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Genetic Diversity in Seedling Populations of Mango

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The genetic improvement of mango (*Mangifera indica* L.), a native of India, has been largely through selection among seedling populations or propagating elite clones and such efforts have resulted in the identification and cultivation of improved commercial varieties. Development of better cultivars by traditional method using morphological traits, although highly heritable, is slow because of long juvenility and being expensive. Farmers of Pusa, Bihar have been conserving high levels of intraspecific diversity of mango in old orchards mostly located along the *Gandak* River. These orchards are often poorly maintained as revenues obtained are small compared to income from other farm activities; however these old orchards harbour a highly diverse population of traditional mango varieties and seedlings, with trees over 80 years old. Hence a survey was conducted in five communities for documenting the mango diversity found in native landraces. Sixteen superior mango varieties of endemic value and importance were evaluated for table, sucking and pickling purposes on the basis of physical appearance and chemical attributes. Out of these, six were found suitable for table, five for sucking, three for pickle and two for dual purpose (sucking and table). Studies revealed a clone from *Bhuskaul* community with fruit weight up to 420.0 g with TSS 27.40^0B and having very thin stone and maturing by the end of August. The present study highlights the need for and demands of diversity rich areas of *Pusa* community in Bihar to conserve and protect seedling mangoes for the benefit of posterity with high value traits for future promotion. Furthermore, the characterization work was captured in fruit catalogues and shared back with the farming community, which might have increased the awareness, interest and appreciation of the available mango diversity and the interest in the continuation of these old highly diverse mango orchards for home use and to explore the commercial potential of these orchards and various types.

Key Words: Accession, Elite materials, Mango, Morphological characterization, Multivariate analysis, Pusa

Introduction

Genetic diversity available in existing germplasm determines the success of any crop improvement programme (Harlan, 1976; Moose and Mumm, 2008). Mango is one of the important fruit crops of India grown on plantations, orchards, and home gardens. Knowledge about the extent of genetic diversity/relatedness in mango germplasm is vital for developing strategies for future gains in productivity. Its improvement through modern methods takes much time and clonal selection, selection from chance seedling and other breeding efforts may results for identification of elite and improved commercial ones. Although, development of better cultivars by conventional method is slow but identification of superior clones on the basis of phenotype, which are generally highly heritable, equally expressed in all environments may shorten breeding cycle. Fruit traits have been the major descriptors for identification of different varieties of fruit crops. However, even in their absence, farmers, breeders and interested stakeholders require to distinguish characters between varieties. Further, while geneticists and plant breeders are particularly interested with diversity at the molecular level (Dempsey, 1996), farmers are more concerned with visible morphological and agronomic variation, which helps them to identify cultivars that are productive and do well in their location specific environment (narrow adaptation as they are unfamiliar

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with the characteristics of the many different cultivars of mango that are now grown and available across the country(Griesbach, 2003).

Agriculturally important place of Pusa, Bihar has diversity of many commercial cultivars of mango *viz.* Kanchan (*Bathua*), *Malda* (Langra), Sukul, *etc.* grown on large scale, Sipia and Bombai are grown by many farmers, and on smaller scale mango varieties like *Paharpur Sinduria*, Fazli, Sunder Langra, Krishanbhog, *Zarda*, *Kerwa*, *Mir Zaffar*, *Zardalu*, *Chinia*, Neelam, Dasheri, Mallika, *Kapuria* and Romani are grown (Baseline Report, 2014). In addition to these varieties, a large population of seedlings of mango is also available in these villages and their number is about 2200 and this is a very good source for the selection of superior clones of mango. It was also thought that selection from superior clones from large number of local variants may have better adaptability and acceptability to the community (Akinnifesi *et al.*, 2007). The variant may be used for different purposes like table, sucking or for pickle preparation.

Morphological characterisation is the first step that should be done before advanced biochemical or molecular studies are carried out (Hoogendijk and Williams, 2001). Hence an effort was made to assess diversity of seedling mangoes in 5 villages of Pusa, Samastipur for selection of superior clones for sucking purposes, table purposes, pickle making or both. The objective of investigation was to document extent of mango diversity found in native land races/farmer's field, its nature and assessment of genetic variability for suitability for table, sucking and pickling purposes based on physical appearance and chemical attributes. Further an assessment of variants grown by the community may lead to identification of promising accession/clone for better quality and late or early maturity over locally acceptable varieties.

