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Molecular Characterization of Maize (Zea Mays L.) Genotypes Using Rapd Markers

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Abstract

The present investigation was undertaken to analyze the genetic diversity in 7 Maize genotypes viz. BTM -15, BTM – 02, BTM -04, BTM – 13, BTM – 11, Phule RAJASHRI, Phule SHAKTI for using RAPD analysis. RAPD amplified polymorphic DNA (RAPD) profile for were generated with 7 primers. Such as OPBF – 01, OPBF – 02, OPBF – 03, OPBF – 04, OPBF – 05, OPBF – 06, OPBF – 07. Seven primers generated 53 amplicons, of which 43 were polymorphic with an average of 5.3 amplicons per primer. A dendrogram was constructed by using the UPGMA that was based on similarity coefficients. The Jacquard's similarity coefficient ranged from 0.46 to 0.82. Among the 7 Maize genotypes, Maximum similarity coefficient 0.82 was observed between BTM-04 and BTM-11 and least similarity coefficient 0.46 was observed between BTM-04 and Phule Shakti. Seven Maize genotype were clustered in two distinct groups that is cluster A and cluster B. Based on the dendrogram, the genotypes can be divided into two major clusters (A and B). Cluster A further splits into sub-clusters A1 and A2, and sub-cluster A2 divides again into two smaller sub-clusters: A2-1 and A2-2. This hierarchical grouping indicates the genetic diversity and relatedness among these Genotypes.

Keywords: *Genetic diversity, RAPD markers, Zea mays, polymorphism, UPGMA clustering, molecular characterization.*

Introduction

Maize (Zea mays L.) is a globally significant cereal belonging to the Poaceae family. Originating from Central America, it has evolved into one of the most adaptable crops, widely grown across diverse environments (Bremer et al., 2003). Known as the "queen of cereals" due to its remarkable yield potential, maize is cultivated in various forms including sweet corn, popcorn, and high oil corn, among others. Beyond its role as a food source, maize is pivotal in several industries, offering immense opportunities for value addition.

Genetic improvement of maize relies heavily on exploring its genetic diversity. Molecular markers, particularly PCR-based techniques like RAPD, have become essential tools in studying genetic variation, constructing phylogenetic relationships, and identifying promising genotypes for breeding programs (Behra et al., 2008; Wilkie et al., 1993). This investigation aimed to analyze genetic diversity among selected maize genotypes using RAPD markers to aid in informed breeding strategies.

Materials and Methods

Plant Material

Seven maize genotypes—BTM-15, BTM-02, BTM-04, BTM-13, BTM-11, Phule RAJASHRI, and Phule SHAKTI—were procured from the Agricultural Research Station, Digraj, Sangli, and MPKV, Rahuri. Laboratory experiments were conducted in the Department of Plant Biotechnology, Loni.

DNA Extraction

Genomic DNA was isolated from young leaves of 9-day-old seedlings using the CTAB method (Doyle & Doyle, 1990). Approximately 20 mg leaf tissue was ground in liquid nitrogen, treated with preheated CTAB buffer, followed by chloroform:isoamyl alcohol extraction. DNA was precipitated using isopropanol, washed with ethanol, treated with RNase, and purified by phenol extraction before dissolving in TE buffer.

PCR Amplification

Seven decamer primers (OPBF-01 to OPBF-07; Operon Technologies) were used for RAPD analysis. PCR was carried out in a 25 µl volume containing 30 ng DNA, primers, and PCR master mix. The thermal cycle included initial denaturation at 94°C for 2 min, 40 cycles of denaturation (94°C, 20 s), annealing (36°C, 1 min), and extension (72°C, 1 min), concluding with a final extension at 72°C for 7 min. Amplicons were separated on 1.2% agarose gel stained with ethidium bromide and visualized under UV light.

Data Analysis

Bands were scored as present (1) or absent (0). Similarity coefficients were calculated using Jaccard's method, and clustering was performed using UPGMA in NTSYS-PC (Rohlf, 1995).

