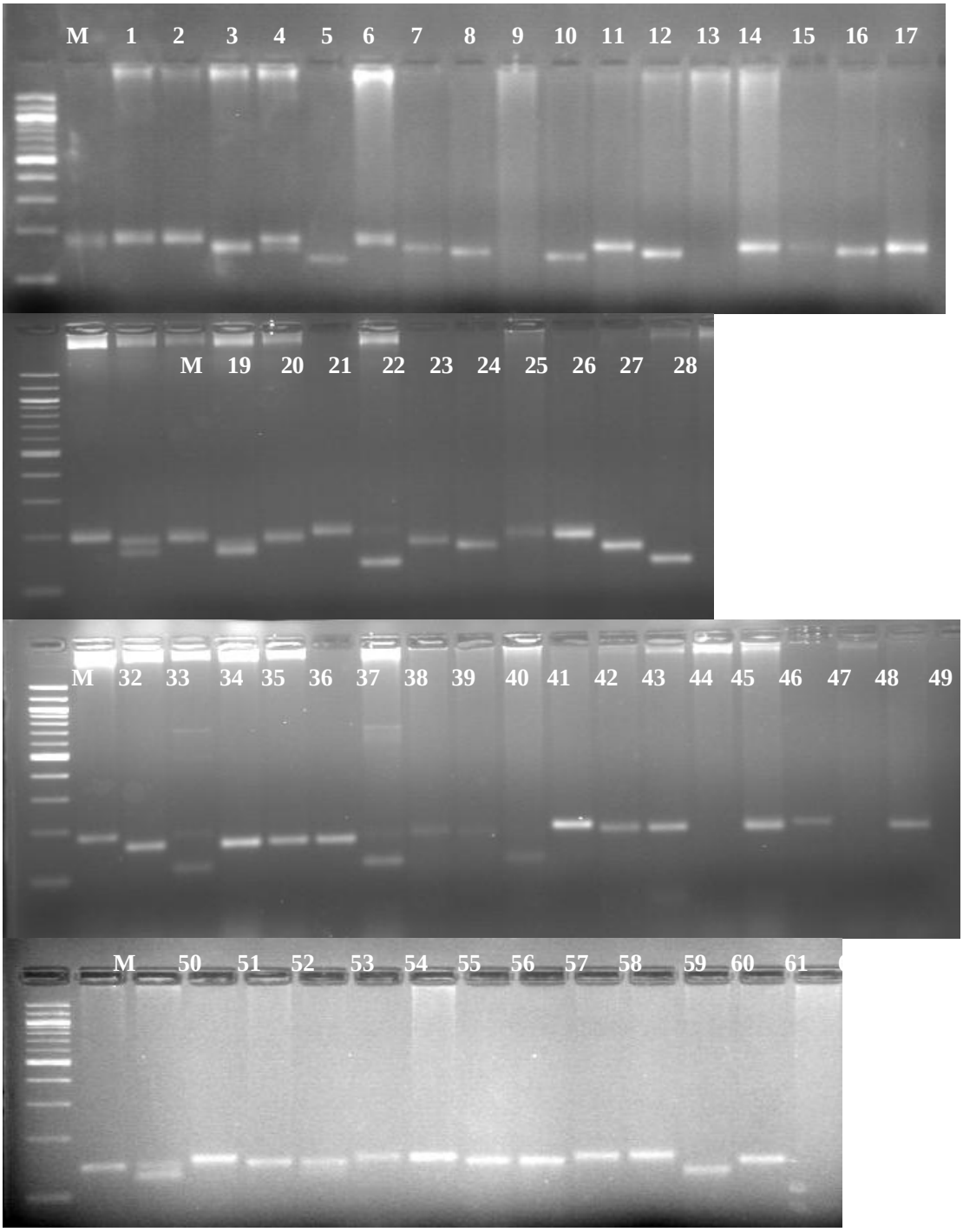

Figure 1. Gel profile showing the amplification of SSR primer *bmc 2136* with all 63 genotypes. M, 100 bp ladder, the number above the lane indicates the genotype number as in Table 1.

