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RESEARCH ARTICLE

Morphological Characterization and SSR Marker Assay of Mango Landraces of West Coast of Kerala, India

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Abstract

Mango is an indigenous crop that originated in North East India-Myanmar region. Local landraces of mangoes found on the South West coastal regions and North East India were found to have many useful traits, including the tendency of polyembryony. To estimate the diversity available in the landraces maintained at ICAR-NBPGR, Regional Station, Thrissur, morphological characterization was carried out using twelve qualitative and ten quantitative traits. To complement the morphological data, the molecular assay was performed using SSR markers. Qualitative leaf traits accounted for less variation, although large variability was observed in all quantitatively measured traits, indicating high genetic variability among local types. The molecular genetic distance derived from the similarity coefficient ranged between 0.12 to 0.96. Twenty genotypes were grouped into two, having a significant degree of polymorphism. This study supports the availability of high variability between landraces, which is an important factor in the effective utilization and *in situ* conservation of genetic resources.

Keywords: *Mangifera indica*, Morphological characters, Molecular characterization, Variability, Yield.

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Introduction

Mango is an important commercial fruit crop of India which is the leading producer in the world (69,110 hectares having yield potential of 5.5 lakh tons) (NMD, 2020). Though monoembryonic types are commercially cultivated to a larger extent, mangoes from tropical regions of Southeast Asia, namely Myanmar, Cambodia, Laos, Malaysia, Singapore, Thailand, Vietnam, coastal parts of Kerala, Karnataka and Goa, are stated to be polyembryonic. These are in high demand as these largely retain their true-to-type nature due to nucellar origin. Polyembryony is common in landraces found in Kerala (Radha and Manjula, 2000). The traditional varieties of Kerala *viz*., Chappikudiyan, ChenkaVarikka, Chandra Karan, Koonan, Kolambu, Kalkandamanga, Karaka manga, Vattan (=Bappakkai, Komanga), Kotturkonam, Kilichundan Tholikaippan, and Muvandan are prized for special preparations. Although commercial mango cultivation is limited to a few hundred hectares in the village of Muthalamada in Palakkad, local mangoes are ubiquitous on farms in Kerala. On the other hand, the mango growing area has decreased considerably due to the change of social and economic status, including a shift in cultivation pattern, which has led to genetic erosion of the state's traditional mango landraces. Therefore, there is a need to document and preserve the traditional landraces that are on the brink of extinction (Simi *et al*., 2013).

Information on the extent of genetic variation for fruit characteristics is crucial in identifying valuable traits that the use of morphological/ molecular markers could achieve. Though morphological markers are easily detectable, inherited, reliable and scored visually, the precise estimate of variability could not be determined due to several environmental factors, including different stages of plant growth and environmental conditions. Thus, the use of molecular markers plays an important role in cultivar identification although expensive, are independent of these external factors (Sennhenn *et al.*, 2014). SSR-based DNA fingerprints, besides their use in selection and mapping (Mc Couch *et al.*, 1997) are reliable markers because of their presence extensively in the genomes, high degree of polymorphism and codominant inheritance.

To protect the precious genetic resources of Kerala from various anthropogenic and environmental threats, ICAR-NBPGR Regional Station, Thrissur, has collected 102 accessions (comprising of about 50 landraces) from various regions of Kerala and adjoining areas over the past 24 years and conserved them in the field gene bank (FGB). The present investigation aimed to characterize Kerala-specific mango types using morphological descriptors and molecular markers that will identify elite trees with high yield, desirable fruit quality and other important economic traits.

Materials and Methods

Experimental Site

Mango germplasm consisted of 20 landraces (Table 1) originally collected from six districts of Kerala along with the allotted indigenous collection (IC) numbers, maintained in the Field Gene Bank of the ICAR-NBPGR, RS, Thrissur, Kerala, India (10.54 N, 76.27 E, 46 m) formed the source of study. Soils of this area are lateritic and a little acidic. Three seedlings for each accession were planted in 1997 at 6 x 6 m spacing and grown under natural conditions without following a package of practices.

Morphological Data

Observations on 12 qualitative and ten quantitative traits were recorded according to the Bioversity international descriptor (IPGRI, 2008) from 2011-2014 and 2019. The qualitative leaf traits and states *viz*., the shape of leaf blade, the shape of leaf apex, the shape of leaf base, margin type, leaf pubescence, leaf texture, leaf attitude in relation to branch, the color of fully developed leaf, leaf fragrance, leaf blade twisting, and thickness of pulvinus were recorded by

Table 1: Indigenous collection number, landrace name and locations of collection from different regions of Kerala*