Results and Discussion

The RAPD primers generated a total of 53 bands, with 43 exhibiting polymorphism, resulting in an average polymorphism rate of ~71%. Table 1 shows individual primer performance, with OPBF-02, OPBF-04, and OPBF-05 achieving 100% polymorphism. The genetic similarity among genotypes ranged from 0.46 to 0.82 (Table 2), highlighting substantial diversity. The dendrogram (Fig. 1) segregated the genotypes into two main clusters (A and B), with sub-clustering reflecting close genetic relationships.

These results demonstrate the effectiveness of RAPD markers in distinguishing maize genotypes and provide valuable information for selecting diverse parental lines for hybrid development.

Tables and Figures

Table 1. Polymorphic amplicons generated by RAPD primers

Primer	Total bands	Polymorphic bands	Polymorphism (%)
OPBF-01	6	1	16.6
OPBF-02	14	14	100
OPBF-03	5	4	80
OPBF-04	6	6	100
OPBF-05	12	12	100
OPBF-06	6	5	83.3
OPBF-07	6	1	16.6
Total	**53**	**43**	**~71%**

Table 2. Jaccard's similarity matrix among maize genotypes

	BTM-15	BTM-02	BTM-04	BTM-13	BTM-11	RAJASHRI	SHAKTI
BTM-15	1.00	0.72	0.52	0.61	0.54	0.56	0.56
BTM-02		1.00	0.59	0.65	0.54	0.64	0.65
BTM-04			1.00	0.79	0.82	0.57	0.46
BTM-13				1.00	0.76	0.62	0.48
BTM-11					1.00	0.65	0.52
RAJASHRI						1.00	0.58
SHAKTI							1.00

DNA Banding Pattern:

