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HORTICULTURE

Clonal variability studies in 'langra' mango (*Mangifera indica* L.) using morphological, biochemical and molecular markers

Ankita Anu[1], Bishun Deo Prasad[2*], Ravindra Kumar[1], Pankaj Kumar[2], Vishwa Bandhu Patel[1] and Rudra Narayan Jha[3]

[1]Department of Hort culture, Bihar Agricultural College, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India.
[2]Department of Plant Molecular Biology and Genet c Engineering, Bihar Agricultural College, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India.
[3]Department of Stat st cs, Mathemat cs and Computer Applicat on, Bihar Agricultural College, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India.
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Corresponding author: dev.bishnu@gmail.com

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Abstract

Mango is one of the most economically important tropical fruits grown in tropical and sub-tropical regions of the world. Being a delicious and widely cultivated fruit, it is regarded as 'the King of fruits' on the Indian subcontinent and the 'national fruit of India' because of the flavour and nutritional qualities. Mango has rich intra-specific diversity, with about 1600 and 1000 cultivars of mango present globally and India, respectively. 'Langra' is one of the leading commercial mango varieties of Northern India including Bihar which is known for mildly fibrous flesh and with a distinct pleasant taste and turpentine flavour. Significant variation exists among the clones of 'Langra' mango with respect to fruit shape, size, colour, quality and taste. Several studies have been made on characterization of intra-varietal variability of many different cultivars of mango. However, clonal variability studies in 'Langra' mango are limited. In this present investigation, an at empt were undertaken to study the clonal variability exit in some 'Langra' mango using morphological, biochemical and Molecular Markers. These results provide evidence that a significant level of genetic variation exits among 10 clones of 'Langra' mango which can be used for mass multiplication of superior clone(s) and can be further utilized in breeding programs.

Highlights

- Ten genotypes of 'Langra' mango were characterized and evaluated for variation in different qualitative and quantitative traits.
- RAPD analysis was performed to discriminate the different clones of 'Langra' mango.
- Based on all the analysis Dudhiya Maldah was found to be the best clone of 'Langra' mango among all the clones analysed.

Keywords: Ascorbic acid, langra, *mangifera indica* L., NTSYS, RAPD, total soluble solids (TSS).

Mango (*Mangifera indica* L.) is one of the most important fruit crops of the Anacardiaceae family. Mango serves as an integral part in human life since it is not only a rich source of nutrients but also a common good shared in culture, life style and religion. It is one of the most popular fruits in the

tropics (Lakshminarayana, 1980). Global production of mango is concentrated mainly in Asia and more precisely in India. It is believed to have originated in Indo-Burma region (Popenoe, 1927; Mukherjee, 1951; Decandolle, 1904). It is now cultivated pantropically throughout the world. In India, mango is distributed throughout the length and breadth of country, except in hilly regions at 915 meters above mean sea level. According to Mukherjee (1951) mango is being cultivated for more than 4000 years in Eastern India and Burma. India is the largest mango producer in the world (Anon, 2013). In India, mangoes are cultivated in an area of 2500 ('000 ha) with an annual production of 18002.4 ('000 MT) and productivity of 7.2 MT/ha (Anon, 2013). Indian mangos occupy 35.8% of the total world's mango area and contribute 22.1% to the total world's mango production.

Mango has rich varietal diversity and there are about 1600 varieties in the world (Pandey, 1998). In India, there are hundreds of mango cultivars, of which about 50 varieties of mango are of commercial importance (Chadha and Pal, 1986; Suresh and Venkateswarlu, 2013). The major mango growing states in India are Maharashtra, Andhra Pradesh, Ut ar Pradesh, Bihar, Karnataka and Gujarat. Bihar contributes 7.6% to the total country's mango production and become the 4th largest mango producer in the country (Anon, 2013). 'Langra' is one of leading commercial mango variety of Northern India including Bihar which is known for mildly fibrous flesh and with a distinct turpentine taste. Langra also has a short season, lasting only from mid-July to the end of the month. It was observed that significant variation exists, among trees of the same clone in an orchard with respect to fruit shape, size, colour and quality, which is ascribed to bud mutation. Asexual propagation enables us to preserve the accumulated mutations which would normally be sieved-out by sexual propagation. Thus, during the course of evolution, number of mutations accumulated in different clones, might have created polymorphism among the cultivated 'Langra' mangoes from different pockets. Therefore, it becomes necessary to establish the phylogenetic relationship and investigate any possible differences at molecular level in the 'Langra' mangoes from different pockets. Further, the identification and characterization of superior clones of 'Langra' are important activities in the management of genetic resources in mango in the context of the present scenario of rapid extinction of such useful material. In the present investigation, efforts were therefore made to study clonal variability's exits in 'Langra' using morphological, biochemical and molecular markers.

Materials and Methods

Plant Materials

Ten genotypes namely Dholi Kothi Maldah, Surajgarha Maldah, Kalkat ia Maldah, Langra/ Maldah, Seso Maldah, Safed Lucknow, Digha Maldah, Kala Maldah, Banarsi Langra and Dudhiya Maldah were used in present investigation. The morphological data were collected from the accession sites of the orchard of Department of Horticulture (Fruit and Fruit Technology), Bihar Agriculture College, Sabour. For molecular characterization leaf material of above mentioned 10 genotypes were collected and experiment were carried out in Central Molecular Biology facilities at Department of Plant Breeding and Genetics, Bihar Agricultural College, Sabour.

Morphological Characterization

Collected fruit samples were characterized and evaluated for variation in different quantitative traits of 'Langra' like fruit length (cm), fruit width (cm), fruit weight (g), peel (%), pulp (%), stone (%), total soluble solids (TSS) (°Brix), ascorbic acid (mg/100g), acidity (%), total sugar (%) and reducing sugar (%) were recorded. Peel and stone of the each genotype were carefully removed and cleaned with distilled water. TSS was recorded with refractometer. Titratable acidity was determined by using standard titration method. The reducing sugar was estimated by Lane and Eyon (1923). Copper titration methods as suggested by Association of Official Analytical Chemists (1975). Total sugars and reducing sugar

was estimated as described by Lane and Eyon (1923). Ascorbic acid content of the juice was determined by titrating freshly extracted juice against 2, 6 Dichlorophenol indophenols dye Association of Official Analytical Chemists (1975). Total carotenoids content of mango fruit was determined by following the protocol of Roy (1973). Quantitative measurement of TSS, acidity, ascorbic acid, total sugar, reducing sugar and carotenoids was analyzed when fruit were naturally ripened.

