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Genetic diversity in unique indigenous mango accessions (Appemidi) of the Western Ghats for certain fruit characteristics

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Mango is one of the choicest fruit crops of the tropical and subtropical regions in the world. Utilization of the conserved germplasm in breeding programmes requires precise information on the genetic relationships between the accessions. Considering the difficulties involved in the traditional divergence studies based on morphological characterization, microsatellites were successfully used for genetic diversity analysis of the indigenous 'Appemidi' type. Also, the major compounds that contribute to the unique aroma of these types were estimated. The materials used in the study consisted of 43 accessions and 14 SSRs developed at the Indian Institute of Horticultural Research, Bangalore. Analysis of sap volatiles was done using GCMS fitted with a DB-5 MS column using helium as the carrier gas. The analysis of 211 bands detected by the 14 Simple Sequence Repeats (SSRs) markers showed unambiguous discrimination of the 43 mango genotypes. The dendrogram resulted in the grouping of accessions into two major clusters, viz. cluster I with highly acidic types and cluster II with less acidic and high TSS group. The aroma of pickle type of mangoes is due to totally different type of terpenes as well as a completely different combination of monoterpenes.

Keywords: Appemidi, aroma compounds, characterization, diversity, mango.

MANGO (*Mangifera indica* L.) is one of the choicest fruit crops of the tropical and subtropical regions in the world. Its popularity and importance can easily be realized by the fact that it is referred to as the 'king of fruits' in the tropical world. Utilization of the conserved germplasm in the breeding programme requires precise information on the genetic relationships among the accessions. Information on the genetic distance among the germplasm accessions will also help avoiding duplicates, thus clearing the nomenclature ambiguity, widening the genetic base of the core collections and ultimately helping in preserving the valuable diversity. Considering the difficulties involved

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in the traditional divergence studies based on morphological characterization[1,2] and new methods based on studies at the DNA level are being incorporated into fruit-breeding programmes because these techniques are reliable, unambiguous in nature and easy to adopt[3,4]. They are widely used for the estimation of genetic diversity and are reported to be more precise than phenotypic markers[5]. A range of DNA markers, viz. AFLP, SSRs, ISSR, RFLP and RAPD have already been in use for exploring the diversity of the global mango gene pool. Simple Sequence Repeats (SSRs) markers are of particular importance to study the variability, identification, germplasm conservation, domestication and movement of germplasm[6]. They have co-dominant inheritance, reproducibility and are easy to interpret[7,8]. In mango, microsatellites have been successfully used for genetic diversity analysis[6,9,10]. In the recent exploration mission carried out by the Indian Institute of Horticultural Research (IIHR), Bangalore, 34 unique types were collected from the Western Ghats which is the hotspot for the pickle mangoes. These indigenous types are known for tender, whole, fruit pickles locally called as 'Appemidi' type. They are specific to the humid, tropical rainforests and are carried away by the rainwater and propagated through the seeds, resulting in rich diversity. These types are unique in the way that they are highly acidic, fibrous and rich in characteristic raw mango flavour, medium to high latex flow and firm pulp with good keeping quality. They are gaining importance in the export market because of their suitability for pickling as whole fruit (tender mangoes), called 'midi' in the local language. Characterization and assessment of diversity is essential to utilize these unique accessions in crop improvement. No work has been done on the characterization and genetic relatedness of these unique types with the commercial varieties, polyembryonic varieties and pickling varieties of other region. Also, there is no report on the major aroma compounds present in these unique types. Hence, an attempt was made to utilize SSR markers to estimate the genetic distances between these accessions and to find out the major compounds that contribute to the unique aroma.

The mango germplasm consisting of 34 pickling accessions collected mainly from Uttara Kannada and Shimoga districts of Karnataka, five commercial accessions, three polyembryonic accessions and a pickling type from Andhra Pradesh (mainly for comparison with the Appemidi type) conserved in the Field Gene Bank (FGB) of IIHR was used in the experiments. Fourteen SSR markers developed at IIHR were used to characterize and estimate the genetic distances among 43 mango accessions.

Freshly mature leaves, free from diseases and developmental deformities were used for DNA extraction. They were brought to laboratory in butter-paper bags and swabbed with 76% ethanol to remove traces of dirt. The DNA from freshly mature leaves of mango cultivars was isolated using CTAB method[11].

The PCR reaction profile and amplification conditions for SSR markers used were according to Ravishankar *et al.*[12]. Electrophoresis was carried out at 75 V for 90 min and the bands were visualized through Gel documentation system for confirmation.