S No.	*Indigenous collections*	*Local name*	*Place of collection*	*District*
1	IC202177-A	Nadashala/ Panchasara manga	Padoor	Palakkad
2	IC202202	Vattan	Cheruthuruthy	Thrissur
3	IC202209	Kolambu	Athani	Thrissur
4	IC212569	Chunayan	Kumbala	Kasaragod
5	IC470613	Muvandan-1	Kumbla	Kasaragod
6	IC470614	Vellarimanga	Nadathara	Thrissur
7	IC470615	Ullitholiyan	Chenganoor	Alappuzha
8	IC470616-A	Mylapoovan (Variant)	Chenganoor	Alappuzha
9	IC470616-B	Kilimukan	Chirayinkeezhu	Thiruvananthapuram
10	IC470621	ValiyaKilichundan	Chirayinkeezhu	Thiruvananthapuram
11	IC470622	Muvandan-2	Vellanikkara	Thrissur
12	IC470627-B	Kilichundan	Vellanikkara	Thrissur
13	IC470656	Nattu maavu-1	Mattathur	Thrissur
14	IC470667	Mallusseri/ Njettikuzhiyan	Karumassery	Ernakulam
15	IC470677	Pulimanga	Karumassery	Ernakulam
16	IC470679	Nattu maavu-2	Mattathur	Thrissur
17	IC470682	Nattu manga	Kuzhikattuserry	Thrissur
18	IC470692	Mailgoa	Cherpu	Thrissur
19	IC470697	Apple manga	Palissery, Annamanada	Thrissur
20	IC548486	Kalletty	Karumasserry	Ernakulam

*Landraces are conserved in the field gene bank at ICAR-NBPGR, Regional station, Thrissur

comparing the specimen with the figures and descriptor states given in the descriptors. The qualitative fruit traits and states, namely fruit shape, the skin color of ripe fruit, fruit pulp color, the quantity of fiber in pulp, fruit apex, fruit skin surface, fruit stalk insertion, fruit beak type, pulp texture of ripe fruit, pulp aroma and slope of fruit ventral shoulder were recorded by comparing the specimen with the figures and descriptor states given in the descriptors. The quantitatively measured leaf traits were leaf blade width (cm), leaf blade length (cm) and petiole length (cm). The fruit characteristics recorded include the weight of the individual fruit (g), fruit length (cm), fruit width (cm), stone length (cm), stone width (cm), stone weight (g), TSS (◦B) and yield/tree. The total yield per tree was recorded in kilograms and rated as high (> 500 kg/tree), medium (100–500 kg) and poor (< 100 kg). Quality of the fruit was measured with sensory evaluation considering the sweetness, flavor, acidity, aroma and astringency of ripe fruits. Based on the sensory evaluation, the taste was rated as excellent (4), good (3), fair (2) and poor (1). About 25 seeds (stone) extracted from mature ripe fruits of each landrace were sown in moist sand beds. Days taken for initiation of germination and number of sprouts per stone were recorded and those accessions with more than one sprout from a stone were considered to be polyembryonic.

DNA isolation and SSR marker analysis

The young, green and just mature leaves were used for DNA isolation using CTAB protocol (Doyle and Doyle, 1990) involving few modifications. DNA quality was estimated on 0.8% agarose gel and the quantity was calculated based on 260/280 spectrophotometer readings. The DNA working solution was prepared to 10 ng/µL and preserved at -20°C. About 50 SSR oligo-nucleotide sequences were taken from the published data by Ravishankar *et al.* (2011) from which 10 were selected based on reproducibility and polymorphism. The 10 polymorphic SSR primers were used for the PCR analysis aimed at generating DNA profiles (Table 4). PCR reactions were performed in 10 µL including 60 ng genomic DNA, buffer (10X with $MgCl_2$), Forward and Reverse primer at 1µM each, 2.0 mM dNTP mix (10 mM) and 0.2 U Taq DNA polymerase (3 U/µL) (Bangalore Genei). PCR assay was carried out in master cycler (Eppendorf) set at 94°C (4 min) initial denaturation, 94°C (1-minute) denaturation, 55°C (45 seconds) annealing and 72°C (1-minute) extension for 30 cycles with a final extension at 72°C (8 minutes). The PCR products were separated on 3% standard agarose (Sanbiomed) gel in TBE (10 mM Tris–borate, 1 mM EDTA) running buffer solution. Amplified products were run on agarose gels and documented after visual examination under UV light. Explicit and reproducible alleles amplified by each of the 20 SSRs were compared with 100 bp DNA ladder for calculating the molecular weights of the amplified products.

Data Analysis

Quantitative traits were analyzed statistically and summary statistics were performed with XLSTAT 2012. Scoring was done using UV tech and allele sizes were obtained as base pairs in integers. These base pairs were converted to binary code for data analysis and interpretation using PAST software. Since accessions exhibited different ploidy levels, SSR integer data were converted into a binary matrix. If the allele is present then one (1), and if absent, it is zero (0). Allelic differences of 1–5 bp difference were considered identical. To compare the effectiveness of SSR markers in diversity analysis parameters *viz.,* number of polymorphic bands, average number of polymorphic bands per unit assay and number of loci per assay unit were considered. Polymorphic information content (PIC) for each SSR primer was calculated using the formula provided by Smith *et al.* (1997). The scored binary data were used to derive similarity coefficients using PAST software (Hammer *et al.* 2001)