In addition to quantitative traits, the fruit samples were also characterized for qualitative traits like fruit shape, skin colour of mature fruit, skin thickness, skin texture, pulp colour, quantity of fibre and eating quality. Qualitative characters were determined by the help of Minimal Descriptors of Agri-Horticultural crops (National Bureau of Plant Genetic Resources).

DNA isolation

Genomic DNA of Mango genotypes were isolated from new young leaves by following CTAB method. Freshly harvested young and tender leaf samples (1gm) were ground in liquid N_2 using mortar and pestle. Approximately 350 mg of the grounded leaf samples were quickly transferred in to 1.5 ml of microcentrifuge tube and equal volume (W/V) (350 µl) of hot (65°C) 2X CTAB buffer was added in to the microcentrifuge tube and mixed thoroughly by vigorous shaking for 2 min. Approximately 700 µl of ice cold chloroform: isoamyl alcohol (24:1) was added into the microcentrifuge tube, mixed well by inversion and centrifuged at 12,000 rpm for 5 min. The top aqueous phase was collected using cut tips into a new microcentrifuge tube and $1/5^{th}$ volume of 5% CTAB solution was added in the microcentrifuge tube and 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 phase was collected using cut tips into a new microcentrifuge tube and equal volume of CTAB precipitation buffer was added, mixed 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 ice cold ethanol (2.5 volumes of the supernant) and mix gently by inversion. Microcentrifuge tubes were centrifuged at 12,000 rpm for 15 min and supernatant was discarded. DNA pellet was washed with 70% ethanol, air dried and dissolved in 30 µl of TE buffer ($T_{10}E_1$).

Data Analysis

RAPD analysis of 10 mango genotypes was conducted by using 11 decamer arbitrary primers obtained from Operon Technologies, California. RAPD amplification was performed in 25 µl volume containing 1X Taq DNA Polymerase buffer, 200 µM dNTPs mixture, 0.5 µM primer, 25 ng of template DNA and 1 U of Taq DNA polymerase (Bangalore Genei, Bangalore, India) in a thermal cycler. The reagents were mixed thoroughly and then placed on a Thermalcycler for cyclic amplification and the conditions for amplification was programmed as follows: The thermal profile set comprised a denaturation step of 5 min at 94°C, followed by 40 cycles of 1 min at 94°C, 30 s at 34°C, an extension at 72°C for 2 min and a final extension at 72°C for 10 min. Amplification products were then subjected to electrophoresis in 1.2% agarose gel using 1 X TBE and detected by ethidium bromide staining, viewed under UV light and photographed with Gel-documentation system. The RAPD markers observed after gel electrophoresis were converted into a matrix of binary data, where the presence of the band was scored as 1 and the absence of band was scored as 0. Using NTSYS software, a dissimilarity matrix was calculated utilising Jaccard's (1908) coefficient. The matrix was converted to a dissimilarity matrix corresponding to the complement (dissimilarity=1−similarity). Cluster analysis based on the dissimilarity matrix, was performed using un-weighted pair-group method arithmetic averages (UPGMA) of the NTSYS–PC version 2.2 (Rohlf, 2005).

Table 1. Morphological characterization of different clones of 'Langra' mango based on quantitative traits.

S. No.	Name of clones	Fruit length (cm)	Fruit Width (cm)	Fruit weight (g)	Peel (%)	Stone (%)	Pulp (%)	Yield of fruit/ plant (kg/plant)
1	Dholi Kothi Maldah	13.35	10.87	843.90	7.25	10.70	82.00	45.14
2	Surajgarha Maldah	10.37	7.45	305.20	12.00	10.11	77.80	106.05
3	Kalkattia Maldah	11.01	7.50	307.00	13.00	11.20	75.78	162.94
4	Langra/Maldah	10.64	7.59	330.30	11.60	10.32	78.02	118.90
5	Seso Maldah	13.66	8.76	492.40	8.34	13.22	78.43	241.27
6	Safed Lucknow	9.35	6.39	202.30	15.21	16.66	68.04	195.92
7	Digha Maldah	10.11	7.58	318.00	8.85	10.34	80.78	358.38
8	Kala Maldah	11.28	7.55	338.00	10.44	12.40	77.10	10.98
9	Banarsi Langra	10.32	7.56	303.60	10.82	12.00	77.18	236.95
10	Dudhiya Maldah	8.58	7.41	182.20	7.27	10.02	52.01	167.80
SE diff. Mean		0.43	0.34	28.82	0.59	0.80	3.88	17.62
CD at 5 %		0.97	0.76	64.21	1.32	1.79	8.64	39.27
CV %		4.00	4.32	7.95	5.45	6.81	5.18	10.76

Table 2. Morphological characterization of different clones of 'Langra' mango using qualitative traits.

