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APPLIANCE OF RAPD MARKERS IN THE ANALYSIS OF GENETIC VARIATIONS IN FIVE MANGO CULTIVARS

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ABSTRACT

The aim of this work was to analyze molecular relationship between five mango (*Mangifera indica*) cultivars *viz*., 'Alphanso', 'Totapuri', 'Langra', 'Kesar' and 'Amrapalli' using molecular RAPD markers. In present study, genomic DNA isolation procedure was standardized to extract DNA from mango pulp. All the samples tested yielded good quality DNA as 260/280 nm ratio was within 1.6-1.8 range revealing that no significant amount of proteins was present in extract. The statistical analysis (ANOVA) indicated $p \leq 0.05$ validating the applicability of optimized protocol. The extracted DNA was subjected to PCR with 39 primers. Thirty six RAPD primers produced distinct and consistent RAPD profiles which revealed 160 bands with decent 87% polymorphism. Neighbour joining tree, constructed based on Nei's genetic distance, displayed splitting of tested cultivars into two separate clades designated as I and II. The method employed in this study highlighted the reproducibility of technique and, therefore, appears to be a useful tool for genetic analysis in mango and other related plant species.

Key words: DNA, mango cultivars, PCR, phylogenetic tree, RAPD

INTRODUCTION

Mango (*Mangifera indica* L.) belongs to family Anacardiaceae and represents a diverse and economically important group of fruit crop having approximately 2000 cultivars. Mango is grown in 85 countries, of which 63 countries annually produce >15,000 t mangoes (NHB, 2018). Globally mango production is mainly concentrated in Asia and precisely in India which ranks first in mango production and accounts for about 50% of world's mango production (NHB, 2018). Mango is distributed throughout the country in India, except in hilly regions at 950 m amsl (Negi, 1999). The country produces about 50 cultivars of mangoes. Region-wise popular cultivars grown in India are 'Alphonso' and 'Kesar' in western India, 'Bangenpalli', 'Totapuri' and 'Neelum' in southern states, 'Langra' and 'Chausa' in northern states and 'Fazli' in eastern states (Anonymous, 2006). In Karnataka the cultivation of 'Alphonso', 'Totapuri', 'Langra', 'Kesar' and 'Amrapalli' is spread across all the districts baring coastal regions. Significant variations exist within and between mango cultivars with respect to fruit shape, size, colour and quality. Some of these cultivars are exported so they require accurate identification (Naidu and Naidu, 2006; Ramakrishnaiah *et al.,* 2018).

The characters of cultivars differ under varying environmental conditions which pose problems in proper identification of cultivars. More reliable molecular methods are now employed to delineate within and among plant cultivars (Williams *et al.,* 1990; Welsh and McCIelland, 1990). The success of molecular study depends on the quality and quantity of DNA isolated. The isolation of high-quality DNA from mango pulp is difficult due to the presence of various secondary metabolites that are co-isolated with genomic DNA. These secondary metabolites interfere in downstream molecular analysis (Moyo *et al.,* 2008) while surplus cell debris and proteins inhibit polymerase chain reaction (PCR) [Das *et al.,* 2009]. Also, RNA contaminants that precipitate along with DNA suppress PCR amplification and interfere in proper priming of DNA templates (Padmalatha *et al.,* 2006; Shahzadi *et al.,* 2010). Hence, there is urgent need to develop an efficient protocol for the isolation and amplification of DNA from mango pulp. RAPD finger-printing techniques have been used to identify horticultural crop cultivars and detect hybrids and clones (Debener, 2000; Collins *et al.,* 2003; Arús, 2006). Phylogenetic relationships within some mango species using RAPD markers have been established (Schnell *et al.,* 1995). In present study, a protocol for DNA isolation from mango pulp was standardized and the isolated DNA was characterized at molecular level using the designed primers.