Results and Discussion

Morphological Characterization

Morphological characterization as per Bioversity International descriptors revealed that moderate variations were observed for the qualitative traits. Leaf texture was leathery (coriaceous) for nine accessions and papery (chartaceous) for the remaining 11 accessions (Table 2). The leaf margin was wavy (9), entire (9), or intermediate (2). However, leaf pubescence was not observed in any of the collections. The color of the leaves ranged from pale green (7) to dark green (2) and green (11). Leaf fragrance (smell emanating from crushing) was mild in 10 accessions and strong in 9 accessions, with one accession, IC202177, showing no leaf scent. The uniqueness of the leaf fragrance is an important feature of Kerala Landraces. Out of 20 accessions, 7 showed twisted leaves to varying degrees and 13 accessions showed no twisting. Variation was observed in the shape of the leaf blade, which was highly variable and ranged from elliptic (6), obovate (8), lanceolate (3), and one accession each in obtuse, oblanceolate and ovate. Leaf apex shape varies from acute (10) to acuminate (10). Leaf base shape was mostly acute in most of the accessions. Leaf attitude in relation to the branch was semi-erect in 14 accessions and, horizontal in 4 and semi drooping in 2 accessions. Leaf pulvinus was thick in 10 accessions, thin in 8 accessions and thin to thick in IC470616-A and IC470622. Earlier findings also revealed large variability for nine leaf traits and nineteen fruit characters among Kuttiatoor mango accessions of Kerala (Dinesh *et al.*, 2015). Joshi *et al.* (2013) examined leaf apex variation among nine mango cultivars. Krishna Pillai and Wijeratnam (2016) were of the opinion that young leaf color could be one of the important traits for classification. With respect to leaf quantitative traits

Table 2: Variability in the mango landraces for qualitative leaf traits with descriptor states

Indigenous collections	*Leaf blade shape*	*Leaf apex shape*	*Leaf base shape*	*Leaf attitude in relation to branch*	*Thickness of pelvinus*	*Leaf blade twisting*	*Leaf Texture*	*Leaf margin*	*Leaf pubescence*	*Color of fully developed leaf*	*Leaf fragrance*
IC202177A	Elliptic	Acute	Acute	Semi erect	Thin	Absent	Chartaceous	Entire	Absent	Green	Absent
IC202202	Obovate	Acute	Obtuse to acute	Semi erect	Thin	Absent	Chartaceous	Entire to wavy	Absent	Pale green	Mild
IC202209	Elliptic	Acuminate	Acute	Semi erect	Thick	Absent	Coriaceous	Wavy	Absent	Dark green	Strong
IC212569	Lanceolate	Acute	Acute	Semi erect	Thick	Absent	Chartaceous	Entire	Absent	Green	Strong
IC470613	Lanceolate	Acuminate	Acute	Semi erect	Thick	Absent	Chartaceous	Entire	Absent	Pale green	Strong
IC470614	Obtuse	Acuminate	Obtuse	Semi erect	thin	Absent	Chartaceous	Wavy	Absent	Green	Strong
IC470615	Obovate	Acute	Acute	Semi erect	Thin	Present	Coriaceous	Wavy	Absent	Pale green	Strong
IC470616A	Elliptic	Acute	Acute	Semi erect	Thin to thick	Absent	Coriaceous	Entire	Absent	Green	Mild
IC470616B	Obovate	Acute	Obtuse	Horizontal	Thin	Present	Coriaceous	Wavy	Absent	Green	Strong
IC470621	Ovate	Acute	Obtuse	Semi erect	Thin	Present	Coriaceous	Entire	Absent	Green	Mild
IC470622	Elliptic	Acute	Acute	Horizontal	Thin to thick	Absent	Coriaceous	Wavy	Absent	Green	Mild
IC470627B	Elliptic	Acuminate	Acute	Semi drooping	Thin	Absent	Chartaceous	Entire	Absent	pale green	Strong
IC470656	Obovate	Acuminate	Acute	Semi erect	Thick	Absent	Coriaceous	Entire	Absent	Green	Strong
IC470667	Elliptic	Acute	Acute	Semi erect	Thick	Absent	Chartaceous	Entire	Absent	Green	Strong
IC470677	Obovate	Acute	Acute to Obtuse	Semi drooping	Thick	Present	Coriaceous	Entire to wavy	Absent	Green	Mild
IC470679	Obovate	Acuminate	Acute	Semi erect	Thick	Present	Coriaceous	Wavy	Absent	Pale green	Mild
IC470682	Obovate	Acuminate	Acute	Horizontal	Thin	Present	Chartaceous	Wavy	Absent	Dark green	Mild
IC470692	Obovate	Acute	Acute	Horizontal	Thick	Absent	Chartaceous	Entire	Absent	Pale green	Mild
IC470697	Lanceolate	Acuminate	Acute	Semi erect	Thick	Absent	Chartaceous	Wavy	Absent	Pale green	Mild
IC548486	Oblanceolate	Acute	Acute	Semi erect	Thick	Present	Chartaceous	Wavy	Absent	Green	Mild

