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**Original Research Article**

# Assessment of Molecular Diversity in Diverse Maize Genotypes Using RAPD and ISSR Markers

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**A B S T R A C T**

Maize (*Zea mays* L.) is one of the most important cereal crop throughout the word. The crop is highly variable and rich in genetic variability. Two DNA based molecular marker techniques, random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR), were used to study the genetic diversity among genotypes of maize. A total of 26 polymorphic primers (16 random and 10 ISSR) were used. Amplification of genomic DNA of 20 genotypes, using RAPD analysis, yielded 79 fragments, of which 58 (73.41%) were polymorphic. The 10 ISSR primers produced 59 bands across 20 genotypes, of which 51 (86.44%) were polymorphic. The similarity coefficient ranged from 0.34 to 0.76 and 0.36 to 0.87 with RAPD, ISSR dendrogram, respectively. The high rate of polymorphism lines revealed by RAPD and ISSR markers indicated that the method is efficient to analyze genetic diversity in maize genotype and that the genetic divergence can be used to establish consistent heterotic groups between maize genotype.

**Keywords**

Maize, molecular marker, RAPD, ISSR, genetic diversity, polymorphism

## Introduction

Maize (*Zea mays* L.) is an important cereal crop belongs to the tribe Maydeae, of the grass family, Poaceae. The plant is native to South America. *Z. mays* L. is the only species in the genus *Zea* with chromosome number 2n=20 (Bremer *et al.*, 2003). Maize is widely cultivated crop throughout the world. The suitability of maize to diverse environments is unmatched by any other crop as the expansion of maize to new areas and environment still continues, as it has range of plasticity. Maize (*Z. mays* L.) is the third most important cereal crop in the world after wheat and rice. Maize in India is known as "Queen of cereals" because of its high production potential and wider adaptability

Maize is used primarily as a food for humans in most areas of the world, in contrast United States where about 85 per cent of the crop is used as cattle feed. Maize grain is extensively used for the preparation of corn starch, corn syrup, corn oil dextrose, corn flakes, gluten, grain cake, lactic acid and acetone which are used by various industries such as textile, foundry, fermentation and food industries. With the development of poultry and livestock industry, its consumption in the feeds has also increased tremendously. It is used in the human diet in both fresh and processed forms. Maize oil has high poly unsaturated fatty acid content and low in linoleic acid (0.7%) and contains high level of natural

flavor. So, it remains a liquid at fairly low temperatures which helps in combating heart diseases.

Maize being a cross-pollinated crop has wide scope for the development of hybrids and composites. In recent years, there has been a perceptible improvement in maize production in India, however production and productivity of India is much less compare to U.S.A. The United States produces almost half of the world's production; other top maize producing countries are China, Brazil, France, Indonesia and South Africa. India ranks fourth in maize production with 21.73 million tones grown on an area of 8.55 million hectares with a productivity of 26.81 quintals per hectare during 2011-12 (Anonymous, 2012). It is mainly grown in Uttar Pradesh, Bihar and Rajasthan because of high yield, ease with which the crop can be cultivated and stability of prices.

Knowledge about germplasm diversity and genetic relationships among breeding materials could be an invaluable aid in crop improvement strategies (Mohammadi and Prasanna, 2003). New molecular tools hold the promise of allowing the identification of genes involved in a number of traits including adaptive traits, and polymorphisms causing functional genetic variation. Conventional breeding techniques which are based on the processes of crossing, back-crossing and selection, proved to be time consuming.

Therefore, molecular technology is increasingly becoming popular as a powerful tool for unambiguous authentication. Molecular techniques for detecting differences in the DNA of individual plants to examine variability in cultivar are useful for identification of potential parental lines. These differences in general are called molecular marker. These molecular markers used for characterization as well as phylogenic analysis in various plant species with reliable and authentic results (Behra *et al.,* 2008). DNA markers provide a direct measure of genetic diversity and go beyond diversity based on agronomic traits or geographic origin (Dreisigacker *et al.,* 2005), thus help in better germplasm management and develop more efficient strategies for crop improvement.

Among the various molecular marker techniques available, polymerase chain reaction (PCR)-based markers, such as randomly amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) have been most popular because of speed, low cost, does not require prior knowledge of DNA sequence and the use of only minute amounts of DNA template for analysis.