S. No.	Name of clones	Fruit maturity group	Fruit shape	Fruit skin colour	Skin thickness	Utility type	Pulp fiberousness	Eating quality	Ripe fruit taste	Pulp colour	Pulp text	Productivity status
1.	Dholi Kothi Maldah	Mid early	Round	Light green with pink base	Medium	Table type	Less Fibrous	Good	Medium sweet	Orange	Firm	High
2.	Surajgarha Maldah	Mid	Round	Light green	Medium	Table type	Less Fibrous	Good	Medium sweet	yellow	Firm	High
3.	Kalkattia Maldah	Mid early	Round	Light green	Medium	Table type	Less Fibrous	Good	Medium sweet	yellow	Firm	Medium
4.	Langra/Maldah	Mid late	Round	Light green	Medium	Table type	Less Fibrous	Excellent	Medium sweet	yellow	Firm	Medium
5.	Seso Maldah	Mid early	Oblong but beak not prominent	Light green	Medium	Table type	Less Fibrous	Good	Medium sweet	yellow	Firm	Medium
6.	Safed Lucknow	Mid	Round	Light green	Medium	Table type	Less Fibrous	Good	Medium sweet	yellow	Firm	Medium
7.	Digha Maldah	Mid	Round	Light green	Medium	Table type	Less Fibrous	Good	Medium sweet	yellow	Firm	Medium
8.	Kala Maldah	Mid	Round	Light green	Medium	Table type	Less Fibrous	Good	Medium sweet	yellow	Firm	Medium
9.	Banarsi Langra	Mid late	Round	Light green	Medium	Table type	Less Fibrous	Good	Medium sweet	yellow	Firm	Medium
10.	Dudhiya Maldah	Mid early	Round	Light green	Thin	Table type	Less Fibrous	Excellent	Medium sweet	yellow	Firm	Medium

Results and Discussion

Morphological characterization

The analysis of variance of 10 clones of 'Langra' identified in this investigation revealed significant differences (Table 1). In this study, the maximum fruit length (13.66 cm) was observed in Seso Maldah and the minimum fruit length was observed in Dudhiya Maldah (8.58 cm) (Table 1). The highest fruit width was observed in Dholi Kothi Maldah (10.87 cm) and the lowest fruit width (6.39 cm) was found in Safed Lucknow. Dholi Kothi Maldah recorded the highest fruit weight (843.9 g) (Table 1). The peel % of Dholi Kothi Maldah was found significantly superior compared to all other clones (Table 1). Dudhiya Maldah possess lowest stone percentage (10.32%) whereas, Safed Lucknow possess maximum stone percentage (16.66%) (Table 1). The highest pulp percentage was rocorded in Dholi Kothi Maldah (82%) on the other hand minimum pulp percentage (52%) was recorded in Dudhiya Maldah. The highest fruit yield (358.83 kg/plant) was observed by variant Digha Maldah lowest yield of 10.95 kg/plant was found in Kala Maldah (Table 1).

Different qualitative traits were analysed (Table 2) as described in Materials and Methods. Dudhiya Maldah, Seso Maldah and Kalkatia Maldah were falls in mid early maturing group (Table 2). Fruit shape was observed to be round in case of all the clones analysed except Seso Maldah. Fruit colour was found to be light green in all the clones analysed except Dholi Kothi Maldah which has light green colour with pink base (Figure 1). The skin thickness was found to be thin in case of Dudhiya Maldah whereas all other clones have medium thickness. The firm texture and less pulp fibrousness were observed in all the clones analysed. The ripe fruit taste was found to be medium sweet in all the clones studied. However, the eating quality was found to be excellent in Dudhiya Maldah among all the clones analysed.

Table 3. Biochemical characterization of different clones of 'Langra' mango.

S. No.	Name of clones	TSS (^{O}Brix)	Acidity (%)	Total sugar (%)	Reducing sugar (%)	Ascorbic acid (mg/100g)	Carotenoids (mg/100g)
1.	Dholi Kothi Maldah	18.60	0.31	10.75	4.59	31.91	4.00
2.	Surajgarha Maldah	18.80	0.29	11.78	4.60	28.55	4.27
3.	Kalkattia Maldah	19.20	0.25	10.78	6.41	26.23	3.13
4.	Langra/Maldah	18.35	0.30	13.67	6.04	28.05	3.82
5.	Seso Maldah	17.00	0.39	13.31	6.55	27.39	4.11
6.	Safed Lucknow	16.25	0.45	10.61	4.65	28.09	1.98
7.	Digha Maldah	18.45	0.32	12.02	6.61	34.34	4.62
8.	Kala Maldah	18.50	0.30	11.58	4.32	24.56	2.19
9.	Banarsi Langra	18.95	0.32	10.62	6.20	30.43	3.35
10.	Dudhiya Maldah	22.10	0.23	10.67	4.65	24.56	3.29
SE diff. Mean		0.93	0.02	0.26	0.11	5.23	0.12
CD at 5 %		2.08	0.03	0.58	0.24	11.66	0.28
CV %		5.01	4.78	2.23	1.96	4.39	3.58