The amplified products were subjected to gene scan analysis for estimation of fragment size using automated DNA sequencer (ABI 3730 DNA analyser) at the Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore. Allelic composition of each accession and the number of total alleles were determined for each SSR locus. The genetic information was assessed only for single-locus SSR using the following parameters: number of alleles per locus (A), observed heterozygosity (Ho, direct count), expected heterozygosity $He = (1 - \Sigma p^2$, where p is the frequency of the ith allele) and polymorphic information content. The computations were performed using the program Cervus 3.0. Band size to binary software was used to convert SSR data to binary data[13]. Genetic similarity between pairs was estimated using Dice coefficient with the help of Windist software[14]. Cluster analysis was performed with NTSYS PC Software, a numerical taxonomy and multivariate analysis software package with UPGMA and a dendrogram was obtained. For testing the consistency or the statistical significance of the genetic relationships between genotypes shown by the dendrogram, bootstrapping analysis was performed using WINBOOT software[14].

For determination of aroma compounds, the sap from the unique accessions, viz. Anantha Bhatta Appe, Isagoor Appe, Adderi Jeerige and Kana Appe was collected and compared with the commercial varieties, viz. Amrapalli and Totapuri. Volatiles from the mango sap were analysed using GCMS fitted with a DB-5 MS column, using helium as carrier gas. Temperature programme was used for GC separation. Volatiles from the sap were extracted using the solvent extraction method. Sap was mixed with pentane–ether mixture and the pentane–ether layer was carefully separated using a separating funnel. Extraction was repeated three times and the pentane–ether was pooled. The pooled extract was evaporated under vacuum and redissloved in dichloromethane before injection to GCMS (Varian 4000). Volatiles were identified using NIST mass library. They were also identified using retention indices of standards.

The analysis of 14 SSR markers revealed that the PCR product size (bp) ranged from 108 (Mi IIHR-23) to 269 (Mi IIHR-17) in 43 mango genotypes (Table 1). It resulted in the detection of a total of 211 alleles, with an average of 15.07 alleles/SSRs, ranging from seven alleles in Mi IIHR-12 and Mi IIHR-13 to 23 alleles/SSRs in Mi IIHR-17. The allele size ranged from 108 to 153 bp in Mi IIHR-23 and from 230 to 269 bp in Mi IIHR-17. The expected heterozygosity ranged from 0.643 in Mi IIHR-18 to 0.931 in Mi IIHR-17. The observed heterozygosity ranged from 0.167 in Mi IIHR-18 to 0.881 in Mi IIHR-17,