RAPD has been the most employed technique in diversity analysis, mapping and genotype identification in number of plant species but low reproducibility is the limit of this technique. ISSR markers overcome the shortcomings of the low reproducibility of RAPD; they produce more reliable and reproducible bands because of the higher annealing temperature and longer sequence of ISSR primers. ISSRs are ideal as markers for genetic mapping and population studies because of their abundance, and the high degree of polymorphism between individuals within a population of closely related genotypes (Singh *et al.,* 2011). Those properties indicate their potential role as good supplements for RAPD-based genome analysis.

So, the present study was designed to access the genetic diversity among 20 diverse maize genotypes using RAPD and ISSR markers to identify potentially useful germplasm for crop improvement.

## Materials and Methods

### Plant material and Experimental site

Twenty (20) maize genotypes were procured from Chaudhary Charansingh Hisar Agriculture Uiniversity, Hisar and Department of Genetics and Plant-breeding, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur. Plants were grown in field, after 20 days of growth leaves, were cut and frozen in liquid nitrogen for DNA extraction. The present study was conducted at Molecular Biology Laboratory at Department of Molecular Biology and Biotechnology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur (India).

### DNA Extraction

Leaves were ground in liquid nitrogen to a fine powder with a chilled mortar and pestle. Genomic DNA was extracted using Doyle and Doyle (1990) cetyl trimethyl ammonium bromide (CTAB) method. The quantity and quality of DNA were determined by spectrophotometer and electrophoresis on 0.8 % agarose gel, respectively. DNA samples were diluted to 50 ng µl-1 for polymerase chain reaction (PCR) amplification.

### RAPD and ISSR-PCR Analysis

RAPD-PCR amplification was performed with 20 random decamer primers obtained from Bangalore Genei Pvt. Ltd., India. Sixteen (16) primers with reproducible and scorable amplifications were chosen for further studies (Table 1). In ISSR-PCR analysis, only 10 primers were selected for further use from 15 ISSR primers obtained from Sigma chemicals Co., USA (Table 2). PCR amplifications for RAPD and ISSR were performed in 20 µl volume containing 2 µl dNTP (250 µM each dNTP), 1µl primer (30 ng µl−1), 1 µl template DNA (50 ng µl−1), 2.5 µl reaction buffer [(10×) 10 mM Tris-Cl pH 9.0, 50 mM KCl], 0.3 µl Taq DNA polymerase [(5 U µl−1) Bangalore Genei Pvt. Ltd., Bangalore, India], 2 µl MgCl2 (25 mM), and 11.2 µl deionized water. PCR reactions were performed with DNA thermal cycler (Eppendorf AG, Germany).

Amplification conditions were as follows: an initial denaturation at 94°C for 5 min followed by 1 min denaturation at 94°C for 36 cycles for RAPD and ISSR, respectively and 1 min at annealing temperature (36°C for RAPD; for ISSR, $22^0$C to $53^0$C it depends upon the primer), 2 min polymerization at 72°C and 2 min final extension at 72°C. After completion of amplifications, 2 µl of gel loading dye (MBI Ferment Inc., USA) was added to each sample and 20 µl volume was resolved on 1.5 and 2.0% (w/v) agarose gel for RAPD and ISSR, respectively in 1× Tris–borate–ethylenediaminetetra acetic acid (TBE) buffer, gels were stained with ethidium bromide. The size of amplified DNA fragments was estimated with standard molecular size markers (100 bp DNA ladder and Lambda DNA/*Eco*R I/ *Hind* III double digest; Bangalore Genei Pvt. Ltd., Bangalore, India). The gels were visualized under UV using gel documentation system (BioRad, USA).

### Reproducibility of amplification patterns

DNA amplifications with each RAPD and ISSR primers were repeated at least thrice to ensure reproducibility. The bands were considered reproducible and scorable only after observing and comparing them in three separate amplifications for each primer.

Clear and intense bands were scored while faint bands against background smear were not considered for further analysis.

## Scoring and data analysis

DNA fingerprints were scored for the presence (1) or absence (0) of bands of various molecular weight sizes in the form of binary matrix. Data were analyzed to obtain Jaccard's coefficients among the isolates by using NTSYS-pc version 2.02e (Rohlf, 1998). The SIMQUAL program was used to calculate Jaccard's coefficients (Jaccard, 1908).

