



MOLECULAR ANALYSIS FOR GENETIC DIVERSITY OF MANGO (*MANGIFERA INDICA* L.) BY RAPD-PCR MARKER

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ABSTRACT

India is the largest producer of *Mangifera indica* L. commonly called mango. “The king of the fruits”, mango is one of the most popular nutritionally rich fruit with anti-diabetic, antioxidant, antiviral, cardiotoxic, hypotensive, anti-inflammatory properties. In India, the phylogenetic relationships among the species of *Mangifera indica* L. are poorly understood. So the present study on “Molecular characterization of Mango is done by adopting RAPD-PCR markers. A total of 99 amplicon levels were produced by 4 primers available for analysis. The highest number of 24 amplicon levels was produced by OPK-04 followed by 23 in OPK-06 and the least of two marker levels was produced by OPB -01. Out of total 99 scorable bands 2 unique bands were produced by primer namely OPB-01. The presence of high genetic diversity (71%) indicates that the population has plenty of scopes for evolution to occur which could be exploited in plant breeding programmes.

KEYWORDS: anti-diabetic, antioxidant, antiviral, cardiotoxic, hypotensive, anti-inflammatory, 24 amplicon.

INTRODUCTION

Mangifera indica L. “the common mango” is an evergreen tree of family Anacardiaceae. It is cultivated in many tropical and subtropical regions. It is native to South and Southeast Asia, from where it has been distributed worldwide to become one of the most cultivated fruits in the tropics. The center of diversity of the *Mangifera* genus is in India. India is the largest producer of Mango with 36% of the world’s population. Mango possesses anti-diabetic, antioxidant, antiviral, cardiotoxic, hypotensive, anti-inflammatory properties. In Hindu religion, Mango tree holds a lot of religious importance. “The king of the fruits”, mango fruit is one of the most popular nutritionally rich fruit with unique flavor, fragrances, taste and health promoting qualities, making it numero-uno among new functional foods, often labeled as “Super fruit”. PCR based molecular markers are widely used in plant species for identification, phylogenetic analysis, population studies and genetic linkage. RAPD has also been widely use in studying the genetic variation, classification of various cultivars and genetic mapping. In India, the phylogenetic relationships among the species of *Mangifera indica* are poorly understood. As traditionally plant identification was relied on morphological characters like flower color, growth habit and other characteristics of plant. So in the present study RAPD-PCR markers are utilized for genetic fingerprinting of Mango.

MATERIALS & METHODS

The present study on “Molecular characterisation of *Mangifera indica* L. using RAPD-PCR marker” was undertaken at GGSDS College, sector 32, Chandigarh by adopting the following methodology: The fresh and young leaves of *M. indica* L. were collected from different locations in and around Chandigarh Table 1. The leaves from each genotype were harvested separately. Mid rib of

leaves were removed and leaf pieces were surface sterilized with absolute alcohol followed by washing with autoclaved distilled water. DNA isolation was done by modified CTAB protocol given by Doyle and Doyle (1990) from the leaves of *M. indica*. The RAPD reaction was performed according to the method developed by McClelland *et al.*, 1995. A set of 30 random decamer primers were purchased from Operon technologies to be used as a single primer for the amplification of RAPD fragments. Primers were screened for the presence of consistent and distinct bands. The reaction was carried out in 25µl volume in a tube using decamer primers of series OPA-01 to OPA-10, OPB-01 to OPB-10 and OPK-01 to OPK-10. Each reaction tube contained 50ng template DNA, MgCl₂ (100mM), dNTP’s (100mM), 3 U/µl of Taq DNA polymerase (Sigma aldrich, USA). The amplification was performed in a DNA thermal cycler (Biorad) 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 5min). (0.5 µg / ml). PCR products were resolved in 1.5 % agarose gel in 1X TBE buffer with 1kb molecular weight marker. The PCR amplified products were visualized and photographed under a U.V transilluminator. Electrophoretic profile were analysed for polymorphism based on the presence and absence of DNA bands on agarose gel. The sizes of DNA fragments were estimated by comparison with standard ladder (100 bp, Sigma aldrich, USA).

Data analysis

For RAPD analysis, the bands with same molecular weight and mobility were treated as identical fragments. The gel pictures acquired through gel documentation system into the computer were processed and scored to obtain binary

data. The amplified products were recorded as present (1) or absent (0) for the individual genotypes. Similarity index was estimated using Jaccard's similarity indices. Cluster analysis was carried out on similarity estimates using the UPGMA method using NTSYS pc, version 2.0.

RESULTS & DISCUSSION

The DNA extracted from ten genotypes of *Mangifera indica* was pure and of a great quality. For accuracy of the results, the high quality and purity of genomic DNA free from secondary metabolites was isolated from these genotypes by modified CTAB method for RAPD reaction, it was necessary to standardize the variables used for the successful amplification of PCR. RAPD amplification is not reproducible below a certain concentration of genomic

DNA. DNA quantifications were performed by visualizing gel under U.V light, after electrophoresis on 1.5 agarose gel at 60 volt for 2hours. DNA isolation gave a good yield of concentration and purity.

30 random decamer primers given in Table No.1 were used for amplification of 10 genotypes of mango. Out of 30 primers, only 4 random decamer primers generated polymorphic bands and rest have produced only monomorphic bands. They showed 99 reproducible bands among 10 genotypes. All the four primers were able to give high polymorphism among the isolates. The size of the obtained amplicons ranged from 300 -800 kb. Association among 10 genotypes revealed by Unweighted Pair- group method with arithmetic mean (UPGMA) cluster analysis is presented in figure 1.