Materials and Methods

Based on preliminary baseline survey of 18 villages of Pusa Block of Bihar and going through Four-cell analysis (Sthapit *et al*., 2006) of four villages, *viz*. *Mahmada*, *Jagdishpur*, *Dhobgama* and *Murliyachak* were identified as project communities. Additionally, two more villages namely *Bhuskaul* and *Dighra* were selected as control communities. The villages were surveyed for assessment of on farm mango diversity and motivations for their continued cultivation by the farmers. Four cell analysis showed that majority of farmers have seedling mango besides local commercial ones (*Kanchan*, *Malda* and *Sukul, etc.*).

These identified mango cultivars were evaluated on site using some of the IPGRI (2006) descriptors for mango. Seventy seven selected trees of seedling mango were analysed from these communities based on popular fruit characters like fruit size, suitability for pickles, sucking and table purposes, peel colour, flesh colour, flavour and storability. The fruits of selected trees were collected from each seedling trees at ripening stage for recording fruit length (cm), fruit width (cm), fruit thickness (cm), fruit weight (g), peel (%), pulp (%), stone weight and total soluble solids (TSS) (°Brix). Physical qualitative characters *i.e.* fruit skin colour (surface colour of ripened fruit was recorded matching with the Royal Horticultural Society, Colour Chart, 1969), flavour and aroma, organoleptic test, and marketability were recorded based on opinion of panel of 5 judges who scored according to hedonic scale suggested by Amerine *et al.* (1965). In addition, the fruit samples were also characterized for other qualitative traits like peel thickness, peel texture, pulp colour, and quantity of fibre and eating quality. Percent peel, pulp and stone were calculated by the weight of the peel, pulp and stone, respectively divided by total weight of the fruit multiplied by 100. In each sample, quantitative traits like TSS was recorded with hand refractometer. Juice acidity was estimated by titrating 2 ml juice against 0.1 *N* NaOH using phenolphthalein as indicator (AOAC, 1980).

Based on phenotypic suitability, feedback from local villagers, organoleptic test, (including taste, sight, colour, smell, and touch etc,) domestic uses and its sensory evaluation, 16 superior seedlings were identified for further evaluation with farmer's descriptors. Genetic divergence among selected trees (accessions) subjected to Principal Component (PCA) and Cluster analysis using the XLSTAT 2012 statistical package. Cluster analyses were carried out on the principal components using the hierarchic ascendant analysis and Euclidian average distance.

Results and Discussion

The results presented in this study are particularly important because they represent morphological traits, which are highly heritable. The use of the trait of fruits obtained from mango seedling plants resulted in the

identification of 77 variants for fruit weight, fruit and pulp colour, TSS, pulp and seed content, utilization purposes *etc.* The data pertaining to physico-chemical characters of mango seedling along with common vernacular name indicates a great diversity exiting at the site (Table 5, 6).

Based on characterization of the 77 mango seedling trees revealed that a large amount of genetic diversity of mango exists in the selected communities. This characterization will result in the identification, conservation and utilization of mango genetic diversity, which in turn will help in maintaining the sustainability of fruit production in the target areas.

Eigen values of principal component axis, percent of total variation and cumulative variation accounted for them obtained from principal component analysis are presented in Table 3. The first four component were identified as major based upon their Eigen values and explained 71.72% of the total variation among seven characters. The first component (fruit weight) has 25.11 % share in total variation, whereas second component contributes 17.79 % (fruit length), third contributes (fruit width) to 14.61 %. The characters contributed the maximum to the divergence should be given greater emphasis for selection in breeding (Jagadev *et al*., 1991).

