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Molecular Genetic Variability in Quality Protein Maize (Zea Mays L.) Hybrids by SSR Markers

Ganga Ram Kohar[a], Balarama Swamy Yadav[a], Jogeswar Gadi [b], Krishna Mohan Kohar [c] and Dil Raj Yadav [d]

[a] Department of Botany, Andhra University, Visakhapatnam 530003 INDIA
[b] Shrijoshika Molecular Diagnostics and Research Centre, Ramangar, Visakhapatnam India.
[c] Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal
[d] Nepal Agriculture Research Council, Nepal

ABSTRACT: Determining genetic variability and its relationship to Quality Protein Maize (QPM) hybrids would enhance and broaden the crop's genetic foundation in improvement programs. In this work, we present the outcomes of ten QPM hybrids using Simple Sequence Repeats (SSR) markers. To measure the genetic diversity and provide work for five primers covering the entire genome, ten QPM hybrids were employed. The NTSYS pc2.1 tool was used to group the hybrids into different clusters, and the Polymorphism Information Content criteria for SSR markers varied between 0.50 and 0.65. According to Bnlg 1194 (0.58) and Umc 1333 (0.5), PIC values were maximal for the primer Phi031 (0.661) and lowest for the primer Phi057 (0.18), as indicated by the mean value of 0.46. The most diverse genotype was maintained by VH141720, which was cultivated by VH141732 and VH141775, according to SSR primer-based distances. Molecular maize breeding improvement programs that seek to capitalize on heterosis and improve genetically diverse populations may be impacted by the study's identification of genotype variability using SSR markers.

Key words: Quality Protein Maize, SSR markers, Dendrogram, molecular Genetic Variability

1. INTRODUCTION

One important grain crop that is utilized for animal feed, human consumption, and numerous industrial uses is maize. However, conventional maize has poor protein quality due to its lack of two essential amino acids, lysine and tryptophan (FAO, 1992). The Opaque -2 (O2) mutation was found to be the most suitable for genetic modification in breeding programs aiming to create maize hybrids with higher levels of tryptophan and lysine. For the Opaque-2 locus, it was shown that maize homozygous for the 02 (recessive) mutation had significantly higher levels of tryptophan and lysine than either heterozygous (O2o2) or homozygous dominant (O2O2).

Genetic homogeneity is the cornerstone of crop development initiatives. The hybrids with more diverse parents are likely to exhibit a wider range of predictability and a higher degree of heterotic countenance in segregating generations. Crop improvement relies heavily on information about elite resource relationships and germplasm homogeneity (Hallauer and Miranda, 1988). By using molecular markers to assign inbred lines to heterotic clusters, a larger number of lines can be described. thereby potentially boosting the success of maize breeding initiatives (Reif et al., 2003). Analysis of diversity Three useful levels of germplasm collections that can be accepted outright are morphological, geographic, and molecular (DNA, Sequence, Gene) (Buckler et al., 2006). Microsatellite or Simple Sequence Repeats (SSRs) are common and highly polymorphic, consisting of tandemly arranged short sequence motifs (between two

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and six nucleotides) found in many eukaryotic genomes, including maize (Powell et al., 1996).

Assessing the usefulness of microsatellites in diversity analysis and determining the genetic variability levels and interrelationships among the ten selected lines are the objectives of the current study.

2. MATERIALS AND METHODS

Ten QPM germplasm lines (Table.1) were supplied for this study by the International Centre for Maize and Wheat Improvement Centre (CIMMYT) at ICRISAT Hyderabad, India. These lines were subsequently sown in the Department of Botany at Andhra University in Visakhapatnam, Andhra Pradesh India.

Table1. Details of QPM hybrids used (Stock ID V-15371)

Sl. No.	Name	Pedigree
1	VH141734	CML171-BBB-1-B*10/CLQRCYQ44-B*4-1-#-B
2	VH141730	(CML161xCLQ-RCYQ31)-B-22-2-B*5/((CML150xCLG2501)-B-31-1-B-1-BBB/CML193- BB-B-2-BB(Q)-B*4
3	VH141782	CML451Q-B*8/CML193-B*7-#-B
4	VH141950	S99TLYQ(HG-AB)-BBB-6-B*7/(CML161xCLQ-RCYQ31)-B-22-2-B*5
5	VH141775	Pop61C1QPMTEYF-39-3-1-2-B-1-BB-#((CML150Xclg2501)-B-31-1-B-1-BBB/CML193-BB)-B-2-BB(Q)-B*4
6	VH141720	(CLQ-RCYQ46xCLQ-rcyq14=(cml164*cml161)-B-1-1-1-BBB)-B-11-1-B*5/((CML150xCLG2501)-B-31-1-B-1-BBB/CML193-BB)-B-2-BB(Q)-B*4
7	VH141732	(CML161xCLQ-RCYQ31)-B-22-2-B*5/G34QC24-BBB-16-B*8-#-B
8	VH133634	(CLQ-RCYQ31xCLQ-RCYQ49=(CML176xCL-G2501)-B-55-2-1-B)-B-10-3- B*5/(CML161X165)-F2-5-2-2-1-B-1-1-BBB-#-B
9	VH141733	CML171-BBB-1-B*10/(CML176xCLG2501)-b-55-1-2-B*4)
10	VH141198 6	(CML161xCLQ-RCYQ31)-B-3-6-BB-3-B*8/(CLQ-6601xCL-02843)-b-26-1-1-BB-1-B*9