MATERIALS AND METHODS

Five agronomically important cultivars namely ‘Alphonso’, ‘Amarapalli’, ‘Kesar’, ‘Langra’ and ‘Totapuri’, used in this study, were sampled in April-June 2016 from five different mango growing regions of Karnataka (India). A total of 25 individuals of each variety from each site were collected by hand. The collected mature mango fruits were subjected to physicochemical characterization following the protocol of Pratibha and Shivashankar (2018).

Extraction and amplification of DNA using RAPD primers

The isolation of genomic DNA was done by using three different protocols: 1) cetyl-tri-methyl ammonium bromide (CTAB) [Porbeski *et al.,* 1997; Pathiran *et al.,* 2018], 2) guanidium isothiocyanate (GITC) [(Schubert *et al.,* 2003], and 3) genomic DNA Mini Spin method (Chromous genomic DNA isolation kit) to purify DNA from isolates. With respect to the first two methods, DNA was isolated using home spun GITC and CTAB extraction buffers, followed by digestion with proteinase K and phenol extraction. For genomic DNA Mini Spin method, 100 mg mango pulp was crushed in a mortar and pestle with 1 mL extraction buffer. The content was centrifuged at 10,000 rpm for 10 min and the clear lysate collected into spin column. About 600 µL of clear lysate was added to 2 mL collection tube and centrifuged at 10,000 rpm for 1 min at room temperature. The spin column was placed again in collection tube containing 500 µL binding buffer and centrifuged at 7,000 rpm for 1 min at room temperature. Spin column was washed with 600 µL washing buffer at 7,000 rpm for 1 min at room temperature. Spin column was placed in 1.5 mL vial containing 50 µL elution buffer and was centrifuged at 7,000 rpm for 1 min at room temperature. The eluted sample was collected and DNA was quantified with spectrometric analysis (Multiskan Sky Micro-plate Spectrophotometer). The amounts of DNA isolated by CTAB, GITC and KIT methods were estimated by measuring the 260/280 nm absorbance ratio spectrophotometrically (Perkin-Elmer Corp., USA). The quality was assessed by running 50 µg DNA on 2% agarose gel in horizontal submarine apparatus (Genei, Bangalore, India) [Sambrook and Russell, 2001]. PCR amplification was performed using the following settings: initial denaturation at 94°C for 5 min, followed by 40 cycles (each cycle at 94°C for 1 min for denaturation; at 47°C for 1 min for annealing; at 72°C for 1 min for extension; and at 72°C for 5 min for final extension). After termination of PCR run, the quality of PCR product was assessed by running on 2% agarose gel in horizontal submarine apparatus (Genei, Bangalore, India) with 10 µL of Gene Ruler 1 kb DNA

ladder (Chromous Catalogue No. LAD02) [Sambrook and Russell, 2001]. PCR and gel electrophoresis were carried-out three times and only reproducible patterns were used for data analysis. The gels were photographed under UV light using Pentax K 100 camera. The images of gels were also taken by documentation system (Bio-Rads Gel Doc XR) for further analysis. RAPD markers across the seven accessions were scored for their presence '1' or absence '0' of bands for each primer. The primer sequences (5'-3') used were: OP-16 (GGTGACTGTG), OPE-18 (GGACTGCAGA), OPE-19 (ACGGCGTATG), OPF-3 (CCTGATC ACC), OPG-19 (GTCAGGGCAA), OPH-17 (AAGCAGC AAG), OPI-6 (AAGGCGGCAG), OPI-18 (TGCC CAGCCT), OPJ-15 (TGTAG CAGGG), OPK-8 (GAACA CTGGG), OPK-18 (CCTAGTCGAG), OPK-19 (CACAGGCGGA), OPL-18 (ACCACCCACC), OPF-19 (CCTCTAGACC), OPH-16 (TCTCAGCTGG), OPI-19 (AATGCGGGAG), OPI-20 (AAAGTGCGGG), OPJ-14 (CACCCGGATC), OPM-10 (TCTGGCGCAC), 1253 (GTTTCCGCCC), OPA-16 (GGTGCGG GAA), SR1 (AGCCAGCGAA), SR2 (GTTTCGCTCC), SR3 (GTAGACCCGT), SR4 (AAGAGCCCGT), SR5 (AACGCGCAAC), SR6 (CCCGTCAGCA), SR7 (GGGCC ACTCA), SR8 (GTGT CGCGAG), SR9 (CCCGCTACAC), OPC09 (CTCACCGTCC), OPB18 (CCACAGC AGT), OPC19 (GTTG CCAGCC), OPA03 (AGTCAGCCAC), OPE-14 (TGCGGCTGAG), OPV19 (GGGTGT GCAG), OPA15 (TTCCGAA CCC), IT-10 (ACGTCCAGAC) and OPV-01 (TGACGCATGG). Binary data tables showing alleles were generated and imported into tab delimited text files, and were analyzed using POPGENE software ver. 1.32 (Yeh *et al.,* 1999). The performance of markers was measured using polymorphic information content (PIC) according to Roldan-Ruiz *et al.* (2000), PICi = 2fi (1-2fi) and Tajimas test (Dt) (Tajimas, 1989). Similarity matrices was generated as per Saitou and Nei (1989) using Free Tree version 0.9.1.50 (Pavilcek *et al.,* 1999). Based on the similarity index **a**n unrooted dendrogram tree was constructed by using MEGA 7 ver. 7.20 (Kumar *et al.,* 2016). The data was statistically analyzed using one-way analysis of variance.

