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Molecular auditing of some selected Indian mangoes (*Mangifera indica* L.)

R. C. Jena and P. K. Chand[Ψ]

Plant Biotechnology Laboratory, Post-Graduate Department of Botany, Utkal University, Vani Vihar, Bhubaneswar - 751 004, Odisha, India

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ABSTRACT

This study describes phenotypic variation in respect of leaf and pomological traits and portrays genetic relationships among 12 selected mango genotypes of India using randomly amplified polymorphic DNA (RAPD) marker technique. The genotypes were phenotypically diverse in respect of 20 different (11 qualitative and 9 quantitative) traits. Traits like sugar/acid ratio, ripe fruit weight and total soluble solids demonstrated higher variations. Many of these traits had two or more than two phenotypic classes with economic importance and thus could be used in breeding to enhance fruit yield and quality. Twenty-three RAPD markers yielded a total of 307 amplified DNA fragments, of which 85.99% were polymorphic, indicating a high degree of genetic diversity. Primers OPA 8, OPA 19, OPG 9 and RPI-10 exhibited 100% polymorphism. Polymorphic information content (PIC) value for RAPD primers ranged from 0.38-0.82 with an average of 0.60. The resolving power varied from 4.83 to 23.5 with an average of 14.35. The average values for Na, Ne, I, He and uHe were calculated from the RAPD data as 1.82, 1.46, 0.41, 0.27 and 0.28 respectively. Twenty-one unique bands were generated which enabled identification of 9 different genotypes. The pair-wise Jaccard's similarity coefficient ranged between 0.55 and 0.81 indicating that the genotypes represent genetically diverse populations. The closest were two hybrids namely 'PKM-1' and 'PKM-2' and the most distant genotypes were 'Pusa Surya', 'Dashehari', 'Neeleshan Gujrat' and 'Sai Sugandh'. UPGMA dendrogram grouped the genotypes into four clusters basing on genetic relatedness/distance which was corroborated in 2D and 3D plots generated from principal component analysis. The study provides information to facilitate marker assisted breeding aimed at genetic improvement of this important fruit crop.

1. Introduction

Mango (*Mangifera indica* L., Family: Anacardiaceae, Order: Sapindales), is one the most important tropical fruit crops of the tropical and subtropical areas of the world. It has been under cultivation since 4,000 years in the Indian subcontinent and its cultivation is as old as Indian civilization (De Candolle, 1884). Representing the largest mango gene pool in the world encompassing over 1000 mango varieties endowed with a high degree of diversity, India is considered to be the center of origin of mango (Mukherjee, 1972; Ravishankar *et al*., 2000). Its place of importance can be understood from its being referred to as 'King of fruits' in the tropical world (Purseglove, 1972).

An ideal mango variety should be dwarf and a regular bearer with medium size fruit (250-300 g). Additionally, it should be highly tolerant of various fungal and bacterial diseases, stable pleasant flavor, attractive colour combined with good keeping quality. Conventional breeding of woody perennial fruit crops like mango based on selection for agro-horticultural attributes is difficult owing to long juvenile phase, self-incompatibility, high degree of cross-pollination and heterozygous nature, polyembryony, meager information on inheritance of important quantitative traits, etc. Existing diverse varieties available in India are not adequate for commercialization; outstanding new varieties in combination with desired superior trait could cater to national and

[Ψ] *Corresponding author; Email:* pkchanduubot@rediffmail.com

international market demand. Furthermore, early and unambiguous identification of plant material is essential for effective germplasm characterization, which is helpful for plant breeders in selecting material for development of new crosses, in solving disputes related to patenting and intellectual property rights and to check bio-piracy, adulteration, etc. (Krishna and Singh, 2007).

Success of crop improvement program depends on proper varietal identification, characterization in combination with nature and magnitude of genetic variability. There is a relatively poor understanding of the pedigree and genetic relatedness of many mango cultivars. There is a considerable confusion in their nomenclature because many of them have unique local and regional names and the spelling and name variants have been translated to the Roman alphabet and that makes tracing their origins and ancestry difficult. Also, the performance of varieties varies under different climatic conditions (Singh, 1978). As in other fruit tree species, mango cultivars are currently identified on the basis of morphological traits based on descriptors (IPGRI, 1989, 2006). Over the last two decades, efforts were made in understanding the extent of variability of mango germplasm based on morpho-physiological traits (Rajwana *et al*., 2011; Bhuyan *et al*., 2007). Undoubtedly, phenotypic characterization forms the basis for germplasm characterization but, it is inaccurate due to the influence of the environment and often limiting number of discriminating traits. Moreover, this mode of identification is complicated with environmental effects on these characters and parallel selection for similar desired traits has often been misleading, labour intensive and time consuming. Many of these complications in characterizing plant germplasm based on phenotype and biochemical analysis can be overcome through direct identification of genotypes using DNA-based diagnostic assay. Compared to morphological markers, DNA markers are unaffected by environmental factors, highly heritable, polymorphic and unlimited in number; hence, they are extremely useful tool for depiction of genetic variability, genome fingerprinting, mapping, evolution, gene localization, population genetics, taxonomy and plant breeding.

Among the techniques used for genetic assessment, randomly amplified polymorphic DNA (RAPD) markers is most frequently used technique for genetic diversity analysis (Gupta and Rustgi, 2004) and proved to be as efficient as other molecular markers based on amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR) and inter simple sequence repeats (ISSR) for a range of plant species. Regardless of advances in DNA marker techniques, RAPD offers speed, simplicity, low cost, whole genome coverage for identification and classification of plants using small amount of DNA (Williams *et al*., 1990). In mangoes a few earlier studies have been carried out using RAPD profiles (Bajpai *et al*., 2008; Jena *et al*., 2010; Rajwana *et al*., 2011; Abou-Ellail *et al.,* 2014). Thus, RAPD could be an efficient technique for molecular auditing of Indian mangoes and to unravel the intraspecific relationships amongst different genotypes of mango. The present investigation is, therefore, aimed at accurate identification and estimation of the genetic divergence among 12 selected mango genotypes from different geographical locations of India using leaf, pomological and RAPD markers.