2.1 DNA Extraction, Quantification and Standard DNA Template Preparation

21-day-old seedling plants were used to extract 200 mg of fresh, healthy leaf tissue. The leaves were finely chopped and ground into a powder using a mortar and pestle. To make a 100 ml volume of extraction buffer with a pH of 8:00, 900 µl of extraction buffer (CTAB 12.5 ml, Tris-HCL 1M-10 ml, EDTA-(0.5M)-5 ml, Nacl (5M)-30 ml, PVP-20 µl, and Distilled water 40 ml) was added to a 2 ml micro centrifuge tube. The container was swirled to gently mix the contents.

When extracting DNA, the CTAB method is used. To summarize, each micro centrifuge tube is filled with 10 µl 2-Mercaptoethanol and 900 µl of preheated CTAB (65^0C), and the tube is repeatedly inverted. After being placed in a water bath set at 65^0C, tubes are left there for 30 to 45 minutes. 800 µl of a chloroform and isoamyl alcohol (24:1) mixture should be added after the water bath. The tube should be repeatedly inverted while the sample is being

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gently mixed. After that, the tubes are centrifuged at 12,000 rpm for six minutes. The supernatant is pipetted into a fresh micro-centrifuge tube, and 700 µl of chloroform: alcohol (24:1) is then added. The tubes were centrifuged for six minutes at 12,000 rpm after several tube inversions. The supernatant is then transferred to 400 µl in a fresh tube. The tubes are centrifuged at 12,000 rpm for 6 minutes. The DNA pellet remains in the tube while the supernatant is discarded. DNA is separated by centrifuging 500 µl of 70% ethanol for five minutes at 12,000 rpm after washing. The ethanol is discarded. DNA-containing tubes are left to dry. The tube used for DNA dilution is placed in a deep freezer after 20µl of elusion buffer (AE) solution has been added.

2.2 DNA Amplification and Gel Electrophoresis

Ten SSR primers are nominated based on diversity analysis and their association with economically important traits (e.g., drought tolerance, disease resistance against Grey Leaf Spot (GLS), Quality Protein Maize (QPM), etc.). We only used five SSR primers for each of the ten QPM hybrid lines because we were able to obtain adequate amplification with just five of these (Table 2). 15 µl of 2X Green Go Taq ® Reaction Master Mix, 4 µl of forward and reverse primers (10 Pico moles of each primer), 2 µl of nuclease-free water, and 10 µl of DNA make up the 29 µl reaction measures used for DNA amplification.

The amplification reaction is shown using the Multi Gene Optic Max, Lab net International, Inc. thermal cycler. A 4-minute hot start and denaturing stage at 95^0C, 35 cycles of denaturation at 95^0C for one minute, annealing temperature (depending on primer type) for one minute, and extension at 72^0C for one minute comprise the basic PCR protocol. The product is maintained at 4^0C following a final extension phase of 5 minutes at 72^0C. The annealing temperature (Ta) is three to fifty degrees Celsius below the primer's melting point (Tm). The Tm of the primer is found using the Oligo calculator.
(http://mcb.berkeley.edu/labs/krantz/tools/oligocalc.html).

Table 1 Details of SSR primers used

Sl. N.	Primer	Sequence	Chr	Ta(Tm), °C	Repeat motifs	Feature	Reference
1	Bnlg1258	F: GGTGAGATCGTCAGGGAAAA20 R: GAGAAGGAACCTGATGCTGC20	2	49 (53)	AG (24)	GLS Disease resistance	Danson et al., 2008
2	Phi057	F: CTCATCAGTGCCGTCGTCCAT21 R: CAGTCGCAAGAAACCGTTGCC21	7	45 (56)	GCC	Opaque-2 and QPM	Jompuk et al., 2006
3	Bnlg1194	F: GCGTTATTAAGGCAAGCTGC20 R:	8	58 (52)	AG (33)	QPM	Krishna et.al, 2012

https://doi.org/10.5281/zenodo.17100693

		ACGTGAAGCAGAGGATCCAT 20					
4	Phi031	F:GCAACAGGTTACATGAGCT GACGA24 R: CCAGCGTGCTGTTCCAGTAG TT22	6	45 (57)	GTA C	Used in Drough t resista nce	Shiri,2 011
5	Umc13 33	F: AGGTAAGCGAGCATCTGAGG GT22 R: TCTGGAGACTCTTCTGGGTG AACT24	7	46 (57)	(CAG)4	Used in Drough t resista nce	Shiri, 2011

