Research Article

Evaluation of Genetic Diversity among Mango (*Mangifera indica* L.) Hybrids and their Parentage using RAPD Markers

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Abstract

Sixteen mango cultivars were screened using Randomly Amplified Polymorphic DNA (RAPD) markers with decamer primers of arbitrary sequence. Out of the 25 primers screened, ten were selected which gave 682 clear and bright fragments. 442 bands were found polymorphic from 682 amplified bands, with an average of 44.20 polymorphic bands per primer. The polymorphism percentage varied from 33.33 (OPA-13) to 100 (OPB-07), with an average of 75.15% polymorphism. PIC value ranged from 0.242 (OPA-09) to 0.412 (OPA-08). A dendrogram based on UPGMA analysis grouped sixteen mango genotypes in the four main clusters, with Jaccard's similarity coefficient of 0.47–0.81. A dendrogram based on Jaccard's co-efficient of similarity implied a moderate degree of genetic diversity among the cultivars used for experimentation. The hybrids which having at least one common parent were placed together. The tendency to use similar parents extensively in a breeding program has lead to concerns about the lack of genetic diversity. Hence, the present study was conducted to assess genetic diversity and genetic relationships among mango cultivars/genotypes. The information will be useful for cultivar identification, genome mapping and initiating marker-assisted selection.

Keywords: Mango hybrids, Parentage, RAPD, Genetic Diversity

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Introduction

Mango (*Mangifera indica* L.), "The King of Fruits", is an important member of the anacardiaceae and is believed to have originated in the Indo-Burma region [1-3]. India has the richest wealth (nearly 1000 varieties) of mango germplasm in the world and it is the ``National Fruit of India''. Mango occupies 1.23 million ha in India with a production of 11 million tonnes, which accounts for 57% of the total world's production [4]. Production of mango in Bihar is 13 lakh tonnes which constitutes around 34% of the total fruit production of the state with productivity of 9.2 MT/ha [5].

The varieties represent potential candidates for any future breeding work designed at producing high yielding, disease-resistant hybrids adapted to the local microclimatic conditions. Genetic resources for potential crop improvement are invaluable, hence their collection, evaluation, characterization and documentation is important. Previously, characterization of mango germplasm was based mainly on morphology [6]. Although morphological characteristics are still extremely useful, these are repeatedly faced with the problems of low penetrance and heritability. Such problems are exaggerated in tree crops for the following reasons: long juvenile period, information being confined to only the maternal parent, anecdotal information on the cultivars and local cultivars bearing different vernacular names having the same original material. On the other hand, molecular markers are highly heritable, available in high numbers and often exhibit sufficient polymorphism to categorize closely related genotypes.

In recent years, extensive molecular work has been carried out to explain the level and pattern of diversity in mango germplasm [7]. DNA-based markers are useful tools for characterizing and studying genetic similarities among land races, varieties and cultivars [8]. Various DNA markers, including restriction fragment length polymorphism (RFLP) [9], random amplified polymorphic DNA (RAPD) [10] and [9], amplified fragment length polymorphism (AFLP) [11] and simple sequence repeats (SSRs) [12] and [13] have been utilized to determine taxonomic identity [13] to estimate genetic diversity [12] and depict the evolutionary histories of mango [11]. RAPD have been used extensively because their application does not need any prior information about the target sequences on the genome. RAPD is simple, ease and fast to access the genetic diversity existing among related individuals. Despite questions about its reproducibility, its utility has been exploited in diversity analysis, mapping, and

identification of mango genotypes [14, 10, 15]. Therefore, in present investigation an assessment of genetic diversity studies was undertaken to understand the level and pattern of diversity in mango hybrids with their parentage developed at Bihar Agricultural University, Sabour based on RAPD profiles.

Materials and Methods

Plant material

Eight mango hybrids and theirs parents which were developed at Bihar Agricultural University, Sabour, Bhagalpur, Bihar (India) through hybridization programme. Leaf sample were collected from the Mango orchards and AICRP of mango (BAU, Sabour) for extraction of DNA. The details of the hybrids with their parentage are given in **Table 1**.