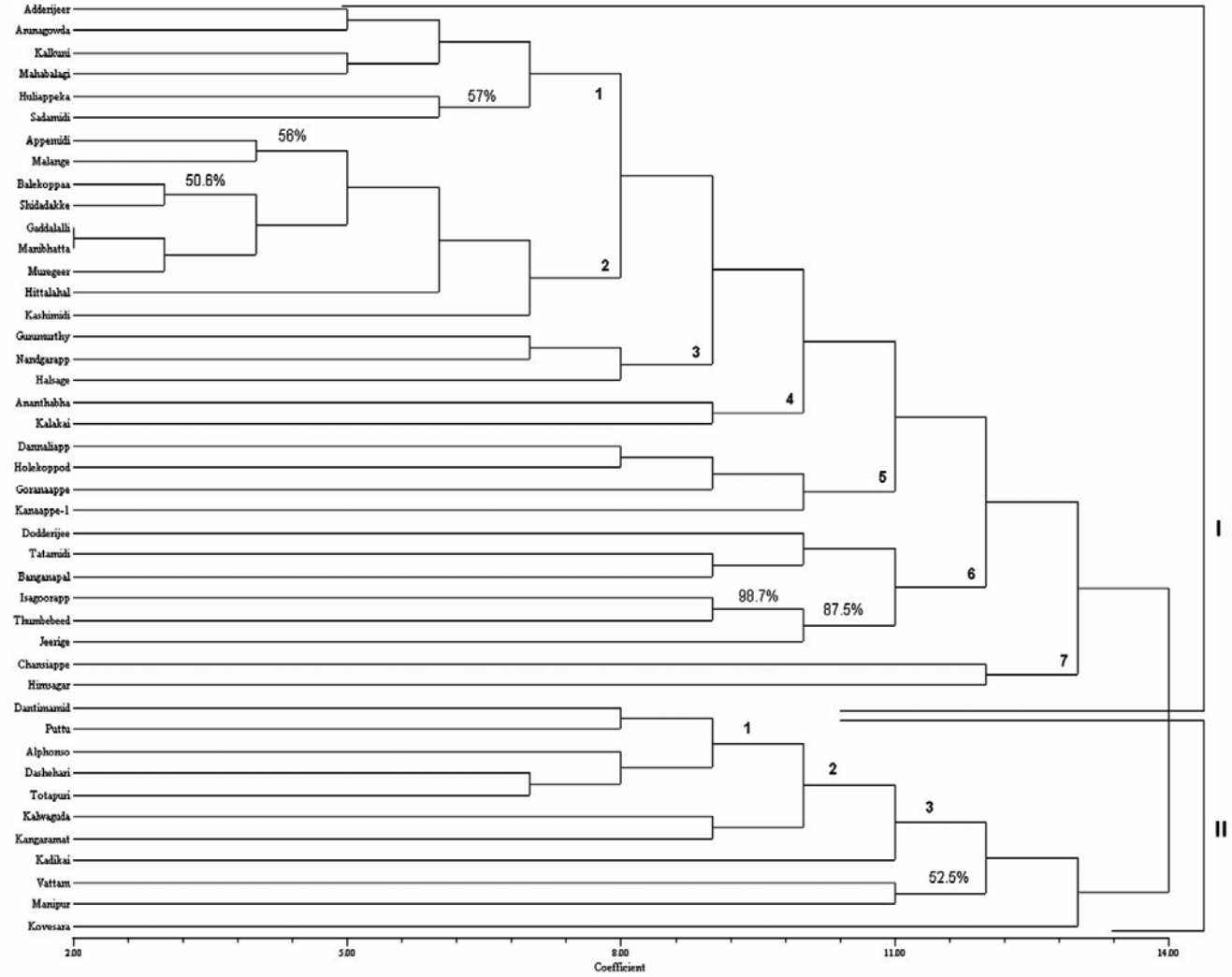

Figure 1. UPGMA dendrogram based on molecular (SSRs) data.

indicating high polymorphism. The polymorphic information content (PIC) value was high (0.915) in Mi IIHR 17 and low (0.617) in Mi IIHR-18. The ability of SSR markers to distinguish mango cultivars was previously reported by several workers[6,9].

The genetic relationships among 43 mango accessions were estimated based on SSRs markers. The molecular dendrogram obtained using Dice coefficient according to UPGMA clustering is shown in Figure 1.

The analysis of 211 bands detected by the 14 SSR markers showed the unambiguous discrimination of the 43 mango genotypes included in the study. It is evident from the dendrogram that mango accessions were grouped into two major clusters, viz. cluster I with highly acidic types and cluster II with less acidic and high TSS group. The bootstrap analysis of tree robustness was also performed and more than 50% bootstrap values were given on the nodes.

Cluster I comprised of 11 accessions and cluster II comprised of 32 accessions. Cluster I was further grouped into seven sub-clusters (i to vii), which were again sub-clustered into two groups. Sub-cluster (i) consisted of six accessions, viz. Sadamidi, Huliappekai, Mahabalagiri Appe, Kalkuni, Aruna Gowda Appe and Adderi Jeerige. The accessions Huliappekai and Sadamidi were closely placed with a bootstrap significance of more than 57%. Sub-cluster (ii) consisted of nine accessions, viz. Appemidi, Malange, Balekoppa Appe, Shidadakke Appe, Gaddalahalli Appe, Mani Bhatta Appe, Muregeer, Hitalahalli Appe and Kashimidi. The accessions grouped under this cluster were moderately acidic (except Kashimidi), with medium to high total soluble solids (TSS) and with moderate levels of sugar (Table 2). A further sub-cluster of (ii) resulted in five groups. The accessions Malange and Appemidi were closely placed with bootstrap significance of 56%, which showed similarity in fruit weight

Table 1. Locus name, length of the amplified fragments and variability parameters in 43 mango cultivars using 14 SSR markers