QPM: Quality Protein Maize; GLS: Grey Leaf Spot; Chr: Chromosome number. Ta: Annealing temperature; Tm: Melting temperature

The amplification reaction is shown using the Multi Gene Optic Max, Lab net International, Inc. thermal cycler. A 4-minute hot start and denaturing stage at 95^0C, 35 cycles of denaturation at 95^0C for one minute, annealing temperature (depending on primer type) for one minute, and extension at 72^0C for one minute comprise the basic PCR protocol. The product is maintained at 4^0C following a final extension phase of 5 minutes at 72^0C. The annealing temperature (Ta) is three to fifty degrees Celsius below the primer's melting point (Tm). The Tm of the primer is found using the Oligo calculator (http://mcb.berkeley.edu/labs/krantz/tools/oligocalc.html).

On a 1.2% Agarose gel, PCR products are separated using 1x TBE buffer (0.78 g agarose in 65 ml 1x TBE buffer). Ethidium bromide (Et Br) is used in the gel and the buffer for DNA staining. After cooling down to between 60 and 70^0C, 4 µl of Et Br is added to the gel. For every sample, 10 µl of the PCR product is added to each well. The main lane is loaded with a 2 µ l 100 bp ladder (HI Media). For 45 to 60 minutes, the gels are run at 80 V, 50 mA, and 5 W. Gels were wrapped in plastic wrap following the run. DNA fragments were created and captured under UV light.

2.3 Band, Scoring and Data Analysis

The optimal and diverse bands that are enhanced by SSR primers are visually planned for their occurrence present (1) or absence (0) of the mimicking band among the ten QPM genotypes. For each unique primer, Power Marker 3.5 calculates the polymorphic information content (PIC) standards. The correspondence matrix was constructed using the SIMQUAL component of NTSYS-pc version 2.02 (Rohlf, 1908). The cluster analysis was conducted using the Jacquard similarity coefficients, and the dendrogram was generated using the Unweighted Pair-Group technique (UPGMA) of the SAHN clustering function of the NTSYS software.

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3. RESULTS AND DISCUSSION

3.1 SSR Profiles

Five primers were used to amplified up the DNA of ten QPM hybrids. Any one of the five primers could separate all ten QPM hybrids. The SSR form of the Bnlg 2158 marker is shown in Fig. 1.

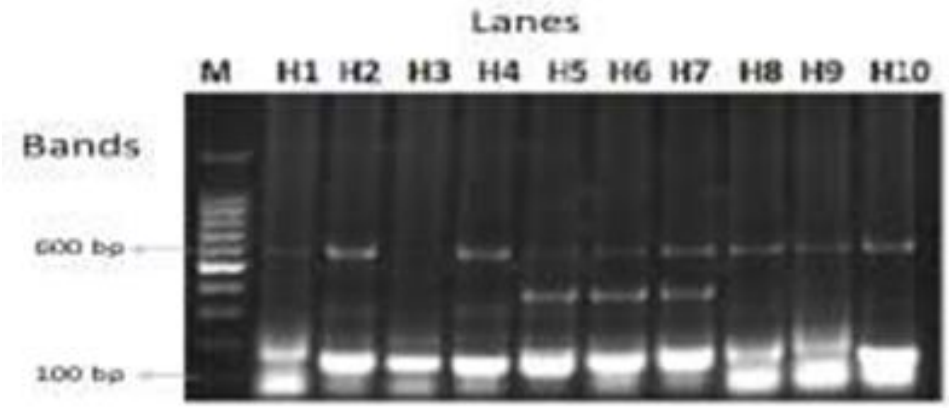

Figure 1 SSR profile of ten QPM hybrids with Bnlg2158 SSR marker in Agarose gel M, 100 bp-1500 bp marker; Lane number is indicated by the number which corresponds with QPM hybrid listed in Table 1.

Table.3 Primer showing nature of polymorphism across QPM hybrids band length

Band No.	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	Band Type
B1	600	600	A	600	600	600	600	600	600	600	Polymorphic
B2	A	A	A	A	400	400	400	**A**	A	**A**	Polymorphic
B3	A	400	A	400	400	400	400	400	A	400	Polymorphic
B4	A	300	300	300	A	A	A	A	300	A	Polymorphic
B5	280	280	280	280	280	280	280	280	280	280	Monomorphic
B6	100	100	100	100	A	100	100	100	100	100	Polymorphic

All of the band loci for the Bnlg 2158 marker are naturally heterozygote. fingerprints of multilocus DNA that are well known for having a high QPM hybridization (Fig. 1). Except for the H3 (VH141782) QPM hybrid, all QPM hybrids had larger size alleles.