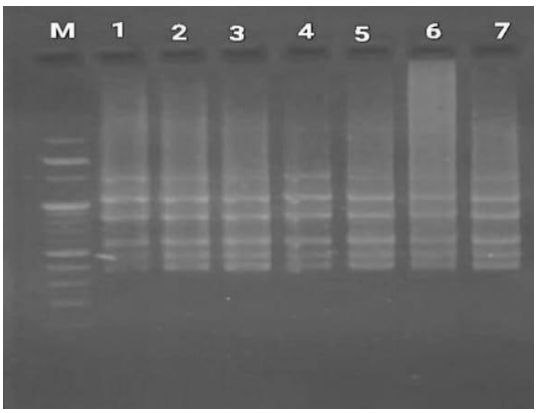

Fig 1:- RAPD Profile by OPBF-01

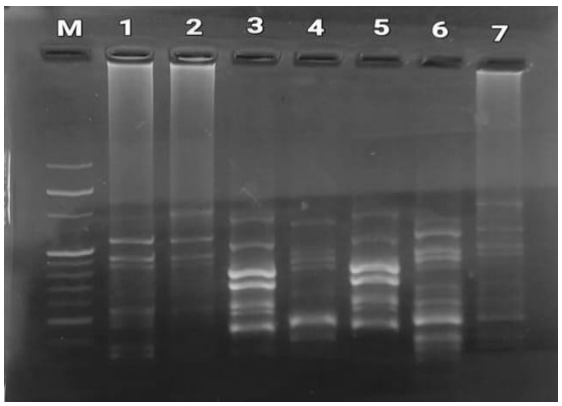

Fig 2:- RAPD Profile by OPBF-02

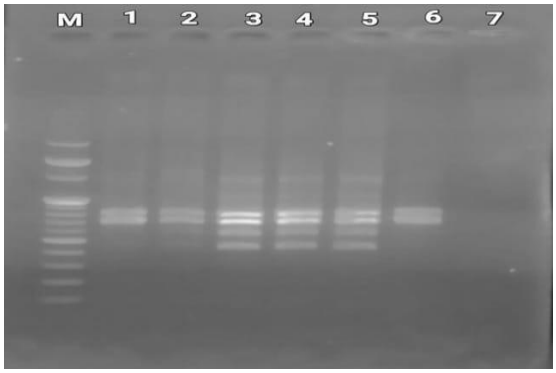

Fig 3:- RAPD Profile by OPBF-03

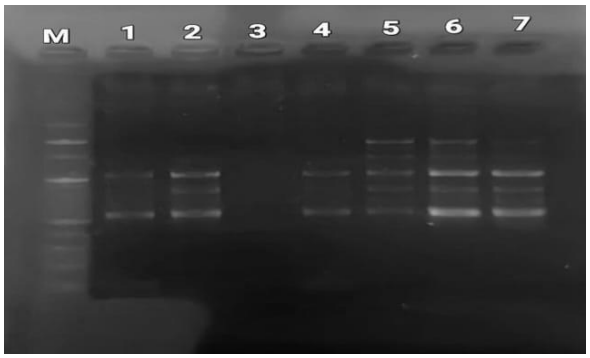

Fig 4:- RAPD Profile by OPBF-04

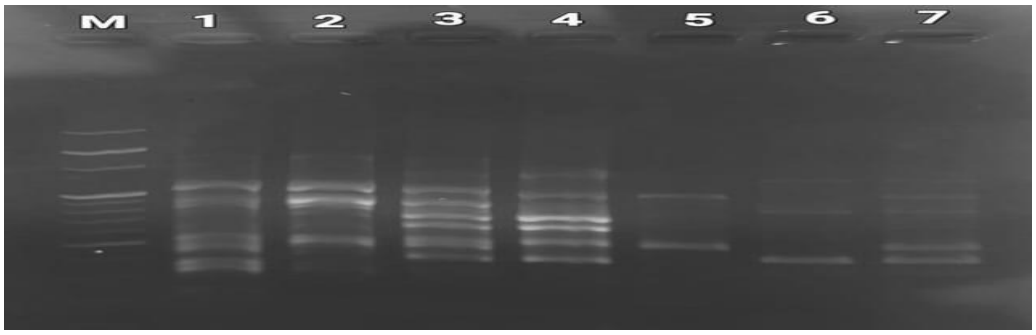

Fig 5:- RAPD Profile by OPBF-05

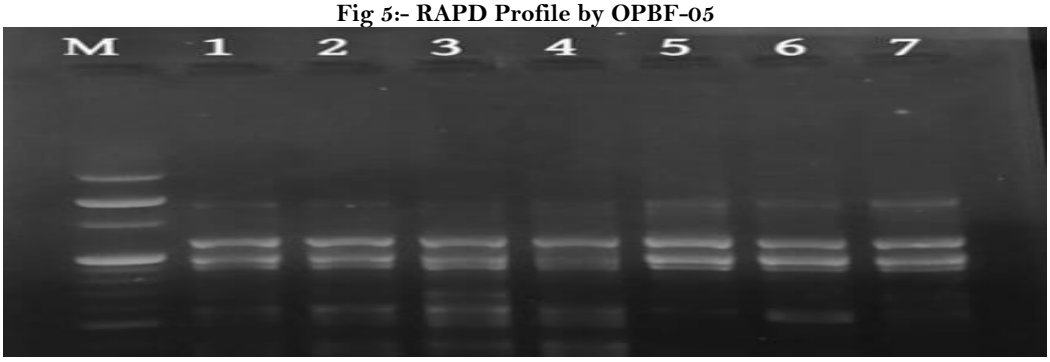

Fig 6:- RAPD Profile by OPBF-06

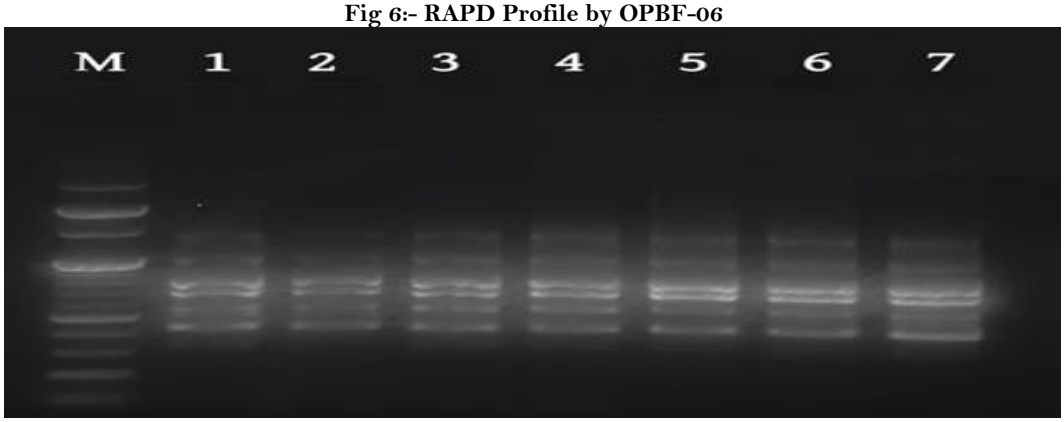

Fig 7:- RAPD Profile by OPBF-07

