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Full Length Research Paper

Characterization of Indian and exotic quality protein maize (QPM) and normal maize (*Zea mays* L.) inbreds using simple sequence repeat (SSR) markers

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Polymorphism analysis and genetic diversity of normal maize and quality protein maize (QPM) inbreds among locally well adapted germplasm is a prerequisite for hybrid maize breeding program. The diversity analyses of 48 maize accessions including Indian and exotic germplasm using 75 simple sequence repeat (SSR) markers yielded 258 scorable alleles, out of which 251 alleles were polymorphic with an average of 3.35 alleles per locus. The polymorphism information content (PIC) values of all the polymorphic primers across the maize genotypes varied from 0.11 to 0.91, with an average value of 0.56. Di-nucleated repeats showed more number of average alleles (4.2) with more mean PIC value (0.61) than tri-, tetra-, penta- and hexa- nucleotide repeats. Gene diversity (*He*) was in the range of 0.11 (*umc*1766) to 0.81 (*mmc*0371 and *bnlg*1600) with an average value of 0.59, while heterozygosity (*Ho*) was observed with an average of 0.19, ranging from 0.02 (*umc*1495) to 0.98 (*umc*1906). Inbreeding coefficient (*F*) varied from 0.06 (*umc*2075) to 1.00 (15 SSR loci). Thus, the present study resulted to the identification of highly polymorphic SSR loci *mmc0371*, *umc*2364, *umc*1568, *bnlg1600*, *phi026*, *umc*2071 and *bnlg1904* by considering the parameters of PIC value (≥0.74), gene diversity (≥0.75), inbreeding coefficient (≥0.62) and polymorphic alleles (≥4). These 7 polymorphic primers can be effectively used in a molecular breeding programs and quantitative trait loci (QTL) mapping studies since they exhibited very high polymorphism over other loci. Genotype pairs VQL 2 and CML 173 was observed to serve as ideal parents for mapping modifiers since they differ significantly for tryptophan content and also showed lowest similarity of 27% between them. Among all the genotypes, V 370 and B 06-7 (22%), V 25 and CM 152 (22%) and V 341 and CM 145 (24%) genotype pairs can be used in maize hybrid breeding programs for producing high yielding hybrids.

Key words: Quality protein maize (QPM), gene diversity, heterozygosity, inbreeding coefficient, SSR markers, allele frequency.

INTRODUCTION

The knowledge of genetic diversity available among the germplasm is of great importance in crop improvement programs in order to exploit heterosis in a hybrid development programme and diversify the end products. In addition, local inbred lines are more useful for the development of breeding materials since they are well adapted to the local environments.

Globally, maize (*Zea mays* L.) is the third most important food crop after rice and wheat, both in terms of area and production; and India is the fifth largest producer of maize in the world contributing 3% of the total global production. Due to its economic necessity, most of the people in the developing countries are overly dependent on maize as staple food. It provides 50% of the dietary

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Abbreviations: QPM, Quality protein maize; **SSR,** simple sequence repeats; **PIC,** polymorphism information content.

protein for humans and can comprise 70% of the protein intake for people in developing countries (Deutscher, 1978). In Africa and some of the Asian countries, almost 90% of maize is grown for human consumption and may account for 80 to 90% of the energy intake. Although, maize is rich in nutrients and minerals, the major limitation is with its endosperm, which is deficient in two essential amino acids, lysine and tryptophan.

Isolation and characterization of *opaque*2 (*o*2) gene showed that this gene is responsible for a transcriptional factor which regulates the expression of *zein* genes and a gene encoding a ribosomal inactivating protein (Schmidt et al., 1990; Lohmer et al., 1991; Bass et al., 1992). The o2 mutation reduces zein content by one-half and enhances the synthesis of a number of non-*zein* proteins including lysine and tryptophan (Damerval and Devienne, 1993; Habben et al., 1993). However, the *o*2 maize was unpopular with maize breeders because of low yields, soft kernel texture and susceptibility to ear rot and stored grain pest (Crow and Kermicle, 2002). Many research efforts at different research institutes, particularly CIMMYT, Mexico led to the development of quality protein maize (QPM) through the introgression of *o*2 modifiers. India is one of the first countries to release QPM composites and hybrids. However, all those composites and hybrids developed were introduced from CIMMYT. Over the years, Vivekananda Institute of Hill Agriculture, Almora, India has developed short duration quality protein maize inbreds (VPKAS News letter, 2009). Those inbreds along with other available inbreds needs to be studied for their effective use in breeding program.

The development of modern plant breeding techniques has greatly facilitated the wider use of a wealth of diversity from many sources including landraces. 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 restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD) (Moeller and Schaal, 1999) and simple sequence repeats (SSR) (Yao et al., 2008; Kalyana Babu et al., 2009; Kamalesh et al., 2009). SSR markers are preferable due to their potential advantages of reliability, reproducibility, discriminating power, standardization and cost effectiveness over other marker technologies (Smith et al., 1997). The SSR markers were also found useful for mapping of *opaque*2 modifiers and introgression of trait of interests using MAS (Dragana et al. 2009). North West (NW) Himalayan region of India harbors vast amount of maize germplasm including normal and QPM maize which plays significant role in the development of high yielding varieties. Knowledge of the amount of diversity existing in the normal and QPM germplasm from NW Himalayan region, other parts of India and exotic inbred lines from CIMMYT, Mexico will give better understanding of the genetic structure and variation and their effective utilization in the maize breeding programs. The present study thus aimed to analyze genetic diversity of QPM and normal inbred lines from NW Himalayan region and other parts of India and CIMMYT; and then identify highly polymorphic SSR loci that will be best suited for diversity and mapping studies.

