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Genetic Diversity and Relationships among Mango Varieties using RAPD Molecular Markers

R. Pruthvish¹ and B.K.Chikkaswamy^{2*}

¹Department of Biotechnology, Acharya Institute of Technology, Soladevanahalli
Hesaraghatta Road, Bangalore, India

²Sigma BioScience Research Center, Indira Nagar Bangalore-560038, India

**Corresponding author*

ABSTRACT

Keywords

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Mango is the genus of tree & bushy tree in the family Mangnoliaceae. Most of the Mango varieties are dioceous and cross-pollinate to produce to produce fertile hybrids suggesting a closer genetic relationship which is not expected at species level. The traditional methods using morphological characters are not successful in establishing the diversity and relationship among 19 different Mango varieties because of the environmental influence. PCR based molecular marker method, RAPD was employed to study the genetic diversity and Inter-relationship among 19 Mango varieties. On an average RAPD analysis generated 5-6 discrete bands/ varieties with 10 nucleotides primers. The size of the amplified products ranged from 100-3500 base pairs in length. With an average of 5-10 bands per primer. Of 21 amplified fragments 50 were polymorphic (80%) with at least one pair wise comparison between 19 varieties. RAPD analysis identified varieties specific amplification products which will be useful in germplasm classification and introgression studies. These results indicated that RAPD based markers are useful for genetic characterization of Mango /varieties/accessions.

Introduction

Genetic fingerprinting has been accomplished traditionally through the use of isozymes, and more recently through reactions fragment length polymorphisms (RELPS), variable number tandem repeats (VNTRs), or a combination of these. While these methods have been very useful in cultivar identification, they have a number of disadvantages, including a limited number of isozyme loci, and the time,

expense, and use of (32 P) for labeling with RELPs and VNTRs. Polymerase Chain Reaction (RAPD) uses arbitrary 10-base primers to amplify random portions of the genome (Welsh and McClelland 1990; Williams et al. 1990). The fragments produced are easily visualized on an ethidium bromide stained gel, and polymorphisms between genotypes reflect heritable differences in the genome. Large

numbers of bands, or loci, can be generated with relative ease. Due to the arbitrary nature of the primers, RAPD markers, unlike RELPs, VNTRs, and isozymes, represent a random sample from the entire genome. They are, however, inherited as dominant markers, requiring larger numbers of loci to be identified and screened to glean the same information as from RELPLs, VNTRs, and isozymes. Amplification of bands in the progeny which were not amplified in either parent has also been reported (Riedy et al. 1992).

The use of RAPDs to determine genetic relationships has been demonstrated in maize (Welsh et al. 1991), conifers (Carlson et al. 1991), cacao (Wide et al. 1992), and various leguminous species (Chalmers et al. 1992, Echt et al. 1992). Within *Mangifera* (mango) species RAPDs have been used to determine phylogenetic relationships (Schnell and Knight 1993). On the basis of the classification of Kostermans and Bom.

RAPD fingerprinting techniques have been used for the identification of horticultural crop varieties, description of cultivars genotypes and for protecting breeder's rights (Williams et al., 1990) enabling assay to be performed at any stage of plant development. RAPD markers have been extensively used to distinguish intraspecific genetic variation in ornamental crops and detection of hybrids and clones (Collins et al., 2003; Arus, 2000; Debener, 2001a).

Ranamukhaarachchi et al. (2001) showed that RAPD markers had the ability to identify pot-plant mango cultivars is crucial to local breeders involved in hybridization programs for varietal development but is currently lacking. In this study, RAPD markers were used to evaluate the extent of genetic variation among some mango cut flower cultivars.

Until recently, several promising species released formally are characterized on the basis of morphological data, mango content and yield potential. Worldwide demands on mango necessitate work on conservation of mango germplasm and their further genetic improvement. Therefore precise characterization of promising species and determination of genetic variation among those are felt necessary.