Table 1 Details of hybrids Mango (*Mangifera indica* L.) with their parentage

Treatments	Name of Hybrids/ parents	Parentage	Year	Special features (Pulp colour)
H_1	Mahmood bahar	Bombai X Kalapadi	1951	Yellowish orange
H_2	Prabhashankar	Bombai X Kalapadi	1951	Light yellow
H_3	Alfazli	Alphonso X Fazli	1980	Light yellow
H_4	Sabri	Gulabkhas X Bombai	1989	Reddish yellow
H_5	Jawahar	Gulabkhas X Mahmood bahar	1980	Yellow
H_6	Sunderlangra	Langra X Sunderprasad	1980	Orange yellow
H_7	Hybrid 140	Langra X Amrapalli	-	Light orange
H_8	Hybrid 60	Sunderprasad X Langra	-	Dull orange
G_1	Bombai			Reddish
G_2	Kalapadi			Yellow
G_3	Alphonso			Reddish
G_4	Fazli			Light yellow
G_5	Gulabkhas			Yellow
G_6	Langra			Light orange
G_7	Amrapali			Brick red
G_8	Sunderprasad			Dull orange

DNA isolation

Freshly harvested young and tender leaf samples were used for DNA extraction as described earlier [16]. 1 g of samples was ground in liquid Nitrogen using mortar and pestle (Worm 65^0C). Approximately, 0.6 ml of the liquid were taken in to 1.5 ml of microcentrifuge and equal volume (W/V) (350 µl) of hot (65^0C) 2X CTAB buffer was added mixed thoroughly by vigorous shaking for 2 min. 700 µl of ice cold chloroform: isoamyl alcohol (24:1) was added, mixed well by inversion and centrifuged at 12,000 rpm for 5 min. The top (aqueous) was collected into a new microcentrifuge tube and $1/5^{th}$ volume of 5% CTAB solution was added mixed well by gentle inversion. Further, equal volume of chloroform: isoamyl alcohol (24:1) was added, mixed well by inversion and centrifuged at 12,000 rpm for 5 min. The top (aqueous) was collected using cut tips into a new microcentrifuge tube and equal volume of CTAB precipitation buffer was added and incubated on ice for 5 min. After incubation, microcentrifuge tube was centrifuged at 12,000 rpm for 5 min and supernatant was discarded. Fifty microlitres of high salt was added into the microcentrifuge to dissolve the pellet. DNA was precipitated by addition of 2.5 vol of (the supernant) ice cold Ethanol and mix gently by inversion. Microcentrifuge tubes were centrifuged at 12,000 rpm for 15 min and supernatant was diascared. DNA pellet was washed with 70% ethanol, air dried, dissolved in 25 µl of 0.1x TE buffer.

RAPD amplification analysis

RAPD analysis of mango hybrids with their parents were conducted using 10 decamer arbitrary primers obtained from Operon Technologies, California. RAPD amplification was performed in 20 µl volume containing 10 µl of Premix Taq® Version 2.0 (Xcelris Lab Ltd. Ahmedabad, Gujarat), 0.5 µM primer and 50 ng of template DNA in a thermal cycler (Agilent Technologies). The PCR cycles comprised an initial 94^0C for 5 min for denaturation followed by 35 cycles of denaturation at 94^0C for 1 min, primer annealing at 34^0C for 30 sec, extension at 72^0C for 2 min and final extension at 72^0C for 3 min. Amplification products were fractionated on 1.2% agarose gel plus 0.5 µml ethidium bromide in 0.1X TAE buffer. The size of the amplified DNA fragments was estimated with 1KB ladders (Gene Rular). The gels were visualized under UV using gel documentation system (UVITEC Combridge).