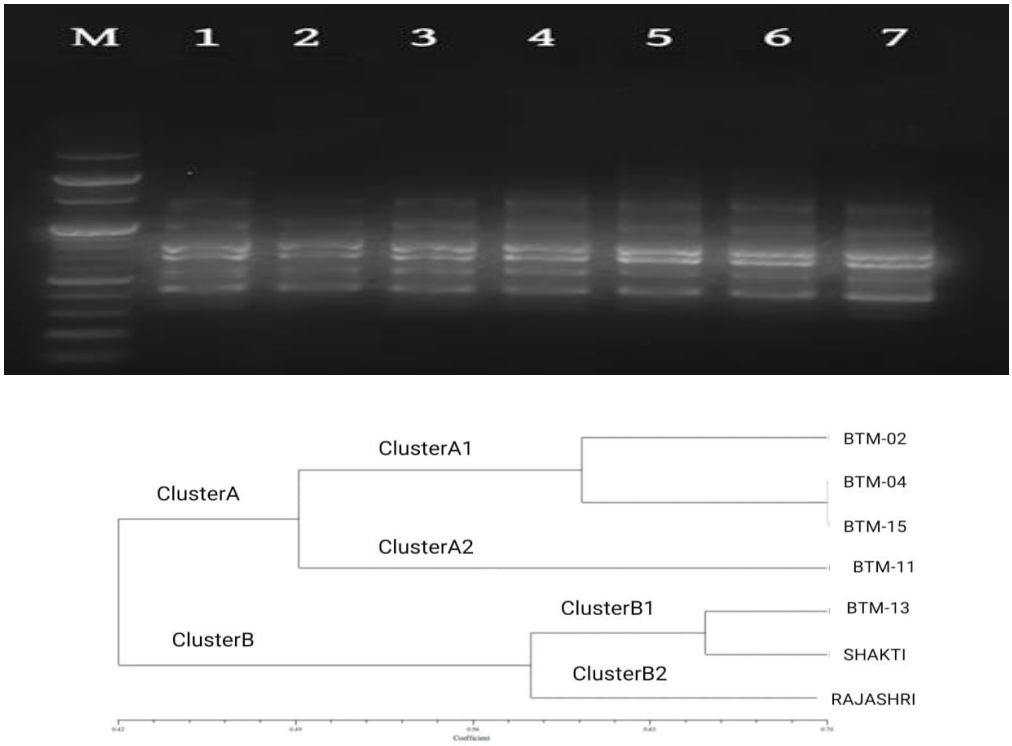

Fig 8:- Dendrogram showing results of RAPD analysis of Maize genotypes.

Conclusion

This study underscores the genetic diversity present among maize genotypes, with RAPD analysis effectively delineating their relationships. Such insights are pivotal for selecting diverse and genetically robust parents to develop superior maize hybrids.

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Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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