The clustering pattern revealed that the varieties collected from same localities did not cluster together. Cluster analysis of 77 accessions using major parameters resulted in 7 clusters (Table 1). 4 clones of mango having TSS more than 25 ^{0}B were identified. These are clone No. 77 (27.40), 56 (27.20), 71 (26.10), 63 (25.30) and 65 (25.00). Mango clone no. 22 has maximum edible portion and pulp: (peel and stone) ratio was recorded up to 3.44. As far as highest fruit weight (more than 420 g) was concerned, the largest mango was found in the clone no. 57 (644.0 g) followed by clone no. 29 (485.2 g), 26 (477.2 g), 54 (470.0 g) and 77 (420.0 g). Twelve mango clones with absolutely no fibre content were identified. They are clone no. 9, 13, 29, 34, 43, 50, 55, 56, 57, 60, 61 and 77. On the basis of cluster analysis of 77 variants, No. 57 was completely different for highest fruit weight (644.00 g) (Fig. 1). The varieties belonging to different clusters with high to moderate genetic distances might be recommended for use in crossing programs to produce new recombinants with desired traits (Majumder *et al*., 2013).

On the basis of cluster analysis of 16 superior clones of mango (Table 2), five clusters were obtained, Pusa mango 1 differs for highest fruit weight, Pusa mango 7 for lightest weight, Pusa mango 12, 4, 1 differs for minimum fruit length, Pusa mango 6, 10, 15, 9 differs for minimum fruit width. Pusa mango 7, 10 had TSS above

Table 1. Cluster formed after assessment of various parameters of 77 varieties

Cluster	Clone No.
I (14)	38, 61, 34, 14, 8, 51, 11, 7, 71, 25, 31, 17, 15, 1
II (11)	48, 64, 45, 74, 5, 46, 65,72, 10, 36, 2
III (12)	62, 44, 39, 16, 56, 55, 12, 19, 40, 27, 13, 9
IV (11)	41, 33, 70, 30, 6, 35, 20, 49, 53, 47, 43, 4
V (14)	68, 58, 42, 46, 69, 66, 50, 24, 75, 60, 52, 32, 18, 8
VI (9)	77, 22, 21, 67, 63, 59, 73, 37, 3
VI (4)	57, 19, 54, 26

Table 2. Cluster analysis of 16 superior clones of mango

Cluster	Clone No.
I (3)	Pusa mango 12, 4, 1
II (5)	Pusa mango 6, 10, 15, 9
III (1)	Pusa mango 7
IV (3)	Pusa mango 16,11, 3
V (4)	Pusa mango 13, 8, 14, 5

Table 3. Principal component analysis (PCA) performed using the XLSTAT (2012) statistical package showing the correlations of the first seven principal components with the variables observed on mango accessions

Principal Component	Varieties	Eigen value (%)	Variation (%)	Cumulative variation (%)
PC1	Fruit weight	1.75	25.10	25.10
PC2	Fruit colour	3.00	17.78	42.89
PC3	Pulp colour	4.02	14.61	57.50
PC4	Pulp: (peel + stone)	5.02	14.21	71.72
PC5	TSS	5.80	11.20	82.93
PC6	Fibre content	6.52	10.27	93.20
PC7	Purpose for suitability (Table, Sucking or Pickle)	7.00	6.79	100.00

Eigen vectors of the most descriptive traits

Table 4. Details of promising clones of mango out of 77 variants of seedling mango