MATERIALS AND METHODS

Plant materials

Seeds of 48 maize genotypes that comprised 14 normal maize inbred lines and 13 QPM inbred lines, developed by Vivekanand Institute of Hill Agriculture, Almora (Uttarakhand), India; 6 normal inbreds from Himachal Pradesh; 11 coordinated maize (CM) inbreds lines (normal) developed by various public sector institutions in India and 4 exotic QPM inbreds from CIMMYT, Mexico were used for the genetic diversity analyses. The details of the maize genotypes along with their maturity, place of release, quality parameter and parentage are given in Table 1. Genomic DNA was isolated from fresh young leaves of the maize using CTAB protocol of Murray and Thomson (1980) with minor modifications like use of chloroform and isoamylcohol (24:1) instead of chloroform/ octanol and also we repeated this step twice. 75 SSR markers were used to detect polymorphism among the 48 maize genotypes, spread evenly over all the chromosomes. The polymerase chain reactions (PCR) and gel documentation were carried out using standard procedures, and the amplified products were resolved on 3.5% agarose gel [Super Fine Resolution (SFR) Agarose; Amresco, USA], while scoring was carried out manually.

Data analyses

The SSR scores were used to create a data matrix to analyze genetic relationships using the NTSYS-pc program version 2.11a (Rohlf, 1992). The dendrogram was constructed based on Jaccard's similarity coefficient (Jaccard, 1908) using the marker data for all the maize genotypes following unweighted pair group method analysis (UPGMA) (Gawel and Jarret, 1992). The polymorphism information content (PIC) was determined as described by Smith et al. (1997), by using the formula PIC = 1 – Σfi2, where *fi* is the frequency of the *i*th allele.

For genotypes showing heterozygosity at a specific SSR locus, the PIC values were calculated after considering each allele as contributing one-half instead of one, as suggested by Narvel et al. (2000). The heterozygosity, gene diversity, allele frequency and inbreeding coefficients were calculated using Power Marker V3.0 software (Liu and Muse, 2005). The number of unique allele was also calculated from power marker software and it indicates uniqueness regarding geographical origin.

RESULTS

Genetic variation of SSR loci among the maize genotypes

The genomic DNA of the 48 maize accessions were amplified using 75 SSR markers and yielded 258 scorable alleles, out of which 251 alleles were found to be polymorphic. All the 75 SSRs were spread across all the chromosomes of maize evenly (Table 2). Out of the 75

Table 1. Details of 48 maize genotypes used in diversity analysis.

S/N	Genotype	Parentage	Type (Normal/ QPM)	Source	Duration
1	V 25	NA	Normal	VPKAS	SD
2	V 335	TZ1-25	Normal	VPKAS	SD
3	V 338	B10 45010	Normal	VPKAS	SD
4	V 341	Mexico ACC. No. 3136	Normal	VPKAS	SD
5	V 353	U15-1 3	Normal	VPKAS	SD
6	V 358	NA	Normal	VPKAS	SD
7	V 359	Syn I	Normal	VPKAS	SD
8	V 364	NA	Normal	VPKAS	SD
9	V 366	NA	Normal	VPKAS	SD
10	V 369	NA	Normal	VPKAS	SD
11	V370	NA	Normal	VPKAS	SD
12	V371	NA	Normal	VPKAS	SD
13	V376	NA	Normal	VPKAS	SD
14	V380	NA	Normal	VPKAS	SD
15	VQL1	CM 212	QPM	VPKAS	SD
16	VQL2	CM 145	QPM	VPKAS	SD
17	VQL3	CM 145	QPM	VPKAS	SD
18	VQL4	CM 212	QPM	VPKAS	SD
19	VQL8	CM 145	QPM	VPKAS	SD
20	VQL12	V 25	QPM	VPKAS	SD
21	VQL15	V 338	QPM	VPKAS	SD
22	VQL20	V 348	QPM	VPKAS	SD
23	VQL21	V 355	QPM	VPKAS	SD
24	VQL22	V 356	QPM	VPKAS	SD
25	VQL25	CM 141	QPM	VPKAS	SD
26	VQL26	V 351	QPM	VPKAS	SD
27	VQL27	V 353	QPM	VPKAS	SD
28	B06-1	NA	Normal	HP	MD
29	B06-6	NA	Normal	HP	MD
30	B06-7	NA	Normal	HP	MD
31	B06-8	NA	Normal	HP	MD
32	B06-19	NA	Normal	HP	MD
33	B06-20	NA	Normal	HP	MD
34	CM126	GCL 33 x Almora local	Normal	VPKAS	MD
35	CM127	GCL 32 x Almora local	Normal	VPKAS	MD
36	CM128	Anantnag Local x (WF9 x M14)	Normal	VPKAS	MD
37	CM129	US 23 x KT 41	Normal	VPKAS	MD
38	CM138	IPA 21-10-f-#-?-15	Normal	IARI	MD
	CM139	(Tarun x MS1)- Y63-1-9-2-1-1-2	Normal	DMR	MD
	CM141	Pool 33 (Alm)	Normal	VPKAS	MD
	CM145	Pop 31C4 HS bulk (Alm)	Normal	VPKAS	MD
	CM152	Pop 31	Normal	VPKAS	MD
	CM153	NA	Normal	VPKAS	MD
	CM212	USA/ ACC No. 2132 (Alm)	Normal	VPKAS	MD
	CML173	CIMMYT Line	QPM	CIMMYT, Mexico	MD to LD
	CML176	CIMMYT Line	QPM	CIMMYT, Mexico	MD to LD
	CML184	CIMMYT Line	QPM	CIMMYT, Mexico	MD to LD
	CML189	CIMMYT Line	QPM	CIMMYT, Mexico	MD to LD

LD, Long duration; MD, medium duration; SD, short duration; VPKAS, Vivekananda Parvatiya Krishi Anusandhan Sansthan; HP, Himachal Pradesh; DMR, Directorate of Maize Research, New Delhi; IARI, Indian Agricultural Research Institute, New Delhi; CIMMYT, International Maize and Wheat Centre, Mexico.