## Results and Discussion

## RAPD band pattern

Information on genetic diversity and relationship among between individuals, population, plant varieties, animal breed and species are important to plant breeders for the improvement of crop plants. Genetic diversity studies can identify alleles that might affect the ability of the organism to survive in its existing habitat, or might enable it to survive in more diverse habitats. This knowledge is valuable for germplasm conservation, individual, population, variety or breed identification and genetic improvement (Duran *et al.,* 2009). Various types of markers such as morphological, biochemical and molecular markers are used for this purpose (Barwar *et al.,* 2008). Twenty RAPD primers having 60% or more GC content were used for the present investigation. Out of 20 primers only, 16 were amplified and showed 73.41% polymorphism. A total 79 amplified bands were obtained of which 58 were polymorphic. The DNA amplicon size and polymorphism generated among various genotypes of *Z. mays* L. using RAPD primers are presented in Table 3. The total number of bands observed for every primer was recorded separately and polymorphic bands was checked subsequently. The total number of amplified bands varied between 2 (primer OPC-08, OPP-04, OPF-17 and OPP-03) and 9 (primer OPD-05) with an average of 4.9 bands per primer. The polymorphism of all 20 genotype *Z. mays* L. were 73.41% and the overall size of PCR amplified products ranged between 180 bp to 2500 bp. Similar to present finding Mukharib *et al.,* (2010) obtained high level of polymorphism of 73.02 per cent among maize inbred lines. Earlier, Bruel *et al.,* (2007) used RAPD molecular markers to analyze genetic diversity between 16 corn lines. Twenty-two primers were used resulting in the amplification of 265 fragments, of which 237 (84.44%) were polymorphic.

Based on RAPD similarity matrix data (Table 4), the value of similarity coefficient ranged from 0.34 to 0.76 i.e. 34-76% or. The average similarity across all the genotypes was found out to be 0.63 showing that genotype were polymorphic genetically. Maximum similarity value of 0.76 was observed between genotypes PM-6 and B. sathi. Similarly minimum similarity value of 0.34 was observed between genotype Arawali and HQPM-5 and this genotype are highly diverse at genetic level.

The RAPD cluster tree analysis of 20 *Z. mays* L. genotype showed that they were mainly divided into main two clusters at a similarity coefficient of 0.45 (Fig 1). Genotype HQPM-5 and EQH-16 was out-grouped from all other genotypes at a similarity coefficient of 0.45 and formed the first cluster. At a coefficient of 0.52 was the second cluster having all remaining genotypes. Genotype PM-3 and C. sathi was again out-grouped and formed another solitary cluster at a similarity coefficient of 0.52.

**Table.1** Details of RAPD primers used in molecular analysis of *Zea mays* L. genotypes

| Sr. No. | Primer* | Sequence 5' to 3' | G: C Content (%) |
|---|---|---|---|
| 1 | OPA-01 | CAGGCCCTTC | 70 |
| 2 | OPC-08 | TGGACCGGTG | 70 |
| 3 | OPD-05 | TGAGCGGACA | 60 |
| 4 | OPD-12 | CACCGTATCC | 60 |
| 5 | OPE-03 | CCAGATGCAC | 60 |
| 6 | OPF-17 | AACCCGGGAA | 60 |
| 7 | OPF-19 | CCTCTAGACC | 60 |
| 8 | OPJ-04 | CCGAACACGG | 70 |
| 9 | OPP-01 | GTAGCACTCC | 60 |
| 10 | OPP-02 | TCGGCACGCA | 70 |
| 11 | OPP-03 | CTGATACGCC | 60 |
| 12 | OPP-04 | GTGTCTCAGG | 60 |
| 13 | OPP-05 | CCCCGGTAAC | 70 |
| 14 | OPP-06 | GTGGGCTGAC | 70 |
| 15 | OPP-07 | GTCCATGCCA | 60 |
| 16 | OPP-08 | ACATCGCCCA | 60 |
| 17 | OPP-09 | GTGGTCCGCA | 70 |
| 18 | OPP-10 | TCCCGCCTAC | 70 |
| 19 | OPP-12 | AAGGGCGAGT | 60 |
| 20 | OPP-16 | CCAAGCTGCC | 70 |