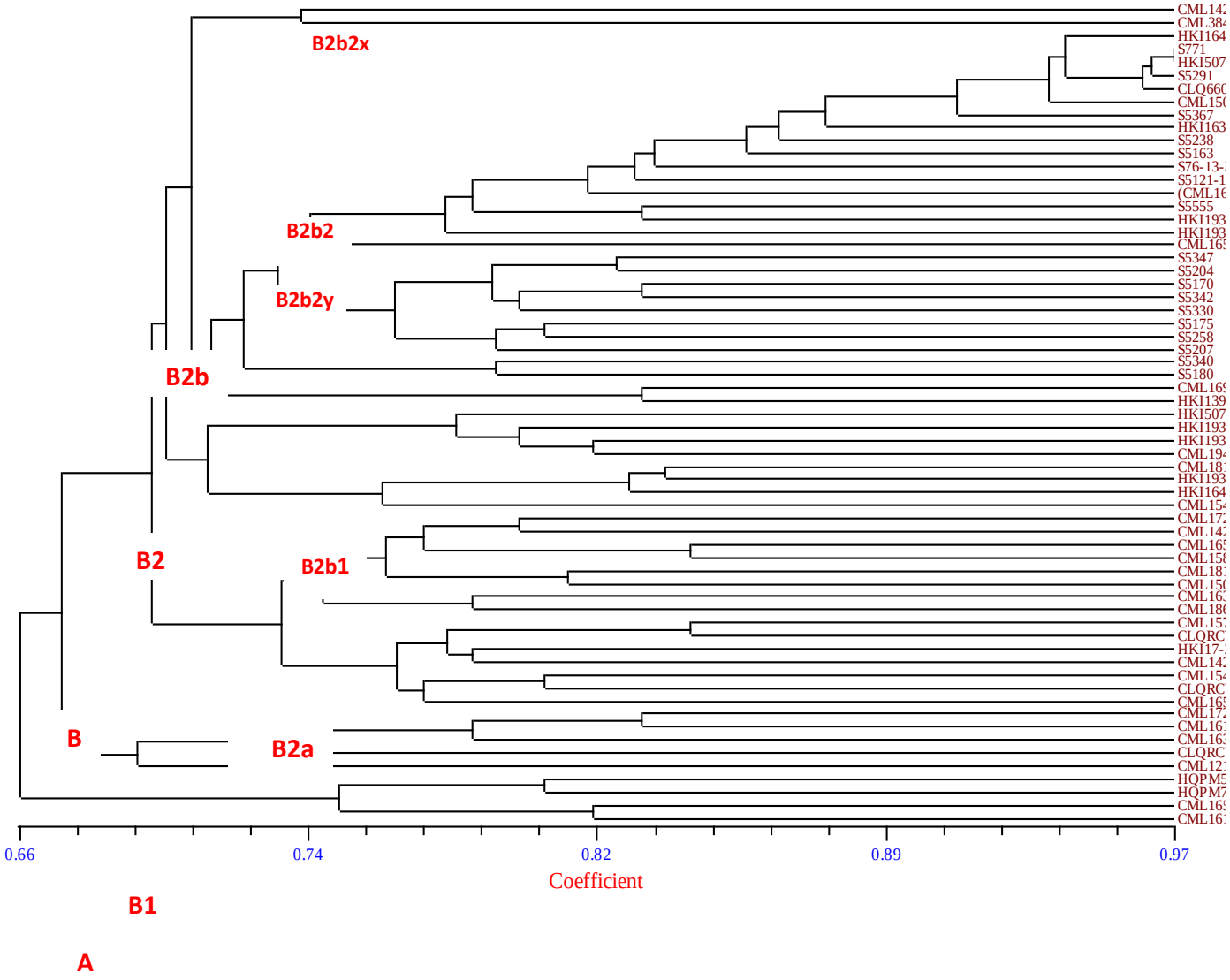

Figure 2. Dendrogram of 63 QPM accessions based on similarity matrix from 37 SSR primers.

DISCUSSION

The presence of genetic diversity in crop populations is not easily detected by morphological characteristics of growing plants. Phenotypic selection of lines on the basis of few morphological characters may result in approval of cultivars with lesser variability because of the influence of environment on growth and development. Molecular markers have been applied in quantification of genetic diversity, genotype identification, gene mapping, association mapping and marker assisted selection (MAS). Assessment of the extent of genetic variation in maize has been carried out based on simple sequence repeats (SSR) (Kalyanababu et al., 2009). The reason for low number of alleles (Na) is that the most microsatellite markers used in the study that is, 37 of 48 were tri and

tetra repeats compared to the other studies in which they have used more di nucleotide repeats (kalyanababu et al., 2012). Moreover, in the present study, we have considered quality protein maize (QPM) inbreds developed in India and Mexico to study the genetic relationship for developing new hybrids. Characterization of genetic diversity is of great value to assist breeders in parental line selection and breeding system design.

The average PIC values, in the present study (0.91) are more than the previous studies of Kamalesh et al. (2009) (0.60). Hoxha et al. (2004) reported SSR markers were shown to be a powerful tool for the detection of genetic diversity in maize population. Marker loci should be chosen uniformly over an entire genome in genetic diversity studies, avoiding biases due to sampling (Bernardo et al., 1997). When the genome was uniformly covered with marker loci, an estimate of standard error (SE) overestimated the actual variance of the GS estimates, providing a sufficiently accurate estimate of GS among maize inbred lines (Dubreuil et al., 1996). In this study, most chromosomes had at least four SSR loci except for chromosome 6 with three SSR loci. The mean SE of individual GS estimates was 0.05 (from 0.04 to 0.06), that is slightly lower than the mean SE (0.06) reported by Enoki et al. (2002).

Based on the above SSR analysis, genetic similarity coefficient measures between the inbred lines that reflect pedigree relatedness ensures a more stringent evaluation of the adequacy of a marker profile data. Inbred CML 142(w) is expected to give better combinations with CML 172, CM 161, CML 163, CLQRCYQ 281, CML 121, HQPM 5, HQPM 7, CML165 and CML 161× CML 165 for the development of hybrids suitable for India.

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