

# Molecular Analysis for Genetic Distinctiveness and Relationships of Indigenous Landraces with Popular Cultivars of Mango (*Mangifera indica* L.) in Andhra Pradesh, India

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## ABSTRACT

Mango (*Mangifera indica* L.) is mostly distributed in the two eco-geographical regions (Coastal and Rayalaseema) of an Indian state of Andhra Pradesh, where large morphological and genetic diversity has been detected due free pollination among plants. A juicy landrace-specific exploration survey covering the two eco-geographical regions was undertaken during 2008 to identify new juicy landraces of mango with high fruit quality. Following a selective sampling strategy, 20 indigenous landraces of mango were selected according to fruit morphology and their fruit and leaf samples were collected for morpho-physiological and molecular characterization, respectively. Morpho-physiological characterization and evaluation based on fruit characteristics revealed that six landraces viz., DM Acc-3, 4, 7, 15, 17 and 18 were elite with respect to fruit characteristics, which were further characterized for their genetic distinctiveness and relationships with the choicest juicy cultivars of mango in Andhra Pradesh ('Peddarasam', 'Chinnarasam', 'Cherukuram', 'Panchadarakalasa' and 'Suvamarekha') at the molecular level, using 109 mango-specific microsatellite markers (SSRs). Jaccard's similarity coefficient ranged from 0.40 to 0.69 signifying wide variability in the landraces of mango to an extent of 31-60%, offering ample scope for selection. Of the 109 SSR markers validated, 57 were polymorphic, of which 10 were highly polymorphic. Diversity was high among 11 genotypes with a total number of 198 alleles displayed. Microsatellite SSR-84 was able to differentiate and/or identify all of the 11 genotypes under study. UPGMA cluster analysis revealed that the six elite landraces viz., DM Acc-3, 4, 7, 15, 17 and 18 clustered according to their geographical origin. Being horticulturally superior and genetically distinct, these six land races can be clonally selected for further testing and multiplication for commercial exploitation.

**Keywords:** exploration survey, fruit quality, genetic diversity, landraces, mango, simple sequence repeats

**Abbreviations:** AFLP, amplified fragment length polymorphism; DM Acc, *desi* mango accession; PIC, polymorphic information content; RAPD, restricted amplified polymorphic DNA; SSR, simple sequence repeat; UPGMA, unweighed pair group method with arithmetic mean; VNTRS, variable number tandem repeat sequence

## INTRODUCTION

Mango (*Mangifera indica* L.) is one of the members of the family Anacardiaceae in the order Sapindales. Mango is believed to have originated in the Indo-Burma region (De Candole 1904). It is known to have been cultivated in Eastern India and Burma for at least 4000 years (Mukherjee 1951). Its cultivation is as old as Indian civilization itself (De Candole 1904). Consequently, it is now one of the most important tropical fruits of the world. The area, production and productivity of mango in India and Andhra Pradesh (AP) are 2.31 and 0.50 million ha, 12.75 and 2.52 million tonnes and 5.50 and 5.10 tonnes ha<sup>-1</sup>, respectively (NHB 2010). Mango occupies 37.84% of total fruit area and contributes 18.62% to total fruit production of India. AP is one of the leading mango-producing states of India with 21.55 and 19.78% of total area and production, respectively (NHB 2010). The top five countries in terms of acreage were India, China, Thailand, the Philippines and Indonesia and in terms of production were India, China, Indonesia, Mexico and Thailand (NHB 2010). India has the distinction of largest mango producing country with 45.59 and 35.70% of total world area and production, respectively (NHB 2010). Its popularity and importance can easily be realized by the fact that it is often referred as 'the King of fruits' in the tropical

world (Purseglove 1972). Mango is considered to be an allopolyploid (2n = 40), most probably amphidiploid and out-breeding species (Mukherjee 1950). Enormous genetic diversity in mango exists in India, which is the primary centre of domestication of this crop. There are nearly 1000 monoembryonic and polyembryonic mango cultivars in India (Negi 2000). High genetic variability of Indian mangoes can be exploited by breeding programmes to produce high quality mangoes.

AP is a leading mango-producing state termed the 'Mango state of India'. In the internal market, mango is highly appreciated by fruit consumers and is produced in the two eco-geographical regions (Coastal and Rayalaseema) of AP. AP is considered as a centre of diversity for mango with a rich diversity of named local cultivars and unnamed local landraces. In this state, landraces of mango from the existing seedling/clonal trees are constantly springing up as chance seedlings and have since been maintained through sexual and clonal propagation leading to the multiplicity of genotypes. These landraces are often distributed in homesteads, avenues and even in commercial orchards. Mango is a highly cross-pollinated species and knowledge of the magnitude of genetic variation among the landraces for fruit characteristics is important because mango consumers are becoming more and more quality-conscious and

thus cultivar conscious. Selection for better quality has been carried out for 4000-6000 years through vegetative propagation for 400 years (Morton 1987). Development of new varieties of mango with improved quality is the engine of market demand. Since breeding and development of high quality superior varieties through hybridization is tedious, resource- and time-consuming, clonal selection of existing superior landraces is a viable alternative, which requires prior quantitative assessment of genetic divergence in the available landraces.

Characterization of diversity is a necessary requirement for crop improvement, use and conservation of plant genetic resources (Krishna and Singh 2007). Further, the scope of crop improvement depends upon the conserved use of genetic variability in plant breeding programmes. Though morphological markers have been used to assess genetic diversity, they had limited application in breeding as they are few in numbers as well as stage specific or dependent. Some of the mango cultivars in India had been identified based on morphological characters by Mukherjee *et al.* (1983) and Subramanyam and Iyer (1989). However, molecular approaches offer an efficient alternative tool to conventional phenotypic diversity. Information regarding genetic variability at the molecular level could be used to help identify and develop genetically unique germplasm that complements existing cultivars. They are very useful for characterizing genetic diversity and relatedness among different mango landraces.