RESULTS AND DISCUSSION

The results of DNA extracted from mango samples using different methods are depicted in Fig. 1. All the samples tested yielded good quality DNA and the 260/280 nm absorbance ratio was found within 1.6-1.8 range indicating that no significant amount of proteins was present in extract (Shivashankar, 2015). The statistical analysis at $p \leq 0.05$ validated the applicability of optimized protocol. Extracted genomic DNA was subjected to PCR with 39 primers, out of which 33 primers produced distinct and consistent RAPD profiles (Fig. 2). One hundred and sixty bands were noticed, of which 140 were polymorphic with a decent polymorphism of 87%. The results are in line with Pruthvish and Chikkaswamy (2016) with 80% polymorphism in mango cultivars using RAPD markers. Similar results were also reported in other fruits and nut tree species such as olive by Fabbri *et al.* (1995), walnut by Shivasankar (2015) and in vegetatively propagated crop like banana by Kaemmer *et al.* (1992) in apple by Schnell *et al.* (1995) and Koller *et al.* (1993)**.** The range of PIC for 140 polymorphic loci averaged 0.94 (Table 1)**.**

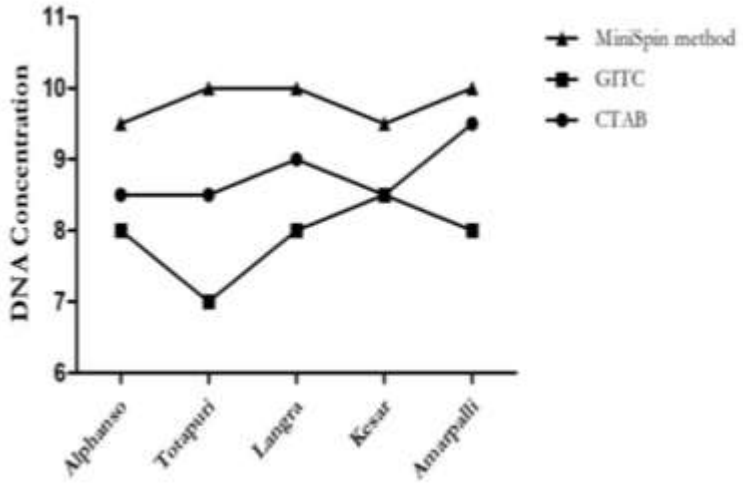

Fig. 1: DNA concentration found by CTAB, GITC and Mini Spin methods for mango cultivars used in efficiency test

Table 1: Genetic variation statistics for all loci

Parameters	PIC value
Na	0.3318
Ne	0.3223
H	0.1564
I	0.2155
P loci	140.0
P loci%	87.5
PIC	94.0
Dt	0.68

