



Genetic diversity in mango cultivars revealed by SSR markers

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ABSTRACT

The existing genetic diversity is the main source of variability in any fruit crop improvement program which serves as a gene pool for identifying superior alleles governing key horticultural traits through allele mining/ association mapping. Therefore the first attempt was made to determine the genetic diversity in 60 mango cultivars using 100 leaf transcriptome sequence derived novel genic SSR markers. A total of 263 alleles were amplified with mean of 3.0 alleles per SSR locus. Size of the amplified alleles ranged from 130 to 300 bp. Mean polymorphic information content (PIC) was 0.45, which demonstrated the presence of moderately high amount of genetic variation between the selected mango cultivars. Neighbour Joining (NJ) tree analysis detected two major groups belongs to North-West and South-East India, Florida, USA and Brazil. The comprehensive molecular characterization of the present set of mango cultivars contributes to the knowledge about the levels and distribution of genetic diversity within these geographically diverse mango cultivars. In addition to that the information generated in this study could be utilized in association mapping and marker assisted selection in mango improvement programme.

Key words: *Mangifera indica*, genic simple sequences repeat, genetic diversity, principal coordinate analysis, AMOVA.

INTRODUCTION

Mango (*Mangifera indica* L.) is most popularly known as “King of fruits” in India due to unique sweet taste, flavour, wide variability, large production volume and variety of end usage. Mango has economic as well as therapeutic value due to its high vitamin, mineral and fibre content. The existing evidences states that this fruit crop has been under cultivation in India for at least 4000 years and is classified within the genus *Mangifera* (Anacardiaceae). This genus includes 73 genera and about 830 species originating in the Northern foothills of the Indian-Myanmar region. Globally, India leads mango production with an annual production of 19.68 million tonnes from an area of 2.26 million hectares (Anonymous, 1), contributing about 56% of the total world production. More than 1,000 mango varieties exist in India today that contributes 39.5% of the total fruit production in the country. Almost all of the existing varieties are chance seedling selections made from naturally occurring open-pollinated population (Dinesh *et al.*, 5) except of some hybrids, which were results of human interventions. In spite of sustained research efforts for increasing the production and productivity over the past four decades, the productivity of mango orchards in India is still low as 8.66 t/ha (Anonymous, 1) and while quality of fruit do not meet local consumer or

export standards. There are several complex factors associated with low productivity and poor fruit quality in mango and the majority of these are governed by complex quantitative traits. For precise genetic manipulation of these complex quantitative traits, understanding the genetic/molecular basis of target traits needs to be investigated thoroughly. In the past, several breeding approaches have been utilized to overcome these yield and quality constraints. The main hurdles limiting the genetic study of mango includes inefficient breeding programme, excessive tree vigour, gigantic size, long juvenile period, single seeded, high fruit drop and highly cross pollinated in nature. In recent times, SSR markers have been widely applied for genetic diversity analysis in mango (Surapaneni *et al.*, 14; Dillon *et al.*, 4) but almost all researchers applied genomic SSR markers. Therefore, there is great scope of utilization of genic SSRs for genetic diversity analysis as these are derived from transcribed genomic regions and specifically target the functional region of the genome and have potential for linkage to loci that may contribute to expressed phenotypes. Therefore, the identified polymorphic genic-SSRs in high value breeding lines can be effectively utilized in marker assisted selection (Varshney *et al.*, 16) and cross-genome comparisons between related crop species because they exclusively aim protein-coding regions. The objective of this study was to determine the genetic diversity among 60 mango cultivars using genic-SSR for identification of diverse

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parents, germplasm management, conservation and determining the future mango breeding strategies.

MATERIALS AND METHODS

Fresh leaf samples of 60 mango cultivars (Table 1) were collected from the scientifically maintained Field Mango Germplasm Block of ICAR- Indian Agricultural Research Institute, New Delhi, India. These mango cultivars includes recently bred hybrids, clonal selections and land races and have been maintained by vegetative propagation. From collected samples the leaves (5 g) of each genotype were used for DNA extraction following CTAB method as described by Murray and Thompson (7) with minor modifications. The isolated DNA was purified and quantified by Nanodrop® (Thermo Scientific, USA) and integrity was checked by agrose (0.8%) gel electrophoresis. For genotyping of 60 mango cultivars, a total of 100 novel genic-SSR primers were designed and synthesized from leaf

transcriptome sequence data of mango (*Mangifera indica* L.) cv. Amrapali. Both PCR reaction master mix composition and temperature conditions were empirically standardized for newly synthesized genic-SSR markers. This exercise involved DNA and primer stock dilution, optimization of PCR mix (nucleotides, buffers, *Taq* polymerase, and DNA concentration), primer concentration and annealing temperature. The PCR reaction was performed at 1 cycle of 3 min at 94°C as initial denaturation, followed by 36 cycles with a denaturation step at 94°C for 30 second, an annealing step for 1 min at respective annealing temperature of each primer in a range of 48.3-53°C and at 72°C for 1 min an initial extension followed by last cycle at 72°C for 10 min for final extension. PCR products were separated on 4% (w/v) metaphor agarose gel by gel electrophoresis. The gel was prepared by using 0.1 µg/ ml ethidium bromide in 1x TBE buffer solution and run at 100 volts for 3 hours. A gel documentation system was used

Table 1. List of mango cultivars undertaken for the study and geographical distribution.