2. Materials and methods

2.1 Plant material

In the present investigation, we have used twelve promising mango genotypes encompassing commercial, local as well as hybrid genotypes obtained from orchards of Orissa University of Agriculture & Technology (O.U.A.T.) and Central Horticultural Experimentation Station (C.H.E.S.), Bhubaneswar, Orissa representing 4 different geographical locations (Eastern, Western, Northern and Southern zones of India) (Table 1). The plants were selected on the basis of their consistency in behavior for the last six years at their growing region for morphological observations as well as for collection of leaf samples for molecular characterization.

2.2 Morpho-biochemical trait evaluation

A total of 20 (11 qualitative plus 9 quantitative) different morphological and biochemical traits pertaining leaf, mature fruit and ripe fruit were assessed for the 12 studied mango genotypes. The qualitative traits were related to leaf (blade shape. leaf margin), mature fruit (skin colour, depth of stalk cavity, presence of neck) and ripe fruit (skin colour, flesh colour, juiciness, table quality, storage life, maturity time) (Table 1). The traits were recorded as per descriptor list (IPGRI, 1989; 2006) and DUS (Distinctness, Uniformity, Stability) guidelines (PPV & FRA, 2008). Evaluation of pomological characters were carried out on samples of 10 randomly chosen ripe fruits per genotype. All leaf related traits were documented from fully expanded mature leaves. Quantitative traits comprised fruit length and width (cm), fruit weight (g), physiological loss (g), peel, pulp and stone (%), total soluble solids (%) and sugar/acid ratio recorded from 10 randomly selected ripe fruits (Table 2). Traits like total soluble solids (TSS) and sugar/acid ratio were calculated using standard methods (Ranganna, 1986; A.O.A.C, 1990).

2.3 Genomic DNA extraction

Emerging young leaves of each of the 12 mango genotypes were collected from which genomic DNA was

isolated, individually frozen in liquid nitrogen and stored at - 80°C until processed. DNA was extracted following CTAB method as originally described by Doyle and Doyle (1990) with minor modifications (Jena *et al*. 2010). The quantity and quality of extracted DNA were determined as per Jena *et al*. (2010).

2.4 Primer screening and RAPD amplification

Initially, a total of 70 RAPD primers were screened with six mango genotypes of which 23 primers revealing clear, distinct, polymorphic and reproducible amplicons were included in the present study for further PCR analysis (Table 3). RAPD primers (OPA, OPC, OPG, and RPI-C Series) were purchased from Operon Technologies (Alameda, California, USA) and Bangalore Genei Pvt. Ltd. (India). RAPD-PCR amplification on each DNA sample was performed in a 25 µl reaction volume containing 1× Taq PCR buffer, 2 mM $MgCl_2$, 200 µM dNTP each, 10 pmoles of single primer, 1U of Taq DNA polymerase (Bangalore Genei, India), 30 ng of template DNA, and the rest sterile nuclease and protease-free water. Reactions without DNA were used as negative control. DNA amplification was carried out in a thermocycler (Applied Biosystems, USA) programmed at an initial pre-denaturation at 94 °C for 3 min followed by 44 cycles of denaturation at 94 °C for 1 min, annealing at 37 °C for 1 min, extension at 72 °C for 2 min with a finally at 72 °C for 7 min.

2.5 Agarose gel electrophoresis

All amplified products were loaded in wells (18 µl of sample + 3 µl of 6 × loading dye) and resolved on 1.5% agarose gel in 1× TBE buffer by electrophoresis at 70 V for 2 h followed by staining with ethidium bromide (1.0 µg/ml). The amplified fragments were photographed using gel documentation system (Bio-Rad, USA) and stored as digital pictures. Low range DNA ruler plus (Bangalore Genei, India) was used as molecular size standard to estimate the size of the fragments.

2.6 Data analysis

For the studied 9 quantitative traits, their descriptive statistics like the maximum, minimum, range, mean, standard error (SE), standard deviation (SD) and coefficients of variation (CV%) were computed using the SPSS® (Statistical Package for Social Studies) software version 17. The results were investigated for statistical significance by one way analysis of variance (ANOVA).

All distinct amplicons were scored visually as discrete variables using 1 for presence and 0 for absence separately for each marker and a binary matrix was obtained for RAPD with final data sets including both polymorphic and monomorphic bands. All amplifications were repeated thrice and only reproducible and unambiguous bands were considered for analysis. From the band patterns obtained with each primer, the genotype-specific bands (if any) along with their sizes were recorded. To determine the suitability and informative ability of RAPD markers, the performance of marker systems was measured using six parameters: polymorphism information content (PIC), effective multiplex ratio (EMR), marker index (MI), resolving power (Rp), genotype index (GI) and Shannon's information index (I). The PIC value for each primer was calculated using the formula PICi =2fi (1 - fi) (Roldan-Ruiz *et al*., 2000). Effective multiplex ratio was evaluated using formula; EMR $= \eta \times \beta$, where η is the average number of fragments amplified by genotype to a specific marker system (multiplex ratio) and β was estimated from the number of polymorphic bands (NPB) and the number of monomorphic bands (NMB); β = NPB/(NPB +NMB). Marker index (MI), which provides an estimate of marker utility for each primer, was calculated using the formula: MI = EMR×PIC. The resolving power (RP) of each primer was calculated as RP = ΣIb, Where $Ib = 1 - (2 \times |0.5 - p_i|)$, p_i being the proportion of genotypes containing the ith band (Prevost and Wilkinson, 1999). Genotype index (GI) represents proportion of genotypes actually distinguished by the primer i.e. the number of genotypes with unique fingerprints divided by total number of genotypes fingerprinted (Sehgal and Raina, 2005). The basic parameters for genetic diversity such as observed number of alleles (Na), effective number of alleles (Ne), Shannon's information index (I), expected heterozygosity (He) and unbiased expected heterozygosity (uHe) were calculated using GenAlEx 6.502 software (Peakall and Smouse, 2012). Pairwise-similarity matrices were generated by calculating Jaccard's similarity coefficient (Jaccard, 1908) to accomplish genetic similarity between the genotypes using NTSYS-pc software version 2.02 (Rohlf, 1993). These similarity coefficients were then subjected for construction of dendrogram by the unweighted pair group method with arithmetic average (UPGMA) and cluster analysis with NTSYS-pc. To further support the clustering results and to obtain a graphical picture of molecular variability 2D and 3D plots were generated from principal component analysis using NTSYSY-pc (version 2.02) (Rohlf, 1993). To estimate the robustness and validity of dendrogram typology and clustering bootstrap analyses were performed of 1000 bootstrap samples using the software WINBOOT (Yap and Nelson, 1996). Mantel's matrix correspondence test (Mantel, 1967) was performed by using MXCOMP algorithm of the NTSYS to compute the cophenetic correlation coefficient, *r*, which determines how well the dendrogram represents the similarity data.