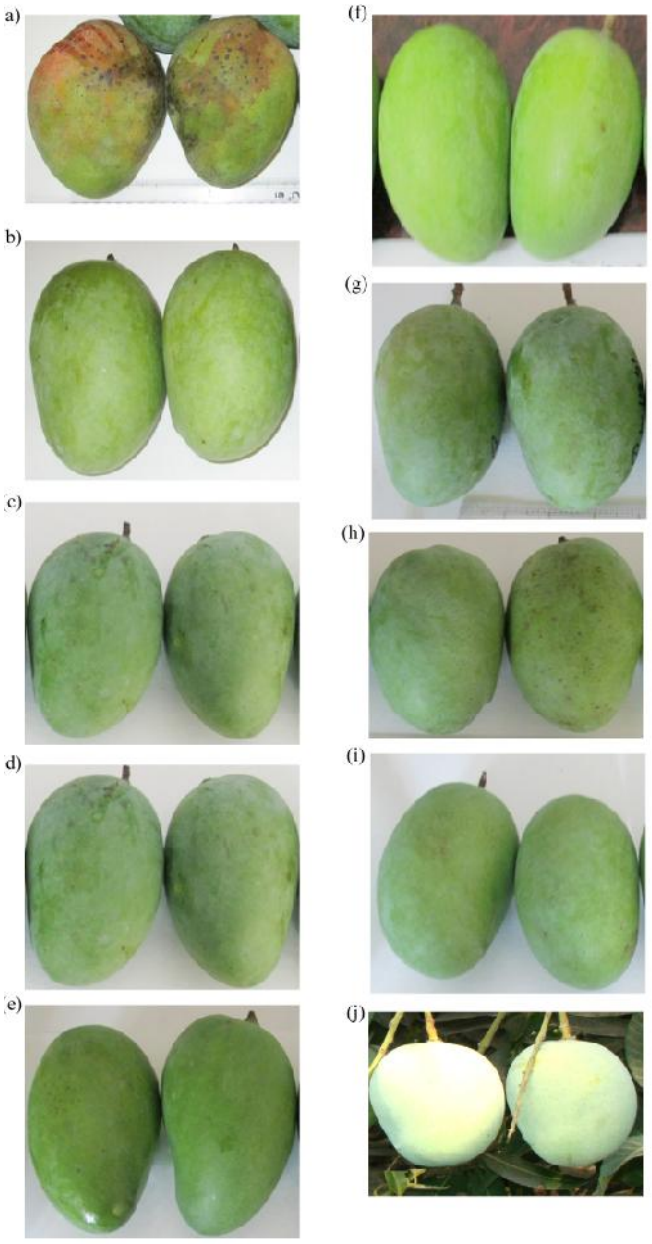

Fig. 1. Morphological appearance of Dholi Kothi Maldah (a), Surajgarha Maldah (b), Kalkattia Maldah (c), Langra/Maldah (d), Seso Maldah (e), Safed Lucknow (f), Digha Maldah (g), Kala Maldah (h), Banarsi Langra (i), and Dudhiya Maldah (j) at maturity stage.

Biochemical Characterization

The TSS contained in the fruit of Dudhiya Maldah (22.10° Brix) was found to be the highest, whereas Safed Lucknow (16.25° Brix) showed the lowest (Table 3). The highest titratable acidity was noted in Safed Lucknow (0.45%) which was significantly higher compared to other clones studied (Table 3). The lowest titratable acidity was observed in Dudhiya Maldah (0.23%) (Table 3). Langra/Maldah had the highest total sugars (13.67%), content whereas, the lowest total sugar content was observed in Safed Lucknow (10.61%) (Table 3). The reducing sugar content was found to be highest in Digha Maldah (6.61 %) which was slightly higher compared to Seso Maldah (6.55%), Kalkat ia Maldah (6.41%), Banarsi Langra (6.20%) and Langra/ Maldah (6.04%). The lowest level of reducing sugar was analyzed in Kala Maldah (4.32%). Interstingly, Kala Maldah produced the highest ascorbic acid (34.34 mg/100 g juice) and proved its superiority over remaining clones analysed. The highest carotenoids content was observed in Digha Maldah (4.62 mg/100 g juice) whereas, Safed Lucknow yielded lowest carotenoids (1.98 mg/100g juice) (Table 3).

RAPD analysis

RAPD analysis was performed to discriminate the different clones of 'Langra' mango by using 11 random primers (Table 4). The amplification profiles of different primers and level of polymorphism observed in different clones of 'Langra' mango were as described in (Table 4 and 5). The primer OPA18 amplified 6 fragment of size ranging from 100 bp to 6.00 kbp (Figure 2a). Out of total 6 bands, 3 polymorphic and 3 monomorphic bands were observed. The percentage polymorphism was found to be 50%. The primer OPB04 amplified 5 fragments (Figure 2b) with 60% polymorphism. The primer OPB07 produced 4 polymorphic bands out of total 6 bands obtained in all the genotypes studied (Figure 2c). The primer OPB08 yielded 5 polymorphic bands (Figure 2d) with 71.42% polymorphism. The primer OPB12 showed 6 polymorphic bands with 60% polymorphism (Figure 2e). The primer OPB16 generated 3 polymorphic (Figure 2f) with 75% polymorphism. The primers OPC07 and OPC08 produced 8 bands with 5 and 7 polymorphic bands, respectively (Figure 3a,b). The percentage