Primers (5′–3′)	Primer number	Allele size range (bp)	Expected heterozygosity	Observed heterozygosity	Polymorphic information content
F: GCCCCATCAATACGATTGTC R: ATTTCCCACCATTGTCGTTG	MiIIHR12	154–177	0.734	0.698	0.680
F: CCCAGTTCCAACATCATCAG R: TTCCTCTGGAAGAGGGAAGA	MiIIHR13	169–190	0.798	0.442	0.757
F: CTAACCATTCGGCATCCTCT R: TCTGTGATAGAATGGCAAAAGAA	MiIIHR15	111–168	0.920	0.476	0.902
F: GCTTGCTTCCAACTGAGACC R: GCAAAATGCTCGGAGAAGAC	MiIIHR17	230–269	0.931	0.881	0.915
F: TCTGACGTCACCTCCTTTCA R: ATACTCGTGCCTCGTCCTGT	MiIIHR18	154–193	0.643	0.167	0.617
F: TGATATTTTCAGGGCCCAAG R: AAATGGCACAAGTGGGAAAG	MiIIHR19	185–209	0.874	0.791	0.850
F: TCTGACCCAACAAAGAACCA R: TCCTCCTCGTCCTCATCATC	MiIIHR23	108–153	0.916	0.567	0.897
F: GCTCAACGAACCCAACTGAT R: TCCAGCATTCAATGAAGAAGTT	MiIIHR24	238–260	0.840	0.581	0.811
F: GCGAAAGAGGAGAGTGCAAG R: TCTATAAGTGCCCCCTCACG	MiIIHR26	131–165	0.877	0.674	0.858
F: AGCTATCGCCACAGCAAATC R: GTCTTCTTCTGGCTGCCAAC	MiIIHR30	190–213	0.887	0.698	0.864
F: TTCTGTTAGTGGCGGTGTTG R: CACCTCCTCCTCCTCCTCTT	MiIIHR31	207–260	0.829	0.600	0.798
F: TGGTGGTGTTTGTTTGCAGT R: ACCACCCGCAGTATTGAAAG	MiIIHR32	177–196	0.786	0.595	0.758
F: CTGAGTTTGGCAAGGGAGAG R: TTGATCCTTCACCACCATCA	MiIIHR34	222–244	0.818	0.674	0.784
F: TCTATAAGTGCCCCCTCACG R: ACTGCCACCGTGGAAAGTAG	MiIIHR36	210–249	0.873	0.698	0.852

(100–111 g) and dense lenticels with strong turpentine flavour. Similarly, the accessions Balekoppa Appe and Shidadakke Appe were placed closely with bootstrap significance of 50.6%, sharing the common trait of oblong fruit shape with smooth skin. The accessions Gaddalahalli Appe, Mani Bhatta Appe and Muregeer formed one cluster and Hitalahalli Appe was placed closer to this subgroup. Yellow–orange pulp with slightly juicy nature combined with moderate sugar (> 6.8%) and medium TSS (> 13.5°B) was noticed as the common traits possessed by these accessions. The accession Hittalahalli Appe also had attractive yellow–orange pulp and was found to have clustered together in sub-cluster (ii). As the accessions evaluated in the present study are unique indigenous types, there are no previous reports to support the present results. However, the genetic relationship in mango was studied using molecular markers[15–17] and it was found that there was considerable diversity in the mango gene pool. The accession Kashimidi was also placed at the end of the cluster. The results obtained indicate a relatively close relationship between the accessions Sadamidi and Huliappekai. Morphologically also, these two share the common quality traits of low sugar (< 2.5%), TSS (< 10.0°B) and pH (> 2.5).

Sub-cluster (iii) consisted of three accessions, viz. Gurumurthy Appe, Nandgar Appe and Halasage. Sub-cluster (iv) had only two accessions, viz. Anantha Bhatta Appe and Kalakai. The fifth sub-cluster consisted of four accessions, viz. Dannalli Appe, Gorana Appe, Holekoppada Appe and Kana Appe-1. The sixth sub-cluster consisted of six accessions, viz. Dodderi Jeerige, Tatamidi, Banganapalli, Isagoor Appe, Thumbeebeedu and Jeerige. Further sub-clustering resulted in two groups where the accessions, viz. Thumbeebeedu and Isagoor Appe were closely placed with bootstrap value of 98.7% significance. The accessions Thumbeebeedu and Isagoor Appe were placed closely indicating a high similarity between these two accessions. Morphologically also they share