DNA fingerprinting is the most dependable testing and documentation technique. It's not always possible to determine a genotype from a fingerprint that only uses one SSR primer. As a result, we used multiple locus SSR markers to create fingerprints. Based on these patterns of allele magnitude, it will also be beneficial to choose different traits associated with genotypes. The status of loci, such as whether homozygotes or heterozygotes are simply strong-minded, can be used to schedule future breeding and maintenance activities. DNA fingerprints

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have been used as an organized technique to document maize hybrids and confirm the genetic integrity of different seed lots (Singh et al., 2014).

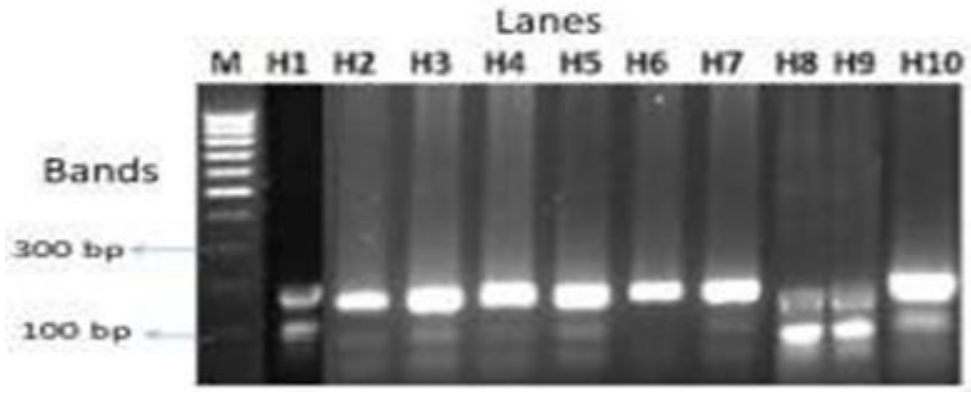

Figure 2 PCR amplification of maize hybrid genomic DNA using the 2Phi057 marker (linked to Opaque-2 and QPM). The total band scores were recorded and polymorphic data was produced. DNA marker M = 100 bp–1500 bp

Table.4-Primer = 2Phi057 showing Polymorphism across QPM hybrids band length

Band No	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	Band Type
B1	180	180	180	180	180	180	180	180	180	180	Monomorphic
B2	120	120	120	120	120	A	120	120	120	120	Polymorphic
B3	80	80	80	80	80	A	A	80	80	80	Polymorphic

Except for H6 (VH141720) for the 2Phi057 marker, all of the band loci are homozygotes. QPM hybrids are distinguished from one another by multilocus DNA fingerprints (Fig. 2). The QPM hybrid, which is made up of both polymorphic and monomorphic bands, had alleles of smaller sizes.

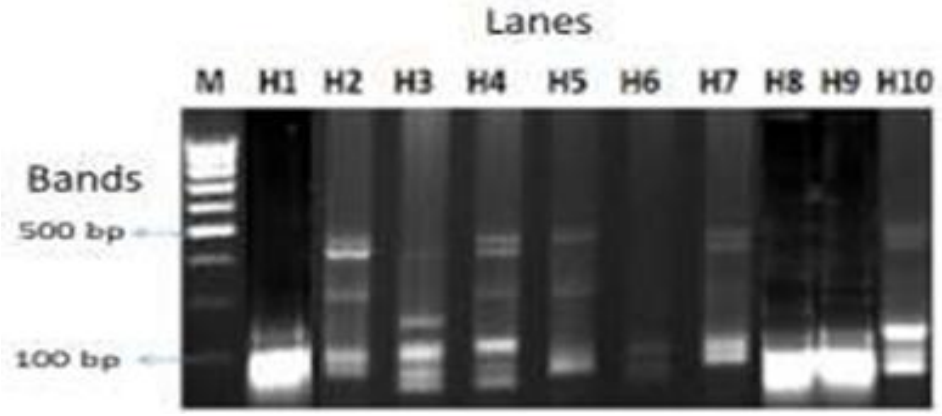

Figure 3 Maize hybrid genomic DNA was amplified by PCR using Primer-3 (Bnlg1194 = related to QPM marker). Polymorphic data is generated and the total band scores are noted. M is the 100 bp–1500 DNA marker.