*na = Observed number of alleles;
*ne = Effective number of alleles (Kimura and Row, 1964);
*h = Nei's gene diversity;
*I = Shannon's Information index (Lewontin, 1972)

Similarity matrices based Nei's genetic distance ranged from 0.431 to 0.964 (Fig. 3). Neighbour Joining tree, constructed on the basis of Nei's genetic distance values (Fig. 4), displayed splitting of tested cultivars into two separate clades designated as I and II. The clade I exclusively included 'Alphonso' while Clade II included 'Totapuri', 'Langra', 'Kesar' and 'Amrapalli'. The clade II was further subdivided into 2 smaller subclades; one sub-clade consisted of 'Totapuri' and 'Langra' while the other subclade included 'Kesar' and 'Amrapalli' (Fig. 4). To conclude, the present study assessed the genetic relationship among five mango cultivars collected from different regions of Karnataka (India). The

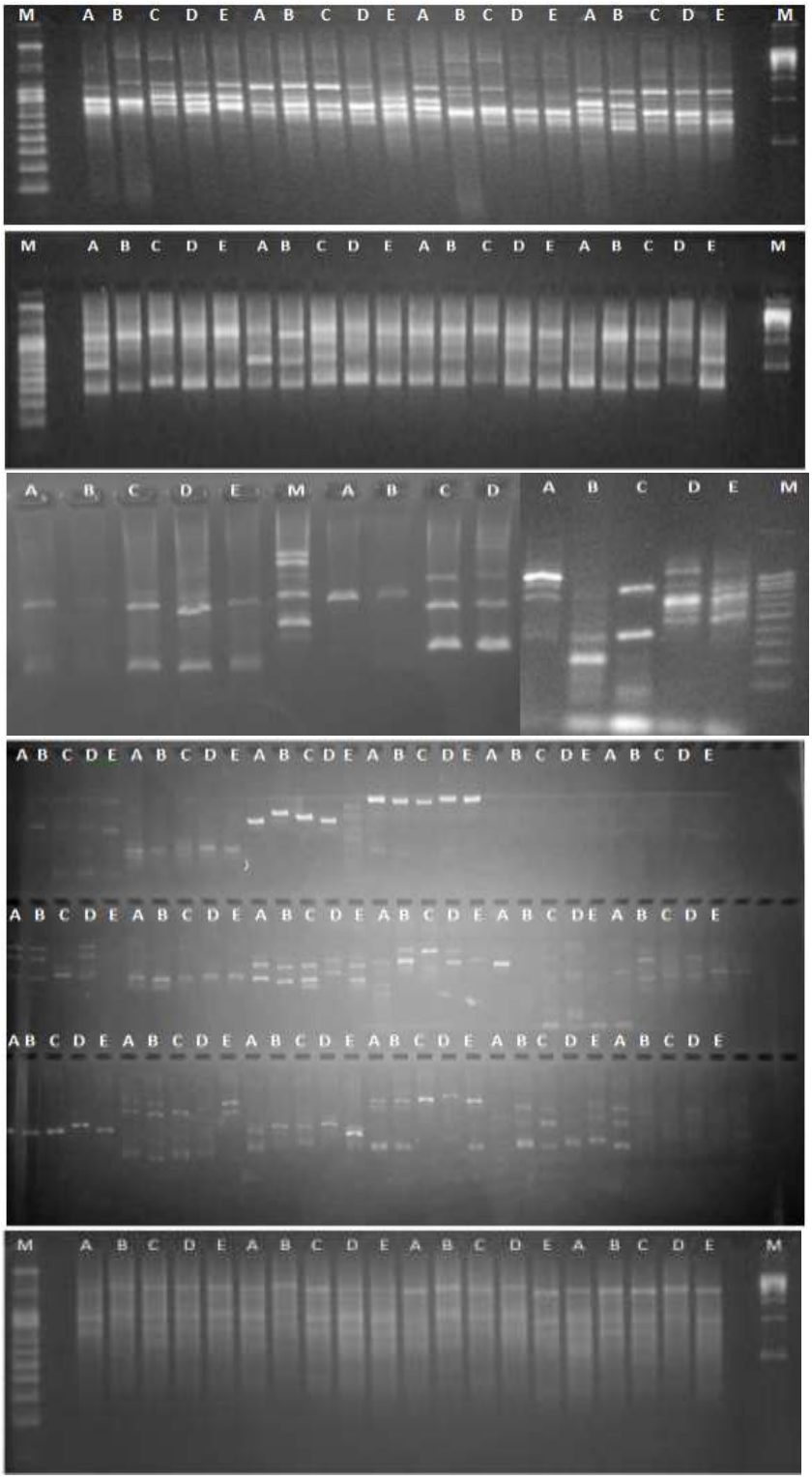