* Operon series code

**Table.2** Details of ISSR primers used in molecular analysis of *Zea mays* L. genotypes

| **S. No.** | **Primers** | **Sequence 5' to 3'** | **G: C Content (%)** | **Tm ($^0$C)** |
|---|---|---|---|---|
| 1 | 810 | ATATATATATATATATT | 0 | 24 |
| 2 | 851 | GTGTGTGTGTGTGTGTYG | 50 | 54 |
| 3 | 802 | ATATATATATATATATATG | 6 | 25 |
| 4 | 852 | TCTCTCTCTCTCTCTCCRA | 44 | 45 |
| 5 | 803 | ATATATATATATATATC | 6 | 24 |
| 6 | 853 | TCTCTCTCTCTCTCTCRT | 44 | 47 |
| 7 | 804 | TATATATATATATATAA | 0 | 23 |
| 8 | 854 | TCTCTCTCTCTCTCTCRG | 50 | 48 |
| 9 | 805 | TATATATATATATATAC | 6 | 22 |
| 10 | 855 | ACACACACACACACACYT | 44 | 52 |
| 11 | 806 | TATATATATATATATAG | 6 | 22 |
| 12 | 856 | ACACACACACACACACYA | 44 | 50 |
| 13 | 807 | AGAGAGAGAGAGAGAGT | 47 | 42 |
| 14 | 857 | ACACACACACACACACYG | 50 | 54 |
| 15 | 808 | AGAGAGAGAGAGAGAGC | 53 | 47 |

**Table.3** DNA amplification profile and polymorphism generated in *Z. mays* L. using 16 RAPD primers

| S. No. | Primer code | MW (bp) | Total no of bands | No. of polymorphic bands |
|---|---|---|---|---|
| 1 | OPA-01 | 350-1400 | 6 | 5 |
| 2 | OPC-08 | 800-2000 | 2 | 1 |
| 3 | OPD-05 | 180-1300 | 9 | 9 |
| 4 | OPD-12 | 450-1500 | 4 | 4 |
| 5 | OPE-03 | 400-1600 | 5 | 4 |
| 6 | OPF-17 | 400-1200 | 2 | 2 |
| 7 | OPJ-04 | 500-1600 | 6 | 3 |
| 8 | OPP-01 | 200-900 | 7 | 5 |
| 9 | OPP-02 | 400-1200 | 4 | 3 |
| 10 | OPP-03 | 400-900 | 2 | 1 |
| 11 | OPP-04 | 650-1000 | 2 | 1 |
| 12 | OPP-05 | 500-1000 | 4 | 4 |
| 13 | OPP-07 | 300-1500 | 6 | 3 |
| 14 | OPP-10 | 200-2000 | 8 | 8 |
| 15 | OPP-12 | 400-1500 | 5 | 2 |
| 16 | OPP-16 | 450-2500 | 7 | 3 |
| Total | | | 79 | 58 |