Table 3: Variability in the quantitative traits studied in the mango landraces

Indigenous collections	*Leaf blade length (cm)*	*Leaf blade width (cm)*	*Leaf blade length-width ratio*	*Petiole length (cm)*	*Fruit length (cm)*	*Fruit width (cm)*	*Fruit weight (g)*	*Stone length (cm)*	*Stone width (cm)*	*Stone weight (g)*	*Pulp content (g)*	*Pulp recovery (%)*	*TSS (° Brix)*
IC202177 -A	16.04	3.83	4.21	1.97	7.10	5.82	126.28	5.92	3.48	34.80	91.48	72.44	21.40
IC202202	15.73	5.23	3.03	3.27	7.16	6.72	141.33	5.57	3.97	26.04	115.29	81.58	11.33
IC202209	23.13	5.87	3.93	3.17	11.60	5.97	135.50	8.12	4.02	24.42	111.08	81.98	17.33
IC212569	21.97	5.07	4.33	4.73	4.92	4.08	41.10	3.86	2.73	10.26	30.84	75.04	14.64
IC470613	18.13	4.63	3.90	2.27	7.02	5.90	131.75	5.17	5.09	19.33	112.42	85.33	13.50
IC470614	25.37	6.93	3.67	4.13	16.92	8.08	428.64	14.06	3.48	37.50	391.14	91.25	24.00
IC470615	13.37	3.97	3.37	2.77	5.92	4.31	67.75	4.90	2.77	14.66	53.09	78.36	23.40
IC470616 -A	16.97	4.53	3.76	2.37	8.30	6.29	132.49	6.25	3.60	20.50	111.99	84.53	19.00
IC470616 -B	20.77	5.67	3.68	4.17	7.58	7.40	167.15	6.40	3.56	24.60	142.55	85.28	13.80
IC470621	11.46	5.57	2.04	1.92	11.54	7.92	323.60	8.53	4.27	45.76	277.84	85.86	12.50
IC470622	20.83	6.63	3.16	2.47	7.28	6.54	157.53	5.33	3.70	18.67	138.86	88.15	14.00
IC470627 -B	17.53	4.68	3.73	2.71	9.42	6.10	166.87	7.96	3.37	25.33	141.54	84.82	13.50
IC470656	27.47	7.87	3.56	5.27	6.38	4.70	80.38	5.28	3.15	12.50	67.88	84.45	15.33
IC470667	26.57	6.07	4.38	6.27	11.55	6.89	183.42	10.38	4.43	35.18	148.24	80.82	13.00
IC470677	24.64	6.12	4.09	2.13	5.80	4.15	57.08	4.08	2.53	10.67	46.41	81.31	18.67
IC470679	28.07	7.21	4.03	2.96	6.54	5.36	95.47	5.12	3.43	20.31	75.16	78.73	16.00
IC470682	29.14	7.83	3.72	6.03	7.18	4.91	99.16	5.91	3.56	23.87	75.29	75.93	16.00
IC470692	23.21	4.23	5.48	3.78	7.13	6.35	147.25	5.63	3.68	23.81	123.44	83.83	19.00
IC470697	25.57	5.43	4.74	2.63	9.07	7.80	263.88	6.33	3.93	27.66	236.22	89.52	16.90
IC548486	18.77	4.33	4.41	2.27	12.63	9.68	658.37	8.66	4.97	78.12	580.25	88.13	16.20
Mean	21.24	5.59	3.86	3.36	8.55	6.25	180.25	6.67	3.69	26.70	153.55	82.87	16.48
Minimum	11.46	3.83	2.04	1.92	4.92	4.08	41.10	3.86	2.53	10.26	30.84	72.44	11.33
Maximum	29.14	7.87	5.48	6.27	16.92	9.68	658.37	14.06	5.09	78.12	580.25	91.25	24.00
Standard deviation	5.10	1.23	0.70	1.33	2.94	1.46	145.42	2.40	0.67	15.15	131.59	4.95	3.54
Standard Error	1.14	0.28	0.16	0.30	0.66	0.33	32.52	0.54	0.15	3.39	29.42	1.11	0.79
CV (%)	24.03	22.03	18.16	39.60	34.38	23.40	80.68	35.97	18.07	56.75	85.70	5.97	21.48

Table 4: Variability in Qualitative parameters of fruits

Indigenous collections	*Fruit shape*	*Skin color of ripe fruit*	*Fruit pulp color*	*Quantity of fibre in pulp*	*Fruit apex*	*Fruit skin surface*	*Fruit stalk insertion*	*Fruit beak type*	*Pulp texture of ripe fruit*	*Pulp aroma*	*Slope of fruit ventral shoulder*
IC202202	Obovoid	Green	Orange	Intermediate	Round	Smooth	Oblique	Perceptible	Soft	Strong	Sloping
iC212569	Ovoid	Greenish yellow	Light yellow	High	Acute	Smooth	Oblique	Pointed	Soft	Mild	Sloping
iC470613	Obovoid	Yellow	Yellow	Intermediate	Acute	Smooth	Oblique	Perceptible	Intermediate	Intermediate	Sloping
iC470614	Oblong	Yellow	Orange	Absent	Acute	Smooth	Oblique	Mammiform	Soft	Intermediate	Sloping
iC470615	Oblong	Greenish yellow	Yellow	High	Acute	Smooth	Oblique	Pointed	Soft	Strong	Ending in a long curve
iC470616-A	Obovoid	Yellow	Dark orange	Intermediate	Round	Smooth	Oblique	Pointed	Firm	Mild	Sloping
iC470616-B	Ovoid	Yellow	Light orange	High	Acute	Smooth	Oblique	Pointed	Firm	Mild	Ending in a long curve
iC470621	Oblong	Greenish yellow	Orange	High	Acute	Smooth	Oblique	Perceptible	Soft	Strong	Sloping
iC470622	Obovoid	Greenish yellow	Yellow	Low	Acute	Smooth	Oblique	Perceptible	Firm	Mild	Sloping
iC470656	Oblong	Greenish yellow	Orange	Intermediate	Acute	Smooth	Oblique	Perceptible	Firm	Mild	Sloping
iC470667	Obovoid	Yellow	Orange	Low	Acute	Rough	Vertical	Perceptible	Soft	Mild	Ending in a long curve
iC470677	Oblong	Greenish yellow	Orange	High	Acute	Smooth	Oblique	Perceptible	Soft	Intermediate	Sloping
iC470679	Obovoid	Green	Light orange	Intermediate	Obtuse	Smooth	Oblique	Perceptible	Soft	Intermediate	Ending in a long curve
iC470682	Oblong	Greenish yellow	Light orange	High	Obtuse	Smooth	Oblique	Perceptible	Firm	Mild	Sloping
iC470692	Obovoid	Greenish yellow	Yellow	Intermediate	Round	Smooth	Oblique	Mammiform	Soft	Intermediate	Ending in a intermediate curve
iC470697	Obovoid	Yellow with red blush	Golden yellow	Low	Acute	Smooth	Oblique	Pointed	Firm	Intermediate	Ending in a intermediate curve
iC202177-A	Oblong	Greenish yellow	Yellow	Intermediate	Obtuse	Smooth	Oblique	Prominent	Soft	Intermediate	Slopping abruptly
iC202209	Oblong	Greenish yellow	Golden yellow	Intermediate	Obtuse	Smooth	Oblique	Pointed	Soft	Intermediate	Ending in a long curve
iC470627-B	Oblong	Greenish yellow	Yellow	Intermediate	Obtuse	Smooth	Oblique	Perceptible	Intermediate	Intermediate	Ending in a long curve
iC548486	Oblong	Greenish yellow	Yellow	Intermediate	Acute	Smooth	Oblique	Perceptible	Intermediate	Intermediate	Rising and then rounded