Until recently, several promising species released formally are characterized on the basis of morphological data, mango content and yield potential. These characters differ under varying environmental condition thereby posing problem identification of species. Unlike morphological markers, cytological (chromosome numbers, nuclear DNA content) and molecular markers (RAPD, AFLP, ISSR etc.) are not prone to environmental influences and characterize the plants portraying the extent of genetic diversity among taxa (Bennett 1987, Bennett and Smith 1991, Waugh and Powell 1992, Chalmers et al. 1994, Das et al. 1998, Rodriguez et al. 1999, Das et al. 2001).

Of the different markers, RAPD has been widely used in the last decade in species identification programmed (Schnell et al. 1995) and in assessing genetic variations among different taxa at DNA level because of its cost effectiveness and simple operation without requiring prior knowledge of species DNA sequences (Williams et al. 1990, Frankel et al. 1995). RAPDs reveal similar patterns of genetic diversity when compared with other marker types and can be performed more rapidly than most other methods (Morell et al. 1995) and can provide vital information for the development of genetic sampling, conservation and improvement strategies (Waugh and Powell 1992, Chalmers et al. 1994). No report has been published so far either on the genetic

characterization or on the extent of genetic variations existing among promising species of mango.

The present study deals with the in situ DNA estimation and RAPD analysis of 19 promising species of Mango to identify and evaluate extent of genetic variation existing among these.

As the efficiency of selection scheme or genetic analysis based on phenotype is a function of heritability of the trait, factors like environment, traits of mutagenic and quantitative inheritance or partial and complete dominance often confound the expression of genetic traits. Many of these complications of a phenotype-based assay can be overcome through direct identification of species with DNA based diagnostic assay. For this reason DNA based genetic markers are being integrated into several plant systems under expected to play an important role in the future plant improvement programmes.

Advent of Polymerase chain reaction (PCR) technology as lead to the development of several novel genetic assays based on selective DNA amplification Krawetz (1989) and Innis et al (1990). RAPD assay detects nucleotides sequence of polymorphism in DNA using only a single primer of arbitrary nucleotide sequence. The protocol is also relatively quick and easy to perform and uses fluorescence instead of radioactivity. Because the RAPD technique is amplification- based assay, only nanogram quantity of DNA is required. One of the strengths of these new assays is that they are more amenable to automation than conventional techniques. It is simple to perform and is preferable to experiment; their species of large number of individuals are to be determined at a few genetic loci. The present investigation will be carried

out on RAPD molecular marker studies in varieties of mango plants with the following objectives. To develop protocol for isolation of DNA from different 19 varieties of mango plants. To develop PCR protocol and to identify RAPD markers to score in various mango plants. To assess the genetic diversity and relationships among mango plants using RAPD molecular markers belonging to family magnoliace.

Matias Kirst et.al.,(2004) studied on DNA marker RAPD,RFLPs and AFLP etc in forest tree species and in particular its application to tree breeding and tree genomics.

Million and Chinnappa (2000) assessed the genetic divergence in forty seven genotypes of

Stellaria longipes (Caryophyllaceae) using RAPD analysis. Datta and Mitrick et.al.,(1997) studied the classification of common bean *Phaseolus vulgaris* from Chile using RAPD data. The study reveals that 95 bean accessions analysed using 25 primers that generated 106 polymorphic bands of RAPD collected from different locations.

Nirupama et.al., (2003). Reported genetic diversity and relationship of 51 accessions of *Vetiver* using RAPD/AFLP analysis. Total of 20 primers were used to generate 5 clusters using UPGMA cluster analysis where as for AFLP analysis nine primers are used to generate 383 unique bands specific to different accessions compared 81 monomorphic bands. In contrast sufficient diversity existing with in the wild and cultivated. Indian germplasm. Raghvendra saxena and Amaresh Sanghamitra Nayak et.al. (2006) studied the 4C nuclear DNA content and RAPD analysis of 17 promising cultivars of turmeric *Curcuma longa* of

zingiberaceae is an important spice and anticancer properties and also showing differential genetic variation among cultivars. The polymorphism ranged from 35.6% to 98.6%.