Table 2. Details of polymorphism and genetic analysis of 75 SSR loci across the 48 maize accessions.

Primer	Polymorphic loci	Polymorphism (%)	PIC	Bin location	Repeat motif	Gene diversity (*He*)	Heterozygosity (*Ho*)	Major allele (bp)	Major allele Frequency	Inbreeding coefficient (*F*)
umc1568	5	100	0.91	1.00	(TAG)4	0.69	0.26	140	0.46	0.62
bnlg182	1	50	0.11	1.02	NA	0.06	0.06	80	0.96	--
umc1917	2	100	0.32	1.03	(CTG)6	0.30	0.10	140	0.81	0.64
umc1906	2	66.6	0.50	1.03	(AGA)6	0.59	0.97	95	0.51	--
umc1292	2	100	0.49	1.04	(TGG)6	0.49	0.04	155	0.54	0.91
umc1397	2	100	0.47	1.05	(ATGCA)4	0.47	0.00	145	0.60	1.00
umc1703	2	100	0.46	1.05	(CTTT)5	0.46	0.00	148	0.63	1.00
umc1661	3	100	0.66	1.07	(GCTCCG)4	0.65	0.48	125	0.42	0.26
umc2195	4	100	0.69	2.00	(CCGC)4	0.66	0.53	152	0.46	0.21
umc2030	3	100	0.65	2.03	(CGA)4	0.65	0.06	135	0.43	0.89
umc1459	3	100	0.62	2.04	(GCAA)7	0.61	0.04	150	0.47	0.93
bnlg2328	4	100	0.58	2.05	AG(33)	0.55	0.30	152	0.61	0.45
phi127	2	100	0.48	2.05	AGAC	0.46	0.15	110	0.64	0.67
mmc0271	4	100	0.58	2.07	(GA)39	0.62	0.00	175	0.55	1.00
umc2184	4	100	0.67	2.07	(GCG)5	0.71	0.30	148	0.40	0.57
phi101049	4	100	0.70	2.09	AGAT	0.72	0.10	248	0.36	0.85
phi96100	4	100	0.63	2.10	ACCT	0.58	0.69	310	0.45	--
umc2071	6	100	0.77	3.01	(ATGT)5	0.78	0.12	165	0.28	0.84
umc1495	4	100	0.61	3.02	(AGGAC)4	0.67	0.02	125	0.45	0.96
umc1528	2	100	0.45	3.04	(TGCG)6	0.44	0.02	98	0.66	0.95
bnlg1904	5	100	0.74	3.04	AG(21)	0.75	0.04	170	0.36	0.94
bnlg1035	5	100	0.64	3.05	AG(13)	0.73	0.00	100	0.35	1.00
bnlg1320	3	100	0.55	3.07	NA	0.57	0.00	110	0.58	1.00
bnlg1350	2	100	0.44	3.08	AG(13)	0.46	0.02	140	0.67	0.95
umc1052	4	80	0.74	3.08	(AAC)5	0.51	0.13	110	0.60	0.80
umc1057	3	100	0.54	3.09	(CGG)6	0.58	0.02	100	0.50	0.96
umc1117	6	100	0.34	4.01	(TCGCA)4	0.79	0.45	140	0.28	0.43
mmc0371	6	100	0.78	4.03	NA	0.80	0.14	260	0.27	0.82
phi026	6	100	0.78	4.04	CT	0.78	0.18	125	0.32	0.77
umc1299	2	100	0.11	4.05	(AAG)5	0.19	0.00	140	0.89	1.00
umc1086	4	100	0.73	4.05	(CT)12	0.61	0.00	100	0.50	1.00
umc1574	3	100	0.53	4.06	(GCC)5	0.57	0.06	175	0.52	0.89
umc1055	4	100	0.41	4.08	(AG)9	0.50	0.09	110	0.68	0.80
umc1276	3	100	0.65	4.09	(GGC)4	0.68	0.48	105	0.41	0.29
umc2206	4	100	0.66	5.00	(GTAC)4	0.70	0.02	95	0.38	0.96