Table 1

Characterization of mango genotypes based on leaf and pomological features[1]

Sl. No.	Genotype and its Geographic distribution		Leaf		Mature Fruit			Ripe Fruit					
			Blade shape	Margin	Skin colour	DSC	Presence of neck	PCS	Flesh colour	Juiciness	Table quality	Storage life	TFM
1	Pusa Surya	NI	Ovate	Wavy	Only green	Shallow	Slightly prominent	Orange	Light yellow	Medium	Excellent	Good	Late
2	Pusa Arunima	NI	Elliptic	Wavy	Green & red	Absent	Absent	Orange	Light orange	Medium	Excellent	Good	Late
3	Ambika	NI	Elliptic	Wavy	Green & purple	Shallow	Slightly prominent	Red & purple	Medium yellow	Medium	Excellent	Very Good	Late
4	PKM 1	SI	Oblong	Wavy	Green & red	Shallow	Slightly prominent	Green & yellow	Light orange	Medium	good	Good	Late
5	Janardhan Pasand	SI	Elliptic	Entire	Green & pink	Absent	Slightly prominent	Yellow & red	Light yellow	High	Excellent	Good	Early
6	PKM 2	SI	Oblong	Wavy	Only green	Shallow	Prominent	Green & yellow	Light yellow	Medium	Interme-diate	Good	Medium
7	Neeleshan Gujrat	WI	Ovate	Entire	Only green	Absent	Very prominent	Green & yellow	Medium yellow	Medium	Good	Good	Late
8	Mahmud Bahar	EI	Elliptic	Entire	Only green	Shallow	Absent	Yellow	Light yellow	Medium	Good	Good	Medium
9	Baramasi	EI	Elliptic	Entire	Only green	Shallow	Slightly prominent	Yellow	Medium yellow	Medium	Poor	Interme-diate	Very late
10	Sai Sugandh	WI	Oblong	Entire	Green & red	Absent	Prominent	Yellow & red	Light yellow	Medium	Excellent	Good	Late
11	Dashehari	NI	Ovate	Wavy	Only green	Absent	Absent	Yellow green	Orange	Medium	Interme-diate	Good	Medium
12	Arka Neelkiran	SI	Oblong	Wavy	Green & red	Medium	Absent	Yellow	Light Orange	Medium	Interme-diate	Very good	Late

EI: Easten India WI: Western India, NI: Northern India, SI: Southern India, DSC: Depth of Stack cavity, PCS: Predominant colour of skin, TFM: Time of fruit maturity.

[1]Features recorded in accordance with DUS (Distinctness, Uniformity, Stability) test guideline (PPV and FRA, 2008) and IPGRI guidelines (1989, 2006)

Table 2

Physico-chemical analysis of mango genotypes based on ripe fruit characteristics

Sl. No.	Genotypes	Fruit length (cm)	Fruit width (cm)	Ripe fruit weight (g)	Physiological loss (g)	Peel (%)	Stone(%)	Pulp (%)	Total Soluble Solids (%)	Sugar: Acid ratio
1	Pusa Surya	11.7	9.1	350.4	12.8	13.87	12.94	73.19	29	82.85
2	Pusa Arunima	11.2	8.2	235.8	18.4	13.18	13.14	73.68	19.8	40.0
3	Ambika	11.0	7.9	318	17.5	10.38	12.26	77.36	24	58.53
4	PKM 1	9.8	7.5	310.5	13.7	15.46	15.78	68.76	31	62
5	Janardhan Pasand	9.95	7.05	246.2	11	15.22	13.76	71.02	14.6	24.4
6	PKM 2	8.1	6.7	177	15.5	13.50	18.62	67.87	27	52.94
7	Neeleshan Gujrat	10.7	9.1	288.7	22.8	11.73	17.03	71.24	18.2	38.1
8	Mahmud Bahar	11.1	8.1	310	19.7	10.24	18.56	71.20	21	47.72
9	Baramasi	8.4	8.3	167.1	13.2	7.94	22.36	69.70	19	42.22
10	Sai Sugandh	15.4	9.2	389.6	28.4	10.49	18.31	71.20	18.6	39.57
11	Dashehari	8.5	5.9	175.3	13.2	12.04	15.76	72.20	27.2	75.55
12	Arka Neelkiran	8.45	7	210.2	20.6	16.41	13.56	70.03	17	37.77
	SE	0.59	0.30	21.30	1.46	0.73	0.88	0.72	1.45	4.88
	SD	2.03	1.03	73.77	5.05	2.52	3.06	2.50	5.02	16.91
	CV %	19.64	13.16	27.85	29.33	20.12	19.11	3.50	22.43	33.68

Table 3

Description and band amplification profile of RAPD primers used in the present study