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Table.5 Primer Bnlg1194 (related to QPM) resistance showing polymorphism across QPM hybrids band length

Lines Bands	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	Band types
B1	A	480	A	480	480	A	480	480	480	480	Polymorphic
B2	A	400	A	400	A	A	400	400	400	400	,,
B3	A	300	A	300	300	A	300	300	A	A	,,
B4	A	A	250	A	A	A	A	A	A	A	Unique
B5	A	A	220	220	A	A	220	A	A	220	Polymorphic
B6	200	200	200	200	200	200	200	200	200	200	Monomorphic
B7	180	180	180	180	180	180	180	180	180	180	,,
B8	100	A	A	A	A	A	A	100	100	A	Polymorphic
B9	80	80	80	80	80	A	80	80	80	A	,,

All of the band loci for the Bnlg1194 marker are naturally heterozygotes. The QPM hybrids differ greatly from one another based on multilocus DNA fingerprints (Fig. 3). All of the QPM hybrid entries had larger size alleles, except for H1 (VH141734), H3 (VH141782), and H6 (VH141720). Six bands showed that they were polymorphic, two showed that they were monomorphic, and one showed that they were unique. The unique band was observed in H3 hybrids (VH141782).

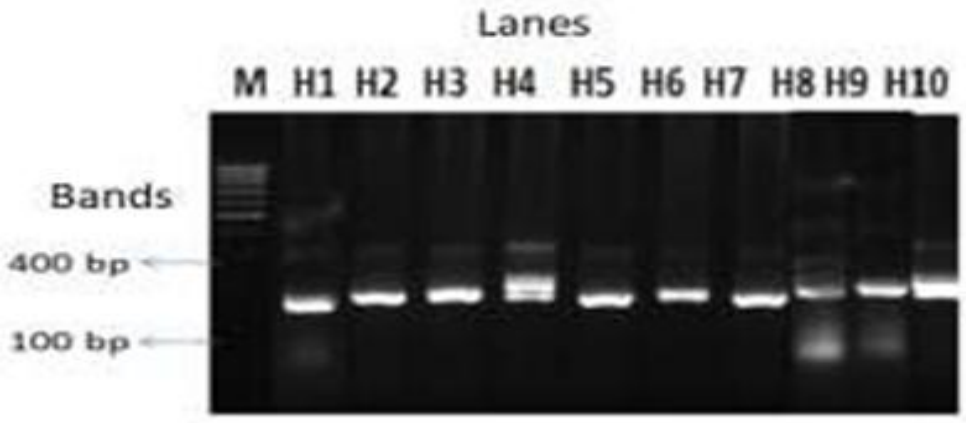

Figure 4 Maize hybrid genomic DNA was amplified by PCR using Primer-4 (Phi031 = drought resistance marker). Polymorphic data was produced and the overall band scores were noted. M is the DNA marker (100–1500 bp).

Table.6- Primer Phi031 = related to drought resistance showing polymorphism across QPM hybrids band length

Bands No.	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	Band type
B1	500	A	A	A	A	A	A	500	500	A	Polymorphic
B2	A	A	A	A	A	A	A	350	A	A	Unique

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B3	300	300	300	300	300	300	300	300	300	300	Monomorphic
B4	A	A	A	220	A	A	A	A	A	A	Unique
B5	200	A	A	200	A	A	A	200	200	200	Polymorphic
B6	180	180	180	180	180	180	180	180	180	180	Monomorphic

All of the band loci for the Phi031 marker were naturally heterozygotes. The QPM hybrid was distinguished from one another by the multilocus DNA fingerprint (Fig. 4). Except for H1 (VH141734), H8 (VH133634), and H9 (VH141733), every allele in the QPM hybrid was smaller. The B1 and B5 bands showed a polymorphic character, the B4 and B2 bands showed a unique nature, and the B3 and B6 bands showed a monomorphic nature. The hybrids H4 (VH141950) and H8 (VH133634) showed the different bands.

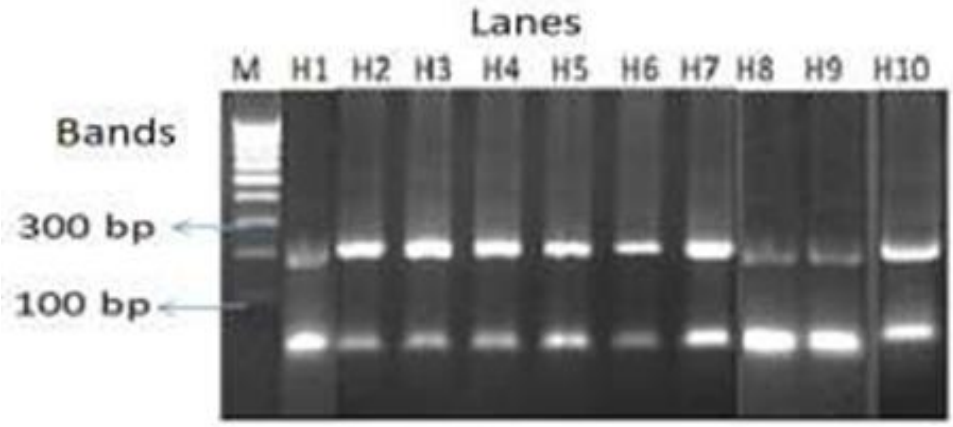

Figure 5 PCR amplification of Maize Hybrid Genomic DNA with Primer-5 (UMC 1333 = Drought resistance marker). The total band scores were recorded and polymorphic data was generated. M= DNA marker (100bp-1500bp)

Table.7- Primer UMC 1333 = (Drought resistance) showing polymorphism across QPM hybrids band length

Bands No.	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	Bands type
B1	180	180	180	180	180	180	180	180	180	180	Monomorphic
B2	50	50	50	50	50	50	50	50	50	50	Monomorphic

All of the band loci for the UMC 1333 marker are naturally homozygote. The QPM hybrid was distinguished from one another by the multilocus DNA fingerprint (Fig. 5). Each of the ten QPM hybrids had a monomorphic allele in both bands, and the alleles were smaller in the QPM hybrid.