Schnell et.al.,(1995) studied the identification of cultivars and genetic relationships in *Mangifera indica* using RAPD technique. 25 accessions of mango were examined using 80 primers. Of the 80 primers 33 did not get amplified 19 were monomorphic and 28 gave

Materials and Methods

The present study selected leaf samples of the 19 mango plants plants that were collected from the conservatory of Biotechnology Centre, Hulimavu, Department of Horticulture, Bangalore, Karnataka, India. Which may represent the wide variation prevalent in the genome The recently matured leaves were collected and used for DNA extraction.

Porbeski et al. (1997) described a relatively quick, inexpensive and consistent protocol for extraction of DNA from expanded leaf material containing large quantities of polyphenols, tannins and polysaccharides. Mature strawberry leaves, which contain high levels of the secondary components, were used as a study group. The method involved a modified CTAB extraction, employing high salt concentrations to remove polysaccharides, the use of polyvinyl pyrrolidone (PVP) to remove phenols, an extended RNase treatment and phenol-chloroform extraction. Average yields ranged from 20-84µg/g, mature leaf tissue for both wild and cultivated octoploid and diploid *Mango*. Results from 19 plants were examined and were consistently amplifiable in the RAPD reaction with as little as 0.5ng DNA per 25µ reaction. Presently this is the first procedure for the

isolation of DNA from mature strawberry leaf tissue that produces consistent results for a variety of different species, both octoploid and diploid, and is both stable and PCR amplifiable before and after extended storage.

Quantification of DNA

DNA quantification can be done by flurometry, spectrometry and agarose gel electrophoresis with standard DNA concentrations (Boiteux et al., 1999). The quality can be assessed by restriction digestion with restriction endonucleases (EcoRI, HindIII etc.) electroporation and spectral properties. Quality is that to what extent the DNA is pure of secondary metabolites and other substances, which hinder further use of DNA in molecular techniques. A good DNA preparation generally exhibits the following spectral properties. It will have A_{230} , A_{230}/A_{260} , A_{280}/A_{260} or A_{260}/A_{280} ratios of less than 0.10, less than 0.45, less than 1.65 or more than 1.80, respectively (Shantha et al., 1998). If a DNA preparation exhibits A_{260}/A_{280} more than 1.80, it shows the presence of RNA and if it is less than 1.65 or less indicates protein contamination (Sambrook et al., 1989).

Results and Discussion

The data obtained in the present study regarding RAPD Molecular Marker studies in 19 Mango varieties The present study reveals that RAPD markers are good choice for assessing the genetic diversity and relationship in Mango varieties with polymorphism levels enough to establish informative fingerprints with a few markers. The information obtained could be of practical use for mapping the mulberry genome as well as for classical breeding. The study also provides a closer basis for

mango breeders to make informative choice on selection of parental materials based on genetic diversity and overcome the problem usually associated with mango crop improvement. The important primers identified will be useful for molecular characterization of gene bank accessions.

19 collections of Mango varieties (Alphonso, Bangalora, Mulgoa, Neelum, Pairi, Banganapalli, Bombay, Bombay Green, Chausa, Dashehari, Fazli, Fernandian, Himsagar, Kesar, Kishen Bhog, Langra, Mallika, Mankurad, Totapuri) which are distributed in different regions Bangalore, are from diverse origin and possibly represent the genetic diversity existing in the species table 1 OPD 1-12.