Mango clones	Place of collection	Local name	Longitude	Lattitude	Altitude (m)	Farmer descriptors
Pusa mango 1	*Jadishpur*, Pusa, Samastipur	Biju mango, Lal Pari	N25^{0}56'47.5"	E085^{0}38'44.8"	14	Very sweet in taste, sucking type, mid maturity. The stone is large and a person can eat many fruits. It has red flesh on maturity. The plant is a regular bearer.
Pusa mango 2	*Jadishpur*, Pusa, Samastipur	Biju mango, Madhukupia	N25^{0}56'56.2"	E085^{0}39'11.1"	152	Very sweet in taste as the name suggests (*Madhu*= honey), big sized fruits for table purpose. Matures by end July. It is not a regular bearer.
Pusa mango 3	*Jadishpur*, Pusa, Samastipur	Biju mango, Sukulia	N25^{0}56'56.3"	E085^{0}39'17.9"	149	Fruit matures after local *Sukul* but fruits are bigger and tastier. Regular and heavy bearer.
Pusa mango 4	*Jadishpur*, Pusa, Samastipur	Biju mango, Sukulia	N25^{0}56'51.1"	E085^{0}39'17.9"	149	Medium size fruit, Red colour at maturity, regular and heavy bearer, Matures by mid July.
Pusa mango 5	*Bhuskaul*, Pusa Samastipur	Biju mango	N26^{0}05'44.6"	E85^{0}26'38.7"	144	Medium sized fruit with a lot of fibre, very sweet and attain red colour at maturity. The skin of the fruits is also edible. It is not a regular bearer and the fruit matures by mid July.
Pusa mango 6	*Jagdishpur*, Pusa, Samastipur	Biju mango	N25^{0}56'47.3"	E085^{0}38'45.1"	145	The fruits are medium sized, very sweet with small stone suitable for table purpose. Shelf life of the fruits is good and the fruits attain an attractive orange colour after maturity. Biennial bearer and the fruit maturity is mid July.
Pusa mango 7	*Jagdishpur*, Pusa, Samastipur	Alphonso biju	N25^{0}56'51.1"	E085^{0}39'70.7"	149	Medium sized fruits, very sweet in taste with lot of fibre and are used for sucking. The fruit attains an attractive red colour after maturity. The fruit shape is similar to *Alphonso*. Biennial bearer and the fruit maturity is mid July.
Pusa mango 8	*Bhuskaul*, Pusa, Samastipur	Malda biju	N25^{0}58'32.3"	E085^{0}38'49.1"	145	The fruits are similar to *Malda* in size and colour, but the maturity is very late, i.e. the latest mango to mature in the month of August-September. The fruits are susceptible to fruit diseases due to the rainy season. Medium sized fruits are very sweet in taste, used for sucking as well as table purposes. It is not a regular bearer and prone to theft due to its late maturity.
Pusa mango 9	*Mahamada,* Pusa, Samastipur	Sona Malda, Malda biju	N26^{0}00'27.8"	E085^{0}41'30.1"	171	The fruits are similar to *Malda* in size and colour, but are elongated and mature in June. The fruits are very shining with orange coloured flesh, not much sweet in taste and are suitable as table fruits. Biennial bearer.
Pusa mango 10	*Mahamada*, Pusa, Samastipur	Sipia biju	N25^{0}59'58.8"	E085^{0}39'05.7"	174	The fruits are similar to *Sipia* in size and colour, but are elongated and mature by July. It is very sweet with almost nil fibre and is suitable for table purpose. Biennial bearer.
Pusa mango 11	*Mahmadpur Kothi*, Muraul, Muzaffarpur	Sukul biju	N26^{0}03'12.9"	E085^{0}31'19.9"	146	The fruits are similar to *Sukul* with lot of fibres, dark yellow colour. Suitable for pickle making and sucking purposes. The fruits can be kept for about a week even after the complete maturity.
Pusa mango 12	*Malinagar*, Pusa, Samastipur	Sipia biju	N26^{0}00'27.8"	E085^{0}41'30.1"	165	The fruits are similar to *Sipia* in size and colour, but are somewhat round in shape and mature in the month of July. The fruits are sweet in taste and are suitable as table fruits. It is not a regular bearer.
Pusa mango 13	*Dhobgama*, Pusa, Samastipur	Biju mango	N25^{0}57'32.7"	E085^{0}38'17.7"	161	The fruits are very attractive and bears colour like *Zarda* mango. Fibre content is very less and the fruits are suitable for sucking and table purpose. The taste is good and sweet.
Pusa mango 14	*Jagdishpur*, Pusa, Samastipur	Sukul biju	N26^{0}00'27.8"	E085^{0}41'30.1"	165	The fruits are similar to *Sukul* in all respects but are having dark yellow or reddish colour on full maturity.
Pusa mango 15	*Jagdishpur*, Pusa, Samastipur	Malda biju	N26^{0}00'27.8"	E085^{0}41'30.1"	165	The fruits are similar to *Malda* in size and colour, but matures by end June. The fruits are sweet in taste, suitable as sucking fruits due to their more fibre content. Biennial bearer and may be suitable for making mango juice.
Pusa mango 16	*Jagdishpur,* Pusa, Samastipur	Mango biju	N25^{0}58'09.6"	E085^{0}38'16.0"	172	The fruits are similar to *Malda* in size and colour, but not much sweet. The fruit are tasty, attractive and are suitable as table fruits due to their almost nil fibre content. It is not a regular bearer and the plant is quite young.

