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Phylogenetic Analysis of *Mangifera* Using RAPD Molecular Markers

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ABSTRACT:

In India, where such a high diversity of mango cultivars originated and exists, ambiguities in cultivar identification and nomenclature are also common especially in case of the cultivars that exhibit prominent similarities in their morphological features. This study used the RAPD method which has advantages over other kinds of DNA-based genetic markers. Total six species of Mango were collected from GKVK Bangalore. Genomic DNA was extracted by Phenol: Chloroform method. The DNA samples were tested qualitatively on 0.8% agarose gel and quantified by using a Nanodrop spectrophotometer (Thermo Scientific). Out of 20 primers, 10 showed amplification. Five primers generated reproducible polymorphic DNA amplification patterns in all the 6 genotypes. These 5 primers yielded a total of 120 scorable bands on amplification and their sizes ranged between 200 bp to 2000 bp. Separation of non-Indian cultivars from the Indian cultivars was revealed in the cluster analysis, however, no such separation was revealed between the north Indian and the south Indian cultivars.

Keywords: Mango, RAPD, PCR, Dendrogram, Diversity

INTRODUCTION:

Mango, the choicest fruit of India is rightly titled as the ‘King of fruits’ because of its wide adaptability, high nutritive value, richness in variety, delicious taste, excellent flavor, attractive appearance and popularity among masses. Over the various mango growing regions, mango breeding attempts are always on for creating better cultivars. Precise information on the genetic relationships within such germplasm diversity is always needed for carrying out efficient breeding programs [3]. Based on the aroma profile, mango cultivars are classified as Indian and Indo-Chinese. Indian type mangos possess intense aroma whereas the Indo-Chinese have mild one. It has already been noted that the people accustomed to the later type, perceive the Indian type as medicinal or having turpentine flavor [2].

There is a considerable confusion regarding cultivar identification, because as presently several mango cultivars of many synonyms in different regions which make identification difficult. Similar cultivars grown in different areas are known by different names. In order to assess the genetic diversity in mango, PCR based DNA markers are considered be the best

tools [1]. Most of the available literature suggested that mango cultivars differ from each other by qualitative as well as quantitative variation. In the similar way, these cultivars also differ in the possession of different chemical classes. Furthermore, each cultivar has been observed to have one quantitatively dominant compound [4]. Few other studies have also reported such basis of diversity in mango [5,6]

A few efforts have been made to unveil the genetic diversity among the mango cultivars. Already, a range of DNA markers AFLP, ISSR, ITS and RAPD have been used for exploring the diversity of the global mango germplasm [7-11]. These analyses have put forth several facts about mango such as mango cultivars have not diverged too much on the genetic scale, mono- and polyembryonic mangos have different genetic bases and mangos from different geographical zones differ genetically. This study use d the RAPD method which has advantages over other kinds of DNA-based genetic markers; it is relatively quick, easy to perform, cheaper, highly informative, need not only prior information of template DNA sequence and synthesis of specific markers.

MATERIALS AND METHODS:

Sample Collection

The leaves of six species of Mango were collected from different parts in and around Karnataka and Andhra Pradesh.

DNA Extraction:

Genomic DNA was extracted by Phenol: Chloroform method. Mango leaf were grinded in extraction buffer and incubated at 60 ^{0} C for 1 hr. DNA was purified once with equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) and once with chloroform: isoamyl alcohol (24:1) and precipitated using 0.8 volumes of chilled isopropanol. The DNA samples were tested qualitatively on 0.8% agarose gel and quantified by using a Nanodrop spectrophotometer (Thermo Scientific).

PCR Amplification:

The polymerase chain reaction was carried out in final volume of 25 µl containing 100 ng DNA, 1 U of Taq DNA polymerase (Chromous Biotech, Bangalore), 2.5 mM MgCl (Chromous Biotech, Bangalore), 2.5 mM each dNTPs (Chromous Biotech, Bangalore) and 100 p mol of primers (GeNei, Bangalore). The DNA amplification was performed in the Corbett RG 6000 thermo cycler using the following conditions: complete denaturation (94°C for 5 min), 10 cycles of amplification (94°C for 45 sec, 35°C for 1 min and 72°C for 1.5 min) followed by 30 cycles of amplification (94°C for 45 sec, 38°C for 1 min and 72°C for 1 min) and the final elongation step (72°C for 5 min). All PCR products were separated on 1.5% (w/v) Agarose gel containing ethidium bromide (0.5 µg / ml). The gel was photographed with HP Alpha-imager.