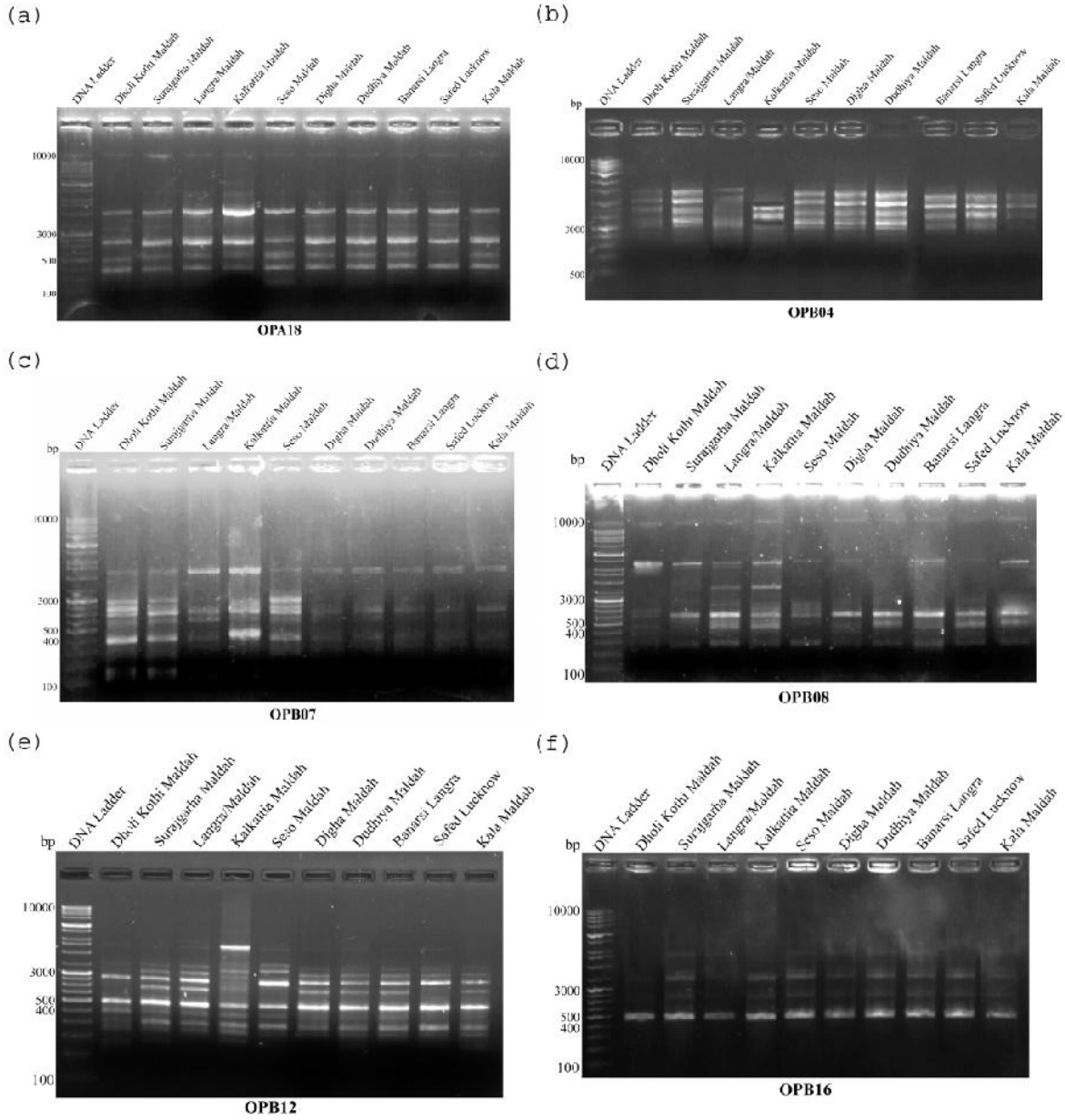

Fig. 2. Molecular profiles of 10 clones of 'Langra' mango obtained using different RAPD markers. RAPD profile of 'Langra' mango using OPA18 (a), OPB04 (b), OPB07 (c), OPB08 (d), OPB12 (e), and OPB16 (f).

polymorphism was found to be 62.5% and 87.50%, respectively. The primer OPC09 generated total 7 bands of size ranging from 250 bp to 8.00 kbp in all the genotypes studied (Figure 3c). Out of the 7 bands generated by OPC09 primer, 6 bands were found to be polymorphic with 85.71% polymorphism. The primer OPC14 generated 6 bands (500 bp to 8.00 kbp) in all the genotypes studied (Figure 3d). Out of the 6 bands generated by OPC14, 5 bands were found to be polymorphic with 83.33% polymorphism (Figure 3e). The primer OPC15 generated 9 bands of size ranging from 250 bp to 4.00 kbp (Figure 3f). Out of total 9 bands, 7 bands were found to be polymorphic with 77.71% polymorphism. The total numbers of bands generated from all the 11 primers used in this study were 553. The number of bands in each mango

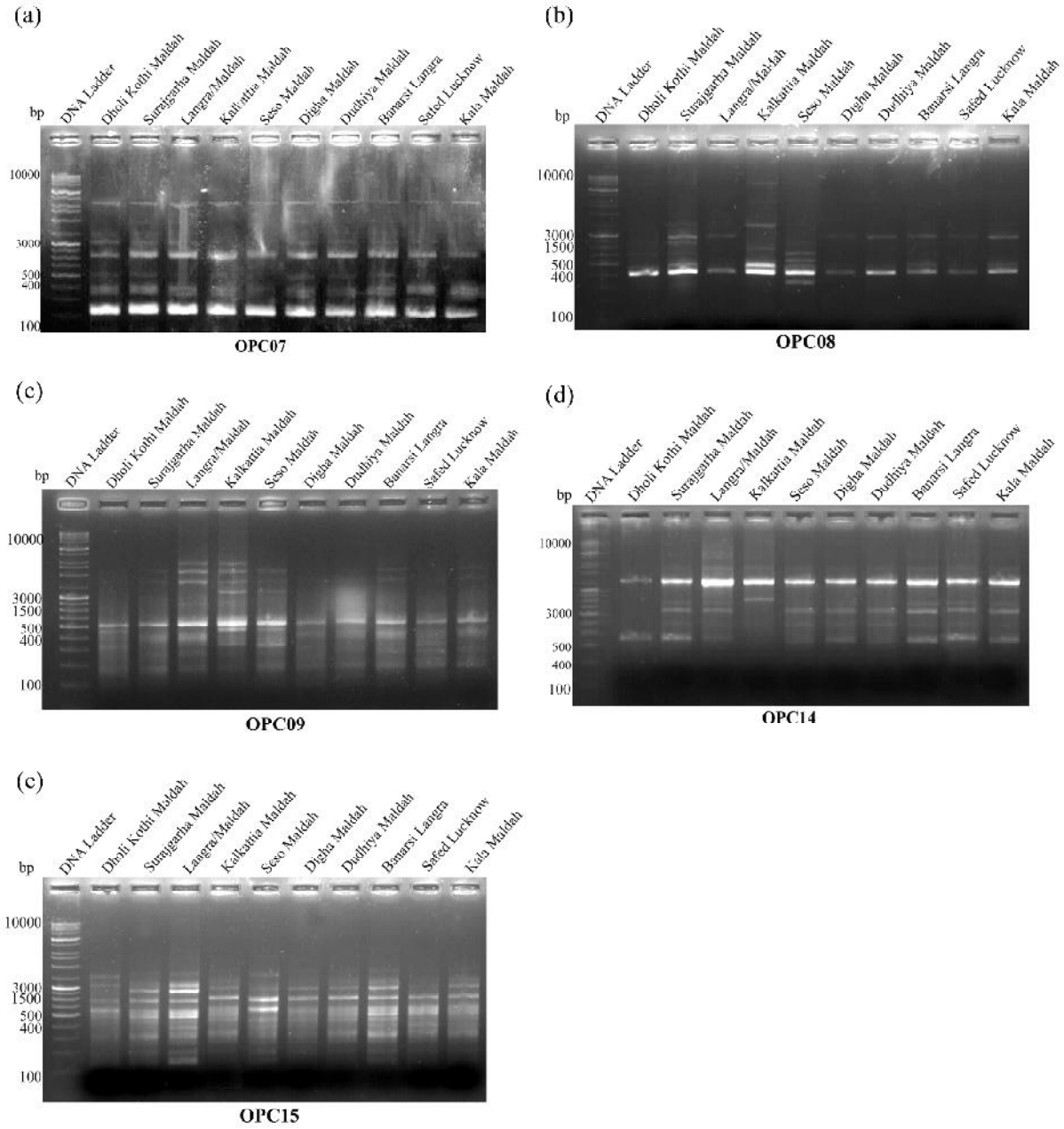

Fig. 3. RAPD profile of ‘Langra’ mango using OPC07 (a), OPC08 (b), OPC09 (c), OPC14 (d), and OPC15 (e).