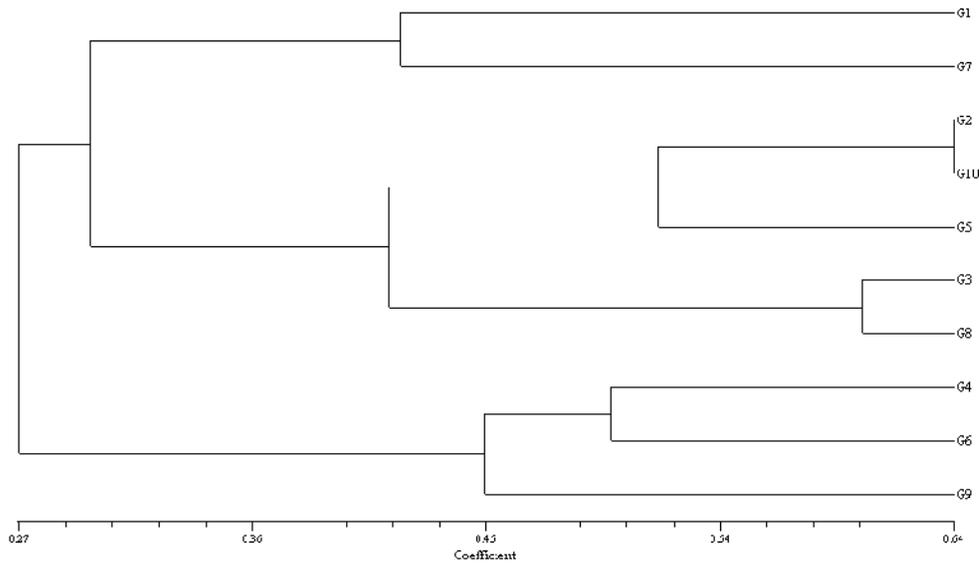


FIGURE 1: Dendrogram showing genetic variation among the ten genotypes of *M. indica* L

Availability and assessment of genetic variation are central to the improvement of any crop species. The similarity coefficients generated from RAPD data were used to construct dendrogram. The cluster analysis comprising that the 10 genotypes showed 27% to 64% similarity index. The main cluster is divided into two subclusters, Subcluster 1 has 7 genotypes which include genotypes G1(sector 46), G7(sector 11), G2(sector 48), G10(sector 49), G5(sector 40),G3(phase 10 Mohali) and G8(sector 4) whereas subcluster 2 includes three genotypes *i.e.* G4 (sector 38), and G6 (sector 49) and G9 (phase 11, mohali). The first subcluster is 27% similar to that of second subcluster which revealed a high level of polymorphism between the genotypes categorized in these two different subclusters. The distributions of populations in different clusters indicates that even though the samples were selected from same geographical areas, the genetic drift, natural and unidirectional selection pressure and human intervention by transferring specimen from one generation to another may be the cause of high level of genetic diversity among different populations (Kiambi *et al.*, 2005, Ward *et al.*, 2005). It is also interested to find out that the samples collected from nearby sectors *i.e.* G3 (phase 10 Mohali) and G9 (phase 11, Mohali) were also

found to be variant. However, G2 sample collected from sector 48 Chandigarh and G6 sample collected from sector 49 Chandigarh were found to be the most related with similarity index of 64 percent. Since *M. indica* is an allogamous crop species, a high inter-population variation is expected (Marshal and brown, 1975).

RAPD was found useful in revealing genetic variation between different species of *M. indica*. All 4 primers clearly differentiate ten mango genotypes that showed 71% polymorphism. All the 4 gels resulting from short-listed primers had maximum number of clear and scorable amplicons in each DNA sample with few ghost or minor bands, which were ignored. A total of 99 amplicon levels were produced by 4 primers available for analysis. The highest number of 24 amplicon levels was produced by OPK-04 followed by 23 in OPK-06 and the least of two marker levels was produced by OPB -01. Out of total 99 scorable bands 2 unique bands were produced by primer namely OPB-01. The maximum numbers (2) of unique bands were produced in G1 collected from sector 46 by OPB-01. This primer should be employed for identification and isolation of elite accessions.

A high level of polymorphism (73%) based on RAPD in accessions of *M. indica* is primarily due to the wild species included in the present study. Gene flow from wild and

domesticated crops remains an important source of variation for their evolution (Jarvis and Hodgkin, 1999). High genetic diversity is necessary for improvement use and conservation of plant genetic resources (Krishna and Singh, 2007). The present studies and similar studies on many crop species *Dactyloctenium aegyptium* (Thakur and Kaur, 2013), in *Bunium persicum* (Majid *et al.*, 2008, pruthvish *et al.*, 2016 and Souza, 2011) in Mango, found RAPD-PCR markers best for analysis of genetic divergence studies. The dendrogram (Figure 1) based on NTSYS-pc version 2 showed distinct separations of the collected populations, which have a fair level of variation at the genetic level. The data present here reflects the utility of RAPD in the analysis of genetic divergence within this super fruit. The presence of high genetic diversity indicates that the population has plenty of scopes for evolution to occur. On the basis of present investigation, it could be concluded that there is tremendous genomic variability and scope of development of new plant varieties of *M. indica* genotypes collected from in and around Chandigarh.

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