(Table 3) wide variations were observed *viz.*, length (11.46 cm in IC470621 to 29.14 cm in IC470682), width (3.83 cm in IC202177A to 7.87 cm in IC470656) and petiole length (1.92 cm in IC470621 to 6.27 cm in IC470667). The ratio of blade length and blade width was maximum (5.48) in IC470692 and minimum (2.04) in IC470621.

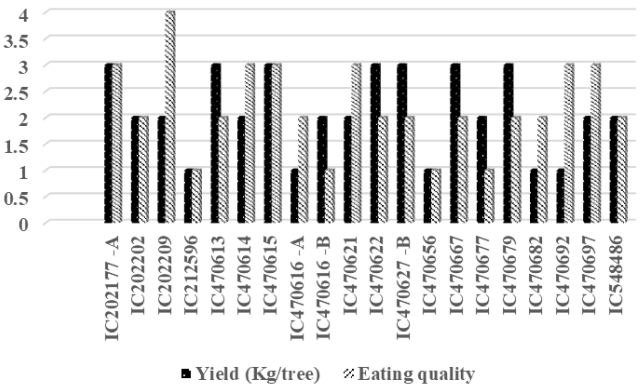
The fruit characters viz., length (4.92 cm in IC212569 to 16.92 cm in IC470614), diameter (4.08 cm in IC212569 to 9.68 cm in IC548486) and weight (41.10 g in IC212569 to 658.37 g in IC548486) also recorded wide variability among the accessions. The pulp recovery and TSS are the important parameters that decide consumer preference. High variation was recorded for pulp recovery percent (72.44 % in IC202177A to 91.25% in IC470614) and TSS (11.33°B in 202202 to 24°B in 470614) among the accessions. The accession IC470614 registered high pulp recovery (91.25 %) coupled with high TSS (24°B). Stone parameters are important in selecting the genotypes with good pulp content. Consumers prefer fruits with the lowest values for stone parameters. Stone weight (10.26 g in IC212569 to 78.12 g in IC548486) and length (3.86 in IC212569 to 14.06 cm IC470614) registered lot of variations. This huge variation in local mango cultivars (Fig. 1) is likely the result of a long period of cultivation, thus providing ample scope for active hybridization and selection mechanisms. According to Rajan *et al.* (2013) north Indian accessions were found to be superior for yield as well as quality attributes compared to accessions of south and western regions. Morphological traits such as leaf length, bearing habit, shape of inflorescence, flowering pattern, the shape of fruit, resistance to diseases, and color of fruit skin serve as morphological markers that can be easily differentiated visually (Khan *et al.*, 2015).

Morphological characterization revealed variations for the fruit qualitative traits (Table 4, Fig. 1). Fruit shape ranged from ovoid (2), obovoid (8) and oblong (10) (Table 4). Fruit apex was acute (12), obtuse (5) or round (3). Skin color was greenish yellow (12) in the majority of the accessions (12), yellow in 6 accessions and green in 2 accessions. Only one accession was yellow with red blush (IC470697). The fruit skin surface was smooth in all accessions except IC470667, where it was rough. Fruit stalk insertion was oblique in all accessions except IC470667 where it was vertical. Fruit beak type was perceptible in most of the accessions (11), pointed in 6 accessions, prominent in IC202177-A and mammiform in IC470692 and IC470614. The slope of the fruit ventral shoulder was slopping abruptly in 10 accessions, ending in a long curve in 8 accessions and rising and then rounding in 2 accessions. A wide spectrum of colors was observed in fruit pulp color. Fruit pulp color was orange (7), yellow (6), light orange (3), golden yellow (2), light yellow (1) and dark orange (1). The quantity of fiber in the pulp was absent in IC470614, low in 3 accessions, intermediate in 10 accessions and high in 6 accessions. The pulp texture of ripe fruit was soft (11), intermediate (3) or firm (6). Pulp aroma varied from mild (7), intermediate (10), to strong (3). Morphological variability may be due to probable genotypic variation among the different cultivars (Mitra, 2016)