S. No.	Genotype	Location	Region	Latitude	Longitude
1	Amrapali	I.A.R.I. New Delhi	North India	28.6195591	77.2979782
2	Pusa Lalima	I.A.R.I. New Delhi	North India	28.6195591	77.2979782
3	Amnesia Hyderabad	Sangareddy, Telangana	South India	17.619416	78.082308
4	Khasulkhas	Saharanpur, Uttar Pradesh	North India	29.967079	77.551017
5	Pusa Shresth	I.A.R.I. New Delhi	North India	28.6195591	77.2979782
6	Bhadaan	Saharanpur, Uttar Pradesh	North India	29.967079	77.551017
7	Rosari	Brasilia	Brazil	-14.235163	-51.925238
8	Chausa	Lucknow, Uttar Pradesh	North India	26.846694	80.946166
9	Ramkela	Punjab	North India	31.147130	75.341218
10	Vanraj	Paria, Gujrat	West India	72.8333	20.36666
11	Ellard	Miami, Florida	USA	25.761680	-80.191790
12	Extrema	Brasilia	Brazil	-14.235163	-51.925238
13	GulabKhas	Sabour, Bihar	East India	78.1166	17.6333
14	Janardan Pasand	Kadium, East Godavari, Andhra Pradesh	South India	81.8333	16.9166
15	Ferandin	Panji, Goa	West India	73.8333	15.48333
16	Irwin	Miami, Florida	USA	25.761680	-80.191790
17	Neelum	Krishnagiri, Tamilnadu	South India	78.23333	12.5333
18	Hybrid165	Delhi	North India	28.6195591	77.2979782
19	Pusa Surya	Miami, Florida	USA	27.664827	-81.51575
20	Pusa Arunima	I.A.R.I. New Delhi	North India	28.6195591	77.2979782
21	Dushehari Sabour	Sabour, Bihar	East India	78.1166	17.6333
22	Mahmood Vikarabad	Lucknow, Uttar Pradesh	North India	26.846694	80.946166

S. No.	Genotype	Location	Region	Latitude	Longitude
23	Smith	Perrine, Florida	USA	25.605106	-80.35366
24	Olour	Thrissur, Kerala	South India	72.2166	10.5166
25	Kurakkan	Thrissur, Kerala	South India	72.2166	10.51666
26	Sonatul	Muzaffarnagar Uttar Pradesh	North India	29.472682	77.708509
27	Langra	Banaras, Uttar Pradesh	North India	78.1166	17.6333
28	Safdar Pasand	Saharanpur, Uttar Pradesh.	North India	29.967079	77.551017
29	Machlli	Lucknow, Uttar Pradesh	North India	26.846694	80.946166
30	GulabJamun	Saharanpur, Uttar Pradesh	North India	29.967079	77.551017
31	Hardil Aziz	Saharanpur, Uttar Pradesh	North India	29.967079	77.551017
32	Mombasa	Saharanpur, Uttar Pradesh	North India	29.967079	77.551017
33	Alphan	Saharanpur, Uttar Pradesh	North India	29.967079	77.551017
34	Kala	Saharanpur, Uttar Pradesh	North India	29.967079	77.551017
35	Ametista	Brasilia	Brazil	-14.235163	-51.925238
36	Primor-de Amoreira	Brasilia	Brazil	-14.235163	-51.925238
37	Ratna	Rantnagiri, Maharashtra	West India	16.990215	73.3120233
38	Alphanso	Rantnagiri, Maharashtra	West India	16.990215	73.3120233
39	Iturba	Brasilia	Brazil	-14.235163	-51.925238
40	Husnara	Sabour, Bihar	East India	78.1166	17.6333
41	Lucknow Safeda	Lucknow, Uttar Pradesh	North India	26.846694	80.946166
42	Tephala	Lucknow , Uttar Pradesh	North India	26.846694	80.946166
43	Dushehari	Malihabad, Uttar Pradesh	North India	26.9400	80.7200
44	Nissar Pasand	Malihabad, Uttar Pradesh	North India	26.94000	80.7200
45	Bombay Green	Kirkee, Maharashtra	West India	18.569936	73.850643
46	Rataul	Saharanpur, Uttar Pradesh	North India	29.967079	77.551017
47	S.B. Alibagh	Saharanpur, Uttar Pradesh.	North India	29.967079	77.551017
48	Pusa Pratibha	I.A.R.I. New Delhi	North India	28.6195591	77.2979782
49	Edward	Perrine, Florida	USA	25.605106	-80.35366
50	Willard	Miami, Florida	USA	27.664827	-81.51575
51	Sensation	Miami, Florida	USA	25.761680	-80.191790
52	Kaleped	Sangareddy, Telangana	South India	17.619416	78.082308
53	Tommy Atkins	Miami, Florida	USA	27.664827	-81.51575
54	Pusa Peetamber	I.A.R.I. New Delhi	North India	28.6195591	77.2979782
55	Mallika	I.A.R.I. New Delhi	North India	28.6195591	77.2979782
56	Totapari Red Small	Krishnagiri, Tamil Nadu	South India	78.2333	12.53333
57	Xavier	Panji, Goa	West India	73.8333	15.4833
58	St. Alexandrina	Brasilia	Brazil	-14.235163	-51.925238
59	Zill	Brasilia	Brazil	27.664827	-81.51575
60	Zardalu	Murshidabad, West Bengal	East India	24.175904	88.280179

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to visualize and document the DNA fragments. The observed amplicon (band) size was determined using 100-bp DNA ladder. To overcome non-amplification or technical failure, PCR was repeated and in case of failure in both, a null allele was recorded. The SSR amplification profiles were scored based on size of most intensely fragments amplified. The mean number of alleles per locus, gene diversity, major allele frequency, heterozygosity, polymorphism information content (PIC) and genetic distance were calculated using Power Marker v3.25 (Liu and Muse, 6) and dendrogram was constructed using MEGA 4.0 software (Tamura *et al.*, 15). Principal coordinate analysis (PCoA) and analysis of molecular variance (AMOVA) was calculated by GenAlEx 6.5 (Peakall and Smouse, 8).

RESULTS AND DISCUSSION

To determine genetic diversity in current study, the selected 100 novel genic-SSR loci were amplified with contrasting alleles across all mango cultivars and showed wide range of diversity. The summary statistics of the 100 genic-SSR loci are given in Table 2. Of the 100 genic-SSR loci tested, 87 SSR loci were observed as polymorphic and 13 SSR loci were monomorphic. The observed 87 polymorphic genic-SSR loci identified a total of 263 alleles. Amplicon sizes generated across all cultivars ranged from 130 and 300 bp which was in accordance to Samant *et al.* (10). The average number of alleles per locus (AN) was ranging from two (MSSR 166, MSSR 172, MSSR 173, MSSR 181, MSSR 188, MSSR 195) to four (MSSR 102, MSSR 108, MSSR 109, MSSR 110, MSSR 119, MSSR 129, MSSR 147, MSSR 153, MSSR 155, MSSR 190). The level of genetic diversity in our study was recorded at a moderately high level, as reflected by the number of average alleles per locus (3.0) but lower than the average alleles estimated for the mango accessions by (Archak *et al.*, 2; Dillon *et al.*, 4 and Ravishankar *et al.*, 9). The gene diversity ranged from 0.286 (MSSR 79) to 0.702 (MSSR 10), with an average of 0.530. Of the 87 SSRs, one (MSSR 107) did not show any heterozygosity, however in the remaining cultivars, it ranged from 0.030 (MSSR 41) to 0.980 (MSSR 18) with a mean value of 0.450. The average gene diversity index and major allele frequency was 0.530 and 0.580 respectively, which represent higher value and supported greater genetic variation among selected cultivars. PIC values, which represent allelic diversity and frequency, had an average value of 0.450. The range of polymorphism information content (PIC) for the SSR loci was observed from 0.260 (MSSR 79) to 0.640 (MSSR 10). The major allele frequency was also calculated for all the 87