Table 5. Physico-chemical character of 16 superior clones of mango

Clone No.	Fruit weight (g)	Fruit length (cm)	Fruit width (cm)	Length : Breadth	Fruit colour	Flesh colour	Fibre content	Skin weight (g)	Pulp (%)	Acidity (%)	Seed weight (g)	TSS (°Brix)	Eating Quality	Special features
Pusa mango 1	140.00	7.66	5.62	1.36	11	8	3	30.00	58.20	0.32	30.00	18.16	3	Attractive fruit and bearing
Pusa mango 2	510.00	12.10	8.50	1.42	1	9	3	60.00	77.00	0.47	50.0	16.40	3	Highly fibrous with small stone
Pusa mango 3	250.00	9.00	6.90	1.30	2	4	3	33.00	71.20	0.75	31.00	18.22	4	Suitable for pickles
Pusa mango 4	160.00	7.60	6.26	1.22	11	2	3	30.00	58.60	0.32	30.00	17.48	1	Attractive appearance
Pusa mango 5	306.00	11.20	7.18	1.56	10	4	4	52.00	68.42	0.32	45.00	15.55	3	Peel is edible
Pusa mango 6	184.00	8.60	6.20	1.39	11	4	5	30.00	63.84	0.23	36.00	19.70	1	Very tasty fruit and attractive fruit appearance
Pusa mango 7	120.00	6.70	5.58	1.19	4	8	2	30.00	43.00	0.32	44.00	21.34	1	Taste is not good
Pusa mango 8	308.00	10.66	6.91	1.56	11	4	5	59.00	64.20	0.23	49.00	19.64	1	Thick peeled
Pusa mango 9	168.00	9.35	6.15	1.15	11	5	5	28.00	68.87	0.32	24.00	12.83	3	Less sweet
Pusa mango 10	170.00	9.83	5.33	1.85	11	4	5	40.00	57.25	0.23	30.00	21.50	1	Suitable for table purposes
Pusa mango 11	243.00	11.32	6.60	1.72	6	5	2	46.00	71.39	0.37	43.00	15.70	1	Suitable for pickle
Pusa mango 12	150.00	7.30	6.16	1.18	11	4	4	30.00	58.00	0.40	30.00	17.06	1	Suitable for juice making
Pusa mango 13	300.00	10.13	7.48	1.36	11	5	5	60.00	61.25	0.20	50.00	18.55	1	Colour is very attractive
Pusa mango 14	300.00	11.42	6.92	1.65	10	3	3	47.00	74.20	0.45	40.00	17.40	1	Good blend of sugar and acids
Pusa mango 15	170.00	8.38	6.00	1.40	1	8	3	40.00	57.40	0.37	40.00	18.44	3	Suitable for sucking
Pusa mango 16	240.00	9.65	6.60	1.46	11	4	6	80.00	70.00	0.43	40.00	17.50	1	Suitable for table purpose

Table 6. Scores for the various quality parameters given to 77 variants of seedling mango

Qualitative parameters	Scores
Fruit colour	1: Green; 2: Greenish yellow; 3: Reddish green, 4: Red, 5: Reddish Orange, 6: Orange, 7: Dark Orange, 8: Light Yellow, 9: Orange Yellow, 10 : Red Yellow, 11: Yellow, 12: Dark Yellow
Pulp colour	1: Red, 2: Reddish Orange, 3: Light Orange, 4: Orange, 5: Dark Orange, 6: Yellow White, 7: Dull Yellow, 8: Yellow, 9: Dark Yellow
Fibre contents	1:Very High, 2: High, 3: Medium, 4: Less, 5: Very Less, 6: Nil
Eating quality	1:Excellent, 2:Very Good, 3:Good, 4:Average
Suitability for purposes	1: Table, 2: Juice or Sucking, 3: Pickle, 4: Table and sucking, 5: Pickling and Sucking