Table 2. Continue

umc1308	2	100	0.143	5.01	(TG)10	0.19	0.04	100	0.89	0.78
umc1766	0	0	0	5.01	(CGCCGG)4	0.11	0.00	150	0.93	1.00
umc2115	5	100	0.53	5.02	(TGCCA)5	0.71	0.06	180	0.43	0.90
umc1155	4	100	0.69	5.05	(AG)20	0.71	0.13	125	0.38	0.82
umc1941	4	100	0.65	5.06	(CTG)10	0.69	0.06	105	0.44	0.90
umc1646	3	100	0.6	5.07	(CTGGA)4	0.66	0.02	80	0.40	0.96
bnlg1766	0	0	0	5.07	NA	0.11	0.00	150	0.93	1.00
phi128	3	100	0.54	5.08	AAGCG	0.60	0.00	110	0.53	1.00
umc2136	3	100	0.64	6.00	(CCT)8	0.45	0.13	150	0.50	0.75
bnlg1600	5	100	0.78	6.00	AG(21)	0.80	0.25	210	0.26	0.68
umc1002	4	100	0.58	6.0-6.01	(TA)10	0.61	0.10	150	0.56	0.83
umc2059	4	100	0.63	6.05	(CAG)8	0.66	0.12	120	0.51	0.81
umc1187	3	100	0.59	6.05	(CCT)4	0.62	0.31	120	0.44	0.50
umc1805	5	100	0.78	6.07	(CT)28	0.78	0.34	110	0.30	0.56
umc1066	3	100	0.66	7.01	(GCCAGA)5	0.53	0.00	135	0.63	1.00
umc1248	4	100	0.68	7.01	(TC)12	0.70	0.02	120	0.37	0.96
bnlg2160	5	100	0.74	7.01	AG(27)	0.69	0.28	140	0.43	0.59
umc2364	4	100	0.79	7.03	(GGA)7	0.74	0.07	250	0.39	0.89
phi114	2	100	0.49	7.05	GCCT	0.56	0.07	155	0.50	0.86
phi045	3	100	0.64	7.05	AAC	0.68	0.16	155	0.42	0.76
pmc1407	5	100	0.78	8.03	(GGC)6	0.77	0.22	100	0.30	0.71
umc1904	4	100	0.63	8.03	(TAAGC)5	0.66	0.88	95	0.50	--
umc1858	5	100	0.74	8.03-8.04	(TA)8	0.75	0.71	160	0.39	0.06
umc2075	4	100	0.50	8.04	(AGCCAG)4	0.47	0.45	85	0.70	0.06
phi015	2	100	0.48	8.04	AAAC	0.53	0.17	95	0.59	0.68
umc1765	3	100	0.63	8.07	(GCT)5	0.65	0.00	110	0.46	1.00
phi119	2	100	0.41	8.08	AG	0.44	0.00	170	0.70	1.00
umc1279	3	100	0.51	9.00	(CCT)6	0.54	0.00	110	0.53	1.00
umc2093	3	100	0.61	9.01	(ACAT)4	0.63	0.00	130	0.43	1.00
umc1732	2	100	0.28	9.02	(CGT)7	0.36	0.04	160	0.77	0.88
phi027	2	100	0.28	9.05	GCGCT	0.32	0.17	140	0.80	0.48
dupssr29	3	75	0.65	9.05	(GA)24	0.64	0.78	95	0.54	--
umc1804	5	100	0.77	9.07	(AG)44	0.77	0.33	110	0.36	0.57
umc1077	4	100	0.78	10.04	(CA)15	0.74	0.20	200	0.36	0.72
umc2163	5	100	0.73	10.04	(AG)28	0.77	0.27	125	0.35	0.64
umc1054	1	50	0.76	10.04	(CAG)6	0.51	0.91	90	0.52	--
umc1506	3	100	0.66	10.05	(AACA)4	0.69	0.48	135	0.36	0.30

Table 2. Continue

umc1645	2	100	0.35	10.06	(CT)10	0.43	0.00	120	0.71	1.00
bnlg1839	2	100	0.48	10.07	AG(24)	0.54	0.00	190	0.55	1.00
umc1061	2	100	0.43	10.07	(TCG)6	0.45	0.04	120	0.68	0.90
Mean	3.35	-	0.56	-	-	0.58	0.18	-	0.52	0.68

NA – not available in the website maizeGDB (www.maizegdb.org).

primers, 73 (97%) were found to be polymorphic and the remaining 2 (3%) were monomorphic (*bnlg*1766 and *umc1766)*. A total of 256 alleles were detected for the 73 polymorphic SSR markers with an average of 3.35 alleles per locus, while it was 3.44 for all the 75 SSR loci including monomorphic markers used in the study. The number of alleles generated with the polymorphic primers ranged from 2 to 6 among the maize genotypes. Four primers *viz., umc2071, umc1117, mmc0371* and *phi026* were found to have maximum number of alleles (6) each; while 11 primers were having five alleles each (Table 2).

The banding pattern of 48 maize genotypes with SSR loci *umc1858* is shown in Figure 1. The PIC values of all the polymorphic primers across 48 maize genotypes varied from 0.11 to 0.91 with an average value of 0.56. Out of the 75 primers, two primers (*umc1568* and *umc2364)* showed highest PIC values of 0.91 and 0.79 respectively, while 6 SSR loci (*bnlg1600, phi026, mmc0371, umc1805, umc1407* and *umc1077)* showed a PIC value of 0.78. The lowest PIC values was observed in primers *bnlg182* (0.11), *umc1299* (0.11) and *umc1732* (0.28) (Table 2). The number of SSR loci based on PIC values, more than the average, was 45 markers. Among them, SSR loci *umc1568, umc2364, phi026 and mmc0371* are noteworthy due to their relatively higher level of polymorphism. A total of 41 SSR markers came under the PIC range of 0.6 to 0.91 with an average value of 0.7, while 34 markers came under the PIC range of 0.00 to 0.59 with an average value of 0.4. The SSRs which had PIC value more than 0.60 also generated more number of loci with an average of 4.02, while it was 2.73 for the SSRs below the PIC value of 0.6 (Figure 2a). Di-nucleated repeats showed more number of average alleles (4.2) with more mean PIC value (0.61) than tri-, tetra-, penta- and hexa- nucleotide repeats ($p<0.05$, correlation is significant). The average PIC values were in the decreasing order from di-nucleotide repeats to hexa- repeats and also same trend was found in case of allele number with the exception of penta- repeat motif. The graphical representation of the pattern of allelic and PIC variation with respect to SSR loci repeats is shown in the Figure 2b.