Data Analysis:

The RAPD profiles were analyzed based on the presence or absence of individual RAPD bands. The genetic distance was calculated by the coefficient of similarity of Jacccard. The matrix of genetic distance was used for grouping the mango cultivars based on the dendrogram constructed by UPGMA (unweighed pair group method with Arithmetic averages)

RESULTS AND DISCUSSION:

In the present work polymerase chain reaction (PCR) based RAPD marker system was used to probe the relationships among 6 mango cultivars that included South Indian cultivars. RAPD stands for Random Amplification of Polymorphic DNA is a type of PCR reaction, but the segments of DNA that are amplified are random. No knowledge of the DNA sequence for the targeted gene is required, as the primers will bind somewhere in the sequence, but it is not certain exactly where. This makes the method popular for comparing the DNA of biological systems in which relatively few DNA sequences are compared. Out of 20 primers, 10 showed amplification. Five primers generated reproducible polymorphic DNA amplification patterns in all the 6 genotypes (Figure 1-5).

In India, where such a high diversity of mango cultivars originated and exists, ambiguities in

cultivar identification and nomenclature are also common especially in case of the cultivars that exhibit prominent similarities in their morphological features. It is essential to authenticate the identities of such cultivars as well as analyze the diversity among the existing cultivars. These 5 primers yielded a total of 120 scorable bands on amplification and their sizes ranged between 200 bp to 2000 bp. Separation of non-Indian cultivars from the Indian cultivars was revealed in the cluster analysis, however, no such separation was revealed between the north Indian and the south Indian cultivars (Figure 6).

CONCLUSION:

RAPD technique is an appropriate technique for determing intra genera relationship. The analysis is proved to be a rapid, inexpensive and useful tool for assessing phylogenetic relationships in plant species and here it is successfully used to reveal the genetic diversity among the 6 different mango cultivars. Not only the DNA-based genetic markers are useful for varietal identification but also further estimation of genetic diversity and relatedness between mango accessions.

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Figure 1: DNA fingerprint for OPA 1