Data analysis

Matrix of RAPD markers was scored from the image of gel electrophoresis in the form of binary data, where the presence of the band was scored as (1) and the absence of band was scored as (0). The polymorphism information content (PIC) was performed as described formerly [17]. Using NTSYS software, a similarity matrix was designed utilising Jaccard's coefficient [18]. Cluster analysis based on the similarity matrix, was performed using un-weighted pair group method arithmetic averages (UPGMA) of the NTSYS–PC version 2.2 [19]. Principal component analysis (PCA) following to construct a correlation similarity matrix afterward Eigen value and Eigen vector matrices. Two dimensional plots were constructed using two principle components selected by the NTSYS-pc software.

Table 2 List of 10 selected RAPD primers used for analysing 8 hybrids of mango with their 8 parentage

Primer (Operon code)	Sequences (5-3)	Fragment size (bp)	Amplified fragment	Mono morphic bands	Poly morphic bands	Poly morphism %	PIC Value
OPA-01	CAGGCCCTTC	500-3000	10	1	9	90.00	0.383
OPA-02	TGCCGAGCTG	200-2000	8	1	7	87.50	0.328
OPA-03	AGTCAGCCAC	200-3000	7	1	6	85.71	0.272
OPA-07	GAAACGGGTG	200-3000	6	3	3	50.00	0.276
OPA-08	GTGACGTAGG	200-3000	8	1	7	87.50	0.412
OPA-09	GGGTAACGCC	200-2000	7	4	3	42.86	0.242
OPA-10	GTGATCGCAG	200-3000	9	1	8	88.89	0.399
OPA-13	CAGCACCCAC	200-1000	3	2	1	33.33	0.305
OPB-06	TGCTCTGCCC	200-4000	7	1	6	85.71	0.348
OPB-07	GGTGACGCAG	200-2000	7	0	7	100.00	0.336
Total			72	15	57	79.17	
Average		230-2600	7.2	1.5	5.7	75.15	

Table 3 Nucleotide sequence of the decamer random primers, the total number of DNA fragments amplified (A) and the number of polymorphic bands (P) for each hybrids with their parentage of individual primers

Primer	Nucleotide sequence	Mango Hybrids with their parentage															
		H-1		H-2		H-3		H-4		H-5		H-6		H-7		H-8	
		A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P
A-01	CAGGCCCTTC	5	4	4	3	3	2	6	5	3	2	3	2	1	0	6	5
A-02	TGCCGAGCTG	6	5	6	5	8	7	6	5	6	5	8	7	5	4	7	6
A-03	AGTCAGCCAC	3	2	7	6	4	3	4	3	4	3	4	3	4	3	5	4
A-07	GAAACGGGTG	4	1	4	1	4	1	5	2	5	2	5	2	5	2	5	2
A-08	GTGACGTAGG	5	4	2	1	6	5	6	5	3	2	5	4	4	3	6	5
A-09	GGGTAACGCC	5	1	5	1	5	1	5	1	5	1	6	2	7	3	5	1
A-10	GTGATCGCAG	6	5	5	4	4	3	3	2	7	6	7	6	3	2	5	4
A-13	CAGCACCCAC	3	1	2	0	3	1	3	1	3	1	3	1	3	1	3	1
B-6	TGCTCTGCCC	6	5	4	3	5	4	2	1	4	3	4	3	5	4	5	4
B-7	GGTGACGCAG	5	5	3	3	5	5	3	3	4	4	5	5	5	5	4	4
Total		48	33	42	27	47	32	43	28	44	29	50	35	42	27	51	36
		G-1		G-2		G-3		G-4		G-5		G-6		G-7		G-8	
		A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P
A-01	CAGGCCCTTC	3	2	8	7	5	4	6	5	1	0	3	2	1	0	1	0
A-02	TGCCGAGCTG	6	5	6	5	4	3	4	3	4	3	4	3	4	3	4	3
A-03	AGTCAGCCAC	2	1	3	2	3	2	2	1	4	3	4	3	2	1	4	3
A-07	GAAACGGGTG	5	2	5	2	5	2	5	2	4	1	5	2	4	1	4	1
A-08	GTGACGTAGG	4	3	7	6	5	4	5	4	2	1	3	2	2	1	2	1
A-09	GGGTAACGCC	5	1	6	2	6	2	6	2	6	2	5	1	6	2	6	2
A-10	GTGATCGCAG	5	4	1	0	2	1	9	8	1	0	2	1	1	0	2	1
A-13	CAGCACCCAC	3	1	3	1	3	1	3	1	3	1	3	1	2	0	2	0
B-6	TGCTCTGCCC	6	5	6	5	4	3	5	4	3	2	2	1	5	4	3	2
B-7	GGTGACGCAG	5	5	1	1	5	5	6	6	4	4	4	4	5	5	5	5
Total		44	29	46	31	42	27	51	36	32	17	35	20	32	17	33	18