loci, which ranged from 0.350 (MSSR 10) to 0.833 (MSSR 79) with an average of 0.580 (Table 2). Thus, a high level of genetic diversity index and major allelic frequency (MAF) justified the sample size taken for the study to estimate the genetic diversity among the cultivars. In this study the average PIC value (0.45) was similar to the values obtained by Singh and Bhat (13) and Begum *et al.* (3). Markers with high PIC values such as MSSR 139, MSSR 184, MSSR 152 and MSSR 165 could be effectively used in inter and intra-cultivar genetic diversity studies on mango. The average values of heterozygosity (0.45) was higher than those reported by Singh and Bhat (13) (0.26) and Surapaneni *et al.* (14) (0.29) but lower than Ravishanker *et al.* (9) (0.624) which can be explained by the fact that the cultivars chosen in their studies were from different geographical regions like Florida (USA), Brazil and India or it may be due to the wide sexual compatibility between mango cultivars and its relative species, the high long history of cultivation, frequency of bud mutations, occurrence of polyembryony etc. noted in most of the cultivars. The moderate to high values of all the measures of diversity indicated allelic richness in the analysed mango germplasm, which can further be utilized in breeding programmes to get desired plant types for commercial cultivation. The loci MSSR 109 and MSSR 190 exhibited the higher alleles and heterozygosity values and could be utilized as valuable marker in genetic investigation on mango germplasm and this able to provide a reliable and reproducible approach for genotype-specific fingerprinting for identification.

The neighbour joining (NJ) cluster analysis (Fig. 1) classified mango cultivars into two major groups, based mainly on their geographical origin, *i.e.* North-West and South-East Indian, Floridian and Brazilian. These major groups further categorised into many sub-clusters based on their genetic dissimilarity matrix and genetic distances. The first group consisted greater than 60% of the cultivars and further categorised in to five sub-clusters. The first cluster consisted of five cultivars, namely, Alphanso, Neelum, Ratna, Ferandin and Vanraj, mostly grown in Western India (Maharashtra, Goa, Gujarat states). This cluster included hybrids and their parents (Alphanso, Neelum, Dushehari). The second cluster included 11 cultivars of mixed origins such as Gulab Khas, Chausa, Rataul, Bhadauran, Ramkela, Nissar Pasand and Alphan from North India, Xavier and Bombay Green from West India, Khasulkhas from East India and Kaleped from South India. This cluster included all the chance seedling originated cultivars and very ancient ones (landraces). The third cluster formed the smallest cluster with six cultivars (Tephala,

Table 2. Details of 87 polymorphic genic-SSR loci, their numbers used in the study, observed band size, major allele frequency, gene diversity, heterozygosity, polymorphism information content and annealing temperature.

Locus	Motif	Primer sequences (5'-3')	Observed bands size range (bp)	Allele number	Major allelic frequency	Gene diversity	Hetero zygosity	Polymorphism information content	Annealing temperature (Ta) °C
MSSR 101	(CT)13	F-GGAGAGAGAAAACCAAGAAAGA R- CCTTCTCCACACAACACTATC	160-180	3.0	0.54	0.55	0.15	0.46	48.3
MSSR 102	(TCT)9	F- AAGACAAAAGCCTGGAGCTA R- GTTGATGGCAACTTTTGTG	150- 180	4.0	0.50	0.64	0.63	0.58	48.3
MSSR 105	(AGA)9	F-ATGTCTTCTCGCACTCTTC R-CTACACCCATAGTCTCCCTCT	150- 170	3.0	0.53	0.53	0.87	0.42	48.3
MSSR106	(TGA)9	F-GTGAAGGACGTTGATTCAT R-GATCCTTTTCGACTGTAATCCT	150- 170	3.0	0.63	0.51	0.57	0.44	48.3
MSSR107	(AAG)9	F-GGCATTATGGTTAAACTTGG R-TTCTTTGTTTACACGCACAC	160- 170	3.0	0.40	0.66	0.10	0.58	48.3
MSSR 108	(AAC)9	F-CTTGAGACCGAAACTTTGAC R-AAAAGCGTCAGAAAACACC	150- 180	4.0	0.57	0.60	0.13	0.55	48.3
MSSR109	(CTT)9	F-CTCTCTCTTTTCCCATTCTG R-GAAAAGAGCTTGAGTTCCATC	150- 180	4.0	0.47	0.59	0.83	0.51	48.3
MSSR110	(TTG)9	F-GAAGCTGTCGATTTTATTGC R-GTGTCTGCACATCCATATAGTA	180- 230	4.0	0.35	0.70	0.33	0.64	48.3
MSSR111	(TTG)9	F-GAAGCTGTCGATTTTATTGC R-GTGTCTGCACATCCATATAGTA	210- 250	3.0	0.42	0.65	0.47	0.57	48.3
MSSR112	(TCT)9	F-AGCAGTTTCTACCTCCAICTT R-GAGGCCTAACCTTAGATCTGT	190- 220	3.0	0.71	0.45	0.38	0.40	48.3
MSSR113	(ATT)9	F-GTAAATCCAACACCAGTTGAG R-CCTAGAAAAGTACGGTCTGTAT	150- 180	3.0	0.61	0.55	0.32	0.49	48.3
MSSR115	(GA)8	F-GACGGATTAGTCTTTTGTGTTG R-ACCATGACCTCCTTCTCC	170- 190	3.0	0.73	0.43	0.22	0.39	48.3
MSSR116	(AAC)9	F-CTCGGCTATGGAGAATAATG R-CGGTACTATAGCCAAAAGGATT	150-160	3.0	0.62	0.54	0.47	0.48	49.8
MSSR117	(CAA)9	F-GGAAGAGGAAATCCATTG R-CCTCTTGAGTATGCGTTTAGA	160- 200	3.0	0.60	0.55	0.17	0.48	49.8
MSSR118	(TTC)9	F-GGTATCGATTATTTGGATGC R-GCATTGTTACTAGCGTTTCA	150- 180	3.0	0.39	0.66	0.98	0.59	49.8
MSSR119	(GAA)9	F-CTCAAGAAATGTTCCGCTACAG R-TGAGCTCAAAGAGATCAGAAG	150- 170	4.0	0.61	0.57	0.48	0.53	49.8
MSSR120	(ATC)9	F-GAGTCCCTAAATGAAATCCTC R-TAGGACCATGACAAAATACCAC	250- 270	3.0	0.53	0.61	0.45	0.54	49.8