Table 2. Fruit characteristics of mango accessions

Accession	Total soluble solids (°B)	Pulp texure	Pulp juciness	Titrable acidity (%)	Quantity of pulp (%)	Vitamin C (mg g^{-1})	pH	Total sugar (%)
Adderi Jeerige	10.5	Light yellow	Intermediate	6.33	44.69	50.8	3.1	2.56
Anantha Bhatta Appe	9	Light yellow	Intermediate	5.43	65.42	65.2	3.08	1.52
Appemidi	15.5	Light yellow	Soft	2.88	63.06	130.23	2.78	4.96
Aruna Gowda Appe	10.3	Light yellow	Soft	7.48	58.25	89.9	3.05	1.4
Balekoppa Appe	16	Golden yellow	Intermediate	0.96	64.72	70.32	3.39	6
Chanshi Appe	17	Light yellow	Soft	0.84	62	24.47	3.17	3.6
Dannalli Appe	8	Golden yellow	Intermediate	0.44	61.63	123.18	3.07	1.83
Dantimamidi	21.4	Yellow orange	Firm	0.38	73.9	30.19	4.9	11.3
Dodderi Jeerige	8.7	Light yellow	Intermediate	6.46	65.53	51.2	2.63	2.81
Gaddalahalli Appe	23.5	Dark orange	Firm	0.57	72.77	39.55	4.12	7.5
Gorana Appe	8.7	Light yellow	Intermediate	3.39	57.98	103.55	3.13	2.22
Gurumurthy Appe	9.1	Light yellow	Soft	3.7	44.95	43.31	2.91	1.77
Halasage	8.85	Golden yellow	Soft	3.78	52.3	137.67	3.08	1.33
Hittalahalli Appe	13.5	Orange	Soft	0.78	63.37	70.85	3.68	6.87
Holekoppada Appe	9.4	Yellow orange	Soft	6.02	45.06	133.45	2.48	2.2
Huliappekai	8.2	Light yellow	Soft	2.69	62.14	67.75	3.67	2.23
Isagoor Appe	12.7	Golden yellow	Intermediate	2.69	68.76	52.33	2.82	7.57
Jeerige	16	Golden yellow	Intermediate	1.47	63.61	53.2	2.99	3.71
Kadikai	15.9	Yellow orange	Firm	0.7	75.65	44.77	4.59	7.8
Kalakai	7.5	Light yellow	Soft	4.48	61.94	41.57	3.08	1.19
Kalkuni	19.7	Golden yellow	Firm	1.57	57.15	95.38	4.62	8.61
Kalwagudda	16.8	Yellow orange	Intermediate	0.25	79.69	62.42	4.88	9.12
Kana Appe-1	22.1	Yellow orange	Intermediate	1.21	46.46	60	3.86	9.37
Kangaramatha	21.2	Orange	Firm	0.26	68.21	24.44	3.51	7.1
Kashimidi	10	Light yellow	Intermediate	7.23	59.13	25.74	3.06	2.77
Kovesara	25.2	Orange	Firm	0.38	75.19	61.83	4.62	9.67
Mahabalagiri Appe	8.5	Light yellow	Soft	6.15	70.59	71.23	2.84	2.25
Malange	13.7	Yellow orange	Soft	0.83	62.84	13.98	3.65	6.55
Mani Bhatta Appe	21.7	Dark orange	Intermediate	0.38	65.94	37.24	4.4	9.8
Muregeer	28.75	Yellow orange	Firm	0.89	51.91	19.54	3.81	10.67
Nandgar Appe	9.6	Light yellow	Soft	5.12	61.68	115.08	2.82	2.18
Sadamidi	9.8	Light yellow	Intermediate	4.13	61.51	70.62	2.71	2.17
Shidadakke Appe	10.8	Light yellow	Soft	2.63	66.98	70.27	3.6	2.7
Tatamidi	26	Dark orange	Firm	0.76	72.97	74.38	4.38	9
Thumbeebeedu	15.1	Golden yellow	Intermediate	1.5	69.54	39.99	3.72	8.57
Alphonso	19	Yellow orange	Firm	0.32	66.9	23.8	4.31	15.3
Banganapalli	18.5	Yellow	Firm	0.12	61.7	19.95	4.84	12.26
Dashehari	18.8	Orange	Firm	0.19	67	12.8	4.81	16.47
Himsagar	19	Yellow orange	Firm	0.11	62.3	18.29	5.61	15.7
Totapuri	17.5	Golden yellow	Firm	0.25	70.5	15.16	3.9	9.8
Manipur	17.9	Dark orange	Intermediate	0.96	69.68	23.03	4.11	6.39
Vattam	16	Golden yellow	Intermediate	0.25	56.34	22.29	5.12	13.13
Puttu	19.05	Yellow orange	Intermediate	0.38	69.21	25.28	4.92	7.61
SE	0.64			0.13	6.16	4.65	0.07	0.36
CD (P = 0.05)	1.27			0.26	12.19	9.2	0.13	0.72

all the traits and look alike, which has been clearly proved at the molecular level. These two accessions also had a relatively close association with Jeerige, where changes in one or two loci have brought out minor changes in morphological trait, which has been explained in the present study. The seventh sub-cluster A_7 consisted of only two accessions, viz. Chansi Appe and Himsagar, which have the common trait of strong turpentine flavour. Thus, it is clear that both qualitative and quantitative traits could be mapped using SSR markers.

Cluster II consisted of three sub-clusters (i–iii). Sub-cluster (i) consisted of Dantimamidi, Puttu, Dashehari, Alphonso and Totapuri. Sub-cluster (ii) comprised of three accessions, viz. Kalwagudda, Kangaramatha and Kadikai. Sub-cluster (iii) consisted of only two accessions, viz. Vattam and Manipur. The accession Kovesara was placed separately at the end indicating that it was genetically diverse. The fruit characters of this accession indicated that the TSS was high (24.2°B) with low acidity (0.38%), and firm, orange pulp with very less fibre indicating its best suitability for fresh fruit consumption.