Eating quality is important which determines the market demand. The accessions IC 202177 A, IC202209, IC470614, IC470615, and IC548486 had good eating quality with good taste (Fig. 2). As yield is a quantitatively inherited trait and its expression is based on cultivar, climatic conditions, crop age, pest and disease incidence and others, the study resulted in the identification of accessions IC202177-A, IC470613, IC470615, IC470622, IC470627B, IC470667, IC470679 with high yield (>500 kg/plant) (Fig. 2). Yield variations among different mango cultivars have been reported earlier (Archit Singh and Sanjay Pathak, 2018).

Since mango exhibits nucellar embryony, the seedlings tend to be true to type. These seedlings serve as good rootstocks. The days taken to germination ranged from 6 (IC470613) to 31 days (IC470679 and IC470677) (Fig. 3). Polyembryony was confirmed if the stone produced multiple seedlings/stone. Based on the number of seedlings per stone, seven accessions were found to be polyembryonic. According to Dinesh *et al*. (2016), polyembryony is a

Figure 1: Variations in fruit shape, size and pulp color of mango landraces used in the study



Yield- High- >500kg/plant, Medium-100-500kg, Poor <100kg/plant
Eating quality: Excellent Good, medium and poor

Figure 2: Variability in yield and eating quality of mango landraces

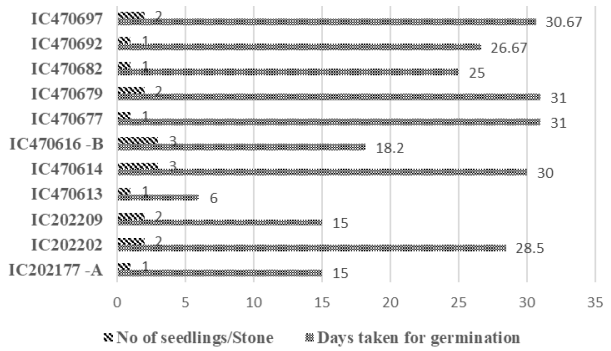


Figure 3: Days taken for germination and number of seedlings/ stones in mango landraces

common trait in mango cultivars of Southeast Asian origin, particularly Kuttiatoor varieties of Kerala, which are regular, heavy-bearing compared to monoembryonic varieties. A similar opinion was expressed by Radha and Manjula (2000) that Kerala, being hot and humid, has both mono and polyembryonic types and collections showed great variation with respect to vegetative and floral characters and the number of seedlings produced per stone (1-6). Unlike monoembryonic types, variability in the polyembryonic cultivars is much less and there is a need to widen the genetic base in polyembryonic varieties. There is a need to create variability by producing new recombinants, which, while retaining the polyembryonic nature, should have other desirable characteristics, either for use as rootstocks or as commercial types. (Sane *et al.*, 2015; Dinesh *et al.*, 2016).

Molecular Profiling using SSR Markers

Out of fifty SSR primers used in the study, ten primers were consistent in producing clear and reproducible bands (Table 6), which were utilized for generating DNA profiles of 20 accessions (Fig. 4). The allele size ranged from 91 bp (MilIHR 18) to 347 bp (MilIHR 24) with a total of 83 polymorphic bands. The alleles per SSR locus were 4 (MilIHR 17) to 11 (MilIHR 24) (Table 6), with an average of 8.3. All the primers have shown 100% polymorphism. Polymorphic information content (PIC) values varied from 0.37 (MilIHR 19) to 0.98 (MilIHR17), with an average value of 0.66. Most of the SSR primers used in the study detected multiple loci, which is in conformity with results reported by and Kumar *et al.* (2013) that were reported to be due to the allopolyploid nature (Mukherjee, 1950). The polymorphism detected varies based on factors like the number of varieties used and the presence of variability among them. By using just three SSR markers, Viruel *et al.* (2005) identified 28 mango genotypes. Dinesh *et al.* (2016) developed DNA barcodes for 10 Kuttiattoor mango accessions for registration with the PPV&FRA.

Cluster Analysis

In our study, the coefficient of similarity based on Jaccard's ranged between 0.12 to 0.96 and grouped 20 individual accessions into two major clusters (Fig. 5). IC470697 and

Table 5: Mango landraces with unique traits

Unique traits	*Indigenous collections*
Good fruit quality suitable for fresh fruits	IC470621, IC470627, IC202177A
Late bearing	IC202177A, IC202209
Regular bearing	IC470613, IC470622
Polyembryony	IC470621, IC470692 IC470622, IC470613, IC470677
Firm flesh	IC470616A
Thin peel, juicy type with good quality fruits	IC470615
Tender mango pickle	IC470656
Culinary preparations (mainly fish curry)	IC470677
Fruits with attractive red skin color	IC470697

