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**RESEARCH ARTICLE**

# MOLECULAR MARKER BASED GENETIC DIVERSITY IN QUALITY PROTEIN MAIZE

**Dr A. Thanga Hemavathy**

Assistant Professor, Department of Pulses, TNAU, Coimbatore

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**ABSTRACT**

Randomly amplified polymorphic DNA markers were used to assess the genetic diversity among thirty three maize inbred lines and seven Quality Protein maize lines of maize. A total of 40 operon decamer primers were screened in duplicate and 14 of these primers were selected for further testing. A total of 112 markers were amplified with 14 primers with an average of 8.0 DNA bands per primer. Among the 112 markers, 94 were found to be polymorphic and the percentage of polymorphism was 83.92 per cent. A total of 12 fragments were amplified with primer OPAW 20 and a minimum number of 3 bands were amplified with primer OPAL-10. The approximate size of the largest fragment amplified was in the range 3.0 to 3.5 kb the smallest easily recognizable fragment amplified was approximately 0.3 kb. Maximum d value of 0.96 was observed between UMI 814 and CML 118, and the least distance was observed between UMI 889 and UMI 57. The nature of relationships among the genotypes as revealed by their genetic distances largely involved similarities with the exception of some pairs, which displayed divergence. The most plausible explanation for the comparatively low genetic distances between the populations is that they might probably have descended from a common ancestral population.



## INTRODUCTION

Maize *(Zea mays L.)* is referred as "Queen of cereals" as it has got the highest potentially among the cereal crops. Maize is important crop after rice and wheat in terms of acreage but rank the second in terms of total production and yield. In India average maize area is 6.93mha, total output is about 13.30 million tonnes and productivity is 2018 kg/ha (Agricultural News, 2008). Maize is used as food, feed and fiber it also has several industrial applications such as manufacture of starch, gluten, germ oil, corn syrup, sugar, corn meal and corn flour. Maize facts and trends report (CIMMYT, 2000) indicated that 17 per cent of maize produced in the world is used as food for human and 66 per cent is used as feed for animals. In India, 77 per cent of maize produced is used for human consumption, while only two per cent is used as feed for animals. This indicates the importance of maize in India, and role plays in meeting the ever-increasing demand for food and also warrants the development of new, high yielding varieties and hybrids of maize.

Genetic progress depends on germplasm quality and breeding methods. The plant breeder's choice of source germplasm methods determines the potential improvement for traits under selection in a breeding programme. The success of any breeding methods depends on the availability of genetic diversity in the base population. Therefore, it is important to characterize and conserve genetic variation in the inbred lines and QPM lines for future breeding programmes.

Molecular markers have been applied to assess genetic diversity in many crops because they are unlimited in number, not affected by the environment and can be organized into linkage maps. There are a number of reports on characterization of maize lines based on isozyme and RAPD markers Tsegaye *et al.* (1996). Among these, RAPD markers have been frequently used for analyzing genetic relationships among QPM lines. Hence, the present study was designed to assess the genetic diversity among 33 inbred lines and seven QPM lines.

## MATERIALS AND METHODS

### *Plant Materials*

Seeds of thirty three maize inbred lines and seven Quality Protein maize lines are listed in **Table 1** were obtained from Maize Breeding unit, Department of millets, Centre or Plant Breeding and Genetics (CPBG), Tamil Nadu Agricultural University (TNAU) and CIMMYT, Mexico. These genotypes were used in molecular analysis of genetic diversity.

### *DNA extraction*

DNA from the 40 genotypes were extracted by following method described by McCouch *et al.*(1998) with sight modification as mentioned below.

**Corresponding author:* **A.Thanga Hemavathy**
Department of Pulses, TNAU, Coimbatore

Five grams of fresh leaves from young seedlings germinated by roll towel method, was freeze thawed using liquid nitrogen.