**Table.4** Jaccards similarity coefficient for RAPD profile generated by Agrose gel electrophoresis

| Genotype | HQPM-1 | HQPM-5 | HQPM-7 | EQH-16 | EQH-63 | PHM-1 | PHEM-2 | PHM-2 | BIO-9637 | HM-8 | Arawali | PM-3 | PM- 4 | PM-5 | Navjot | B. sathi | PM-6 | K. Malan | EC-3161 | C. Sathi |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HQPM-1 | 1.00 | | | | | | | | | | | | | | | | | | | |
| HQPM-5 | 0.41 | 1.00 | | | | | | | | | | | | | | | | | | |
| HQPM-7 | 0.72 | 0.42 | 1.00 | | | | | | | | | | | | | | | | | |
| EQH-16 | 0.49 | 0.49 | 0.51 | 1.00 | | | | | | | | | | | | | | | | |
| EQH-63 | 0.71 | 0.47 | 0.71 | 0.58 | 1.00 | | | | | | | | | | | | | | | |
| PHM-1 | 0.74 | 0.40 | 0.67 | 0.54 | 0.75 | 1.00 | | | | | | | | | | | | | | |
| PHEM-2 | 0.67 | 0.43 | 0.65 | 0.46 | 0.64 | 0.65 | 1.00 | | | | | | | | | | | | | |
| PHM-2 | 0.71 | 0.41 | 0.67 | 0.47 | 0.68 | 0.68 | 0.72 | 1.00 | | | | | | | | | | | | |
| BIO-9637 | 0.67 | 0.40 | 0.60 | 0.44 | 0.59 | 0.63 | 0.71 | 0.76 | 1.00 | | | | | | | | | | | |
| HM-8 | 0.68 | 0.36 | 0.65 | 0.42 | 0.61 | 0.60 | 0.61 | 0.73 | 0.75 | 1.00 | | | | | | | | | | |
| Arawali | 0.53 | **0.34** | 0.52 | 0.47 | 0.56 | 0.61 | 0.50 | 0.56 | 0.61 | 0.58 | 1.00 | | | | | | | | | |
| PM-3 | 0.48 | 0.51 | 0.48 | 0.47 | 0.47 | 0.51 | 0.61 | 0.53 | 0.60 | 0.49 | 0.41 | 1.00 | | | | | | | | |
| PM- 4 | 0.59 | 0.36 | 0.65 | 0.43 | 0.59 | 0.54 | 0.55 | 0.61 | 0.68 | 0.73 | 0.57 | 0.52 | 1.00 | | | | | | | |
| PM-5 | 0.67 | 0.41 | 0.64 | 0.49 | 0.63 | 0.62 | 0.68 | 0.66 | 0.74 | 0.62 | 0.60 | 0.57 | 0.72 | 1.00 | | | | | | |
| Navjot | 0.68 | 0.36 | 0.72 | 0.44 | 0.61 | 0.64 | 0.67 | 0.69 | 0.66 | 0.63 | 0.53 | 0.52 | 0.63 | 0.70 | 1.00 | | | | | |
| K.Sathi | 0.65 | 0.41 | 0.68 | 0.44 | 0.60 | 0.60 | 0.65 | 0.66 | 0.67 | 0.73 | 0.54 | 0.52 | 0.69 | 0.69 | 0.71 | 1.00 | | | | |
| PM-6 | 0.65 | 0.42 | 0.66 | 0.47 | 0.65 | 0.64 | 0.65 | 0.72 | 0.73 | 0.76 | 0.61 | 0.53 | 0.65 | 0.71 | 0.71 | **0.76** | 1.00 | | | |
| K. Malan | 0.61 | 0.44 | 0.68 | 0.47 | 0.59 | 0.58 | 0.61 | 0.66 | 0.61 | 0.69 | 0.53 | 0.47 | 0.61 | 0.61 | 0.65 | 0.69 | 0.74 | 1.00 | | |
| EC-3161 | 0.53 | 0.40 | 0.53 | 0.44 | 0.55 | 0.55 | 0.61 | 0.57 | 0.58 | 0.59 | 0.54 | 0.42 | 0.48 | 0.57 | 0.54 | 0.61 | 0.60 | 0.53 | 1.00 | |
| C. Sathi | 0.54 | 0.43 | 0.57 | 0.48 | 0.54 | 0.53 | 0.58 | 0.53 | 0.55 | 0.47 | 0.42 | 0.61 | 0.49 | 0.55 | 0.58 | 0.54 | 0.57 | 0.56 | 0.49 | 1.00 |

**Table.5** DNA amplification profile and polymorphism generated in *Z. mays* L. using 10 ISSR primers

| S. No. | Primer code | MW (bp) | Total no. of bands | No. of polymorphic bands |
|---|---|---|---|---|
| 1 | Primer-851 | 30-1150 | 6 | 4 |
| 2 | Primer-852 | 350-1200 | 8 | 8 |
| 3 | Primer-854 | 300-1150 | 7 | 4 |
| 4 | Primer-803 | 250-1350 | 8 | 8 |
| 5 | Primer-805 | 300-1150 | 5 | 4 |
| 6 | Primer-808 | 100-1200 | 2 | 2 |
| 7 | Primer-853 | 300-1150 | 4 | 2 |
| 8 | Primer-855 | 400-1600 | 8 | 8 |
| 9 | Primer-857 | 250-1100 | 7 | 7 |
| 10 | Primer-856 | 500-2200 | 4 | 4 |
| Total | | | 59 | 51 |