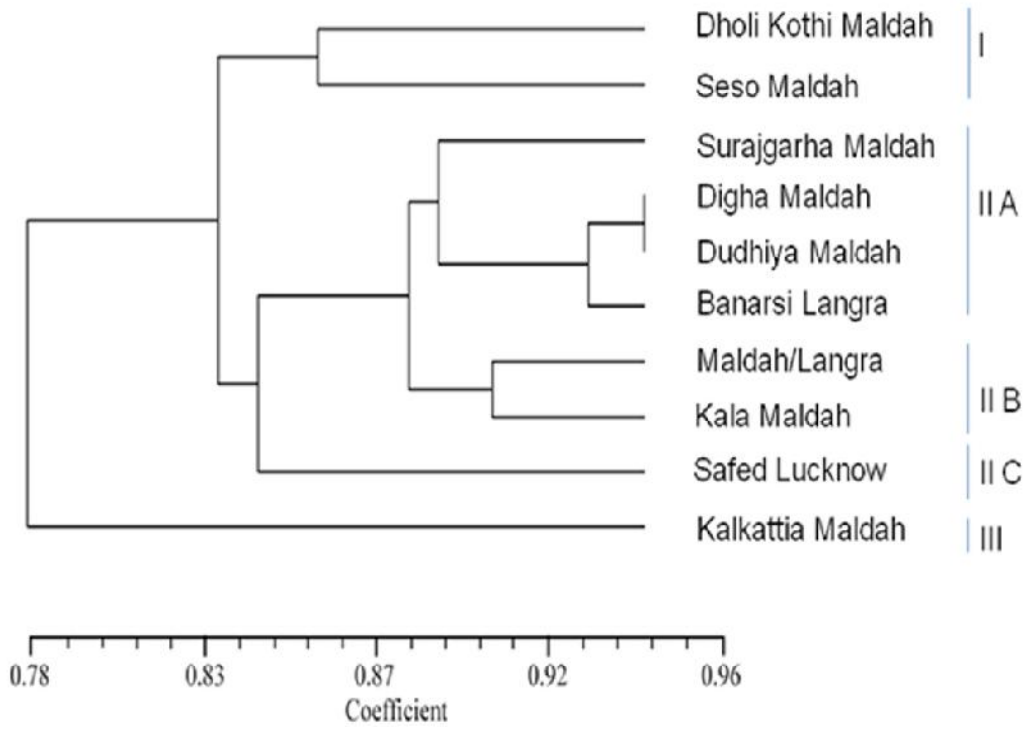

Fig. 4. Dendrogram of 10 clones of 'Langra' mango based on RAPD data.

clones studied was ranging from 43-67. The minimum and maximum number of bands was recorded in Safed Lucknow (43) and Surajgarha Maldah (67), respectively. The highest number of polymorphic bands was observed in Surajgarha Maldah (47) whereas, the least number of polymorphic bands were observed in Safed Lucknow (24) (Table 5). The total number of polymorphic bands was found to be 356 (64.37 % polymorphism). The primer OPB12 yielded maximum number of bands whereas, OPC08 primer showed maximum polymorphism in present investigation (Table 4).

RAPD data analysis

The data obtained from RAPD study were analyzed as described in Materials and Methods. Considering the bands obtained using all the primers, the similarities coefficient were ranged from 0.379 to 0.862 (Table 6). In this study, Dudhiya Maldah and

Table 4. Sequences and analysis of RAPD markers used for characterization of different clones of 'Langra' mango.

S. No	Primers	Primer Sequence 5'-3'	Total number of bands	Number of poly-morphic bands	Polymor-phism (%)
1.	OPA18	AGGTGACCGT	6	3	50.00
2.	OPB04	GTTGCGATCC	5	3	60.00
3.	OPB07	GGTGACGCAG	6	4	66.66
4.	OPB08	GTCCACACGG	7	5	71.42
5.	OPB12	CCTTGACGCA	10	6	60.00
6.	OPB16	TTTGCCCGGA	4	3	75.00
7.	OPC07	GTCCCGACGA	8	5	62.50
8.	OPC08	TGGACCGGTG	8	7	87.50
9.	OPC09	CTCACCGTCC	7	6	85.71
10.	OPC14	TGCGTGCTTG	6	5	83.33
11.	OPC15	GACGGATCAG	9	7	77.71

Digha Maldah were found to be the most similar (86%), while Kalkat ia Maldah and Safed Lucknow were observed to be the most divergent (38%) clones of 'Langra'. Further these similarity matrixes obtained were used to generate dendrogram (Figure 4). Three different clad were observed in this dendrogram. Clad I includes Dholi Kothi Maldah and Seso Maldah. Clad II were further sub divided into 3 sub clad. Clad IIA includes Surajgarha Maldah, Digha Maldah, Dudhiya Maldah and Banarsi Langra. Clad IIB includes Langra/Maldah and Kala Maldah. Clad IIC include Safed Lucknow. Clad III include Kalkat ia Maldah which was observed distinctly apart from all the clones of Langra studied.

The present investigation showed the presence of abundant genetic variation on the basis of morphological, biochemical as well as molecular level in the genotypes investigated. Historically

Table 5. Level of polymorphism observed in different clones of 'Langra' mango.