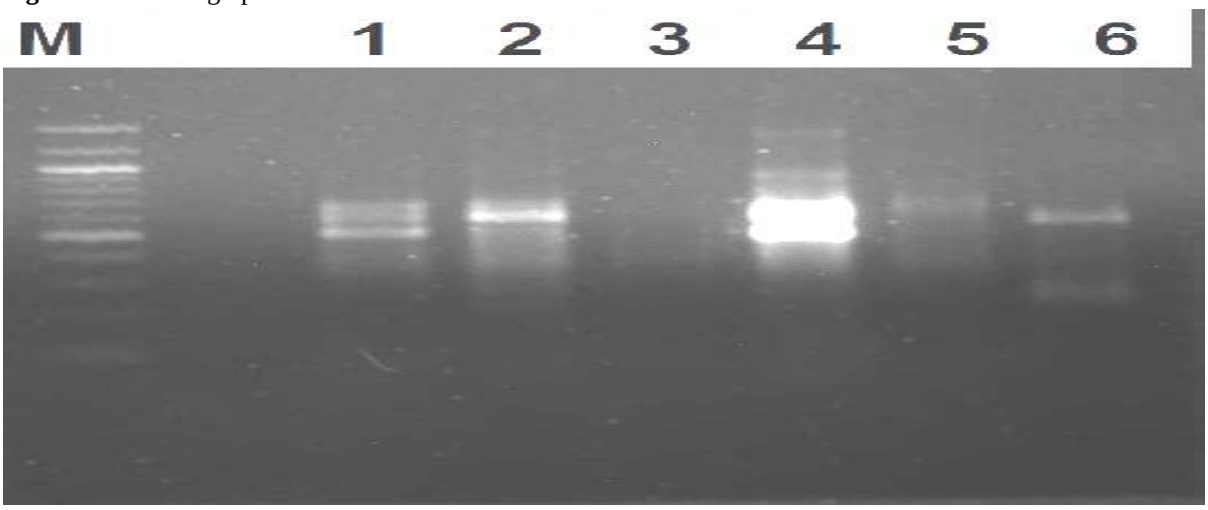

Figure 2: DNA fingerprint for OPA 5

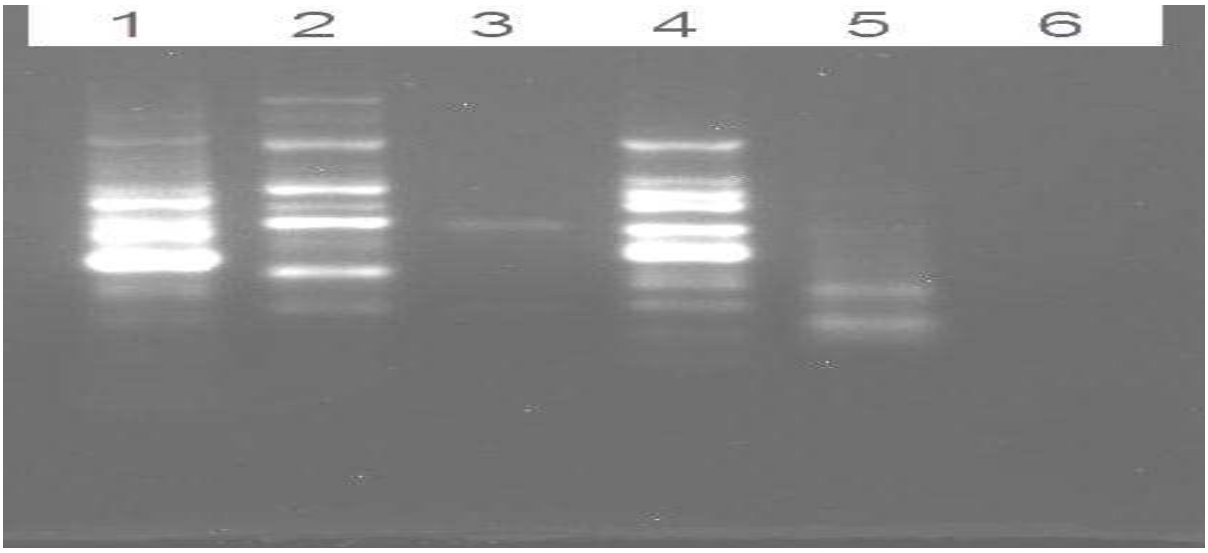

Figure 3: DNA fingerprint for OPA 11

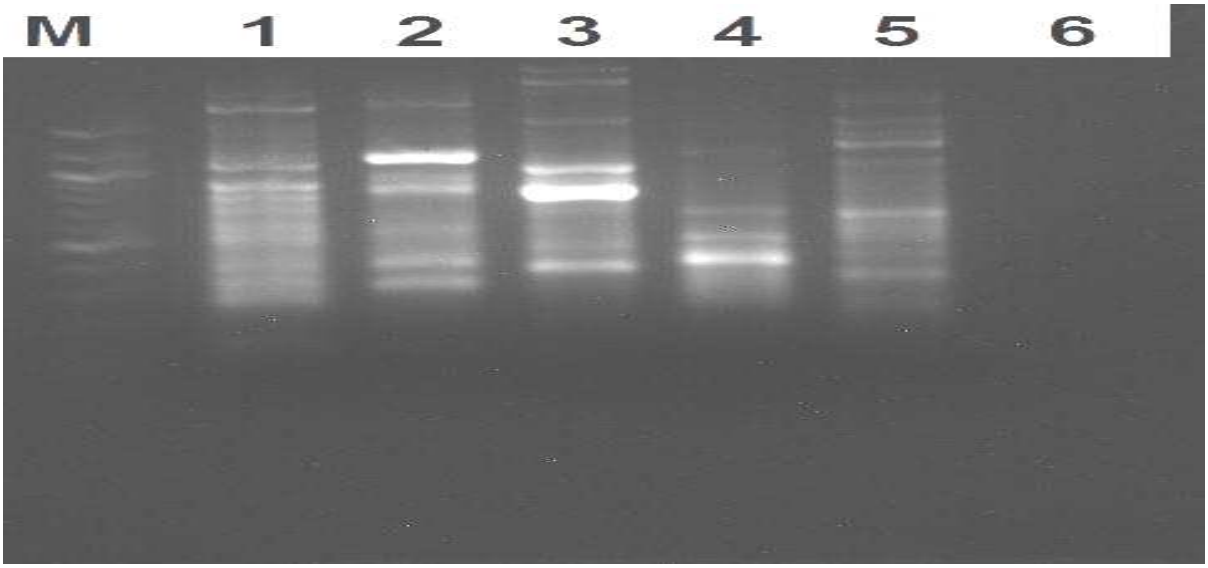