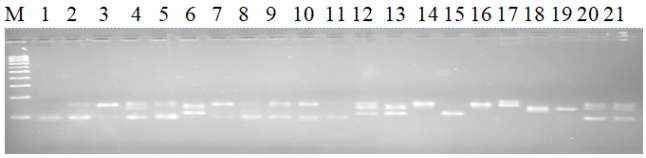


Figure 4: DNA profile of 20 landraces with MilIHR18. M- 100 bp ladder, 1- IC470622, 2- IC IC470616A, 3- IC470677, 4- IC470656, 5- IC470621, 6- IC 870393*, 7- IC470613, 8- IC470692, 9- IC202177A, 10- IC202209, 11- IC470614, 12- IC548486, 13- IC202202, 14- IC470679, 15- IC212569, 16- IC470616 B, 17- IC670682, 18- IC470667, 19-IC470627, 20- IC470615, 21- IC470697
*- Not included as phenotypic data is not available

IC470621 exhibit maximum genetic similarity (0.48). According to Simi *et al.* (2013), similarity coefficient values varied between 0.217 and 0.83, estimated by RAPD in 30 mango varieties. Each major cluster was split into sub-clusters primarily based on a high similarity coefficient. The first major cluster contained 12 accessions and was divided into three sub-clusters. The first sub-cluster consisted of 3 genotypes, IC202209, IC548486 and IC470679 (Kolambu, Kalletty and Nattumaavu-2, respectively). IC202209 (Kolambu) was late bearing, and produces good quality medium size fruits. IC548486 (Kallety) fruits were very big and were originally collected from saline areas. Nattumavu (IC470679) is used for pickle-making and curry purposes. The IC470679 (Nattumaavu/Nattumanga) were highly heterogeneous, where tree-to-tree variation for fruit quality traits was very high. In this group, the majority of them are pickling types, bunch-bearing, containing high latex, and soft, juicy types with high variation for sweetness and flavor.

The second sub-cluster consists of 5 accessions viz., IC202202, IC212569, IC470616-B, IC 470682 and IC470677. Accessions IC202202 and IC212569 (Vattan and Chunayan, respectively) produced round fruits and were very prolific

Table 6: Properties of primers, primer sequences and amplification products used in the study

Primer ID	*Sequence (5'-3')*	*Repeat motif*	*Expected range (bp)*	*Allele range (bp)*	*No. of alleles per locus*	*No. of polymorphic alleles*	*Polymorphic information content (PIC)*
MiIIHR 12	F: GCCCCATCAATACGATTGTC R: ATTTCCCACCATTGTCGTTG	$(GA)_{11}$	178	175-219	6	6	0.67
MiIIHR17	F: GCTTGCTTCCAACTGAGACC R: GCAAAATGCTCGGAGAAGAC	$(GT)_{13}GAGT(GA)_{10}$	244	166-193	4	4	0.98
MiIIHR18	F: TCTGACGTCACCTCCTTTCA R: ATACTCGTGCCTCGTCCTGT	$(GT)_{12}$	168	91-123	9	9	0.48
MiIIHR 19	F: TGATATTTTCAGGGCCCAAG R: AAATGGCACAAGTGGGAAAG	$(AC)_{11}$	187	96-289	10	10	0.37
MiIIHR 23	F: TCTGACCCAACAAAGAACCA R: TCCTCCTCGTCCTCATCATC	$(GA)_{17}GG$ $(GA)_6$	144	142-312	10	10	0.71
MiIIHR 24	F: GCTCAACGAACCCAACTGAT R: TCCAGCATTCAATGAAGAAGTT	$(CA)_9TACC(CATA)_6$	246	209-347	11	11	0.71
MiIIHR 26	F: GCGAAAGAGGAGAGTGCAAG R: TCTATAAGTGCCCCCTCACG	$(GA)_{14}$ GGA $(GAA)_2$	166	106-180	10	10	0.57
MiIIHR 31	F: TTCTGTTAGTGGCGGTGTTG R: CACCTCCTCCTCCTCCTCTT	$(GAC)_6$	229	191-295	7	7	0.88
MiIIHR 34	F: CTGAGTTTGGCAAGGGAGAG R: TTGATCCTTCACCACCATCA	$(GGT)_9$ $(GAT)_5$	237	123-172	8	8	0.65
MiIIHR 36	F: TCTATAAGTGCCCCCTCACG R: ACTGCCACCGTGGAAAGTAG	$(TC)_{17}$	246	212-273	8	8	0.57
Average number of alleles					8.3	83	

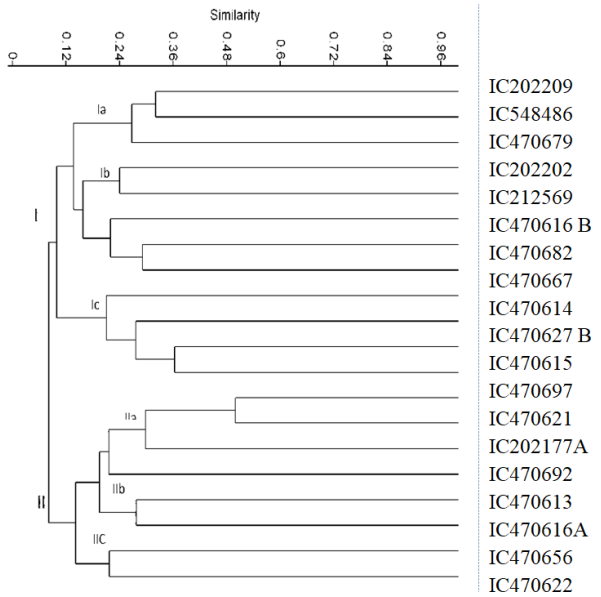


Figure 5: Dendrogram depicting genetic relationships 20 mango landraces analyzed with 10 SSR loci

bearers. Kilimukan (IC470616-B) produces fruits with more fiber content without fruit fly incidence. Here, the control measures were not adopted for controlling the fruit fly population and fruit fly incidence was not observed under natural epiphytotic conditions. IC470682 (Nattu manga) exhibited a bunch-type bearing habit; IC470677 (Puli manga) was acidic and used in culinary preparations. The common factor in this sub-cluster was that all of them were used for culinary purposes.