Table 7. Best accessions for the important horticultural traits

S.No	Traits	Accessions
1.	Fibre contents	Pusa mango 3, 11, 12, 14, 15
2.	Eating quality	Pusa mango 2, 4, 5, 6, 8, 9, 10, 16
3.	Suitability for purposes	Pickles (Pusa mango 3, 14); Table (Pusa mango 2, 4, 5, 6, 8, 9, 10,16); Sucking (Pusa mango 1, 7); Table and sucking (Pusa mango 13); Sucking and pickles (Pusa mango 11); Sucking and juice making (Pusa mango 12, 15)

21^0B, least stone weight (<30.0 g) was found in Pusa mango 1, 3, 9, 10 and 12 and highest pulp percentage (> 70.0 %) was available with Pusa mango 2, 3, 11, 14 and 16 (Table 6, Fig. 3).

Clone no. 77 (*Malda* seedling) (Fig. 1) from *Bhuskaul* community had almost similar fruit characteristics to *Malda* with very late maturity (August end). This is potentially a high value trait as by this time fruits from other varieties would have already stopped coming to market. It had a maximum of six desirable characteristics

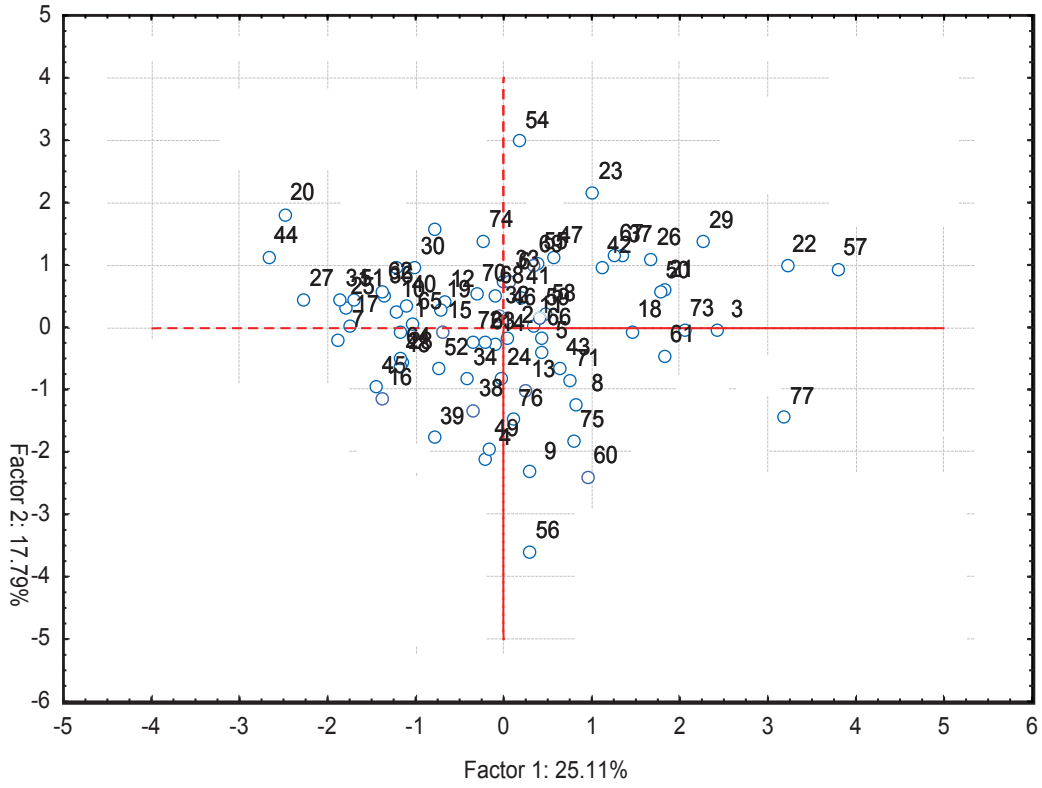

Fig. 1. Distribution of varieties in PC1 (Fruit weight) and PC2 (Fruit colour), 25.11% and 17.79%, respectively