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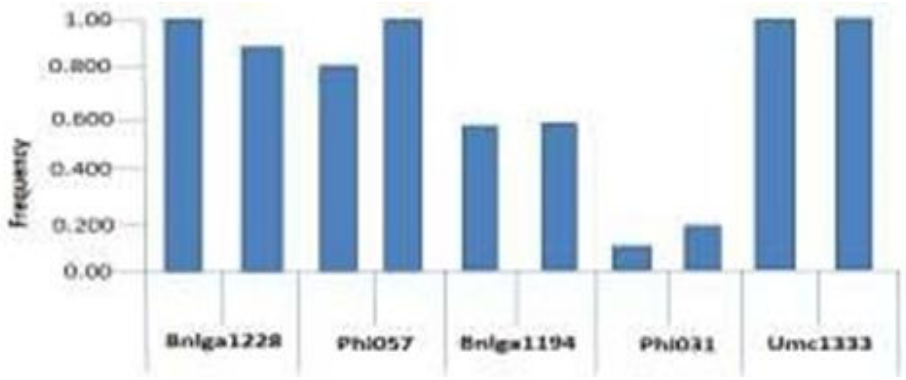

Figure 6 Allele frequency of five SSR markers based on ten QPM hybrids

3.2 SSR Markers Diversity

Five SSR primers are used to analyze ten QPM hybrids for molecular diversity and relationships. Only four primers generated polymorphic profiles, despite the fact that all of them produced unique banding patterns. A total of 26 alleles were identified; 3 (unique) bands displayed clear size differences, 7 (26.92%) were monomorphic bands, and 15 (57.69%) were polymorphic bands (Table 8). Alleles in the hybrids ranged from 2 to 9, with an average of 2.6 alleles per locus. Of the polymorphic markers, two produced at least five and no more than six alleles each, and two produced two alleles each. The number of alleles did not differ significantly among the genotypes that were part of this study. Nepolean et al. (2013) found 111 polymorphic alleles with an average of 3.17 alleles per locus, whereas Kumar et al. (2016) reported 59 alleles with an average of 2.62 alleles per locus. However, 76 different alleles were found by Patel et al. (2017), with an average of 4.47 alleles per locus. The number of alleles developed between studies may vary depending on the size and scope of the study samples, the techniques used to find polymorphic markers that influence allelic differences, or the uniformity based on pedigrees.

The polymorphism information content (PIC) value ranges from 0.183333 to 0.661667 with an average of 0.461, which is relatively low when compared to the polymorphism information content reported by Ranatunga et al. (2009) (PIC = 0.53 to 0.99), Adeyemo et al. (2011) (PIC = 0.17 to 0.84), Sserumaga et al. (2013) (PIC = 0.16 to 0.91), and Patel et al. (2017) (PIC = 0.46 to 0.86). Our results, however, are in line with those of Kumar et al. (2016), who used the same set of primers to analyze 13 genotypes of maize and found that the PIC value ranged from 0.142 to 0.497, with an average value of 0.304. The low PIC value in the current study is due to the high frequency of two alleles.

The highest PIC values are found in primers Phi 031 (0.661) and Bnlg 1194 (0.587), while the lowest PIC values are found in primers Phi 057 (0.183) and Bnlg 1258 (0.408). Kumar et al. (2016) reported that the maximum PIC value for primer phi050 was 0.497. PIC shows how informative the SSR loci are and how well they can detect genotype changes based on their genetic relationships. The primers Phi031 (0.661) and Bnlg1194 (0.587) are identified as the most suitable markers for genetic diversity research based on the highest PIC value of 0.661. We discovered that there were few allelic differences and diversity values in the SSR marker. Tri/tetra/penta-nucleotide primers may have been specifically consumed, or the QPM genotypes used in this study may have had a weak genetic basis.

https://doi.org/10.5281/zenodo.17100693

Table.8 Primer showing polymorphic across QPM hybrids.