Sl. No.	Primer ID	Sequence (5'-3')	% GC	Tm[1] (°C)	TNB	NPB	NMB	Range (bp)	PB (%)
1	OPA 6	GGTCCCTGAC	70	34	15	13	2	200-2000	86.66
2	OPA 7	GAAACGGGTG	60	32	16	14	2	100-2500	87.5
3	OPA 8	GTGACGTAGG	60	32	12	12	0	100-2500	100
4	OPA 9	GGGTAACGCC	70	34	21	20	1	100-2000	95.24
5	OPA 12	TCGGCGATAG	60	32	18	17	1	100-2000	94.44
6	OPA 17	GACCGC TTGT	60	32	11	10	1	200-2000	90.91
7	OPA 19	CAAACGTGGG	60	32	17	17	0	100-2000	100
8	OP C 2	GTGAGGCGTC	70	34	4	3	1	600-1500	75
9	OP C 5	GATGACCGC C	70	34	9	5	4	300-2000	55.55
10	OP C 8	TGGACCGGT G	70	34	9	8	1	150-2500	88.88
11	OP C 11	AAAGCTGCG G	60	32	12	9	3	300-3000	75
12	OP C 15	GACGGATCA G	60	32	12	10	2	100-2500	83.33
13	OP C 18	TGAGTGGGT G	60	32	10	8	2	300-1815	80
14	OP C 20	ACTTCGCCA C	60	32	12	10	2	300-2000	83.33
15	OPG 2	GGCACTGAGG	70	34	11	9	2	100-2500	81.82
16	OPG 9	CTGACGTCAC	60	32	14	14	0	200-2000	100
17	OPG 15	ACTGGGACTC	60	32	15	12	3	200-2000	80
18	OPG 17	ACGACCGACA	60	32	16	12	4	100-2000	75
19	RPI 1	AAAGCTGCGG	60	32	17	14	3	200-2500	82.35
20	RPI 2	AACGCGTCGG	70	34	15	14	1	300-3000	93.33
21	RPI 4	AATCGCGCTG	60	32	11	10	1	200-2500	90.91
22	RPI 7	ACATCGCCCA	60	32	14	7	7	200-3000	50
23	RPI 10	ACGATGAGCG	60	32	16	16	0	200-2000	100
TOTAL				307	264	43			
AVERAGE					13.35	11.48	1.87		85.99

[1]Tm: Melting temperature, TNB: Total number of bands; NPB: Number of polymorphic bands; NMB: Number of monomorphic bands; PB (%): Polymorphic band percentage

Table 4

Amplification performance and diversity parameters of mango genotypes as revealed by RAPD markers

Sl. No.	Primer ID	PIC	EMR	MI	Rp	GI	Na	Ne	I	He	uHe
1	OPA 6	0.53	5.73	3.04	18.33	0.08	1.87	1.53	0.46	0.31	0.32
2	OPA 7	0.66	5.50	3.41	18.67	0.08	1.88	1.53	0.46	0.31	0.32
3	OPA 8	0.75	6.58	4.08	13.17	0.00	1.83	1.69	0.54	0.37	0.39
4	OPA 9	0.82	4.38	3.29	17.33	0.03	1.95	1.47	0.48	0.28	0.30
5	OPA 12	0.66	5.17	3.14	17.50	0.05	1.83	1.50	0.45	0.30	0.31
6	OPA 17	0.63	5.36	2.77	11.83	0.00	1.91	1.51	0.47	0.31	0.32
7	OPA 19	0.77	6.29	3.90	17.83	0.03	2.00	1.59	0.50	0.33	0.35
8	OP C-02	0.58	4.25	2.47	4.83	0.00	1.75	1.49	0.44	0.29	0.31
9	OP C-05	0.49	1.56	0.76	10.33	0.00	1.56	1.16	0.17	0.10	0.10
10	OP C-08	0.62	3.11	2.40	6.67	0.00	1.89	1.31	0.34	0.21	0.22
11	OP C-11	0.60	3.17	1.90	12.33	0.03	1.75	1.33	0.31	0.20	0.21
12	OP C-15	0.52	5.67	2.95	15.33	0.00	1.83	1.60	0.44	0.33	0.34
13	OP C-18	0.51	4.30	2.19	11.17	0.00	1.40	1.31	0.28	0.19	0.19
14	OP C-20	0.66	3.83	2.53	11.67	0.00	1.83	1.41	0.38	0.25	0.26
15	OPG 2	0.58	5.09	2.95	13.33	0.00	1.83	1.41	0.40	0.26	0.27
16	OPG 9	0.62	4.50	3.69	10.50	0.03	2.00	1.50	0.47	0.31	0.32
17	OPG 15	0.63	3.27	2.83	14.17	0.00	1.80	1.31	0.30	0.19	0.20
18	OPG 17	0.38	5.81	2.87	23.50	0.00	1.75	1.53	0.43	0.29	0.31
19	RPI 1	0.49	5.76	2.85	22.33	0.03	1.82	1.55	0.46	0.31	0.33
20	RPI 2	0.63	5.67	3.38	16.17	0.05	1.93	1.63	0.51	0.35	0.36
21	RPI-4	0.57	4.91	2.74	11.00	0.03	1.91	1.52	0.46	0.30	0.32
22	RPI-7	0.44	1.71	2.70	18.00	0.05	1.50	1.19	0.20	0.12	0.13
23	RPI 10	0.73	5.25	2.65	14.00	0.08	2.00	1.57	0.48	0.32	0.33
	TOTAL	13.87	106.88	65.46	329.99	0.55	41.83	33.63	9.42	6.24	6.51
	AVERAGE	0.60	4.65	2.85	14.35	0.02	1.82	1.46	0.41	0.27	0.28

PIC: Polymorphism information content; EMR: Effective multiplex ratio; MI: Marker index; Rp: Resolving power; GI: Genotype index, Na: Observed number of alleles, Ne: Effective number of alleles, I: Shannon's information index, He: Expected Heterozygosity, uHe: Unbiased Expected Heterozygosity

Table 5

Genotype-specific RAPD markers for mango genotypes audited

Sl.No.	Marker[1]	Presence (+) / Absence (-)	Genotype identified	Sl.No.	Marker[1]	Presence (+) / Absence(-)	Cultivar identified
1	OPA – 6_{300}	(+)	Pusa Surya	12	OPG – 9_{840}	(-)	Baramasi
2	OPA – 6_{560}	(+)	Sai Sugandh	13	RPI – 1_{2000}	(-)	Ambika
3	OPA – 6_{1420}	(+)	PKM 2	14	RPI – 2_{650}	(+)	Mahmud Bahar
4	OPA – 7_{250}	(-)	PKM 2	15	RPI – 2_{2500}	(+)	Sai Sugandh
5	OPA – 7_{1000}	(+)	Sai Sugandh	16	RPI – 4_{2500}	(+)	Baramasi
6	OPA – 7_{1520}	(+)	Dashehari	17	RPI – 7_{500}	(-)	Ambika
7	OPA – 9_{500}	(+)	PKM 2	18	RPI – 7_{700}	(+)	Arka Neelkiran
8	OPA – 12_{150}	(+)	Neeleshan Gujrat	19	RPI – 10_{450}	(+)	Pusa Surya
9	OPA – 12_{1650}	(+)	Arka Neelkiran	20	RPI - 10_{550}	(+)	Pusa Surya
10	OPA – 19_{1000}	(+)	PKM 2	21	RPI – 10_{900}	(+)	Pusa Surya
11	OPC – 11_{630}	(+)	Ambika				