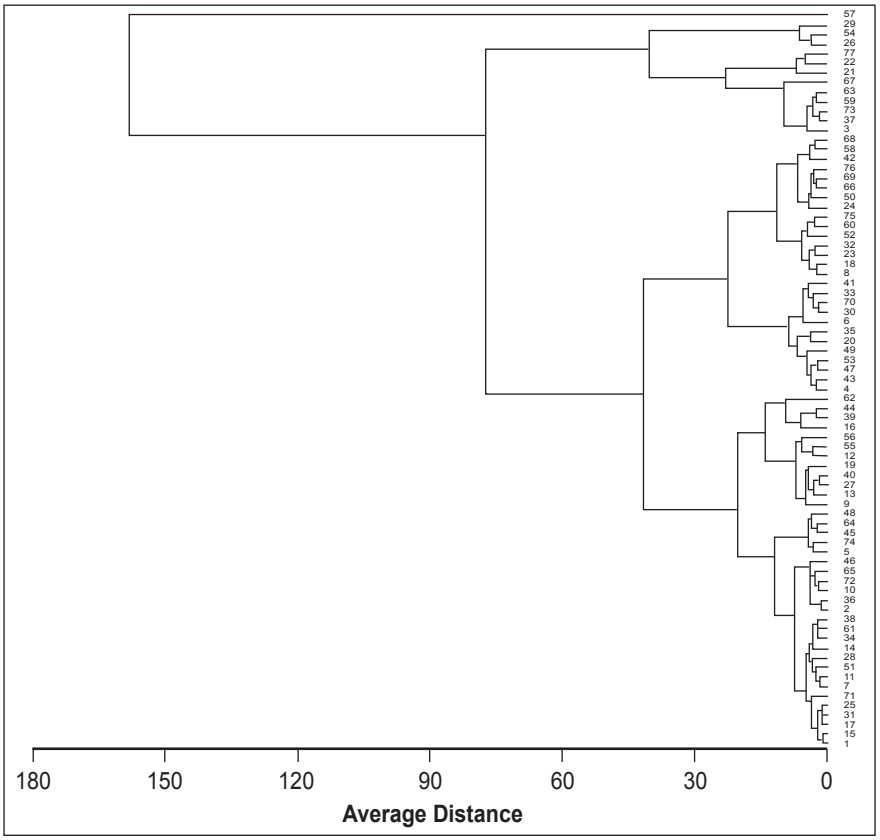

Fig. 2. Dendrogram based on morphological characters of 77 mango accessions from the Pusa, Bihar