Statistical analysis of genetic diversity

Gene diversity also known as 'expected heterozygosity' (*He*) was in the range of 0.11 (*umc*1766) to 0.81 (*mmc*0371 and *bnlg*1600) with an average value of 0.59. The heterozygosity, known as 'observed heterozygosity' (*Ho*) was observed with an average of 0.19 and range of 0.02 (*umc*1495) to 0.98 (*umc*1906). Major alleles with highest frequency were observed in *bnlg*182 (80 bp allele) at 97% followed by the loci *umc*1706 (150 bp) at 94%. A total of 11 unique alleles were found with the SSR loci *viz*., *umc*1568, *umc*2206, *umc*1187, *umc*1858, phi027, *umc*1706, *umc*2115, *umc*1057, *umc*1117, *umc*2184 and *phi*9610. Inbreeding coefficient (F) varied from 0.06 (*umc*2075) to 1.00 (15 SSR loci) (Table 2). It ranges theoretically from −1 to 1. It is one only if landraces are genetically heterozygous. Based on the above SSR analysis by considering the parameters of PIC value (≥0.74), gene diversity (≥0.75), inbreeding coefficient (≥0.62) and polymorphic alleles (≥4), 7 most highly polymorphic SSR loci *mmc*0371, *umc*2364, *umc*1568, *bnlg*1600, *phi*026, *umc*2071 and *bnlg*1904 were observed (Table 3).

Associations among maize genotypes

Jaccard's similarity coefficient between all the 48 maize genotypes ranged from 22 to 87.6%. The maximum similarity (92%) was found between the genotype pairs VQL 1 and VQL 4 (87.6%), followed by VQL 2 and VQL 3 (81%) and V 380 and VQL 1 (77%). The above results on similarity were also substantiated by the dendrogram (Figure 3). However, the lowest similarity was found between the genotype pairs V 370 and B 06-7 (22%), V 25 and CM 152 (22%), and V 341 and CM 145 (24%). The similarity coefficients were used as input data for the cluster analysis using NTSYSpc 2.11 program and the resulting dendrogram is shown in Figure 3.

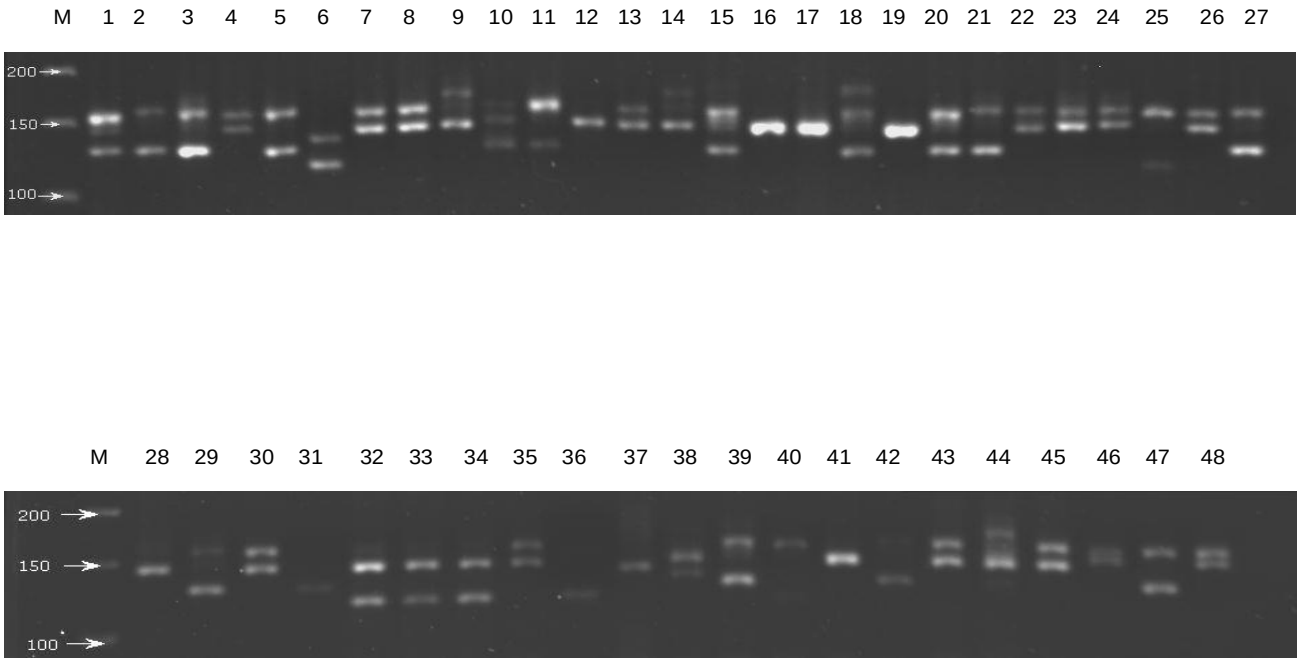

Figure 1. The SSR banding profile of 48 maize accessions used in the study. Lane 1: M- 50 bp marker; Lanes 2 to 48, maize genotypes used in the study (for labels, please refer to Table1).