Figure 4: DNA fingerprint for OPA 12

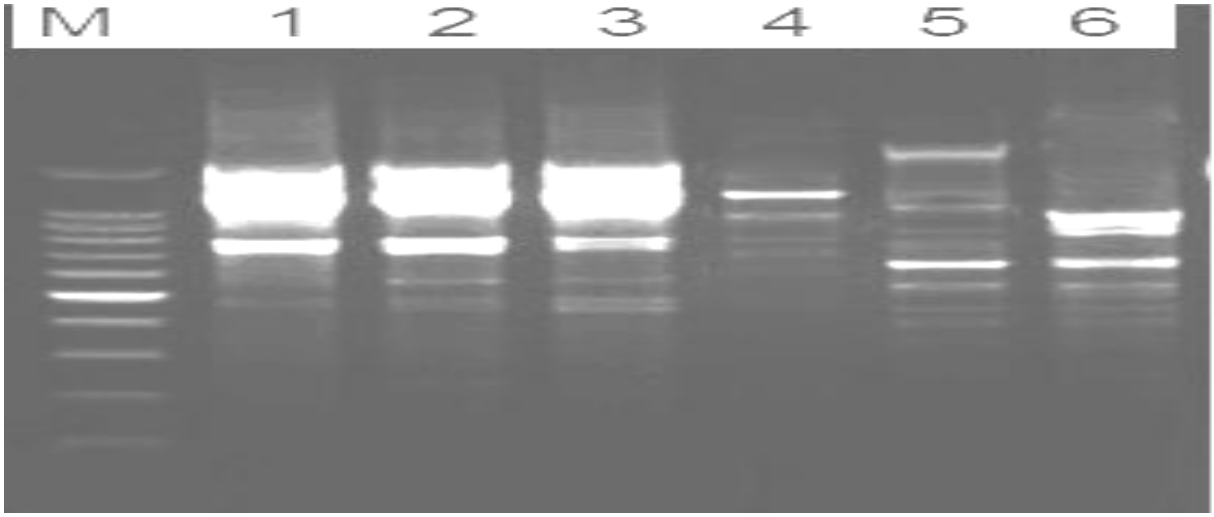

Figure 5: DNA fingerprint for OPA 18

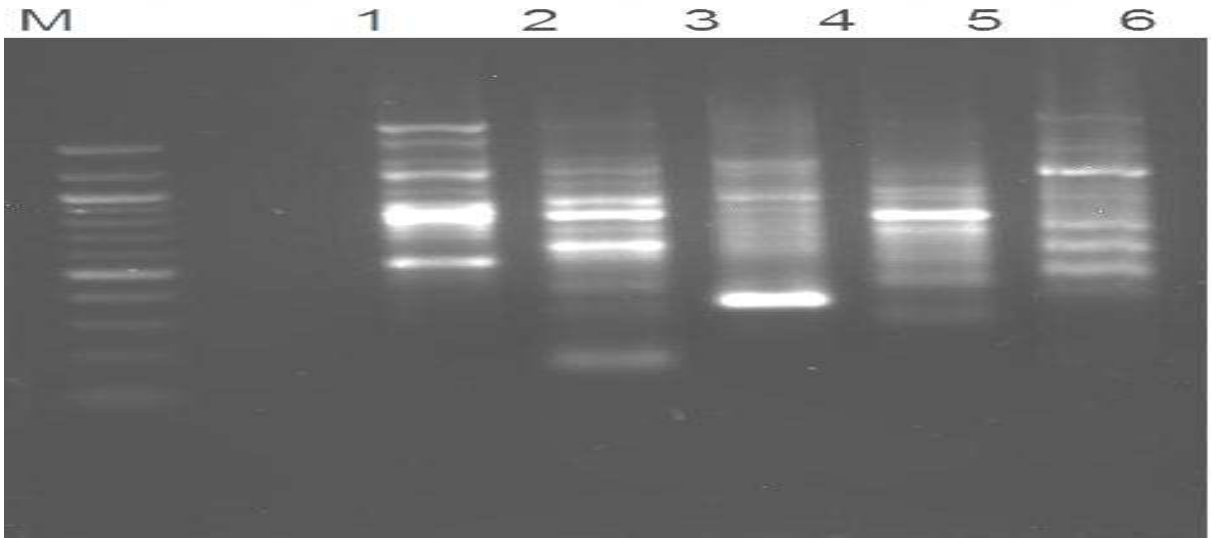

Figure 6: Dendrogram for all samples