**Table.6** Jaccards similarity coefficient for ISSR profile generated by Agrose gel electrophoresis

| Genotype | HQPM-1 | HQPM-5 | HQPM-7 | EQH-16 | EQH-63 | PHM-1 | PHEM-2 | PHM-2 | BIO-9637 | HM-8 | Arawali | PM-3 | PM- 4 | PM-5 | Navjot | K.Sathi | PM-6 | K. Malan | EC-3161 | C. Sathi |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HQPM-1 | 1.00 | | | | | | | | | | | | | | | | | | | |
| HQPM-5 | 0.68 | 1.00 | | | | | | | | | | | | | | | | | | |
| HQPM-7 | 0.80 | 0.52 | 1.00 | | | | | | | | | | | | | | | | | |
| EQH-16 | 0.64 | 0.61 | 0.58 | 1.00 | | | | | | | | | | | | | | | | |
| EQH-63 | 0.80 | 0.66 | 0.72 | 0.69 | 1.00 | | | | | | | | | | | | | | | |
| PHM-1 | 0.78 | 0.61 | 0.67 | 0.67 | 0.78 | 1.00 | | | | | | | | | | | | | | |
| PHEM-2 | 0.75 | 0.55 | 0.64 | 0.51 | 0.74 | 0.69 | 1.00 | | | | | | | | | | | | | |
| PHM-2 | 0.75 | 0.65 | 0.67 | 0.58 | 0.78 | 0.69 | 0.75 | 1.00 | | | | | | | | | | | | |
| BIO-9637 | 0.69 | 0.56 | 0.54 | 0.52 | 0.62 | 0.66 | 0.66 | 0.73 | 1.00 | | | | | | | | | | | |
| HM-8 | 0.56 | 0.53 | 0.60 | 0.45 | 0.54 | 0.47 | 0.48 | 0.59 | 0.60 | 1.00 | | | | | | | | | | |
| Arawali | 0.48 | 0.46 | 0.39 | 0.56 | 0.52 | 0.59 | 0.45 | 0.45 | 0.54 | **0.36** | 1.00 | | | | | | | | | |
| PM-3 | 0.62 | 0.51 | 0.57 | 0.53 | 0.61 | 0.68 | 0.65 | 0.69 | 0.75 | 0.55 | 0.56 | 1.00 | | | | | | | | |
| PM- 4 | 0.54 | 0.47 | 0.54 | 0.50 | 0.55 | 0.48 | 0.46 | 0.54 | 0.61 | 0.73 | 0.42 | 0.66 | 1.00 | | | | | | | |
| PM-5 | 0.73 | 0.59 | 0.62 | 0.68 | 0.72 | 0.70 | 0.67 | 0.61 | 0.64 | 0.45 | 0.54 | 0.66 | 0.58 | 1.00 | | | | | | |
| Navjot | 0.80 | 0.56 | 0.76 | 0.55 | 0.76 | 0.71 | 0.80 | 0.72 | 0.66 | 0.54 | 0.46 | 0.68 | 0.55 | 0.73 | 1.00 | | | | | |
| B. sathi | 0.68 | 0.57 | 0.70 | 0.47 | 0.64 | 0.59 | 0.65 | 0.62 | 0.57 | 0.68 | 0.40 | 0.56 | 0.59 | 0.54 | 0.71 | 1.00 | | | | |
| PM-6 | 0.67 | 0.62 | 0.62 | 0.55 | 0.69 | 0.67 | 0.58 | 0.61 | 0.64 | 0.63 | 0.51 | 0.57 | 0.51 | 0.62 | 0.70 | 0.70 | 1.00 | | | |
| K. Malan | 0.66 | 0.73 | 0.68 | 0.53 | 0.65 | 0.56 | 0.51 | 0.60 | 0.52 | 0.65 | **0.36** | 0.48 | 0.56 | 0.54 | 0.63 | 0.73 | 0.71 | 1.00 | | |
| EC-3161 | 0.55 | 0.48 | 0.56 | 0.47 | 0.57 | 0.52 | 0.62 | 0.59 | 0.48 | 0.50 | 0.40 | 0.41 | **0.36** | 0.50 | 0.59 | 0.54 | 0.56 | 0.51 | 1.00 | |
| C. Sathi | 0.75 | 0.58 | 0.76 | 0.57 | 0.74 | 0.72 | 0.71 | 0.72 | 0.69 | 0.56 | 0.48 | 0.71 | 0.54 | 0.67 | **0.87** | 0.75 | 0.73 | 0.66 | 0.58 | 1.00 |