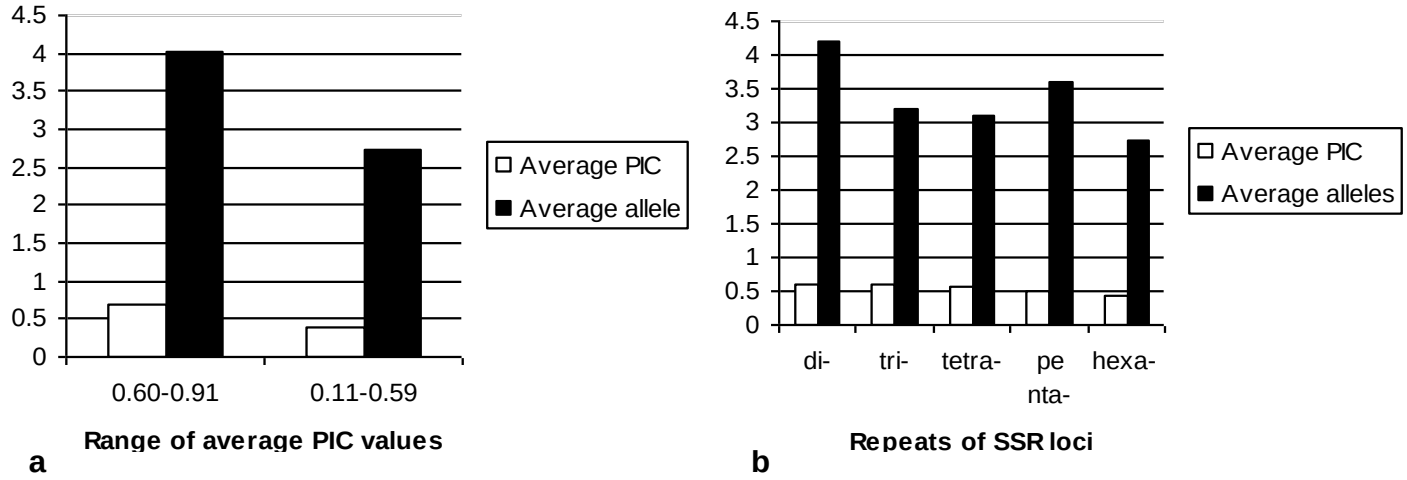

Figure 2. (a) Pattern of allelic variation and average PIC values with respect to range of PIC values; (b) Graphical representation of pattern of allelic and PIC variation with respect to SSR loci repeats.

Table 3. Parameters for genetic analysis of most highly polymorphic primers.

Marker	Gene diversity	PIC	Inbreeding coefficient	Polymorphic alleles
*mmc*0371	0.805	0.78	0.82	6
*phi*026	0.79	0.78	0.77	6
*bnlg*1600	0.80	0.78	0.69	5
*umc*2071	0.79	0.77	0.84	6
*umc*2364	0.75	0.79	0.89	4
*umc*1568	0.69	0.91	0.62	5
*bnlg*1904	0.75	0.74	0.94	5