Results and Discussion

Initially total 25 RAPD primers were screened, out of which 10 primers responded with three or more reproducible bands were included in the present study. Total 682 DNA bands were produced in all 16 genotypes by PCR amplification of DNA. Amplified fragments varied from three (OPA-13) to ten (OPA-01) in number, with the size range of 230-2600 bp (**Table 2**). Out of 682 amplified bands, 442 bands were found polymorphic, with an average of 44.20 polymorphic bands per primer (**Tables 3** and **4**).

Table 4 Level of polymorphism observed in hybrid mango and their parentage using RAPD primers

Treatments	Number of total fragments	Average fragments per primer	Number of poly morphic fragments	Average polymorphic fragments per primers
H-1	48	4.8	33	3.3
H-2	42	4.2	27	2.7
H-3	47	4.7	32	3.2
H-4	43	4.3	28	2.8
H-5	44	4.4	29	2.9
H-6	50	5.0	35	3.5
H-7	42	4.2	27	2.7
H-8	51	5.1	36	3.6
G-1	44	4.4	29	2.9
G-2	46	4.6	31	3.1
G-3	42	4.2	27	2.7
G-4	51	5.1	36	3.6
G-5	32	3.2	17	1.7
G-6	35	3.5	20	2.0
G-7	32	3.2	17	1.7
G-8	33	3.3	18	1.8

The polymorphism percentage varied from 33.33 (OPA-13) to 100 (OPB-07), with an average of 75.15% polymorphism. PIC value ranged from 0.242 (OPA-09) to 0.412 (OPA-08) (Table 2). The amounts of polymorphism detected among mango genotypes as observed by RAPD primer are shown in **Figure 1**. A dendrogram based on UPGMA analysis grouped sixteen mango genotypes in the four main clusters (**Figure 2**), with Jaccard's similarity coefficient of 0.47–0.81 (**Table 5**). Hybrids grouped within the same cluster in the dendrogram were basically related to the original sources of the hybrids. In general, the hybrids sharing common parents tend to group together (Table 1). Relationship among the 16 genotypes was also detected by PCA based in the dendrogram were occupying the same positions in the two dimensional scaling also (**Figure 3**).