**Fig.1** Dandrogram constructed with UPGMA clustering method of 20 genotypes of *Z. mays* L. using RAPD primers

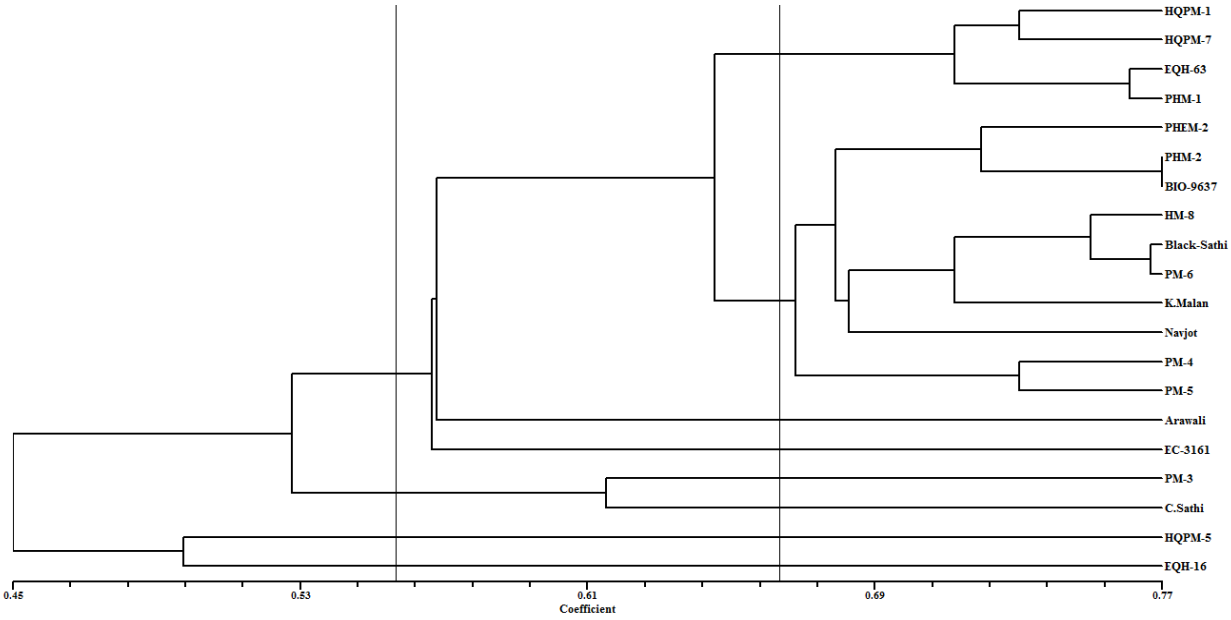
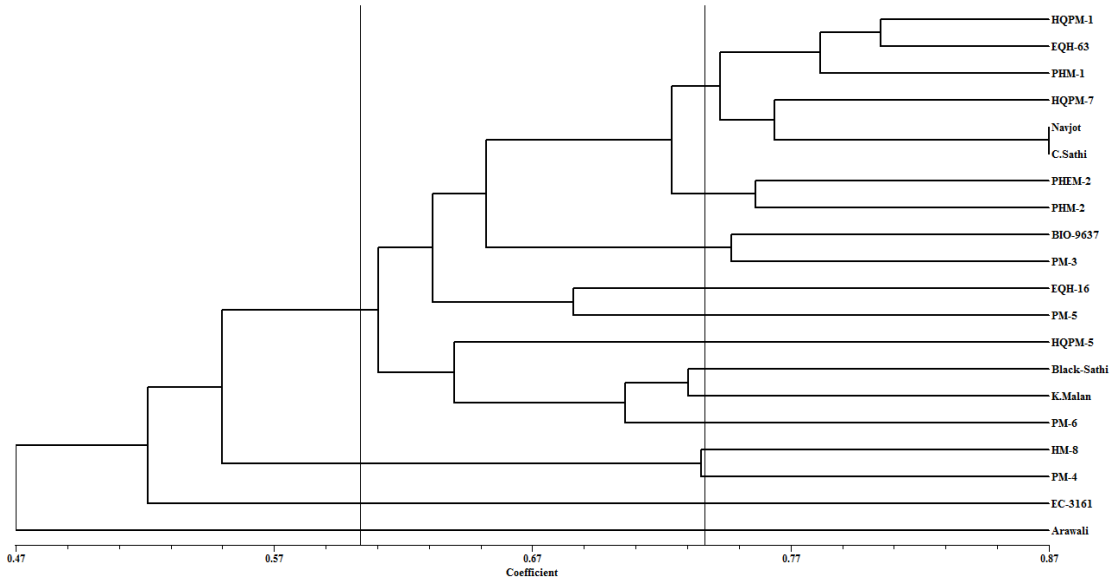