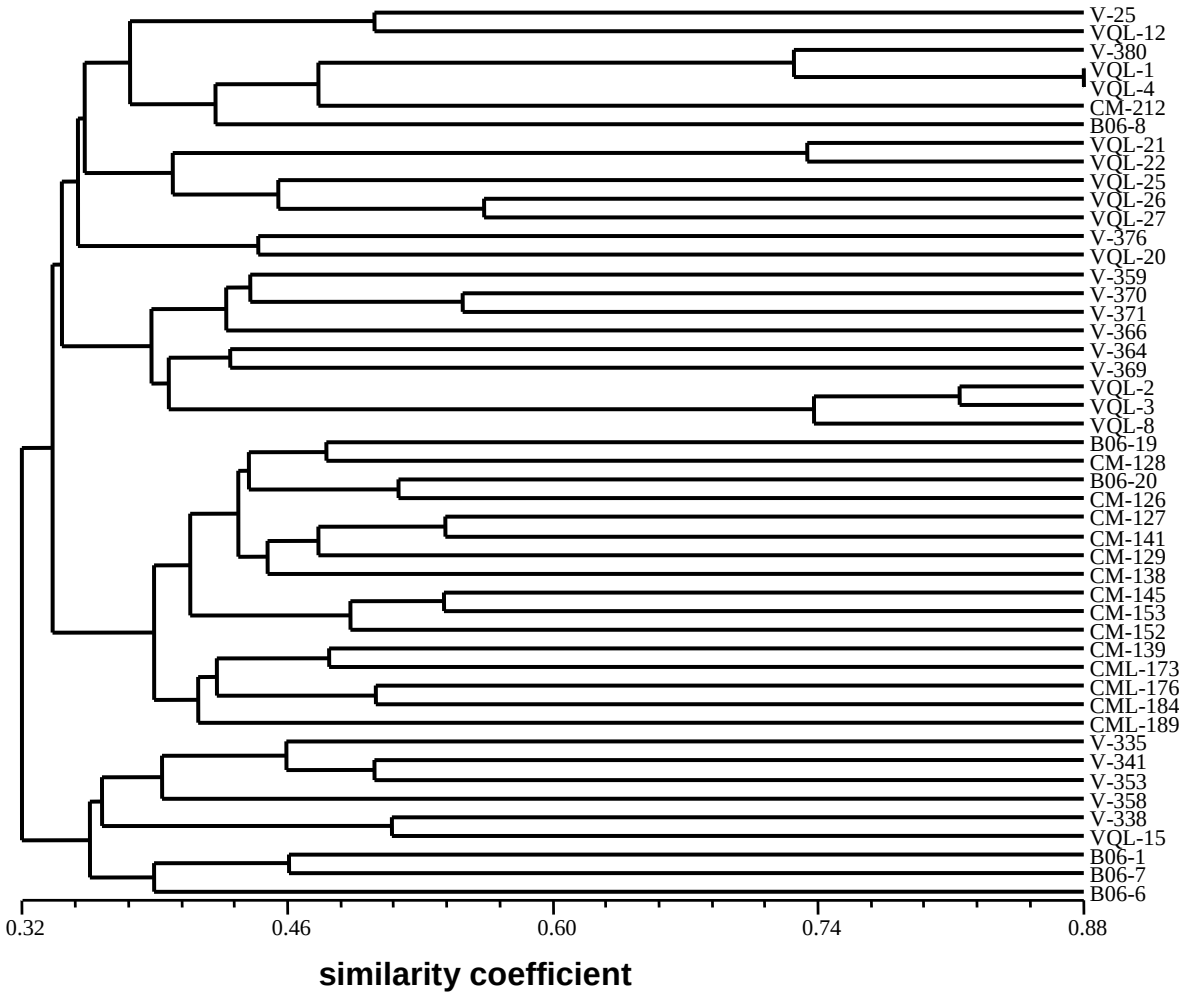

Figure 3. Dendrogram of 48 maize accessions based on similarity matrix from 75 SSR primers.

The dendrogram generated through UPGMA analysis grouped all the 48 maize genotypes into three major groups: A, B and C. The major group A comprised 23 genotypes, while the cluster B and C comprised 19 and 9 genotypes respectively. Cluster 'A' included both QPM and non-QPM inbreds while all the CM inbreds were grouped under the cluster B. It is interesting to note that the CIMMYT lines were grouped along with CM lines. The cluster C consisted of all the Vivek genotypes from VPKAS and B 06-1, B 06-7 and B 06-6 which were from Himachal Pradesh (HP). The analysis of grouping based on the quality parameters (normal and QPM) is correlated to some extent; however, they spread randomly across the three clusters. The cluster A comprised all the QPM lines of India (except VQL 15) and 9 normal inbreds (V25, V 380, V 376, V 359, V 369, V 364, V 366, V 371 and V 370) developed by VPKAS. However, VQL 15 was grouped along with other VQL inbreds in the 2D analysis of principal component (PCA). In cluster A, genotype VQL 1 showed greatest similarity with genotype VQL 4 with similarity value of 87%, while genotype CML 173 and V 25 (22%) showed lowest similarity. A total of 14 normal genotypes developed by VPKAS were clustered in group A and C, of which group C consisted of 5 genotypes. All the coordinated maize lines (normal) were grouped along with CIMMYT lines (QPM) except CM 212 in group B. CM 212 is originally V 212, which was renamed as CM 212 by the national system of India (AICRP on maize). The normal lines of Himachal Pradesh were scattered across all the three groups. The genotype B06-8 clustered along with CM 212 with a similarity of 47% in the group A, while the genotypes B 06-19 and B06-20 under the group B. The inbred line B 06-19 showed close relationship with CM 128 (48%), where as B 06-20 showed closeness with CM 126 (52%).

DISCUSSION

Cultivation of few genotypes in any crop including maize leads to narrow genetic base. The crop is prone to many biotic and abiotic stresses. Improvement of maize

breeding requires identification of highly diverse germplasm and highly polymorphic molecular markers, and their effective utilization in the identification of quantitative trait loci (QTLs) for agronomically important traits. In the present study, 75 primers yielded 258 scorable alleles, out of which 251 were found to be polymorphic with an average of 3.44 alleles per locus. Similar results have been observed with Legesse et al. (2007), where they used only 27 SSR markers among 56 highland and mid-altitude maize inbred lines obtained from CIMMYT programs in Ethiopia and Zimbabwe. Bantte and Prasanna (2003) also reported similar results where they found an average of 3.25 alleles using 36 SSR loci. Whereas Yao et al. (2008) reported 6.4 alleles per locus using 45 SSR loci among 124 maize landraces of Wuling mountain region in China. This higher number of average alleles was due to wide range of land races used in the present study, where landraces are expected to exhibit high diversity. Out of the total 75 primers used, all the primers amplified among all the genotypes. However, 73 primers (97%) were found to be polymorphic and the remaining 2 (3%) were monomorphic. This high level of polymorphism existing among the selected genotypes has great potential for use in breeding programs. In the present study, we found close proportionate relationship between the number of alleles and the PIC values of SSR loci. PIC demonstrates the in-formativeness of the SSR loci and their potential to detect differences among the inbred lines based on their genetic relationships. The primers having more PIC values showed more number of alleles and the relationship was shown in Figure 2. The SSR loci having more number of alleles and highest PIC values, *umc*1568, mmc0371 and *phi*026 are very useful for further genetic and mapping studies. Di-nucleotide SSR loci identified the largest mean number of alleles (4.2) and mean PIC (0.61) as compared to tri, tetra and penta nucleotide repeats during study, which is also in close agreement with previous observations in maize (Senior et al., 1998; Enoki et al., 2002; Legesse et al., 2007). A conclusion may be derived that the loci with more number of alleles can be very useful in the assessment of genetic diversity. Similar results were also shown by Legesse et al. (2007) where they found that di-nucleotide repeats had a mean number of allele of 4.8 and 0.67 average PIC that was higher than tri-, tetra- and penta- nucleotide repeats in 56 highland and mid-altitude maize inbred lines; obtained from CIMMYT programs in Ethiopia and Zimbabwe; that were genotyped using 27 SSR loci. The PIC values of all the polymorphic primers across 48 maize genotypes in the present study were in the range of 0.11 to 0.91 with an average value of 0.56. The number of SSR loci with higher than average PIC values was found to be 45. The average PIC value determined in our investigation agreed with earlier findings reported based on SSR marker in maize inbred lines (Senior et al., 1998; Vaz Patto et al., 2004). PIC demonstrates the informativeness of the SSR loci and their potential to detect differences among the inbred lines based on their genetic relationships. Legesse et al. (2007) also reported an average PIC value of 0.58, but their highest PIC value was 0.71 and also number of SSR loci having highest PIC values was very low. The high level of polymorphism is due to diverse germplasm and more number of SSR loci used in the present study. Among the SSRs, *umc1568, umc2364, phi026* and *mmc0371* are noteworthy due to their relatively higher polymorphism, high PIC values and more number of polymorphic alleles per locus and they can effectively be used in genetic diversity studies. A total of 11 unique alleles were found with the SSR loci namely: *umc*1568, *umc*2206, *umc*1187, *umc*1858, *phi*027, *umc*1706, *umc*2115, *umc*1057, *umc*1117, *umc*2184 and *phi*9610. The unique alleles identified will be useful for tagging of cultivars with special characters.