Grouping of accessions in cluster II was mainly based on the qualitative traits like high TSS, low titrable acidity, low pH and medium to high sugar, making these

Table 3. Aromatic profile of unique accessions in comparison with commercial varieties

Compound	RI	Anantha Bhatta Appe		Kana Appe		Totapuri		Adderi jeerige		Amrapali		Isagoor Appe	
		µg/ml	Area (%)	µg/ml	Area (%)	µg/ml	Area (%)	µg/ml	Area (%)	µg/ml	Area (%)	µg/ml	Area (%)
Ethylbenzene	846	1.173	0.011	2.103	0.034	2.174	0.020	2.189	0.018	5.608	0.744	2.165	0.020
Tricyclene	924	N.D.	N.D.	N.D.	N.D.	2.213	0.020	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
α-Thujene	928	N.D.	N.D.	N.D.	N.D.	2.209	0.020	0.290	0.002	0.301	0.040	1.042	0.010
α-Pinene	946	12.324	0.084	4.630	0.076	2.369	0.022	11.860	0.096	0.355	0.047	9.237	0.085
(–)-β-Pinene	962	690.589	4.584	264.430	4.225	1323.003	11.987	574.154	4.582	182.201	23.995	957.919	8.594
Camphene	951	7.877	0.053	3.173	0.052	23.783	0.218	7.689	0.062	2.528	0.335	13.725	0.126
Sabinene	976	36.356	0.247	N.D.	N.D.	95.024	0.870	18.767	0.152	1.140	0.151	43.255	0.396
α-Phellandrene	1007	10.153	0.069	4.209	0.069	144.328	1.322	8.743	0.071	22.079	2.930	70.109	0.642
δ-3-Carene	1009	214.871	1.357	98.010	1.503	4615.583	41.273	209.667	1.695	378.220	49.561	182.286	1.586
β-Phellandrene	1029	9127.100	60.904	3775.378	60.345	N.D.	N.D.	7499.576	59.897	N.D.	N.D.	6402.618	57.349
Limonene	1034	2292.011	15.454	942.565	15.315	37.859	0.347	2124.823	16.867	4.059	0.539	1636.907	14.332
α-Terpinene	1056	1982.372	13.322	871.766	14.157	9.752	0.089	1416.833	10.998	1.902	0.252	1145.579	10.215
cis-Ocimene	1039	N.D.	N.D.	N.D.	N.D.	2741.542	24.989	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
trans-Ocimene	1048	N.D.	N.D.	N.D.	N.D.	240.546	2.203	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
(E, Z)-2,6-dimethyl-2,4,6-octatriene	1046	N.D.	N.D.	N.D.	N.D.	173.841	1.592	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
α-Terpinolene	1085	32.858	0.223	10.079	0.165	16.345	0.150	27.772	0.225	N.D.	N.D.	26.603	0.244
Allo-ocimene	1127	N.D.	N.D.	N.D.	N.D.	791.072	7.245	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
α-Campholenal	1155	9.559	0.065	N.D.	N.D.	0.000	0.000	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
α-Cubebene	1345	N.D.	N.D.	N.D.	N.D.	6.262	0.057	N.D.	N.D.	N.D.	N.D.	20.886	0.191
α-Copaene	1375	N.D.	N.D.	N.D.	N.D.	45.368	0.416	N.D.	N.D.	N.D.	N.D.	226.045	2.070
β-Elemene	1388	N.D.	N.D.	N.D.	N.D.	13.101	0.120	6.734	0.054	N.D.	N.D.	N.D.	N.D.
α-Gurjunene	1411	N.D.	N.D.	N.D.	N.D.	1.705	0.016	N.D.	N.D.	10.437	1.385	N.D.	N.D.
Iso-caryophyllene	1413	5.844	0.040	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
β-Caryophyllene	1418	7.843	0.053	34.739	0.568	135.286	1.239	120.700	0.976	63.021	8.256	92.721	0.849
(Z)-CIS-α-bergamotene	1421	17.879	0.121	0.990	0.016	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
α-Guaiene	1424	N.D.	N.D.	N.D.	N.D.	46.877	0.429	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
ῑ-Gurjunene	1444	1.761	0.012	N.D.	N.D.	28.372	0.260	N.D.	N.D.	N.D.	N.D.	1.044	0.010
(+)-Aromadendrene	1447	13.747	0.093	50.662	0.829	40.066	0.367	219.648	1.577	9.139	1.213	3.317	0.030
α-Humulene	1454	3.134	0.021	18.529	0.303	68.445	0.627	63.373	0.512	35.141	4.489	52.928	0.485
β-Farnesene	1458	3.876	0.026	N.D.	N.D.	3.063	0.028	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Germacrene D	1464	N.D.	N.D.	N.D.	N.D.	14.021	0.128	3.321	0.027	N.D.	N.D.	2.097	0.019
ῑ-Muurolene	1475	0.263	0.002	0.623	0.010	2.525	0.023	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Cadina-1,4-diene	1479	N.D.	N.D.	N.D.	N.D.	8.040	0.074	N.D.	N.D.	N.D.	N.D.	2.458	0.023
Viridiflorene	1492	N.D.	N.D.	1.933	0.032	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
α-Muurolene	1494	1.343	0.009	5.367	0.088	3.063	0.028	23.569	0.191	N.D.	N.D.	N.D.	N.D.