**Table1** List of genotypes and their parentage

| Accession No. | Parentage | Source |
|---|---|---|
| UMI 9 | MS-9 | MBS, Coimbatore |
| UMI 10 | MS-10 | MBS, Coimbatore |
| UMI 17 | CM-202 | MBS, Coimbatore |
| UMI 21 | CM 420 | MBS, Coimbatore |
| UMI 27 | CM 105 x CM 104 C | MBS, Coimbatore |
| UMI 29 | CM 500 x CM 201 | MBS, Coimbatore |
| UMI 35 | THl DMR-5x Taiwan comp.$DeF_2$x (CM 202x CM 111) | MBS, Coimbatore |
| UMI 37 | P.DMR-5 x Cuprico $F_3$ x (CCM 202x CM 111) | MBS, Coimbatore |
| UMI 42 | P.DMR-5 x Taiwan comp. $MSC_1$ $F_4$ x (CM 202 x CM 111) | MBS, Coimbatore |
| UMI 48 | PHIL DMR-2 | MBS, Coimbatore |
| UMI 51 | PHIL DMR-5 | MBS, Coimbatore |
| UMI 57 | Taiwan DMR-3 | MBS, Coimbatore |
| UMI 61 | Taiwan DMR-13 | MBS, Coimbatore |
| UMI 64 | Bagor Comp. – 10 | MBS, Coimbatore |
| UMI 70 | Puerto Gurad-2 | MBS, Coimbatore |
| UMI 76 | Chain cross | MBS, Coimbatore |
| UMI 79 | Pioneer-102 | MBS, Coimbatore |
| UMI 86 | Amber | MBS, Coimbatore |
| UMI 113 | YUZP-SC-48 A (UMI 113/A white kernels) | MBS, Coimbatore |
| UMI 118 | YUZP-206 | MBS, Coimbatore |
| UMI 128 | PKT-1 | MBS, Coimbatore |
| UMI 131 | PKT-4 | MBS, Coimbatore |
| UMI 189 | 2407 | MBS, Coimbatore |
| UMI 226 | South African Tall x Akbar comp. | MBS, Coimbatore |
| UMI 266 | Malan local (Rajasthan Udaipur) | MBS, Coimbatore |
| UMI 285 | Suwan-1 (Indonesia composite) | MBS, Coimbatore |
| UMI 420 | (UMI29) x (UMI 51) | MBS, Coimbatore |
| UMI 426 | (UMI 47) x (UMI 134) | MBS, Coimbatore |
| UMI 427 | (UMI 25) x (UMI 51) | MBS, Coimbatore |
| UMI 524 | 96123 (Sarhael x Suwan 1) x (Suwan 1) | MBS, Coimbatore |
| UMI 620 | (Sakathi x CM 111) x $F_4$ | MBS, Coimbatore |
| UMI 814 | Diara EVF –10 | MBS, Coimbatore |
| UMI 841 | LODANA 8929 MEX/2441 | MBS, Coimbatore |
| UMI 889 | Plot No 1332 | MBS, Coimbatore |
| CML 141 | Pob 62C 5HC 24-5-3-2-1-B-B-2-B-B-# | CIMMYT, Mexico |
| CML 142 | Pob 62 C 5HC 93-5-6-1-3-B-B-B-7-B-B-# | CIMMYT, Mexico |
| CML 143 | Pob 62C 6HC 88-1-1-B-B-B-10-B-B-# | CIMMYT, Mexico |
| CML 144 | Pob 62 C 5 HC 182-2-1-2-B-B-B-3-1-#-# | CIMMYT, Mexico |
| CML 145 | Pob63cOHC181-3-2-14#-2B-B-B-B-#-# | CIMMYT, Mexico |
| CML 146 | AC 8563 MH 35-3-1-B-2-1-B-B-1-B-B-# | CIMMYT, Mexico |
| CML 147 | Pob63c2HC53-1-1-B-B-B-9-B-B-# | CIMMYT, Mexico |

The thawed leaves were ground to powder and transferred to a 50 ml centrifuge tube. Twenty milliliter of warm ($65^0$C) extraction buffer (100 mM Tris HCL, $p^H$ 8.0; 500 mM Nacl; 1.25 % sodium dodecyl sulphate (W/v); 3.8 gm sodium bi sulphate per liter) was added and the tubes were incubated at $65^0$C for 20 minutes with intermittent shaking at five minutes interval. After 20 minutes, 10 ml of 5 molar potassium acetate was added and the tubes were shaken vigorously and placed in an ice pack on a shaker for 15 minutes. After 15 minutes, the contents were centrifuged at 3000 rpm for 20 minutes at room temperature. The clear supernatant was collected in a separate tube by filtering through a miracloth.