[1]Each RAPD marker is represented by the primer number and the band size (bp)

Table 6

Jaccards Similarity Matrix generated by RAPD primers for mango genotypes audited

	Pusa Surya	Pusa Arunima	Ambika	PKM 1	Janardhan Pasand	PKM 2	Neeleshan Gujrat	Mahmud Bahar	Baramasi	Sai Sugandh	Dashehari	Arka Neelkiran
Pusa Surya	1.00											
Pusa Arunima	0.58	1.00										
Ambika	0.67	0.72	1.00									
PKM 1	0.72	0.66	0.68	1.00								
Janardhan Pasand	0.63	0.72	0.68	0.71	1.00							
PKM 2	0.77	0.70	0.72	0.81	0.77	1.00						
Neeleshan Gujrat	0.64	0.70	0.72	0.75	0.77	0.78	1.00					
Mahmud Bahar	0.60	0.73	0.68	0.72	0.75	0.74	0.78	1.00				
Baramasi	0.68	0.70	0.66	0.70	0.61	0.68	0.63	0.68	1.00			
Sai Sugandh	0.66	0.61	0.60	0.71	0.60	0.68	0.55	0.60	0.63	1.00		
Dashehari	0.55	0.68	0.63	0.69	0.68	0.72	0.74	0.72	0.63	0.57	1.00	
Arka Neelkiran	0.60	0.69	0.67	0.74	0.79	0.79	0.74	0.75	0.64	0.68	0.70	1.00

3. Results and discussion

3.1 Leaf and pomological diversity based on morpho-biochemical traits

Description of mango germplasm through morpho-pomological parameters was a necessary prelude to biochemical or molecular characterization (Litz, 2004). In the present investigation twelve mango genotypes of India were evaluated with respect to 11 qualitative and 9 quantitative traits to determine the genotypic diversity through morphological and biochemical traits. A high degree of variation was observed among genotypes based on qualitative and quantitative characters related to leaf and fruit (Table 1, Table 2). Most of the qualitative traits as given for the leaf, mature fruits and ripe fruits were polymorphic showing more than two phenotypes in mango genotypes. The leaf blade shape was highly polymorphic among mango genotypes; ovate in 'Pusa Surya', 'Neeleshan Gurat' and 'Dashehari', elliptic in 'Pusa Arunima', 'Ambika, 'Janardhan Pasand', 'Mahmud Bahar' and 'Baramasi' while oblong in four hybrid genotypes ('PKM-1','PKM-2','Sai Sugandh' and Arka Neelkiran'). Two phenotypic classes for leaf margin were recorded; 'Janardhan Pasand', 'Neeleshan Gujrat','Mahmud Bahar', 'Baramasi' and 'Sai Sugandh' were with entire margin, whereas for the rest of the genotypes the leaf margin were wavy. A high variability in leaf characteristics was reported in indigenous mangoes of Pakistan (Rajwana *et al*., 2011).

Mature fruit skin colour was a highly polymorphic trait, varied from green to greenish red, purple and pink. 'Pusa Surya',' PKM 2', 'Neeleshan Gujrat', 'Mahmud Bahar', 'Baramasi' and 'Dashehari' had green skin colour, 'Sai Sugandh', 'Arka Neelkiran', 'Pusa Arunima' and 'PKM 1' had 'green and red' skin colour, 'Ambika' and 'Janardhan Pasand' had greenish purple and greenish pink skin colours respectively. Stalk cavity were absent in 'Pusa Arunima', 'Janardhan Pasand', 'Neeleshan Gujrat', 'Sai Sugandh' and 'Dashehari' where as it was 'medium' in only one genotype 'Arka Neelkiran' and 'shallow' in the rest of six genotypes. Ripe fruit skin and flesh colour are consumer preference traits. High variations were noticed in ripe fruit skin colour which ranged from orange, yellow, 'green and yellow', yellow green, 'yellow and red', 'red and purple' at the ripening stage. Interestingly, fruits of the genotype 'Ambika' possesses very attractive and unique 'red and purple' skin colour. A significant variation in ripe fruit skin colour was reported in mangoes (Barholia and Yadav, 2014; Sennhenn *et al*., 2014).

Our results also revealed a significant variability in flesh colours of fruits with more than three phenotypic classes. Least variation was observed for juiciness; only one genotype 'Janardhan Pasand' was highly juicy whereas the remaining genotypes were with medium range juiciness. Higher peel percentage were recorded in 'Arka Neelkiran', 'Janardhan Pasand', 'PKM 1', 'Pusa Surya', 'Pusa Arunima', 'PKM 2' and 'Ambika'. A high percentage of peel may be responsible for good storage life of the fruits. Fruit storage life is also a very important trait for packaging and transportation; most mango collections under study had 'very good' to 'good' storage life except 'Baramasi' which had 'intermediate storage life. A single mango genotype namely, 'Janardhan Pasand' was early maturing; 'PKM 2', 'Mahmud Bahar' and 'Dashehari' had mid-season maturity whereas the remaining eight genotypes showed late-very late fruit maturity. As these categorical morpho-pomological characters are discrete these could be used for varietal discrimination.

Significant differences were also obtained for the all 9 quantitative ripe fruit traits. The recorded mean values for each of the quantitative traits with summary statistics at $p \leq 0.05$ are presented in Table 2. Traits such as sugar: acid ratio, physiological loss, ripe fruit weight and TSS displayed high CVs (>20%) while the traits like peel percentage, fruit length and stone percentage had intermediate CV values. The remaining traits such as fruit width and pulp percentage presented comparatively low CV values (<15%). Galvez-Lopez *et al.* (2010) reported a similar range of CV % for fruit length, width and weight for native mangoes of Mexico.