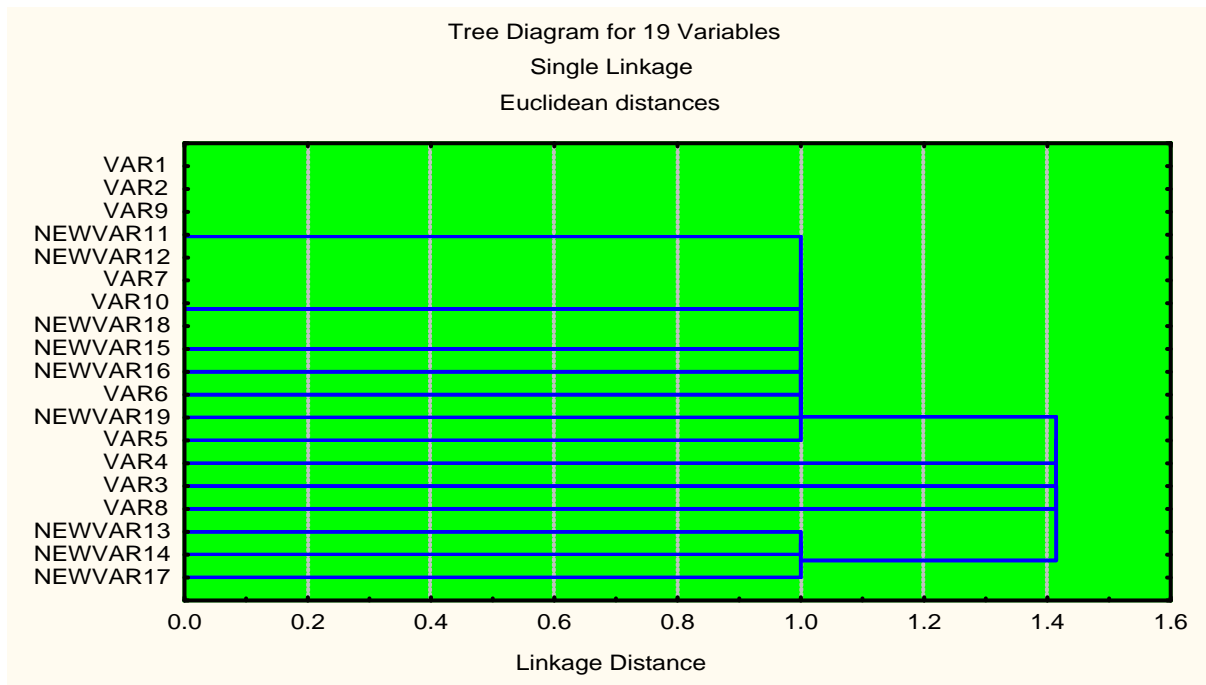
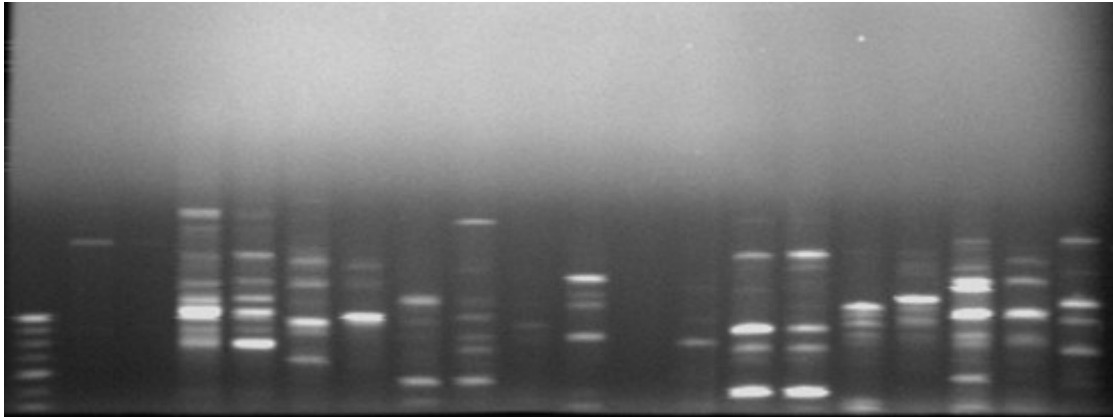
The RAPD analysis: The random primers amplified with genomic DNA of different Mango varieties generated 55 RAPD bands in the size ranged from 1000 base pairs in length. The number of bands obtained per primer ranged from 5-6 with an average of 11 with exception in some of the lanes where no amplification and bands formation has not taken place was observed. A total of 80% bands was polymorphic. The complete amplification details are presented in the table 2. DNA profiles generated by OPD 1-12 are shown in fig 1