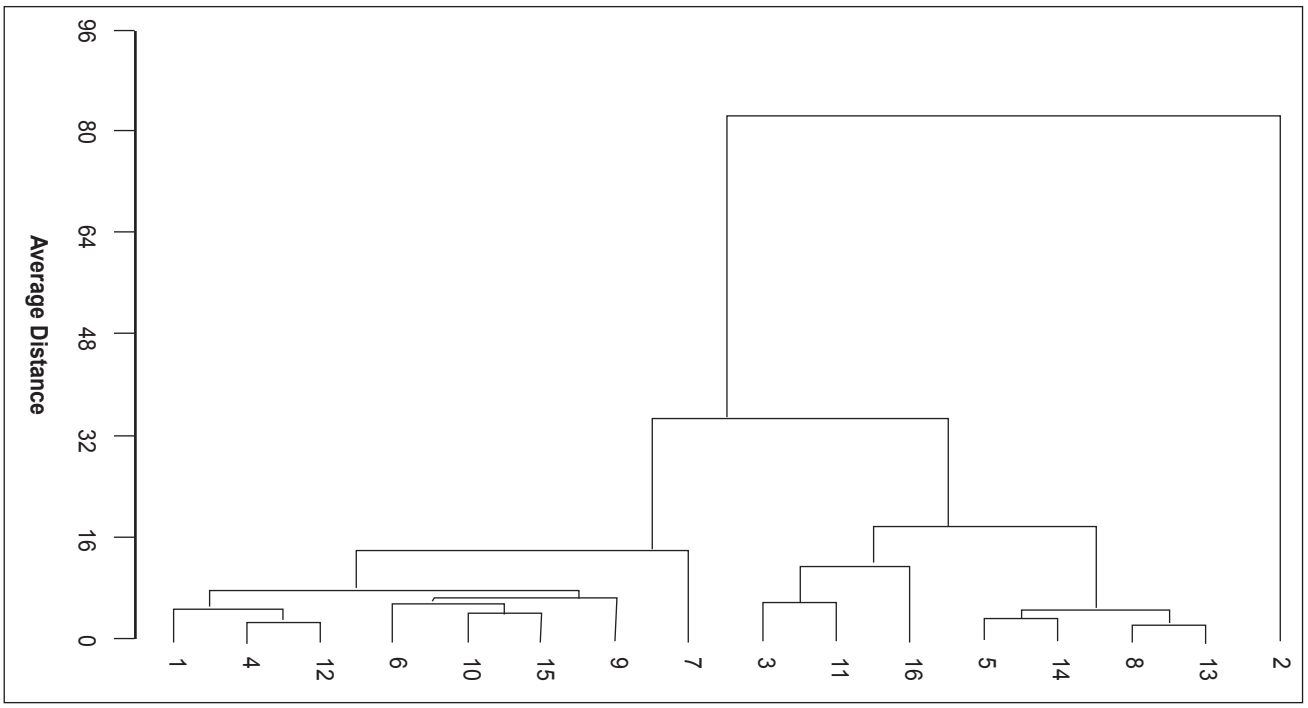

Fig. 3. Dendrogram constructed based on morphological characters of 12 mango superior clones of *Pusa*, Bihar

Fig. 4. Clone 77 matures by September, other parameters are similar to popular *Malda* mango (whole and cut fruit)

of mango such as better fruit weight (420.0 g), dark orange peel and pulp colour, pulp: peel and stone ratio (2:23) with highest TSS (27.40 °B) without any fibre. This is most suited for table purpose; very late maturity may lead to staggered availability. The clone no. 50 and 60 were also at par with clone no. 77 but had lesser fruit weight (300 g, and 159 g) and less sweet (22.20 and 21.30 °B). The clone no. 2, 3, 6, 47 and 76 were observed as having four desirable fruit characteristics. Similarly, nine clones viz. 29, 34, 43, 53, 55, 57, 58, 60 and 73) were possessing three desirable characteristics.

According to Genu and Pinto (2002), in the majority of tropical developing countries, the natural abundance of fresh mango fruits frequently brings an excess in local demand, which is true in the case of Pusa site as well. In fact, mango fruit used for processing should have firm texture, high yield, medium size, high sugar content, bright colour, good flavour and aroma after

ripening, low acid content, no fibre and low tannin content. In this investigation, on the basis of uses of fruits, mango varieties were classified into four groups *i.e.*, table, sucking, pickling, table and sucking varieties. Out of sixteen superior strains of mango (enjoying local importance) and evaluation based on physical appearance and chemical attributes, eight were found suited for table, two for pickling, two for sucking, one for table and sucking, one for sucking and pickle, two were suited for sucking and juice making purposes (Table 7). Kundu *et al*., (2009) also found lot of variation among less known mango varieties grown in West Bengal, India *viz. Nabab bhog, Mithua and Khota Lagga* mango for yield and fruit quality and *Baro Langra Guti, Subodh Guti, Dudh Kumar, Ghia, Gour* and *Durgabhog* as table variety for good quality.

The characterization of the Clone no. 77 (Fig. 4) from mango seedlings indicated, a large amount of genetic diversity is available in the selected communities. This characterization of seedling population helps in the identification, conservation and utilization of mango genetic diversity, and also encourages growers to maintain the sustainability of fruit production.

This study revealed that great variability exists among different mango seedling progenies and this can be exploited for the selection of elite genotypes in future after evaluating their performance.

In the present survey, it was felt that demand for table, sucking and pickles type has been escalated in the region due to decrease in seedlings mango population and awareness about indigestion problems generally experienced after consumption of grafted mangoes.

Therefore, 16 identified clones can be used for table, sucking, juice making and pickling purposes. These accession's characterisation and evaluation inculcate the communities to go for conservation and development efforts required for conservation of unique varieties.

These accessions can be subjected to further selection procedures so that superior planting materials can be made available to the growers. Those who are maintaining maximum number of unique varieties may be nominated for plant saviour community awards. The registration of farmer's varieties with PPVFRA, New Delhi may lead to strengthening our genetic resources of mango and can be future breeding options either for one or more quality attributes.

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