The fruit length varied from 8.1cm ('PKM-2') to 15.4 cm ('Sai Sugandh'); fruit width from 5.9 cm ('Dashehari') to 9.2 cm ('Sai Sugandh'); fruit weight from 167.1 g ('Baramasi') to 389.6 g ('Sai Sugandh'); physiological loss from 11 g ('Janardhan Pasand') to 28.4g ('Sai Sugandh'; peel percentage 7.94% ('Baramasi') to 16.41% ('Arka Neelkiran'); stone percentage from 12.26% ('Ambika') to 22.36% ('Baramasi'); pulp percentage from 67.87% ('PKM-2') to 77.36% ('Ambika'); TSS from 14.6% ('Janardhan Pasand') to 31% ('PKM-1') and sugar/acid ratio from 24.4 ('Janardhan Pasand') to 82.85 ('Pusa Surya'). The results on fruit length and diameter were in accordance with the variation level detected in Mexican mangoes reported by Galvez-Lopez *et al*. (2010). Variation range for traits like pulp and stone %, TSS and sugar/acid ratio etc. corroborated with the findings of Anila and Radha on Indian mangoes (2003)

Most of the traits studied like red blush on skin, orange to dark-orange coloured flesh, 'excellent' table quality, 'very good' storage life, medium sized fruit, low amount of stone and high pulp percentage, high TSS and sugar/acid ratio had potential economic interest especially those related to fruit quality. They could thus serve as target traits for mango

growers and breeders. In the present study, high substantial variance between the genotypes for above traits coupled with high CV values and the existence of two or more phenotypic classes for each qualitative traits shows that Indian mango germplasm is a rich source of genetic variation for characters of commercial interest. Our investigation revealed that the genotypes like 'Ambika', 'Dashehari', 'Janardhan Pasand', 'Sai Sugandh' and 'Pusa Surya', possessing excellent fruit quality characters of consumer's preference, could be considered as promising candidates for selection of parents for breeding program. Two of these genotypes namely 'Janardhan Pasand' and 'Dashehari', have already been used for the development of different mango hybrids while the remaining three warrant immediate attention.

The results from the current research support the view that leaf-pomological traits and biochemical contents in fruits can be used efficiently for cultivar discrimination as well as for estimating the genetic relationships across large and diverse groups of mango genotypes. These findings are in accordance with other studies indicating that both quantitative and qualitative traits are very helpful in the identification and evaluation of cultivars in mango germplasm (Sennhenn *et al*., 2014; Khan *et al.,* 2015).

3.2 Genetic polymorphism and RAPD patterns

The knowledge of genetic variation and the genetic relationship between plant individuals can be an important consideration for efficient rationalization and utilization of germplasm resources. Besides morphological traits, a high degree of polymorphism was also observed at the molecular level. Initial screening of 70 RAPD primers yielded 23 primers with clear and reproducible banding patterns (Table 3). A total of 307 distinct bands were produced in different size ranging from 100 to 3000 bp with an average of 13.35 bands per primer, of which 264 (85.99%) were polymorphic and only 43 (14.01%) were monomorphic (Table 3). The total number of bands was found to range from 4 (OPC 2) to 21 (OPA 9) and the number of polymorphic bands ranged from 3 (OPC 2) to 20 (OPA 9). The percentage of polymorphism ranged from 50% (RPI 7) to 100% (OPA 8, OPA 19, OPG 9 and RPI 10) with an average of 84.75% polymorphism per primer. The banding patterns of 12 mango genotypes using OPA 6, OPA 7 and RPI 10 primers are displayed in Fig. 1a, b, c. The results of the present study were close to findings from genetic diversity studies on Indian mango genotypes using RAPD (Bajpai *et al*., 2008; Karihaloo *et al*., 2003). However, compared to our results, a low to moderate level of polymorphism with RAPD primers was observed in earlier experiments in Indian mangoes (Ravishankar *et al*., 2000) and Egyptian mangoes (Abou-Ellail *et al*., 2014). This disagreement between various studies may be ascribed to differences in the number of primers and genotypes used along with their diverse genetic backgrounds. High PIC value of 0.82 (OPA 9) and low PIC value of 0.38 (OPG 17) with an average value of PIC per primer 0.60 were obtained (Table 4). The highest EMR value of 6.58 was observed with the primer OPA 8 and the lowest EMR 1.56 was observed with the primer OPC 5 with an average of 4.65 per primer. Marker index (MI), which reflects the overall usefulness of a given marker system was found to be highest with the primer OPA 8 (4.08) and lowest in the primer OPC 5 (0.76), with an average of 2.85 per primer. Resolving power (RP), the discriminatory potential of the primer, was the highest with the primer OPG 17 (23.5) and the lowest with the primer OPC 2 (4.83) with an average of 14.35 per primer. The genotype Index (GI) ranged from 0.00-0.08 with an average of 0.02. The genetic diversity values based on Shannon index ranged between 0.17 (OPC 5) to 0.54 (OPA 8) with a mean of 0.41. Average values of observed number of alleles (Na), effective number of alleles (Ne), expected heterozygosity (He) and unbiased expected heterozygosity (uHe) of 1.82, 1.46, 0.27 and 0.28 (Table 4) respectively was recorded with RAPD markers. Most informative RAPD markers based on PIC, marker index and Shannon's index were identified as OPA 7, OPA 8, OPA 9, OPA 19, RPI 2 and RPI 10.