The genetic analysis of RAPD markers based on Ward's method of Euclidean distance showed genetic similarity among the collection of Mango varieties Fig 2. The collection of Alphonso & Bangalora was closest with the maximum similarity (95%) whereas Mulgoa and Neelum forms separate cluster than other varieties. Euclidean Ward's method clustering based on RAPD data showed clear separation of collections fig 2. Some of the Mango varieties are

available abundantly with in 19 Mango species even though a few species of Mango occur naturally in India and Karnataka many cultivated species of Mango varieties do not find their origin outside the country of some of the cultivated Mango accessions which are classified as different species, cross pollinate with each other and produced fertile offspring showing no signs of sexual incompatibility characteristics of species. This fact suggests a close genetic relationship among the cultivated Mango species. The present investigation involving 19 Mango varieties and its RAPD analysis further supports this view.

The high level of polymorphism (80%) in Mango varieties reflects out crossing nature on par with results obtained with RAPD in other fruits and nut tree species such as Pistachio (Hormaza 1995), olive (Fabbri et.al.,1995),walnut (Nieise et.al.,1998) and vegetatively propagated crop such as banana (Krammer et.al.,1992) and apple (Koller et.al.,1993).Similar results was reported by Schnell et.al., (1995) Karinaloo et.al., (2003) & Maria cristina et.al., (2006).The molecular analysis using RAPD markers did not show corelation between the ploidy and number of polymorphic bands suggesting the redundancy of genome complements in these species. The ploidy level of a plant does not appear to influence the number of fragments per primer (Wolf and Peter Vanrijn 1993).In Mango varieties percentage of similarity based on F value ranged 80 to 30 most of the Mango pairs had shown similarity value some of the morphological variation and improvement in agronomic traits, leaf yield have been achieved through selective cultivation and breeding programmes, there appears to be effort has been made to utilise the useful trait found in Mango varieties.

Fig.1 Gel Diagram of Mango Using Opd 1-12 for 19 Mango Varieties



The present study has made an attempt to assess the genetic diversity among 10 collection from different region of Bangalore. Morphological traits are greatly influenced by the environment. It has always been difficult to accurately assess the diversity and interrelationship among Mango varieties similar report was reported by (Awasthi et al., 2004) on mulberry plant. DNA marker such as RAPD and ISSR have become a handy tool for quick and reliable estimation of diversity for crop

improvement and conservation programme in mulberry (Sharma et al., 2000).

The present results also showed number of RAPD bands such as 2,2,0,20,4,4,2,2,46 respectively in Mango varieties, Mango 1 and Mango 2 further Ward's method of genetic distance by Squared Euclidean distances dendrogram revealed maximum similarity between Alphonso & Bangalora Whereas Mulgoa and Neelum forms separate cluster than other varieties.

The mean genetic similarity was less in the case of *Mango officinalis* and *Mango malabaricum* indicating a diverse genetic background of the former. This conclusion is also supported by the large variability exhibited in phenotypic traits reported by Ravindran et al., 1997 in mulberry plants. The RAPD data separated the varieties into three distinct groups confirming their taxonomic status unambiguously which are geographically isolated and thriving in comparatively different habitats. Due to high heterozygosity of the species it is not possible to conserve the seeds unresisting condition and is maintained in the vegetative form in the field gene bank. Most of the primers used for DNA profiling of the Jamine collections generated polymorphisms.

The present study gives an insight into the broad genetic structure of these varieties. The information generated from the study will be useful for further in-depth analysis of these varieties towards utilization in mango improvement and conservation programmes.

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