Genetic Diversity in Mango Cultivars Revealed by SSR Markers

Locus	Motif	Primer sequences (5'-3')	Observed bands size range (bp)	Allele number	Major allelic frequency	Gene diversity	Hetero zygosity	Polymorphism information content	Annealing temperature (Ta) °C
MSSR121	(AAG) ⁹	F-GTGCTCTCTAGCTTTTCTC R-TATTATCCCACCTCCTCAGAT	190- 250	3.0	0.72	0.42	0.37	0.36	49.8
MSSR122	(CAT) ⁹	F-CTTCTTTACATCCTCAACAC R-GTCAAATTTTCCAACGGTAG	200- 220	3.0	0.52	0.62	0.17	0.55	49.8
MSSR123	(AT) ¹⁴	F-ACAAAACGATCAGATCACAGA R-TTACTTGGCATGCTACACTCT	150-180	3.0	0.50	0.62	0.47	0.55	49.8
MSSR124	(TACA) ⁷	F-GGTAAGAGGATGCTTCTGAT R-TCAGATAGTGATGGAAGGAGA	180-200	3.0	0.71	0.45	0.38	0.40	49.8
MSSR125	(AT) ¹⁴	F-CTGTCAGGTTAAGCTGTAAA R-TTCTCATCTGACTAACCTTCAG	150-170	3.0	0.35	0.67	0.47	0.59	49.8
MSSR129	(TC) ¹⁴	F-TTCGTCTATGTTGTTGCTGA R-GGAAACCAGATATACCAAACC	140- 180	4.0	0.63	0.53	0.55	0.47	49.8
MSSR130	(AT) ¹⁴	F-ATGCACTCATGGTACTTGG R-CCTGTACTACCCGGTTACTC	250-300	3.0	0.59	0.55	0.58	0.48	49.8
MSSR131	(TA) ¹⁴	F-TGATGATAGTCCACACAGTTG R-CACACGTGCCAGTACATTT	130- 180	3.0	0.55	0.57	0.67	0.49	49.8
MSSR132	(AC) ¹⁴	F-AAGCACGTCTGAATGAGAAT R-CTTACAAAATCGGGTATGCTG	140- 180	3.0	0.63	0.52	0.47	0.46	51.0
MSSR133	(GA) ¹⁴	F-GCGAGCTAGATAAGTTTCGATA R-CATTCACCCGGTAGATGTTAAG	140-170	2.0	0.73	0.39	0.13	0.31	51.0
MSSR134	(AG) ¹⁴	F-ACCTCTTGGAGTCGCATTTA R-GTTAGTCGTATGGCAAGTTGA	250-300	3.0	0.64	0.49	0.62	0.41	51.0
MSSR135	(GA) ¹⁴	F-CTTCTCCGACATGAATGAAT R-GCTTCCCACTTCACTGTTTAC	260-300	3.0	0.44	0.64	0.45	0.56	51.0
MSSR136	(AG) ¹⁴	F-ACCTCTTGGAGTCGCATTTA R-GTTAGTCGTATGGCAAGTTGA	250-300	3.0	0.43	0.64	0.27	0.56	51.0
MSSR137	(ACAT) ⁷	F-CCCTGGTACTCTACAACATA R-GAGGCTAAATAATGCTTCCCTG	130-160	2.0	0.65	0.49	0.37	0.41	51.0
MSSR138	(AT) ¹⁴	F-TCTACTGACAACCTCGAGCAC R-ACTGTCCAAAATTCCTCCTTT	180- 300	3.0	0.74	0.42	0.28	0.38	51.0
MSSR140	(TTTC) ⁷	F-ACTATAAAGGGCGCATACAA R-CTACCCGTGGTATTGGAATGA	250- 290	3.0	0.63	0.50	0.60	0.43	51.0
MSSR141	(TC) ¹⁴	F-GCACTGAGTAATCACCAAAG R-GGTCCTGTGTAGTTTTGAAAG	230- 260	3.0	0.68	0.45	0.03	0.38	51.0
MSSR142	(TCTCT) ⁵	F-CTCTTTGGCCTTCTCTTCTC R-CAAATTTTCGTTGGTCAAGAG	140-190	3.0	0.70	0.46	0.20	0.41	53.0