3.3 Genotype specific diagnostic markers

Using RAPD technique a total of 21 unique bands were generated which identified 9 ('Pusa Surya', 'PKM-2', 'Sai Sugandh', 'Dashari', 'Neelshan Gujrat', 'Arka Neelkiran', 'Ambika', 'Mahmud Bahar' and 'Baramasi') out of the 12 total genotypes audited (Table 5). Two genotypes namely 'Pusa Surya' and 'PKM 2' were the unique genotypes each possessing maximum of four RAPD specific loci. Two most informative primers OPA 6 & OPA 7 were identified each of which were able to generate the highest number (3) of unique bands individually for identification of three different genotypes. It was interesting to note that even the absence of a specific band has capacity for discriminating genotypes 'PKM-2' (250 bp), 'Baramasi' (840 bp) and 'Ambika' (500, 2000 bp). These genotype specific amplicons identified with RAPD marker systems will play essentially important roles in characterization, conservation, and utilization of mango germplasm. Similar type of presence/absence of specific loci(s) were reported in a number of plants with RAPD for rice (Raghunathachari *et al*., 2000) and cashew (Jena *et al*., 2016). The unique amplicons can be developed to SCAR markers for marker-assisted selection and other trait-specific analysis. Based on the ability to detect unique bands, OPA 6, OPA 7 and RPI

10 were recognized as efficient primers which would be useful for detecting mixtures and duplicates of mango seedlings in the future. This kind of marker tagging will contribute to the efficient selection and hybridization in mango breeding programs as the source of new and novel alleles aiming at genetic improvement of this fruit crop.

3.4 Genetic similarity and cluster analysis

The genetic similarity coefficient was evaluated by calculating the Jaccard's similarity coefficient based on the proportion of shared bands. Jaccard's similarity coefficient ranged from 0.55 to 0.81 with a mean value of 0.68 (Table 6). The high values of cophenetic correlation coefficient, r = 0.85 between the similarity matrix and co-phenetic matrix obtained from UPGMA dendrogram indicated good illustration of relationships between genotypes in the cluster analysis. The most closely related genotypes were 'PKM 1' vs 'PKM 2' with the highest similarity index (0.81) closely followed by 'PKM 2' vs 'Arka Neelkiran' and 'Janardhan Pasand' vs 'Arka Neelkiran' (0.79). On the other hand, most distantly related genotypes were 'Dashehari' vs 'Pusa Surya' and 'Sai Sugandh' vs 'Neeleshan Gujrat', 'with the lowest similarity index (0.55) followed by 'Dashehari' vs 'Sai Sugandh' (0.57) and 'Pusa Surya' vs 'Pusa Arunima'(0.58) representing most diverse varieties (Table 6). The similarity range detected in the present study using RAPD markers was higher than those reported by Karihaloo *et al*. (2003).

Assigning a cut-off point of 0.70 the UPGMA clustering algorithm of RAPD marker analysis separated the 12 genotypes into four major clusters (I - IV) spanning an index length of 0.63-0.81 (Fig. 2). Cluster I, II and IV included 2, 2, and 1 genotype respectively where as Cluster III comprised maximum 7 number of genotypes. The two most diverse genotypes 'Pusa Surya' (North India) and 'Baramasi' (East India) were grouped together in Cluster I. 'Pusa Surya' is a selection from an exotic cultivar 'Elden' of Brazil and 'Baramasi' is a novel land race as it bear fruits throughout the year. In Cluster II, two hybrids namely 'Pusa Arunima' ('Amrapali' × 'Sensation') and 'Ambika' ('Amrapali' × 'Janardhan Pasand') were included with a coefficient similarity of 0.72. These two hybrids have 'Amrapali' as the Female parent. Cluster III was divided into two sub-clusters (IIIA and IIIB) with a similarity of 71%. Sub-cluster IIIA comprises six genotypes of which five are hybrids. Sub-cluster IIIA was further divided to two sub-sub clusters, $IIIA_1$ and $IIIA_2$, the former included four genotypes (3 hybrids, 1 selection). Interestingly in this, the three hybrid genotypes 'PKM-1' ('Chinnaswarnarekha' × 'Neelum'), 'PKM-2' ('Neelum' × 'Mulgoa') and 'Arka Neelkiran' ('Alphonso' × 'Neelum') shared commonalities in their pedigree i.e. having same parent 'Neelum' as well as morphological features namely oblong leaves with wavy margins, good storage life, fruit lengh and weigth, pulp and peel %. Clustering of mango genotypes with 'Neelum' as a parent was also reported by Vasanthaiah (2009). The second sub-sub cluster $IIIA_2$ contained two hybrids 'Neeleshan Gujrat', and 'Mahmud Bahar' sharing 0.78 % similarity. At a similarity coefficient of 0.71, single genotype 'Dashehari' formed a distinct sub cluster IIIB. Interestingly, a single genotype namely 'Sai Sugandh', a cross-bred of two diverse genotypes 'Kesar' × 'Totapuri', formed a separate cluster IV. It is a distinct variety with large and long fruit having deep sinus with good storage life and excellent eating quality character. 'Totapuri' is a regular South Indian variety with large fruit with low fruit quality whereas 'Kesar' is a Western Indian variety with best quality small fruit. The relationship established for all genotypes in the cluster analysis presented in form of dendrogram was also mirrored in the two dimensional (2D) and three dimensional (3D) principal component analysis (PCA) of the mango genotypes audited (Fig. 3 and 4).

Classification of diversity in germplasm collections is important for plant breeding. In this study, we investigated genetic diversity in mango genotypes based on leaf and pomological characteristics along with a DNA-based molecular marker. There is much environmental influence accounting for the morphological variability observed. Therefore, compared to molecular techniques, morphometric traits are relatively less reliable and inadequate for precise discrimination of closely related genotypes and analysis of their genetic relatedness/distance. Nevertheless, phenotypic variables are useful for preliminary, fast, simple, and inexpensive varietal identifications and can be used as a general approach for assessing gross genetic diversity among genotypes. Many traits recorded in this study are with high economic importance and, therefore, they serve as target traits for selection by mango growers and breeders. RAPD analysis has been shown to be an useful technique for providing information concerning the degree of polymorphism and diversity parameters of mango. In addition, this technique can be exploited for efficiently identifying and characterizing mango germplasm with respect to specific agro-pomological traits. Understanding and structuring of the genetic diversity among mango genotypes will be a major foot step to accelerate linkage analysis, association mapping, marker assisted selection and cross breeding programs, which would aid strategies aimed at germplasm characterization, management, conservation and improvement of this important fruit crop.