Fig. 2: Gel profile of RAPD Primers: A) OP-16, OPE-18, OPE-19, OPF-3; **B)** OPG-19, OPH-17, OPI-6, OPI-18; **C)** OPI-6, OPI-18, OPJ-15, **D)** OPK-8, OPK-18, OPK-19, OPL-18, OPF-19, OPH-16, OPI-19, OPI-20, OPJ-14, OPM-10, 1253, OPA-16, SR1, SR2, SR3, SR4, SR5 **E)** SR6, SR7, SR8, SR9 and OPC09

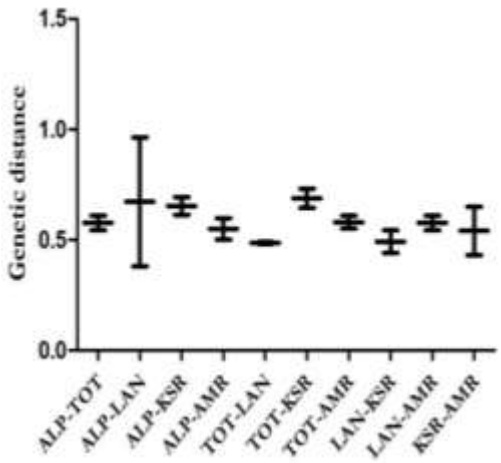

Fig. 3: Scatter plot of RAPD estimates based on genetic distance between mango cultivars

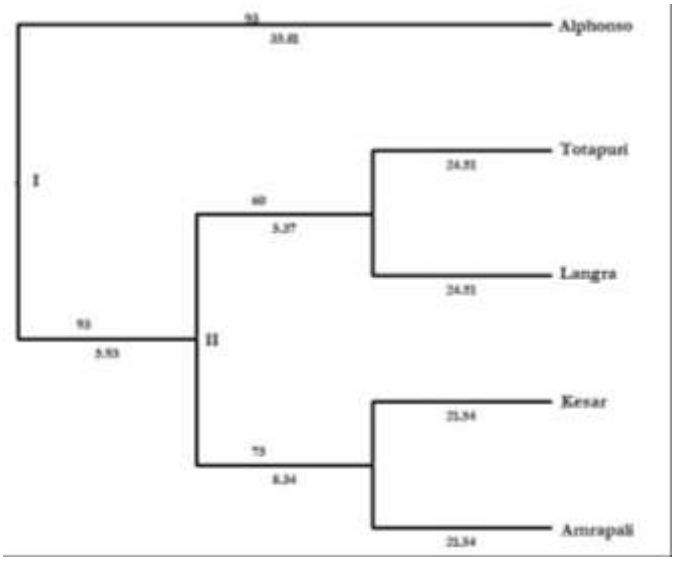

Fig. 4: Dendrogram of five mango cultivars based on RAPD profiles

optimized protocols among mango cultivars using RAPD markers were invaluable; hence, their collection, evaluation found to be simple, reliable and inexpensive. Further, the genetic resources for potential crop improvement are invaluable; hence, their collection, evaluation and documentation are important in order to efficiently collect and maintain the germplasm. The present work will be useful in conservation biology.

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