Locus	Motif	Primer sequences (5'-3')	Observed bands size range (bp)	Allele number	Major allelic frequency	Gene diversity	Hetero zygosity	Polymorphism information content	Annealing temperature (Ta) °C
MSSR143	(GA)15	F-ACCACATACATCAAGCTCAAC R-ACCAGATCCACTTCAAAAATC	250-300	3.0	0.43	0.64	0.30	0.56	53.0
MSSR144	(TTG)10	F-CATGGTAATGATGTTGGTCA R-GAACCAGACTTAGAGGTTG	250-300	3.0	0.53	0.54	0.48	0.44	53.0
MSSR145	(AT)15	F-CGTTTCAGGGGATTTATGTC R-GGAAAAGCCTTGAAGTTTCT	280-300	3.0	0.56	0.53	0.68	0.43	53.0
MSSR146	(GA)15	F-CTCTCCTTCATCTCATCGTTA R-TCCTTAGATTGCAGAGAAAACC	260-300	3.0	0.51	0.62	0.05	0.55	53.0
MSSR147	(TTC)10	F-CAGATCTTCTACAAACAACAGC R-GGGAGACTGAGAGTTTGTGTTT	180-250	4.0	0.66	0.52	0.43	0.47	53.0
MSSR148	(GAA)5	F-TGATATTTAGGCATGGCAAC R-ACCAGAGGGAACAGAGACTA	250-280	3.0	0.59	0.56	0.42	0.50	53.0
MSSR150	(TA)15	F-AGGATGTTGCAGTTGAAAGT R-CTCCCTATTAACAGTATGCTC	210-280	3.0	0.50	0.56	0.43	0.46	53.0
MSSR151	(AT)15	F-TGCTAGAGGACAACAAGAAC R-CAGATTCCTTGATCGTGTGT	150-180	3.0	0.54	0.56	0.65	0.47	49.3
MSSR152	(CTT)10	F-GTAAATCGGAGGGGATAATG R-CCCCCTGTAGTTTATTCTGA	150-180	3.0	0.45	0.59	0.37	0.49	49.3
MSSR153	(GGA)10	F-TTGGTTGTAGTTACTTCAGCAG R-TGAGATAGATGTGAGGCTTATG	160-190	4.0	0.58	0.58	0.53	0.53	49.3
MSSR154	(TCATCT)5	F-GAAGTAGAGGAAGGCATCTGT R-GGATCTTTCACCTTCTTCCG	150-180	3.0	0.64	0.48	0.62	0.39	49.3
MSSR155	(TCATCT)5	F-TGGCTTGAGTCAGAAGTTTT R-AACCATCGTGAGTAATTTGG	180-220	4.0	0.61	0.53	0.55	0.46	49.3
MSSR156	(CTGATT)5	F-TCCTGATCTCTTTAGCTCCTT R-GAGTTCTCGAACACCAATTTCT	230-270	3.0	0.61	0.54	0.25	0.47	49.3
MSSR157	(TGG)10	F-ACAGAGACGATACTGATGATGA R-GTGTGGAGCTGAGTATGTGTC	240-280	3.0	0.38	0.66	0.15	0.59	49.3
MSSR158	(TGACCC)5	F-TCTCCACCTCCTGTACTAATG R-ATCCTCAAAGGTCAGTTTC	150-170	3.0	0.59	0.53	0.48	0.44	49.3
MSSR159	(GAA)10	F-TGTCGGTAGTTGACTTGAGAG R-AGCAGAATTGTGACGAGACT	140-180	3.0	0.75	0.39	0.37	0.34	49.3
MSSR160	(TCTTTC)5	F-CGGATCTTTCACTTACTCTCA R-CATTGGTCAAAGGAAGAAGA	140-170	3.0	0.71	0.44	0.38	0.38	49.3
MSSR161	(ACGATG)5	F-GAAAGAACACTACCGATGAGGAT R-TACTCTGAATCACCAGACCAC	130-170	3.0	0.49	0.53	0.68	0.42	49.3

Genetic Diversity in Mango Cultivars Revealed by SSR Markers

Locus	Motif	Primer sequences (5'-3')	Observed bands size range (bp)	Allele number	Major allelic frequency	Gene diversity	Hetero zygosity	Polymorphism information content	Annealing temperature (Ta) °C
MSSR162	(TCATCT) ₅	F-CAACTTATCTATCAGCCCTTG R-TTATCTGACCCCTTCTTTAC	150-170	3.0	0.50	0.52	0.37	0.40	49.3
MSSR163	(CAAACA) ₅	F-AGAATCACATCTAGGGCACTT R-CTCATCGGTTCCAGTCTATTT	250- 300	3.0	0.68	0.48	0.45	0.43	49.3
MSSR164	(CAA) ₁₀	F-AGTGCCAGTTCATCATCATAC R-AAGCGGCAAACTATGATCT	190-230	3.0	0.47	0.64	0.33	0.57	49.3
MSSR165	(TGCTGG) ₅	F-ACCTTCTGATTCGGCTATTGA R-GCTGCTCAAGTATCAGGTGTA	140-170	3.0	0.49	0.58	0.55	0.49	49.3
MSSR166	(CAA) ₁₀	F-AGTGCCAGTTCATCATCATAC R-AAGCGGCAAACTATGATCT	180-210	2.0	0.69	0.43	0.55	0.34	49.3
MSSR167	(CAAAA) ₆	F-AATAATGTGTGCACCCCTACG R-AAACCCGGTGAGAGTAGTGG	220-250	3.0	0.53	0.53	0.73	0.42	49.3
MSSR168	(TCTTCT) ₅	F-GAGCACTTGAGTAGCAAGAGA R-TTCACCAAGAAGCAGAGAAT	140-170	3.0	0.60	0.49	0.67	0.39	49.3
MSSR169	(TCAAC) ₆	F-CACCCCTTTCTCTTCTCTC R-AGAAAGCGTGTGAGTGAGT	140- 160	3.0	0.51	0.54	0.55	0.44	49.3
MSSR170	(TGA) ₁₀	F-TTGTGACCCTACAACCTTCTGT R-GAAATGAAGTACCCTTGTGAG	140-180	3.0	0.51	0.52	0.68	0.40	49.3
MSSR171	(TA) ₁₅	F-GGAAAACCTGTTAGCTCAGAT R-AGACAAGTAAGCCAATGGAA	210- 280	3.0	0.52	0.52	0.67	0.40	49.3
MSSR172	(GAA) ₁₀	F-CATTAGCAGTAATGGGACAG R-TCTTTTGTGCTTCCGAACT	230- 280	2.0	0.62	0.47	0.43	0.36	49.3
MSSR173	(TC) ₉	F-TCAGTCTCTCATTTCACCTTC R-ACTCTGAGGTGATGAACAAGA	250- 290	2.0	0.63	0.47	0.45	0.36	49.3
MSSR174	(ACCACA) ₅	F-ATGGGGTAGTCAGGTCTACAT R-GACATATGTGCAGTGGTGTG	140- 170	3.0	0.76	0.38	0.38	0.33	51.7
MSSR176	(TA) ₁₆	F-GCGTTCGAACCTAAGAGAA R-TACCCCTGCTTGCTCTCATAC	150- 190	3.0	0.71	0.43	0.25	0.36	51.7
MSSR177	(TTTC) ₈	F-GGGTGTGATGTTATACTTGG R-ATTCAGATAGCCCTTGATTCC	220- 280	3.0	0.56	0.55	0.68	0.47	51.7
MSSR178	(TC) ₁₆	F-ACCTTGAATAGCTGCCTATCC R-TTGCTCTCTTAAAGGCTCTC	160- 180	3.0	0.71	0.43	0.32	0.36	51.7
MSSR179	(AAAT) ₈	F-CACACTCACAGAACCCTCA R-GAAGAAAGTGAGGTAAGATGC	140- 170	3.0	0.83	0.29	0.27	0.26	51.7