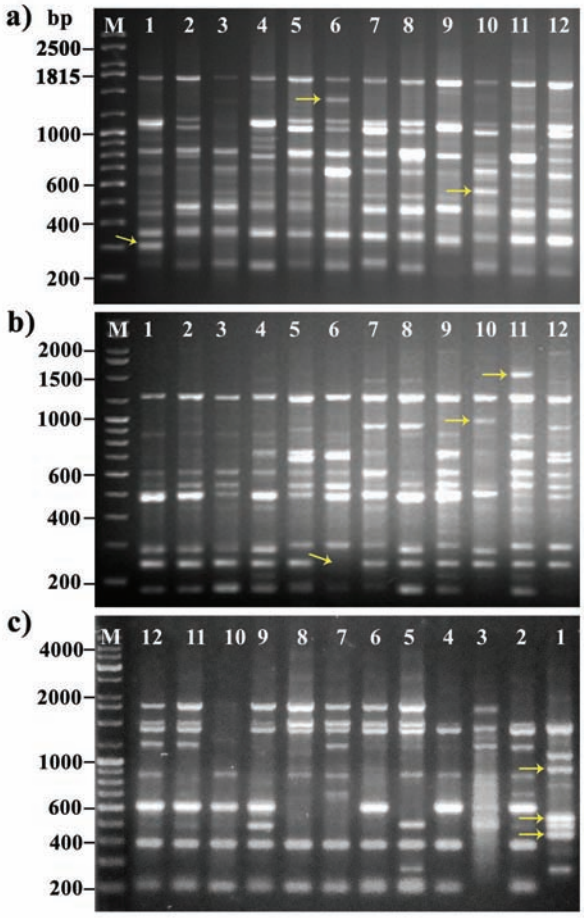

Fig. 1. RAPD profiling of 12 selected mango genotypes using primer OPA 6 (a), OPA 7 (b), and RPI 10 (c).Lane M : Low Range DNA Ruler Plus. Lanes 1-12 correspond to the mango genotypes (Listed in Table 1). *Arrows denote the presence of unique bands.*

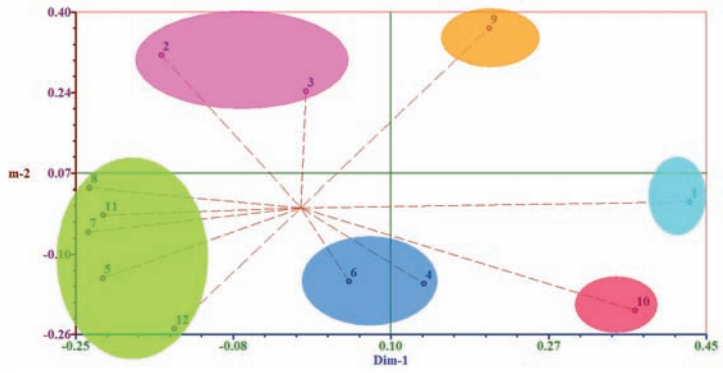

Fig. 3. Two dimensional plot generated from Principal Component Analysis (PCA) using RAPD markers. *(Numbers [1-12] plotted represent individual genotypes as listed in Table 1).*

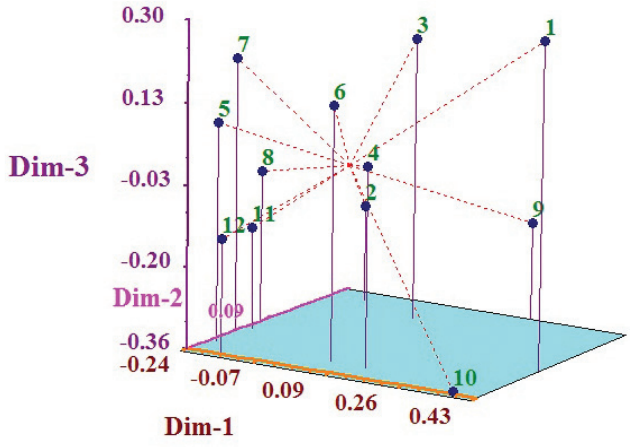

Fig. 4. Three dimensional distribution of mango genotypes based on PCA analysis based on RAPD markers. *(Numbers [1-12] plotted represent individual genotypes as listed in Table 1).*

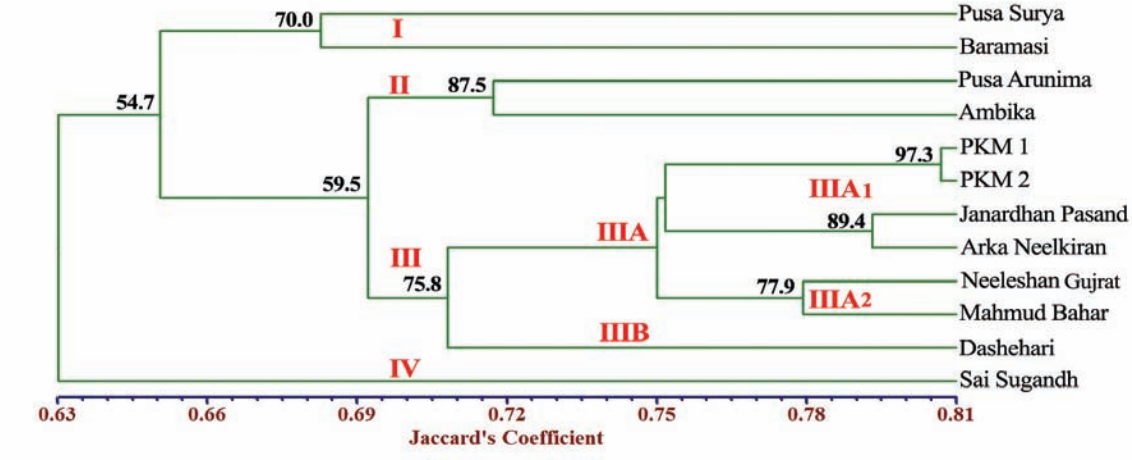

Fig. 2. UPGMA dendrogram constructed from RAPD based Jaccard's Similarity Coefficient depicting phylogenetic relationship among 12 selected mango genotypes. *(Values at the nodes correspond to bootstrap support [1000 replications])*

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