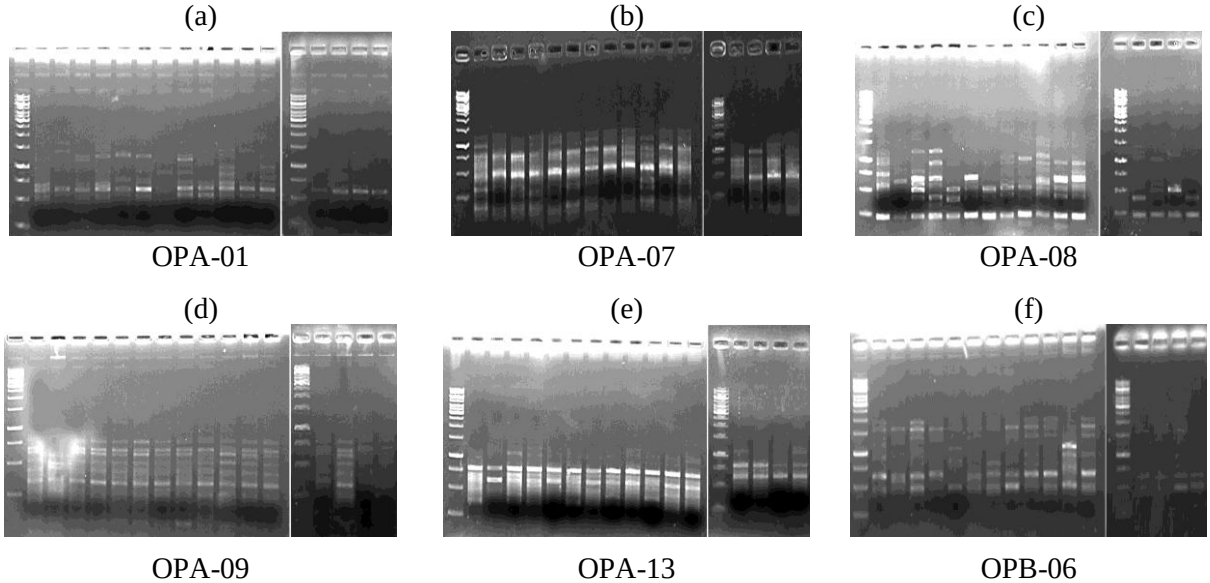

Figure 1 Molecular profiles of hybrid mango with their parents obtained using different RAPD markers. RAPD profile using OPA-01 (a), OPA-07 (b), OPA-08 (c), OPA-09 (d), OPA-13 (e) and OPB-06 (f)

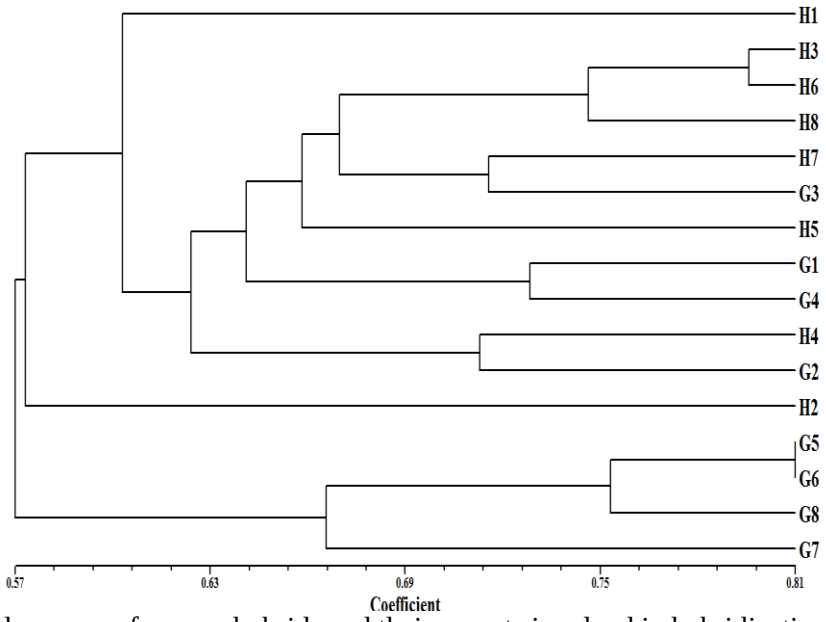

Figure 2 UPGMA dendrograms of mango hybrids and their parents involved in hybridization programme based on Jaccard's similarity

Table 5 Jaccard's similarity coefficient calculated from RAPD data among mango hybrids and their parents