The observed and expected heterozygosity within the genotypes showed obvious deviations from Hardy–Weinberg expectations. Gene diversity (*He*) was in the range of 0.11 to 0.81 with an average value of 0.59 which is highly correlated with earlier findings. Yao et al. (2008) observed gene diversity at 0.58 among the 124 maize landraces which showed that the markers used during the present study showed maximum polymorphism among 48 genotypes. The heterozygosity, was observed with an average of 0.19, ranged from 0.02 (*umc*1495) to 0.98 (*umc*1906) which is lesser than the findings by Yao et al. (2008), where they observed an average value of 0.39.

Inbreeding coefficient (F) varied from 0.06 (*umc*2075) to 1.00 (15 SSR loci) (Table 3). Consequently, selection practiced during the development of hybrids, could have led to the deficit of heterozygous individuals. The *F* values implied that the selected genotypes showed a typical mixed mating system along with good amount of heterozygotes which could find high variation among the genotypes.

Based on the above SSR analysis by considering the parameters of PIC value (≥0.74), gene diversity (≥0.75), inbreeding coefficient (≥0.62) and polymorphic alleles (≥4), the present study reported 7 highly polymorphic SSR loci *mmc*0371, *umc*2364, *umc*1568, *bnlg*1600, *phi*026, *umc*2071 and *bnlg*1904 (Table 3). These polymorphic primers can effectively be used in molecular breeding programs and QTL mapping studies since they exhibited very high level of polymorphism over other loci.

Associations among maize genotypes

The ability to provide distance measures between the inbred lines that reflect pedigree relatedness ensures a more stringent evaluation of the adequacy of a marker profile data. The fact that minimum genetic distance revealed during the study is a good indication confirming

the power of SSR markers to distinguish between closely related inbred lines, which was also reported by Smith et al. (1997). The average gene diversity existing among all the inbred lines were relatively high (58%), indicating existence of high levels of polymorphisms among the inbreds. These results are in close agreement with the findings reported among the maize inbreds using a SSR marker system (Yao et al., 2008).

The dendrogram constructed using the UPGMA clustering algorithm grouped the inbred lines into three clusters. These groupings, in most instances, revealed evidence of associations related to their pedigree records and place of release. It is because most of them were derived involving limited number of parents. This is in agreement with earlier investigators (Reif et al., 2003), who demonstrated the correspondence of SSR marker distance with pedigree information in maize. Alternatively, the 48 maize genotypes were broadly grouped into QPM and normal groups with several exceptions since the markers used in the study are not *o*2 specific. The cluster 'A' comprised all the QPM lines from India except VQL 15 and 9 normal inbreds (V25, V 380, V 376, V 359, V 369, V 364, V 366, V 371 and V 370) developed by VPKAS. The QPM inbred VQL 15 showed close relationship with V 338 and found under single cluster C. This is because, VQL 15 is the QPM version and isogenic to V 338. The genotypes VQL 1 and VQL 4 were clustered along with CM 212, since both were sister lines derived from CM 212 normal inbred line. A total of 14 normal genotypes developed by VPKAS were clustered in group A and C, of which group C consisted of 5 genotypes. The normal maize inbreds of Himachal Pradesh were scattered across all the three groups. All those inbreds were derived from different sources. Besides, the pedigree and effects of selection, the diverse range of genes affecting QPM traits in maize is also a key factor that affect in diversification of maize germplasm and establishing the genotypes in different clusters.

The salient findings of the present study were identifications of highly diverse genotype pairs among the normal and QPM genotypes. The genotype pairs VQL 2 and CML 173 will serve as ideal parents for mapping modifiers since they differ significantly for tryptophan content (0.52 and 1.00%, respectively) and also had the lowest similarity of 27%. However, among all the genotypes V 370 and B 06-7 (22%), V 25 and CM 152 (22%), and V 341 and CM 145 (24%) genotype pairs can be used in maize hybrid breeding programs for developing highly productive hybrids. There is also a great scope to derive further improved materials by hybridizing with distant lines identified during the study and for selection of the desired line.

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