To the supernatent, $2/3^{rd}$ volume of chilled iso-propanal was added and maintained at room temperature for one hour. After an hour's time, the DNA, which was floating as slimy clear mass was hooked out and transferred to another centrifuge tube with 5ml of TE buffer ($p^H$ 8.0).

After the complete dissolving of DNA in the buffer, $1/8^{th}$ volume of ammonium acetate was added, followed by addition of twice the volume-chilled ethanol. The slimy mass of DNA was again hooked out and 500 µl of buffer ($p^H$ 8.0) and maintained at $-20^0$C as stock.

***DNA quality and quantity check***

Electrophoresis of genomic DNA was performed on 0.8 per cent agarose gel to check quality and quantity of DNA. DNA concentration for Polymerase chain reaction (PCR) amplification was estimated by comparing the band intensity of a sample with that of intensities of known dilutions that gave good amplification. The dilutions were approximately made by taking one volume of crude genomic DNA and diluting it with required volume of TE buffer ($p^H$ 8.0). The quantity of TE buffer required was fixed based on band intensity.

***RAPD analysis***

DNA from the 40 genotypes were amplified using a set of 14 arbitrary oligonucleotide decamer primers obtained from operon technologies Inc. Alameda, CA, USA amplification reaction mixtures were made up to 20 µl with 10 mM Tris HAL ($p^H$- 9.0), 50 mM KCL, 1.5 mM $Mgcl_2$, 0.001 per cent gelatin, dATP, dCTP, dTTP an dGTP- 0.1 mM each, 1.0 pg of primer, 20-30 ng of genomic DNA and 0.5 unit of Taq DNA polymerase (Bangalore Genei PVT, Ltg., Bangalore).

Amplification was performed in 200 µl thin walled Tarsone PCR tubes in a PCR- 300 (Perkin Elmer) thermal cycler programmed for 40 cycles 1 minute at $92^0$C, 1 minute at $34^0$C and 2 minute $72^0$C preceded and succeeded by 2 minutes at $92^0$C and 10 minutes at $72^0$C respectively. PCR amplification products (20 µl) were subjected to electrophoresis in 1.4 per cent agarose gel in 1X TBE buffer at 120V for 3.5 hours using Hoefer super submarine electrophoresis unit (Pharmacia biotech). The electronic image of ethidium bromide stained gel was captured using kodak digital science DC-120 zoom digital camera (Eastman kodak co, Rochester, Ny) and the gel was documented using electrophoresis documentation and analysis system (EDAS-120) ID image analysis software (Scientific imaging systems, Eastman Kodak company, N.Y). List of primers used for RAPD analysis and their sequence information are mentioned in **Table 2.**

***Scoring***

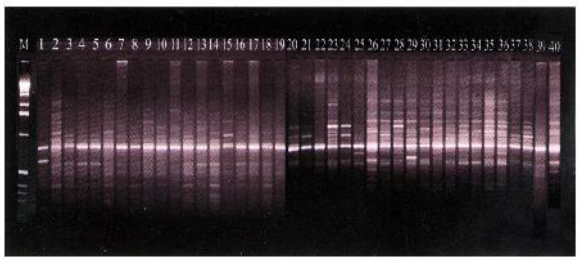
**Table 2** List of primers used and their sequence

| Sl.No | Primers code | Sequence 5' to 3' |
|---|---|---|
| 1 | OPAW-09 | ACTGGGTCGG |
| 2 | OPAW-10 | GGTGTTTGCC |
| 3 | OPAW-18 | GGC*GC*AACTG |
| 4 | OPAW-19 | GGACACAGAG |
| 5 | OPAW-20 | TGTCCTAGCC |
| 6 | OPAK-4 | ACTTGGCCGT |
| 7 | OPAK-12 | AGTGTAGCCC |
| 8 | OPAK-17 | CGCTTTAATT |
| 9 | OPAL-2 | ACCCTGTGGG |
| 10 | OPAL-09 | CAGCGAGTAG |
| 11 | OPAL-10 | AAGGCCCCTG |
| 12 | OPAL-19 | TCTGCCAGTG |
| 13 | OPAB-08 | GCTTGGCCTA |
| 14 | OPAB-18 | CTAGTCCTCA |