(*Contd*)

Table 3. (*Contd*)

Compound	R.I	Anantha Bhatta Appe		Kana Appe		Totapuri		Adderi jeerige		Amrapali		Isagoor Appe	
		µg/ml	Area (%)	µg/ml	Area (%)	µg/ml	Area (%)	µg/ml	Area (%)	µg/ml	Area (%)	µg/ml	Area (%)
β-Selinene	1495	N.D.	N.D.	N.D.	N.D.	10.340	0.095	N.D.	N.D.	0.387	0.051	1.528	0.014
Germacrene B	1495	N.D.	N.D.	N.D.	N.D.	1.903	0.017	N.D.	N.D.	0.461	0.061	4.358	0.040
(–)-Zingiberene	1496	3.124	0.021	0.290	0.005	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
α-Selinene	1497	0.769	0.005	4.893	0.080	26.878	0.246	16.301	0.132	2.347	0.311	5.653	0.052
Valencene	1499	0.396	0.003	2.004	0.033	7.891	0.072	11.694	0.095	N.D.	N.D.	0.507	0.005
β-Guaiene	1499	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	6.933	0.056	N.D.	N.D.	0.472	0.004
δ-Guaiene	1505	1.631	0.011	N.D.	N.D.	97.413	0.892	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
β-Bisabolene	1507	216.321	1.367	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
α-Bisabolene	1511	3.816	0.026	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
ĩ-Cadinene	1513	N.D.	N.D.	1.049	0.017	1.580	0.014	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
δ-Cadinene	1515	N.D.	N.D.	3.678	0.060	29.748	0.272	9.109	0.074	1.697	0.225	19.495	0.179
(–)-α-Amorphene	1532	N.D.	N.D.	1.312	0.021	2.438	0.022	N.D.	N.D.	0.772	0.102	1.317	0.012
Guaiol	1591	N.D.	N.D.	N.D.	N.D.	4.435	0.041	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
ĩ-Cadinol	1614	N.D.	N.D.	N.D.	N.D.	3.302	0.030	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
(–)-δ-Cadinol	1646	N.D.	N.D.	N.D.	N.D.	0.859	0.008	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Viridiflorol	1590	N.D.	N.D.	N.D.	N.D.	17.015	0.156	4.990	0.040	N.D.	N.D.	N.D.	N.D.
Guaiol acetate	1712	N.D.	N.D.	N.D.	N.D.	3.570	0.033	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Methyl hexadecanoate	1885	1.604	0.011	1.355	0.022	1.800	0.016	1.627	0.013	4.713	0.625	1.743	0.016
Cembrene	1929	N.D.	N.D.	N.D.	N.D.	39.431	0.361	N.D.	N.D.	8.720	1.157	N.D.	N.D.
(–)-Kaurene	2033	N.D.	N.D.	N.D.	N.D.	16.344	0.150	N.D.	N.D.	2.869	0.381	N.D.	N.D.
Methyl octadecanoate	2128	43.331	0.294	10.703	0.175	9.052	0.083	2.264	0.018	15.583	2.068	6.173	0.057
Total		14,743.925	98.488	6114.471	98.200	10,911.836	98.689	12,392.627	98.429	753.681	98.919	10,934.188	97.653

accessions suitable for fresh fruit consumption. The genetic identity of polyembryonic cultivars has been justified from the present study. It is clear from the results that SSR analysis is efficient to prove the genetic distances between mango accessions at the genomic level. This is well justified from a cluster where all the acidic types are clustered together. The ability of SSR markers in distinguishing different species of mango was previously reported by several workers[6,9,10,18–20]. Interestingly, molecular characterization also revealed no correlation between geographical origin and clustering pattern for the varieties studied here. Probably if more varieties from different regions are studied together, then the pattern may change[11]. Clustering of genotypes belonging to different geographic region suggests that they might have evolved from the existing mango gene pool from which they were selected by local people to domesticate them in different areas for cultivation. Pandit *et al.*[21] observed no geographic separation of North and South Indian mangoes, also, it was reported that the cultivar pool from North and South India is considerably homogeneous[22]. However, what needs to be noted here is that a large number of varietal assessment cannot be compared with the varietal assessment taken from a small geographic region.