SSR locus	Approximate product size amplified (bp)	Total number ofalleles	Total number of Polymorphic alleles	Total number of Monomorphic alleles	Total numberof Unique band	PIC Value
Bnlg1258	100-600	6	5	1	0	0.408
Phi 057	80-180	3	2	1	0	0.183
Bnlg1194	80-480	9	6	2	1	0.587
Phi031	180-500	6	2	2	2	0.661
Umc1333	50-180	2	0	1	0	0.5
total		26	15	7	3	0.468

3.3 QPM hybrid Maize diversity

All QPM hybrids, except for VH141782, have allele sizes between 50 and 300 bp (Table 9). The QPM hybrids have allele sizes ranging from 50 to 600 bp. The average number of alleles per locus is 1.53. The VH141720 has two alleles per locus, compared to just 1.24 alleles per locus for VH133634 and VH141950.

Table 9. Summary of Genetic Statistics of QPM hybrids based on 5 SSR markers

SN	QPM hybrid	Allele size range, bp	Allele/ Locus, n	Major Allele frequency	Gene Diversity	Heterozygosity	PIC value
1	VH141734	50-600	1.6	0.5	0.5	0.8	0.7296
2	VH141730	50-600	1.4	0.7	0.3	0.6	0.5376
3	VH141782	50-300	1.73	0.6	0.4	0.7	0.64
4	VH141950	50-600	1.24	0.8	0.2	0.4	0.36
5	VH141775	50-600	1.63	0.6	0.4	0.7	0.64
6	VH141720	50-600	2	0.5	0.5	0.8	0.7296
7	VH141732	50-600	1.44	0.6	0.4	0.6	0.5904
8	VH13364	50-600	1.24	0.9	0.08	0.2	0.1536
9	VH141733	50-600	1.44	0.6	0.4	0.6	0.5904
10	VH1411986	50-600	1.53	0.7	0.3	0.4	0.4224
	Average		1.53	0.65	0.35	0.58	0.5394

VH141720 has the lowest major allele frequency, while the two QPM hybrids, VH133634 and VH141950, have the highest major allele frequencies. The average values for PIC, heterozygosity, and gene diversity are 00.5394, 0.35, and 0.58, respectively. VH133634, VH133634, and VH133634 have the lowest levels of gene diversity, heterozygosity, and PIC, the three measures of genetic diversity. The genes with the highest PIC values, heterozygosity, and gene diversity are VH141720 and VH141734.

It is not unusual to find reports of the diversity of all markers. For a number of reasons,

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diversity above QPM hybrids that incorporate all of the factors considered may be even more beneficial than direct selection of QPM hybrids. We have found that VH141720 and VH141734 are more diversified than other QPM. Some of these QPM hybrids may be suitable candidates for pooled composition for population mixing and conservation since comparable values suggest genetic closeness.

Similar genetic statistics were found in several studies. According to Gurung et al. (2010), the Arun-4 variety had a maximum heterozygosity of 54.64%, while the average heterozygosity was 45.07% at the variety level. The highest level of heterozygosity was displayed by Phi109188 (81.03%). The variety-level gene diversity varied from 0.51 in Manakamana-2 to 0.56 in Arun-4 and Khumal Yellow. According to Reif et al. (2003), the gene diversity of the tropical maize population was 0.56. PIC ranged from 0.50 to 0.95 for 34 SSRs on QPM. Bnlg1401 had the highest PIC and Bnlg1506 the lowest, per Krishna et al. (2012). A total of 19 polymorphic alleles were found in 8 maize genotypes using nine polymorphic SSR primers with 0.297 PIC and 0.373 gene diversity values (Kumari et al., 2018). The best markers for genotype identification were phi064 and phi053, according to PIC values (0.367). A total of 40 alleles (bands) with an average of 3.33 alleles per locus were discovered in 38 maize hybrids using 12 SSR markers (Shiri, 2011). The polymorphism information content (PIC) of the 12 SSR loci ranged from 0.23 (Phi080) to 0.79 (UMC2359), with a mean of 0.53.

3.4 Cluster analysis

Dendrograms of ten QPM hybrids are constructed using 26 polymorphic loci generated by each of the five SSR markers. The similarity coefficients ranged from 0.5200 to 0.8800, as shown in Table 4. The hybrids VH141734, VH141733, VH141775, and VH141732 have a high degree of similarity and may be descended from the same parents, as indicated by the similarity coefficient's maximum value of 0.64. With the lowest similarity coefficient values (0.5200), the hybrids VH141720 and VH133634, as well as VH141732 and VH141733, are the least similar to one another. This might be because they are descended from different parents, have different morphological features and traits, or originate from different places.