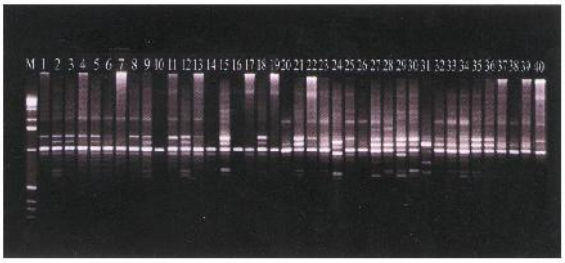
**Table 3** Level of polymorphism detected in RAPD

| Total number of primers used | 14 |
|---|---|
| Number of markers | 112 |
| Number of polymorphic markers | 94 |
| Number of monomorphic markers | 18 |
| Percentage of polymorphism | 83.92 |
| Average number of markers | 8 |
| Range pf markers | 3 |

**RAPD profile generated by primer OPAW - 20**



**RAPD profile generated by primer OPAB - 08**



Amplification profiles of all the 40 genotypes were compared with each other and bands of DNA fragments were scored as present (1) or absent (0). The data of the primers were used to estimate the similarity on the basis of the number of shared amplification products (Nei and Li, 1979). Similarity coefficients were utilized to generate a dendrogram by means of Unweighted Pair Group Method of Arithematic Means (UPGMS).

## RESULTS AND DISCUSSION

The 40 genotypes were subjected to RAPD analysis with 14 primers and the result of the analysis are presented in the Table 13. A total of 112 markers were amplified with 14 primers with an average of 8.0 DNA bands per primer. Among the 112 markers, 94 were found to be polymorphic and the percentage of polymorphism was 83.92 per cent (Table.3). A total of 12 fragments were amplified with primer OPAW 20 (Plate.1) and a minimum number of 3 bands were amplified with primer OPAL-10. More over the size of the DNA fragments varied with different primers **(Plate 2.).** The approximate size of the largest fragment amplified was in the range 3.0 to 3.5 kb the smallest easily recognizable fragment amplified was approximately 0.3 kb. The genomic DNA of the UMI 427 genotype amplified the maximum number of DNA bands (98) while the minimum numbers of DNA bands (80) were amplified by genomic DNA of the genotypes (UMI 27).

The first estimate of genetic similarity was based on one third of the molecular marker data. By sequentially adding the remaining marker data, it was observed that the estimates did not significantly change. Therefore, this convergence suggests that these are accurate estimates of genetic similarity.

Binary scores were generated based on the presence or absence of markers and the data matrix was converted to Dice's (1945) similarity matrix. Maximum d value of 0.96 was observed between UMI 814 and CML 118, and the least distance was observed between UMI 889 and UMI 57. Dendogram generated by sequential agglomerative hierarchical non-overlapping clustering (SAHN) method with UPGMA distances based on the similarity matrix (Figure 1) indicated the genotypes could be broadly classified into six clusters. The genotype UMI 27 was found to be a solitary member of one of the clusters, similarity co-efficient at molecular level was highest between UMI 814 and IMI 86 followed by CML 146 and UMI 118 (Table 14).Among the three methods of analysis of genetic diversity, molecular markers are widely considered accurate because they are at least affected by environment.

RAPD analysis resulted six clusters of genotypes, similar concurrence within and between clusters of morphogenetic traits and molecular markers was reported by Cross *et al.* (1992), Tsegaye *et al.* (1996), Doldi *et al.* (1997), Kraic *et al.* (1998) and Ravi (2000). According to Tsegaye *et al.* (1996) the lack of concordance between different descriptors indicated the influence of different evolutionary forces on the two categories of descriptors *viz.*, morphological and molecular. They also opine that morphological traits are the primary target of artificial selection, and traits like maturity have significant adaptative value under the growing condition at the site of origin of the populations. According to them, clustering based

on morphological performance of the populations could serve as a guide in predicting their segregation potential and adaptation.

The results of RAPD cluster analysis markers are more reliable and environmentally neutral (Lanza *et al.*, 1997) and can contribute directly to development of classification schemes and also to identification of diverse parents for hybridization.

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