A comparison was also made using both morphological and molecular methods to arrive at a clear understanding about the nomenclature. Both these methods were able to identify the acidic pickling types from the commercial and polyembryonic types. The genetic identity of the accessions, Dantimamidi, Puttu, Alphonso, Dashehari, Totapuri, Kalwagudda, Kangaramatha, Kadikai, Vattam, Manipur and Kovesara has been revealed by way of clustering into a single group in both the methods.

As in the case of morphological clustering[23], the accessions Isagoor Appe, Thumbeebeedu and Jeerige were grouped under a single cluster in the molecular dendrogram indicating their genetic identity. A casual observation of leaf, flower, fruit and stone characters indicated that Isagoor Appe, Thumbeebeedu and Jeerige may be duplicate accessions. Although the varieties Thumbeebeedu and Isagoor Appe are synonyms, the interesting observation is that both belong to different places according to the place of collection, which promotes the notion that the varieties spread within a locality by single plant selection by the farmers.

In morphological analysis, the accessions Appemidi and Malange were grouped under the same sub-cluster, which was also proved by molecular markers. Similarly, the accessions Balekoppa Appe and Shidadakke Appe by the way of their acidic nature were clustered in a single group in the morphological dendrogram and was confirmed by molecular markers. The accession Kashimidi was closely related to Hittalahalli Appe, which in turn clustered with Muregeer. Even though the accession Kashimidi was morphologically different in terms of size and shape, it proved to be genetically similar in the present study. Thus, the present study clearly proves that the morphological characterization can be complemented by molecular characterization. Similar results were reported by Weerarante *et al.*[24]. Higher efficiency of SSRs markers in molecular characterization of mango compared to morphological markers is well demonstrated[6,9,10,18–20]. Considering the above points, it is concluded that Thumbeebeedu, Isagoor Appe and Jeerige are very closely related.

The volatile compounds separated from the water fraction of the sap (Table 3) indicated that there is a clear distinction between the mango types in volatile profiles. The sap constituted mainly terpenoid compounds in all the varieties. Total concentration of aroma compounds was maximum in Anantha Bhatta Appe (14.743 mg/ml), followed by Adderi Jeerige (12.39 mg/ml), Isagoor Appe (10.93 mg/ml) and Totapuri (10.91 mg/ml). Aroma concentration was minimum in Kana Appe (6.11 mg/ml) and Amrapali (0.75 mg/ml). Total aroma concentration indicates that Anantha Bhatta Appe has a very strong odour when compared to the other genotypes. Amrapali has a very weak odour. Aromatic profile of compounds showed that the relative percentage of β-phellandrene, limonene and α-terpinene was about 81–89% of the total volatiles in Anantha Bhatta Appe, Adderi Jeerige, Isagoor Appe and Kana Appe. However, in Totapuri δ-3-carene, (–)-β-pinene, cis-ocimene and allo-ocimene were the major compounds amounting to 83% of the total volatiles; in Amrapali, δ-3-carene, (–)-β-pinene, cis-ocimene, caryophyllene and humulene amounted to 85% of the volatiles. This indicates that the aroma of pickle type of mangoes is due to totally different type of terpenes compared to the other two fruit-type mangoes. Results also indicated that pickle types have a large quantity of terpenoids as well as a completely different combination of monoterpenes for their typical aroma. These may contribute for longer shelf life and higher preference by consumer. The volatile compounds are responsible for odour and contribute to overall flavour of fresh and processed fruit[25]. More than 285 volatile compounds have been reported from various mango cultivars, including monoterpenes, sesquiterpenes, esters, aldehydes, ketones, alcohols, acids, aliphatic hydrocarbons and aromatics[26,27]. Aroma of mango fruit has been reportedly influenced by various factors like cultivars[26–28] and fruit maturity[29,30].

The study resulted in the identification of the highly acidic pickling types (cluster I) from the less acidic and high TSS types (cluster II). The clustering pattern was mainly based on qualitative traits like TSS, titrable acidity, pH and sugar. The volatile compounds separated from the water fraction of the sap indicated that there is a clear distinction between the fruit types and the pickling types in volatile profiles. The results also indicated that pickle types have a large quantity of terpenoids as well as

completely different combination of monoterpenes for their typical aroma.

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