Locus	Motif	Primer sequences (5'-3')	Observed bands size range (bp)	Allele number	Major allelic frequency	Gene diversity	Hetero zygosity	Polymorphism information content	Annealing temperature (Ta) °C
MSSR181	(TAT)11	F-GGAGTAGGCATTCAAAATGA R-CTGTGGATTCGCAATAGTTT	140- 170	2.0	0.56	0.49	0.48	0.37	51.7
MSSR182	(CTT)11	F-AGACCCCTACGCTCAGCTTATT R-TGCTGCTAGTATGAAGAATCC	140- 160	3.0	0.81	0.32	0.08	0.28	51.7
MSSR185	(AG)17	F-TTTGAGAGGGAGGTACAAAGT R-CCTCGTCTTAATCCCTTACAC	170- 190	3.0	0.52	0.54	0.37	0.44	51.7
MSSR186	(AG)17	F-AACACCCAGATCTTACCAAAC R-CAGCAGTAGCAGCTCATAGTT	230- 300	3.0	0.54	0.53	0.32	0.43	51.7
MSSR187	(TA)17	F-CTTAATCCGTTACGCCATA R-ATGGCTTTCATACCAATGAC	260- 290	3.0	0.63	0.48	0.52	0.38	51.7
MSSR188	(AG)17	F-GAGAGTCCACAAAAATGGAA R-GAAAGGTTGGAGATTACCT	140- 160	2.0	0.56	0.49	0.52	0.37	51.7
MSSR189	(ACAT)9	F-GGTAACCAAAGTGAGGGATTA R-CCATTTGAAGCTGAGGTG	250-300	3.0	0.51	0.56	0.87	0.46	51.7
MSSR190	(TA)18	F-GCCCTAAAGAATCTGTAAACC R-ATGCATGCAGACACAACCTTA	160- 220	4.0	0.52	0.56	0.83	0.47	51.7
MSSR191	(CTTTCT)6	F-CCTCGTTATACAAGAAGATCG R-ATGGTTATACAGGATGTGTGG	180- 230	3.0	0.63	0.54	0.32	0.48	51.7
MSSR192	(GTG)6	F-GGAAGATTGATGCTGAGAAG R-GGAAAACCTCAGGAGTGACA	140- 180	3.0	0.56	0.55	0.32	0.47	51.7
MSSR193	(TCT)12	F-CTTCCCTCCTTCCCTCTTCTT R-GAGACTTTAATCAGGCGATCT	180- 200	3.0	0.62	0.49	0.43	0.38	51.7
MSSR194	(AC)19	F-AGGTACAAAAGACTTCCAACC R-CACTGTCTGCACAGATTTTTC	200- 240	3.0	0.67	0.46	0.47	0.37	51.7
MSSR195	(TCA)13	F-CACCTTCTCTGCTTGCACCTAC R-GAACTTGCATAGTGTCCCATTA	250- 270	2.0	0.51	0.50	0.38	0.37	51.7
MSSR196	(ATC)14	F-AAGTCCACGCTCACTCACCTTA R-ATGGGGAGGATAAATTGAAT	140-160	3.0	0.55	0.51	0.60	0.40	51.7
MSSR197	(TAT)14	F-CTTCCAATCCCACCTAAAAT R-GAGAGTATAAGCAGCATGGAG	150- 180	2.0	0.53	0.50	0.72	0.37	51.7
MSSR199	(TGA)5	F-GATAGTAGCTCCTCAGGTTCC R-CTTACGTCCTTGTGGCATTTC	170- 190	3.0	0.54	0.51	0.62	0.40	51.7
MSSR200	(TA)25	F-GTTGGACCATGTACTTGTCTG R-GGCTACCTAGATTATCCTCTTG	270-290	3.0	0.61	0.51	0.42	0.42	51.7
-	--	--		3.0	0.58	0.53	0.45	0.45	