The third sub-cluster consists of four accessions, namely, IC470614, IC470627, IC470615 and IC470697 (Vellari manga, Kilichundan, Ullitholiyan, and Apple manga, respectively). IC470614 (Vellari manga) fruits were long and cucumber type; IC470627 (Kilichundan) fruit quality was good and used for fresh fruit purposes. IC470615 (Ullitholiyan) fruits had a thin peel, juicy with good quality fruits. IC470697 (Apple manga) was a variant of Muvandan with good bearing and the fruit peel was red in color. Here all of them produced good quality fruits with good eating quality.

The second major cluster II had eight genotypes and was subdivided into three sub-clusters. The first sub-cluster had IC470621, IC202177A, IC470692 and IC470613 (Valiya Kilichundan, Nada Shala/Panchasaramanga, Mailgoa and Muvandan, respectively). The fruit quality of IC470621 (Valiya Kilichundan) was good and used for table purposes; IC202177A (Nada Shala/Panchasaramanga) fruits were late bearing, sweet, firm flesh type and used for table purposes. IC470692 (Mailgoa) was originally collected from Goa; IC470613 (Muvandan) is a regular bearing type. In this sub-cluster, all three are polyembryonic types and also suitable for fresh fruit with good eating quality. The

second sub-cluster included IC470616A and IC470677 (Mylapoovan-variant, Puli manga, respectively). IC470616A (Mylapoovan) was firm flesh type, and IC470677 (Puli manga) was polyembryonic. The third sub-cluster had IC470656 and IC470622 (Nattu manga, Muvandan, respectively). IC470656 (Nattu manga), appemidi type, was used for tender mango pickle; IC470622 (Muvandan) was a typical regular bearing variety. Two Muvandan types, *viz*., IC470613 and IC470622 collected from two different places, have broadly grouped in the same cluster but in different sub-clusters.

In this study, we observed that there was no clear separation of landraces belonging to different regions of Kerala. Ward *et al.* (2005) also reported that there was no partition of genotypes based on their geographical vicinity because the populations studied originally were seedlings and not vegetative propagated ones. He attributed it to high variability, which was the result of the allogamous breeding. Simi *et al.* (2013) also got a similar result that the grouping of varieties did not follow any regular pattern with respect to geographical location in 30 local varieties of mango collected from the four districts of southern Kerala. The genotypes of the same cluster have a high similarity coefficient based on genetic distance, but morphologically, they are different with respect to fruit traits.

According to Karihaloo *et al.* (2003), in mangoes, due to heterozygous nature and outcrossing nature, high diversity is prevalent in regions in India among naturally produced seedlings. The commercial cultivars were selected from the existing seedling population based on consumer preferences. Kerala does not consider mango to be a commercial crop, although mango trees are a necessary part of every homestead there. Kerala's production of mangoes is distinguished for its earliness. The first mango fruits of the season arrive from Kerala in Indian markets. Due to early bearing habit (the harvesting in March to April), they fetch the highest price due to the great demand for fruits in the major marketplaces in other parts of the country (Radha and Nair, 2000)

In the current study, we looked at the quantitative and qualitative characteristics of mango and ascertained that there was a noticeable difference between the 20 mango accessions that were taken from various eco-geographical regions of Kerala. With regard to leaf and fruit characteristics, the majority of mango landraces are unique morphologically. Furthermore, there is a lot of genetic variation in mangoes, both within and across landraces and well-known cultivars, providing room for development. All of the native landraces that were gathered from six districts in Kerala were found to be morphologically unique from the commercial cultivars, indicating their potential for use either directly or through breeding programs.

Also noteworthy is the presence of significant cultivar sources for enhancement based on regular bearing habits, pickle and curry type mangoes, besides juicy and good quality fruits for fresh consumption in this germplasm collection (Table 5). Thus, the morphological variability data, along with molecular data and genotype grouping, should aid breeders in choosing genotypes for crosses to create new cultivars appropriate to the current tropical high, humid climate. The polyembryonic accessions with specific traits would help in enhancing the genetic base of polyembryonic types and those with desirable traits could be utilized as parents in crop improvement programs.

Authors' Contribution

Conceptualization of research (AS&MRD); Designing of the experiments (AS&MRD); Contribution of experimental materials (JJ &KP); Execution of field/lab experiments and data collection (AS&CV); Analysis of data and interpretation (AS, JJ & KP); Preparation of the manuscript (AS&CV).

Declaration

The authors have declared that they do not have any conflict of interest. The submissions are original and unpublished and are not under consideration for publication elsewhere. Authors also declare that the manuscript is based on their own original work and the manuscript is checked for plagiarism.

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