S. No	Name of clones	Number of total fragments	Average fragments per primer	Number of poly-morphic fragments
1.	Dholi Kothi Maldah	52	4.73	32
2.	Surajgarha Maldah	67	6.09	47
3.	Langra/Maldah	60	5.45	40
4.	Kalkattia Maldah	50	4.55	30
5.	Seso Maldah	60	5.45	40
6.	Digha Maldah	56	5.09	36
7.	Dudhiya Maldah	52	4.73	32
8.	Banarsi Langra	61	5.55	41
9.	Safed Lucknow	43	3.91	24
10.	Kala Maldah	52	4.73	34

mango genotypes have been characterized using morphological markers. In the present investigation clones of Mango 'Langra' has been characterized using different morphological parameters like fruit length (cm), fruit width (cm), fruit weight (g), peel (%), pulp (%) and stone (%). These morphological traits have widely been used to characterize mango genotypes (Gan *et al*. 1981). Fruit length, fruit width and fruit weight were found to be the significantly higher in Dholi Kothi Maldah than other clones of Langra. The fruit weight was observed to be the lowest in Dudhiya Maldah. The peel % was highest in Safed Lucknow. All these traits were positively associated with morphological variability in mango germplasm total soluble solids (TSS) (°Brix), ascorbic acid (mg/100g), acidity (%), total sugar (%) and reducing sugar (%) were recorded.

Fruit morphology is a purely varietal character which is influenced by environments and locations also. The variation observed in case of fruit size in our studies might be because of environmental and location effect. Such variations were also reported by different workers (Singh *et al*. 1997; Lodh *et al*. 1974; Badyal and Bhutani, 1989 and Chat erjee *et al*. 2005). The fruit size of mango cultivar 'Mallika' reduced significantly when grown in Sabour, Bihar compared to fruit sized obtained when grown in Abohar, Punjab (Sharma and Josan, 1995; Kumar, 1998). The possible cause of differentiation in fruit morphology was due to the variation in characters of the pericarp like cell size, laticiferous canals intercellular space etc. It has also been suggested that the increase in fruit size, weight and other parameters are due to increase in the cell size and intercellular spaces and due to accumulation of carbohydrates. The growth of fruit in the later stage was due to osmotic accumulation of food substances and water (Combe, 1960).

In Table 1 the heaviest fruit (843.9 g) was produced by Dholi Kothi Maldah while the lowest fruit weight was observed in Dudhiya Maldah (182.2 g). It was found that some cultivars when grown in different localities showed less variation in their weight while the other cultivars showed much variation. Langra showed less variation in fruit weight when grown in different locations of India like Punjab (fruit weight-224.0g; Bakshi and Bajwa, 1959), Bangalore (fruit weight-224.0g; Lodh *et al*. 1974) and Sangareddy (fruit weight-200.0g; Yadav *et al*. 1984). Amrapali showed maximum variation *i.e.* 208.00 g in Punjab

Table 6. Correlation coefficient similarity matrix of RAPD banding pattern obtained from different clones of 'Langra' mango.

	Kala Maldah	Safed Lucknow	Banarsi Langra	Dudhiya Maldah	Digha Maldah	Seso Maldah	Kalkattia Maldah	Langra / Maldah	Surajgarha Maldah	Dholi Kothi Maldah
Kala Maldah	1									
Safed Lucknow	0.614	1								
Banarsi Langra	0.806	0.645	1							
Dudhiya Maldah	0.661	0.691	0.794	1						
Digha Maldah	0.698	0.644	0.857	0.862	1					
Seso Maldah	0.609	0.507	0.635	0.556	0.611	1				
Kalkattia Maldah	0.403	0.379	0.442	0.478	0.432	0.486	1			
Langra/ Maldah	0.762	0.578	0.779	0.647	0.681	0.600	0.549	1		
Surajgarha Maldah	0.676	0.473	0.767	0.690	0.722	0.707	0.513	0.707	1	
Dholi Kothi Maldah	0.493	0.525	0.548	0.600	0.588	0.647	0.500	0.514	0.644	1

(Sharma and Josan, 1995), and 143.00 at Delhi (Majumdar *et al*. 1982). Thus it is clear that fruit weight is a varietal character which is influenced by environment also. In the present study a wide range of fruit weight from 181.33 to 604.10 was recorded. Similarly, huge variations (50 g to 640 g) were observed in mango fruit when 60 cultivars were studied under Punjab conditions (Bakshi and Bajwa, 1959). Similar observations were reported by Kumar (1998) under Sabour conditions. Other workers had also reported variations from locality to locality of the mango cultivars (Rao *et al*. 1972; Lodh *et al*. 1974; Kulkarni & Rameshwar 1981; Yadav & Singh, 1985; Singh, 1990; Chat erjee *et al*. 2005).

In this study the lowest peel % was observed in Dudhiya Maldah (7.27%) while highest was obtained in Safed Lucknow (15.21%). Differences in peel % among varieties from one location to another location even at the same place from one season to another were reported previously (Teaotia and Shrivastava, 1961; Kulkarni and Rameshwar, 1981; Badyal and Bhutani, 1989; Kumar 1998; Dhillon *et al*. 2004; Sinha *et al*. 2007; Bains and Dhillon, 1999). The possible cause of variation might be due to the facts that mango is the most heterozygous crop and its variable nature is found from place to place (Prasad, 1977).

The pulp % is an important criteria for the evaluation of cultivars in a particular area because this is the part of fruit, which in finally utilized by the people. The cause of differences in pulp percentage is the same as discussed in stone percentage of the fruit i.e. heterozygous nature of the cultivars. In this study different variant of langra showed marked variation in pulp %. The range of pulp % varied from 33.90 to 77.50% in different cultivars in the Punjab (Bakshi and Bajwa, 1959). Kumar, (1998) studied 101 mango cultivars at Sabour (Bihar) and observed a range of pulp % (56.70% in Safeda Malihabadi to 85.00 % in Put u). The differences in pulp % observed from place to place which might be due to environmental and seasonal variations (Singh and Maurya, 1986; Badyal and Bhutani, 1989; Kumar and Singh, 2005; Chat erjee *et al*. 2005; Sinha *et al*. 2007). Similarly, variations in stone % among the different mango varieties has also been reported previously (Teaotia and Shrivastava, 1961; Badyal and Bhutani, 1989; Dut a *et al*. 2008).