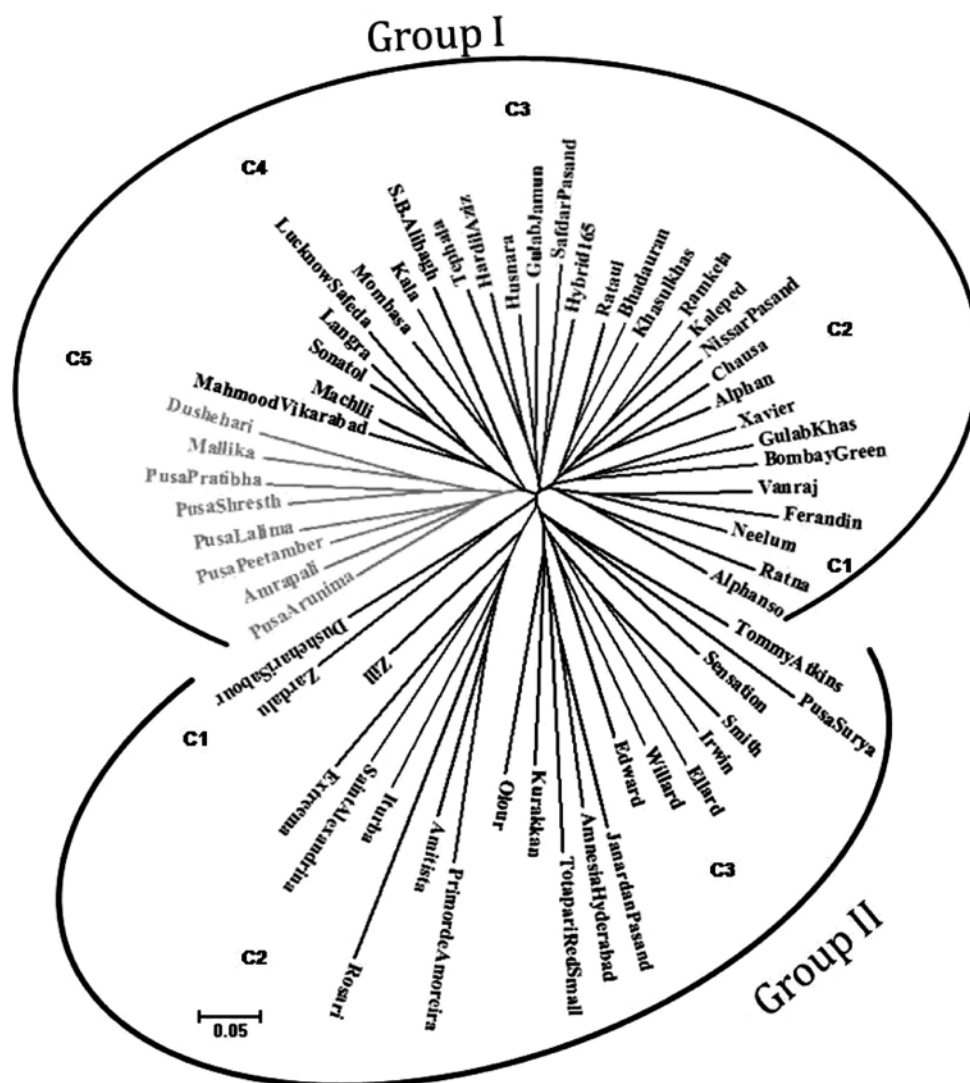


Fig. 1. Neighbour Joining (NJ) dendrogram based on dissimilarity index depicting phylogenetic relationships among 60 mango genotypes using of 87 polymorphic genic-SSR loci.

Hardil Aziz, Husnara, Gulab Jamun, Safdar Pasand and Hybrid 165), with the majority having a tendency of regular bearing and coloured fruits. The fourth cluster comprised of a total eight cultivars, namely, Mahmood Vikarabad, Machlli, Sonatol, Langra, Mombasa, Kala, Lucknow Safeda and S.B. Alibagh which are supposed to be originated strictly from the Northern India as seedling selections, majority having the tendency of alternate bearing and belong to Lucknow and Saharanpur districts of Uttar Pradesh state of India. The fifth cluster formed by 8 cultivars (Pusa Arunima, Amrapali, Pusa Peetamber, Pusa Lalima, Pusa Shresth, Pusa Pratibha, Mallika and Dushehari), which were genetically very close and included inter-varietal hybrids and their parents, which belong to North India and bred at IARI, New Delhi.

The major group I formed by cultivars of two different geographical locations, *i.e.*, North India and West India. Since the West and North India is having geographically more closer distance and there were frequent and considerable exchange of planting material in present and past, therefore it seems logical to have overlapping and closeness amongst the studied germplasm beside this majority of these cultivars were selected by farmers as superior chance seedlings from the wild, based on their fruit characters and propagated vegetatively by grafting. As these seedlings have evolved from local germplasm available in that geographical region, they showed genetically proximity also (Ravishankar *et al.*, 12). The West Indian cultivars grouped in to one cluster, *i.e.* Group 1 (cluster 1) and

that was as expected given that Alphonso derived from Neelum and Ratna and share common genetic makeup. The ICAR-IARI evolved hybrids were grouped together in the group 1 (Cluster 5) such as, Pusa Shresth, Pusa Arunima, and Pusa Pratibha sharing similar parentage (Amrapali x Sensation) and Pusa Lalima (Dushehari × Lal Sunderi). Hybrids namely Amrapali and Mallika sharing Dushehari as parent were clustered together. Moreover, Amrapali and Mallika were result of reciprocal cross Dushehari and Neelum and thus showed a high degree of similarity. The tendency of clustering among mango hybrids revealed that they had stronger affinity towards female parent Amrapali. The similar types of clustering of IARI hybrids and their parents were also reported by Samant *et al.* (10) and Singh *et al.* (12), while investigating genetic diversity among mango accessions using different molecular markers. The group II consisted 40% of total cultivars and further categorised in to three broad clusters and consisted cultivars from Eastern India, Southern India, Florida, USA and Brazil. The first cluster was the smallest and consisted only two cultivars, belonging to Eastern parts of India (Dushehari Sabour and Zardalu). The second cluster composed of a total seven cultivars (Primor de Amoreira, Amitista, Rosari, Iturba, Saint Alexandrina, Extreema and Zill) exclusively of Brazilian origin, whereas third cluster consisted of 13 cultivars belonging to Southern India (Kurakkan, Olour, Amnesia Hyderabad, Janardan Pasand, Totapari) polyembryonic types and Flordian types (Willard, Edward, Smith, Irwin, Ellard, Tommy Atkins, Pusa Surya syn. Eldon and Sensation) grouped together and as they belonged to coloured type mango having common parentage from Florida, USA, respectively.

On the basis of embryo types, polyembryonic cultivars of South Indian origin (Olour and Kurakkan) were grouped together which justify the diverse genetic base with different geographical origins of polyembryonic and monoembryonic mango cultivars. The embryony nature based grouping of mango cultivars has also been reported by Ravishankar *et al.* (9) who reported inter-crosability of both monoembryonic and polyembryonic cultivars and single dominant gene inheritance pattern of polyembryony trait. The mango cultivars of Brazilian origin (Primor de Amoreira, Ametista, Rosari, Iturba, St. Alexandrina, Extreema and Zill) formed a separate cluster (Cluster 2 of Group II) due to their origin in that particular geographical location, whereas, Floridian (Tommy Atkins, Pusa Surya syn. Eldon, Sensation, Smith, Irwin, Ellard, Willard and Edward) and South Indian cultivars (Janardan Pasand, Amnesia Hyderabad, Totapari Red Small, Kurakkan,

Olour) grouped into Group II (Clusters 3). In Group II (Cluster 3), the genetic closeness among mango cultivars was obvious because they are related with each other by their pedigree. The seedling cultivars Haden originated from Indian Mulgoba cultivars gave rise to several Floridian mango varieties like 'Tommy Atkins', 'Edward' and 'Eldon. Florida (USA) served as a secondary geographical origin of several cultivars as Pusa Surya (Eldon), Sensation, Smith, Irwin, Ellard, Willard and Edward and these cultivars were grouped together owing to their common geographical origin (Schnell *et al.*, 11). The separation based on their geographical location was inevitable and apparent in current set of mango cultivars. However, some of the cultivars in Group I and Group II did not differentiated according to geographical pattern and hence admixtures in the populations can be attributed primarily due to cross-pollination, gene flow, especially orchards situated in close proximity, chance seedling and exclusive vegetative propagation.