**Fig.2** Dandrogram constructed with UPGMA clustering method of 20 genotypes of *Z. mays* L. using ISSR primers




The second subcluster of cluster II was further divided into two main clusters at a similarity coefficient of 0.58 and out grouped genotype EC-3161, from all other genotype. The subcluster of cluster II was further divided into major cluster at a coefficient of 0.59. Here, first cluster contain only a single genotype Arawali, and second cluster are the major cluster and separated into two minor groups at a coefficient at 0.64. Minor I group contain 10 genotypes *viz*. PHEM-2, PHM-2, Bio-9637,

HM-8, B.sathi, PM-6, K.malan, Navjot, PM-4 and PM-5. While group II contain four genotypes, HQPM-1, HQPM-7, EQH-63 and PHM-1.

## ISSR band pattern

Fifteen ISSR primers were used for the present investigation, ten primers showed amplification in all genotypes except primer-802, primer-804, primer-806, primer-807 and primer-810. The 10 ISSR primers, total 59 amplified bands were observed of which 51 were polymorphic.

The total number of bands observed for every primer was recorded separately and polymorphic bands percentage was calculated subsequently (Table 5). The total number of amplified bands varied between 2 (primer-808) and 8 (primer-852) which an average 5.9 per primer. The polymorphism percentage ranged from as low as 50% (primer-853) to as high as 100 % in six primers (Primer-852, Primer-803, Primer-808, Primer-855, Primer-857, Primer-856). Average polymorphism across all the 20 genotype of *Z. mays* L. was found to be 86.44%. Overall size of PCR amplified products ranged between 100bp to 2200bp.

ISSR similarity matrices of 20 *Z. mays* L. genotype revealed the relationship among them (Table 6). The similarity indices between different genotypes ranged from 0.36 to 0.87 i.e. 36-87%. The average similarity across all the genotypes was found out to be 0.61, showing that genotypes were highly polymorphic. Maximum similarity value of 0.87 was observed in C.sathi and Navjot. While minimum similarity range was observed in three genotype group *viz*., Arawali, HM-8, K.malan, Arawali and EC-3161, PM-4. The low ranged of similarity showed that genotypes are genetically more diverse and highly polymorphic.

The ISSR cluster analysis of 20 *Z. mays* L. genotype showed that they were mainly divided into two major clusters at similarity coefficient of 0.47 (Fig. 2). Genotype Arawali was out-grouped from all other genotype at a similarity coefficient of 0.47 and formed the first cluster. At a coefficient of 0.52 was the second cluster having all other genotypes. Genotype EC-3161 was again out grouped and formed another solitary cluster at a similarity coefficient of 0.52. The second subcluster of cluster II was further divided into two main cluster at a similarity coefficient of 0.58 and out grouped genotype HM-8, PM-4, from all other genotypes. The subcluster of cluster II was further divided into two clusters at a coefficient of 0.62. Cluster first contained only four genotype *viz*. HQPM-5, B.sathi, K.malan and PM-6 and second cluster was further separated into two groups- major and minor, at a coefficient of 0.64. Minor group consisted genotype EQH-16 and PM-5. Major group consisted of the remaining 10 genotypes.

The association amongst different genotype presented in the form of dendogram, the genotype which lay nearer to each other in dandogram was more similar to one another then those lying apart. The dandogram also showed the relative magnitude of resemblance among different genotype of *Z. mays* L. used in current investigation.

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