The TSS of fruit juice gives a rough idea of the sweetness because TSS includes all type of soluble solids. In this study the Dudhiya Maldah and Kalkat ia Maldah showed high TSS compared to other clones studied. The improvement in TSS content of fruits may be due to the increased

hydrolysis of polysaccharides into sugars and also due to enhanced mobilization of carbohydrates from organic acids. The results of the present investigation showed close conformity with the findings of Kalra *et al.* (1981); Kulkarni and Rameshwar (1981); Singh and Maurya (1986); Kumar and Singh (2005) and Sengupta *et al.* (2006).

Acidity of the fruits gives a blend with sweetness. The combination of acidity and flavour provide quality to the fruits. The acidity of the fruit is directly related to ripening of the fruit though it is a genetical character of the individual variety. In this study the maximum acidity (0.45%) was obtained in Safed Lucknow which was significantly followed by Seso Maldah (0.39%) whereas minimum value was observed in Dudhiya Maladh (0.23%) followed by Kalkatia Maldah (0.25%) and Surajgarha Maldah (0.29%). Similar results were observed by previous studies (Kalra *et al*. 1981; Kulkarni and Remeshwar, 1981)

The total sugars are considered as an important factor for the quality of fruits. In this study highest total sugar was recorded in Langra/Maldah (13.67%) which showed parity with Seso Maldah (13.31%), Digha Maldah (12.02%), Surajgarha Maldah (11.78%) and Kala Maldah (11.58%). The variation in total sugar content was also observed in different genotypes of mango (Gangwar and Tripathi, 1973; Lodh *et al*. 1974; Kalra *et al*. 1981; Kulkarni and Rameshwar, 1981; Patkar *et al*. 1984; Prasad *et al*. 1984; Singh and Maurya, 1986). Some variation in equality was observed which might be due to change in environment which was supported by Sengupta *et al.* (2006).

In this study a wide range of ascorbic acid content (24.56 to 34.34mg/100g of juice) in different cultivar of mango was observed. Such value of ascorbic acid in Langra was also reported by many workers Singh and Chadha, (1961); Gangwar and Tripathi, (1973); Lodh *et al.* (1974) and Yadav *et al.* (1982); Chatterjee *et al.* (2005) and Sengupta *et al.* (2006). Although mango is not a rich source of ascorbic acid but the ascorbic acid content is Langra is very high compared to other genotypes. The carotenoids content in different clones of Langra ranged from 1.98 to 4.62mg/100g of juice. Similar kind of variations were reported previously (Ornelas-Paz *et al*. 2008; Robles-Sánchez *et al*. 2009) in 'Manila' and 'Ataulfo' mangoes, respectively.

From the results of this morphological study, a vision is obtained about the range of intravarietal heterogeneity in 'Langra' mango conserved in coordinated garden and horticulture garden in the collection sites. Conventionally also, the intracultivar heterogeneity of mango has been characterized mostly at the morphological level by several researchers (Gan *et al*. 1981; Naik, 1948, 1971; Singh *et al*. 2009). The prime advantages of morphological traits are simplicity and rapid, inexpensive assays, even from herbarium specimens and other dead tissues. Although morphological traits are very useful, they have several disadvantages. They are often limited in number. They suffer from lack of decisiveness. They face heritability problems as they may be controlled by epistatic and pleiotropic gene effects. Morphological characterizations are error prone due to environmental variations affecting expression of these characteristics. In addition, these observations are time consuming and this mode of identification is slow because of long juvenile periods. Thus, these morphological characters may not adequately represent the genetic heterogeneity among accessions of a cultivar. Hence, characterization of intra-varietal heterogeneity based on morphological traits needs complementation with molecular markers as they can contribute greatly to the utilization of intra-varietal heterogeneity through descriptive information of structure of genotypes, analyses of relatedness, the study of identity and location diversity. The study of genetic heterogeneity has been greatly facilitated by the advent of DNA marker technology in the 1980s, which offered a large number of environmentally-insensitive genetic markers that could be generated to follow the inheritance of important morpho-agronomic traits. A number of DNA markers,

such as restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP) and randomly amplified polymorphic DNA (RAPD) are available for detection of genetic polymorphism (Semagn *et al*. 2006). RAPD are among the most commonly used DNA markers for studying genetic polymorphism. Several molecular markers has been used in the analysis of the genetic diversity of mango (Viruel *et al*. 2005; Pandit *et al*. 2007; Santos *et al*. 2008; Singh and Bhat, 2009; Gálvez-López *et al*. 2009), several of which have employed the RAPD technique (Karihaloo *et al*. 2003; Ravishankar *et al*. 2004; Souza and Lima, 2004; Srivastava *et al*. 2004; Rajwana *et al*. 2008; Faleiro *et al*. 2009; Jena *et al*. 2010; Souza *et al*. 2011). The large percentage (87.50%) of polymorphic bands (OPC08) detected in the present study clearly indicates that RAPD fragments are highly polymorphic and particularly informative in the estimation of genetic relationships. Similar levels of polymorphism associated with RAPD markers have been reported in earlier studies involving mango genotypes (Rahman *et al*. 2007; Anju *et al*. 2008; Rajwana *et al*. 2008; Souza *et al*. 2011). The large amplitude (0.379 to 0.862) of the genetic similarity coefficients established in the present study confirms the occurrence of considerable genetic variability among *M. indica* cultivars, as previously observed in other RAPD analyses (Karihaloo *et al*. 2003; Viruel *et al*. 2005; Rajwana *et al*. 2008; Souza *et al*. 2011). However, the variation amplitude presently determined was larger than that reported by Karihaloo and co-workers (2003) for Indian mango cultivars (range 0.32-0.75; average 0.56. It is evident, therefore, that the clones of Langra exhibit an extremely high level of variability.

The large amplitude of the genetic similarity coefficients (0.379 to 0.862) observed in the present investigation confirms the occurrence of considerable genetic variability among different clones of Langra. Similarly, large amplitude of the genetic similarity coefficients was observed in different mango cultivars as previously observed by RAPD analyses (Karihaloo *et al*. 2003; Viruel *et al*. 2005; Rajwana *et al*. 2008; Souza *et al*. 2011; Karihaloo *et al*. 2003). These finding suggests that the clones of Langra exhibit an extremely high level of variability.

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