In order to further demonstrate the genetic distribution pattern, Principal Coordinate Analysis (PCoA) analysis was used to generate a scatter plot. It is showed that two distinct groups containing almost the entire population were identified and was closely agreed with the Neighbour Joining tree. The first three coordinate axes accounted for 18.93% of the variation observed (Fig. 2). The first axis explained 8.44% of genetic variation followed 5.50% by second axis. This may be mainly due to frequent introduction of genetic material from South India to Florida and Brazil for development of varieties and vicinity of South and East Indian regions. In addition, an analysis of molecular variance (AMOVA) procedure was used to estimate the partitioning of genetic variance among and within populations (Table 3). According to genic-SSR markers, percentage of genetic variation among populations was 7%, and within population 93%, which further categorised among individuals 9% and within individuals 84% (Fig.3). The results indicate that the major proportion (84%) of variation was exhibited within the individual which is obvious due to highly cross-pollinated nature of mango crop. The distribution of variation among and within population was found proportional with earlier study as reported by Samant *et al.* (10). Our study showed that diverse allelic combinations were exist within cultivars and exchange rates of alleles were very high within cultivars than among themselves, which showed the presence of high genetic variability among cultivars within a region than the cultivars between regions which are in congruence with the PCoA and AMOVA results. Based on information generated by the genetic divergence study in the current study,

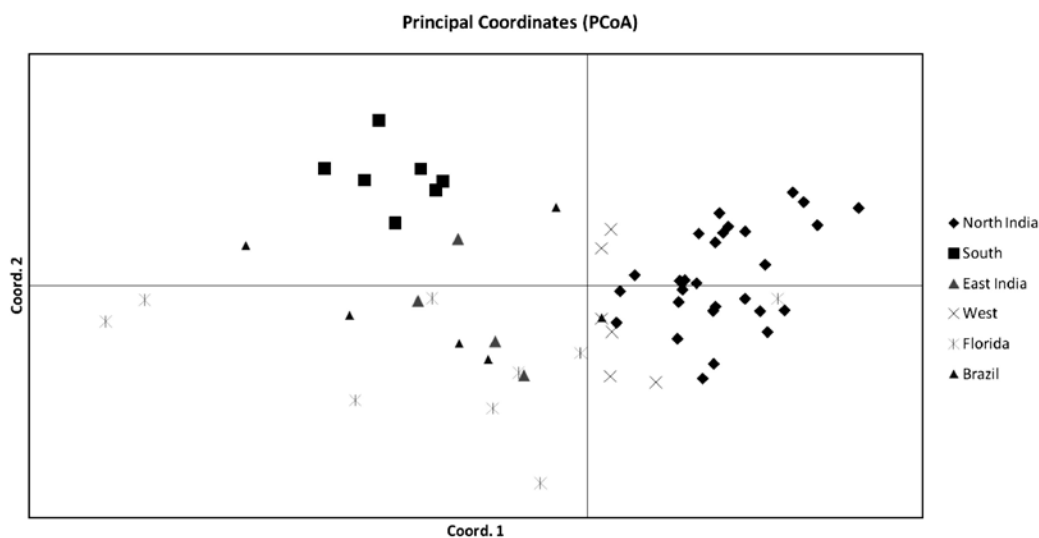


Fig. 2. Principal coordinates analysis (PCoA) of 60 mango genotypes based on 87 polymorphic genic SSR loci.

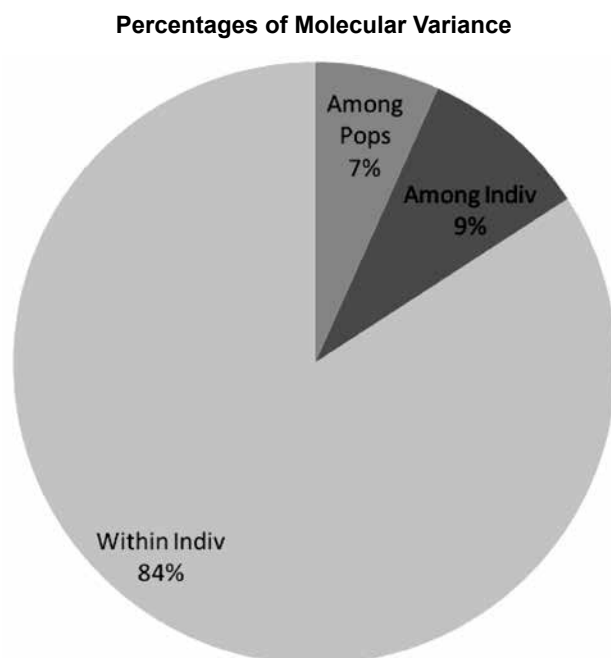


Fig. 3. Analysis of molecular variance of 60 mango genotypes based on 87 polymorphic genic-SSR.

Abbreviation (Pops: populations, Indiv: individual)

the mango cultivars that showed high magnitude of genetic relatedness can be eliminate or abandon to develop true association panel and core collection with diverse representatives for future breeding programme such as QTL and association mapping studies for targeting traits of interest.

This study is the first report of a comprehensive set of genic-SSR markers, used in mango cultivars diversity analysis. The NJ clustering analysis broadly detected two major groups within these cultivars, which largely correlate with the state-wise grouping as well as based on genetic constitution of the cultivars. Results state that the selected cultivars possibly have evolved from an existing mango gene pool across the geographical location and the generated information can be further utilized for the germplasm management and to improve the current mango breeding strategies. Furthermore, the findings will be useful to predict approaches such as classical mapping population development, association analysis; parental line selection in mango improvement programs and desired cultivar development for exploiting the existing genetic variation exists in this population, ultimately save time, cost and resources.

Table 3. Analysis of molecular variance (AMOVA) summary for 87 polymorphic genic-SSR loci.

Source	df	SS	MS	Est. Var.	% Variation
Among Pops	5	255.005	51.001	1.564	7
Among Individual	54	1291.728	23.921	2.135	9
Within Individual	60	1179.000	19.650	19.650	84
Total	119	2725.733		23.349	100

df: degree of freedom, SS: Sum of square, MS: Mean sum of square, Est. Var.: Estimated variance, Fixation index (F_{st})= 0.05

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