Table 10: Jacquard's Similarity coefficient matrix for ten QPM hybrids based on SSR marker analysis

QPM hybrids	VH141734	VH141730	VH141782	VH141950	VH141775	VH141720	VH141732	VH133634	VH141733	VH141986
VH141734	1.0000									
VH141730	0.6800	1.0000								
VH141782	0.7600	0.7600	1.0000							
VH141950	0.7200	0.8000	0.7200	1.0000						
VH141775	0.6800	0.7600	0.6000	0.7200	1.0000					
VH141720	0.7600	0.6800	0.6800	0.6400	0.7600	1.0000				
VH141732	0.6400	0.8000	0.6400	0.7600	0.8800	0.8000	1.0000			
VH133634	0.6000	0.6800	0.6000	0.8000	0.6000	0.5200	0.6400	1.0000		
VH141733	0.8800	0.7200	0.8000	0.6800	0.5600	0.6400	0.5200	0.6400	1.0000	
VH141986	0.6800	0.7600	0.6800	0.8000	0.6800	0.6000	0.7200	0.8400	0.6400	1.0000

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The phylogenetic tree divided the hybrids into three clusters according to the values of the similarity coefficients. Cluster II had a maximum of four hybrids, while cluster I had three and cluster III had three (Fig. 7). The most diverse hybrid is VH 141720, which is followed by VH 141732 and VH 141775. Therefore, the most genetically diverse parents can be used in breeding programs. Ranatunga et al. (2009) classified 45 genotypes of maize into two major clusters, each of which was further subdivided into several smaller clusters. Kanagarasu et al. (2013) used ten SSR markers to examine the diversity of 27 inbred lines of maize. Five major clusters with 0.62 similarity were revealed by the cluster analysis results.

Based on similarity coefficient values, the hybrids were grouped into three clusters by the phylogenetic tree. Cluster I had three hybrids, Cluster III had three hybrids, and Cluster II had a maximum of four hybrids (Fig.7). VH 141720 is the most varied hybrid, followed by VH141732 and VH141775. Thus, in a breeding program, the most diverse genotypes can be used as parents. 45 maize genotypes were grouped by Ranatunga et al. (2009) into two main clusters, each of which was further subdivided into a number of smaller clusters. Five major clusters with 0.62 similarity coefficients were found by Kanagarasu et al. (2013) using ten SSR markers for diversity analysis of 27 maize inbred lines. Patel et al. (2017) produced a dendrogram that divided eight genotypes into three groups based on similarity coefficient values.

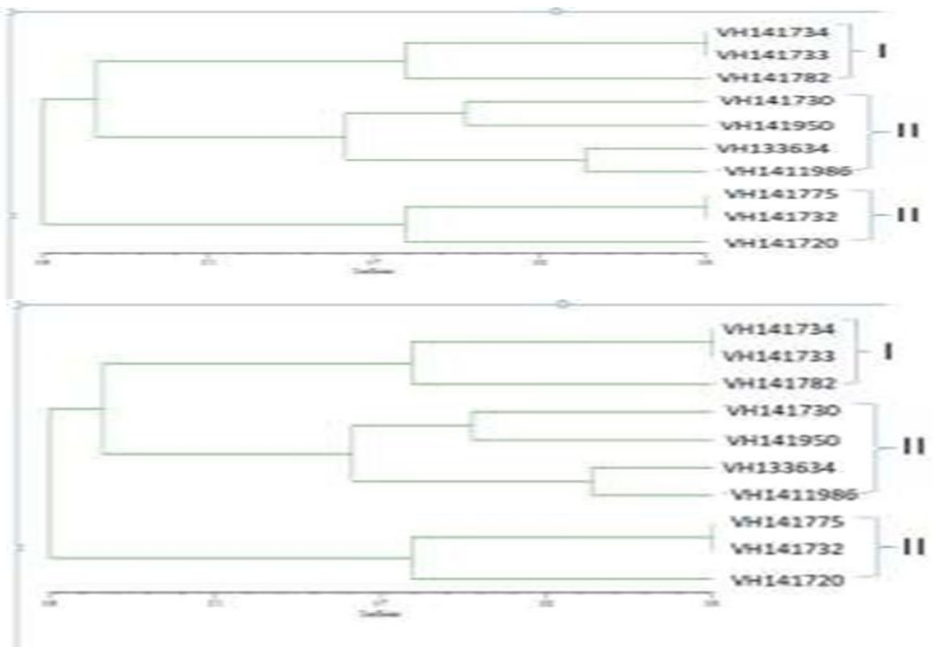

Figure 7 Dendrogram showing clustering of ten QPM hybrids based on SSR marker analysis

4. CONCLUSIONS

The study discovered molecular diversity and interrelationships among ten QPM genotypes. Polymorphism information content (PIC) values show that Bnlg1194 and phi031 are the best SSR markers for detecting QPM hybrids. The hybrids VH141720, VH141732, and VH141775 were distinguished from the other hybrids in the cluster analysis using SSR markers. Breeders of maize can easily assign hybrids to heterotic groups by using SSR markers to guide the selection of parents for the creation of novel hybrids. They are therefore useful for accurately defining QPM hybrids at the molecular level. Additionally, SSR markers provide a means of uniquely identifying particular hybrids according to their distinct allelic patterns, a function that is increasingly important in varietal protection.

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