Hybrids/ Genotypes	H-1	H-2	H-3	H-4	H-5	H-6	H-7	H-8	G-1	G-2	G-3	G-4	G-5	G-6	G-7	G-8
H-1	1.00															
H-2	0.55	1.00														
H-3	0.67	0.62	1.00													
H-4	0.60	0.67	0.70	1.00												
H-5	0.53	0.56	0.65	0.64	1.00											
H-6	0.60	0.61	0.80	0.66	0.68	1.00										
H-7	0.55	0.56	0.74	0.60	0.65	0.74	1.00									
H-8	0.68	0.63	0.75	0.68	0.64	0.74	0.63	1.00								
G-1	0.64	0.54	0.62	0.58	0.63	0.65	0.65	0.67	1.00							
G-2	0.57	0.54	0.60	0.71	0.55	0.57	0.60	0.70	0.67	1.00						
G-3	0.55	0.47	0.62	0.60	0.65	0.64	0.71	0.63	0.65	0.66	1.00					
G-4	0.60	0.50	0.61	0.52	0.64	0.68	0.58	0.67	0.73	0.59	0.60	1.00				
G-5	0.48	0.57	0.61	0.63	0.65	0.58	0.72	0.54	0.58	0.56	0.61	0.51	1.00			
G-6	0.54	0.54	0.64	0.62	0.65	0.55	0.67	0.56	0.55	0.53	0.57	0.51	0.81	1.00		
G-7	0.51	0.54	0.58	0.50	0.58	0.55	0.64	0.51	0.62	0.56	0.54	0.51	0.68	0.60	1.00	
G-8	0.47	0.53	0.60	0.52	0.57	0.57	0.67	0.53	0.54	0.49	0.56	0.47	0.80	0.70	0.71	1.00

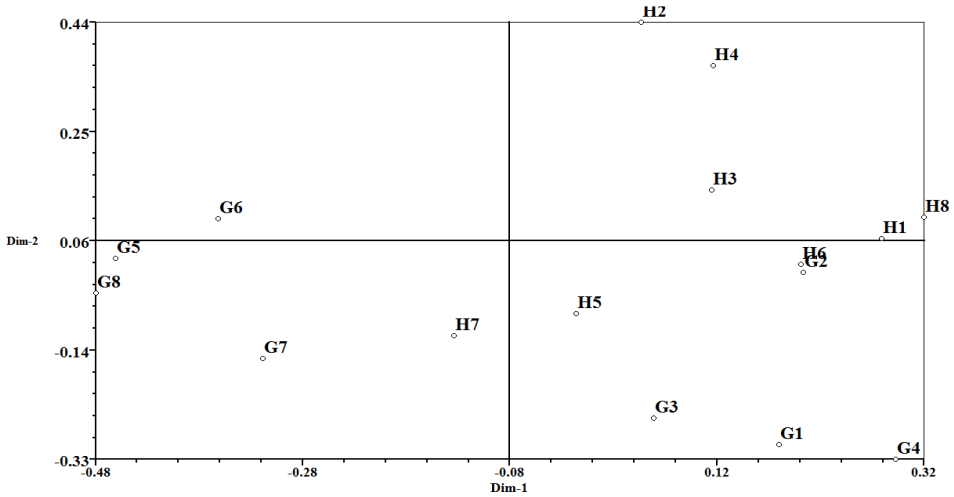

Figure 3 Principal coordinate analyses of RAPD primer data for mango hybrids with their parentage

The use of different types of RAPD molecular markers has proven useful to distinguish and to determine the genetic relationship among different mango genotypes [10, 9]. The percentage (79.17%) of polymorphic bands detected in the present study clearly indicates that RAPD fragments are polymorphic and particularly informative in the evaluation of genetic relationships. Similar levels of polymorphism associated with RAPD markers have been reported in earlier studies involving mango [20]. The genetic similarity coefficients were in the range of 0.47– 0.81. This is compatible with earlier studies that reported genetic similarities of 0.61–0.95 using RAPD analysis [21]. While in another study, relatively low estimates of similarity coefficients (0.32–0.72) were reported among 29 Indian cultivars [10]. The dendrogram generated by this studied classified the whole genotypes into four major clusters. Our findings are also in accordance with the previous studies which showed dendrogram grouped the genotypes into three clusters, which correspond well with their pedigree relationship [20].

Conclusion

The present study is the footstep for estimation of genetic diversity among mango hybrids with their parentage. The genetic analysis based on amplification signals employing RAPD markers is fairly strong to evaluate the genetic relationships. This finding will considerably facilitate marker validation for agronomically important characters, genome mapping and recombination breeding programmes aiming at the development of new cultivars/hybrids.

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