Although there are some constraints in conventional mango breeding programmes, molecular markers open up new opportunities to produce new cultivars with improved characteristics that will improve productivity, fruit quality and the economic competitiveness of the mango industry (Bally *et al.* 2009). Molecular markers are useful for identifying cultivars and landraces and for studying genetic similarities among them (Duneman 1994). Different DNA markers *viz.*, random amplified polymorphic DNA (RAPD) (Jayasankar and Litz 1998; Bally *et al.* 1996; de Souza and Lima Souza 2004; Diaz Matallana *et al.* 2009; Fitmawati *et al.* 2010; Souza *et al.* 2011), restriction fragment length polymorphism (RFLP) (Eiadthong *et al.* 1999a), variable number of tandem repeats (VNTRs) (Adato *et al.* 1995), amplified fragment length polymorphism (AFLPs) (Yamanaka *et al.* 2006) and simple sequence repeats (SSRs) or microsatellites (Eiadthong *et al.* 1999b; Honsho *et al.* 2004; Duval *et al.* 2005; Schnell *et al.* 2005; Viruel *et al.* 2005; Hirano *et al.* 2010; Wahdan *et al.* 2011) have been employed in mango to study monogenic and polygenic traits. Of these, microsatellite markers are of particular impor-

tance to study genetic relatedness and distinctiveness of mango germplasm (Viruel *et al.* 2005). Microsatellites are an efficient type of molecular marker based on tandem repeats of short (2–6 nucleotides) DNA sequence (Charters *et al.* 1996) and have advantages over other types of molecular markers, i.e., their abundance in most genomes, uniform distribution, hypervariability, codominance and polymerase chain reaction (PCR)-based protocols (Li *et al.* 2002).

As in other fruit tree species, mango cultivars are currently identified on the basis of morphological traits based on descriptors (IPGRI 2006). DNA fingerprinting using SSRs has been applied to accession identification programmes and for genetic analyses of a broad range of agricultural and horticultural crops (Pedersen 2006). However, DNA-fingerprinting based on SSRs, in addition to their usefulness in mapping and breeding (McCouch *et al.* 1997), has become the marker of choice, because of their widespread occurrence throughout the genomes of all eukaryotic species, their co-dominant inheritance and the high level of polymorphism observed due to variations in repeat lengths. The high discriminatory power of SSRs is also important for analyzing variation in a crop gene pool (Powell *et al.* 1996). SSR analysis shows great potential for mango improvement and can be performed for variety identification and estimation of genetic variation in existing populations (Brettell *et al.* 2002). DNA fingerprinting and studies on genetic diversity of mango cultivars and *Mangifera* species are also being conducted at several research stations (Kumar *et al.* 2001; Karihaloo *et al.* 2003). Kumar *et al.* (2001) screened 50 commercial mango cultivars using RAPD markers to estimate the genetic diversity and observed a high degree of genetic variation among the cultivars and the variety 'Mulgoa' was found to be very distinct. Karihaloo *et al.* (2003) reported a high diversity within regions in India and confirmed that this is not surprising given that mango is a cross-pollinated plant and selecting superior strains according to taste among naturally produced seedlings has given birth to the commercial cultivars and the observed appreciable range of variation. Landraces are adapted to local climates. As genetic erosion of landrace diversity is increasing, evaluation has become a necessity for on-farm and *ex-situ* conservation, at least for most of the important types. After on-farm characterization of these landraces, they should be evaluated for better utilization.

Our objectives were to determine genetic distinctiveness of land races and their genetic relatedness with the popular juicy cultivars of mango in AP, using microsatellite markers. Landrace identification would enhance current

**Table 1** Collection sites of desi mango accessions.

Accession	Collection site				
	Latitude	Longitude	Village	District	Region
DM Acc-1	16°45' N	80°32' E	Burugagudem	Prakasam	Coastal
DM Acc-2	16°52' N	80°44' E	Reddygudem	Prakasam	Coastal
DM Acc-3	16°42' N	80°38' E	Pondugala	Prakasam	Coastal
DM Acc-4	16°42' N	80°38' E	Pondugala	Prakasam	Coastal
DM Acc-5	16°42' N	80°38' E	Pondugala	Prakasam	Coastal
DM Acc-6	16°42' N	80°38' E	Pondugala	Prakasam	Coastal
DM Acc-7	16°42' N	80°38' E	Pondugala	Prakasam	Coastal
DM Acc-8	16°42' N	80°38' E	Pondugala	Prakasam	Coastal
DM Acc-9	16°43' N	80°38' E	Gururajapalem	Prakasam	Coastal
DM Acc-10	16°43' N	80°38' E	Gururajapalem	Prakasam	Coastal
DM Acc-11	16°43' N	80°38' E	Gururajapalem	Prakasam	Coastal
DM Acc-12	16°43' N	80°38' E	Gururajapalem	Prakasam	Coastal
DM Acc-13	16°43' N	80°38' E	Gururajapalem	Prakasam	Coastal
DM Acc-14	16°43' N	80°38' E	Gururajapalem	Prakasam	Coastal
DM Acc-15	16°52' N	80°44' E	Reddygudem	Prakasam	Coastal
DM Acc-16	15°18' N	78°12' E	Yagantipalle	Kurnool	Rayalaseema
DM Acc-17	14°13' N	78°37' E	Konampet	Kadapa	Rayalaseema
DM Acc-18	15°18' N	78°12' E	Yagantipalle	Kurnool	Rayalaseema
DM Acc-19	16°43' N	80°38' E	Gururajapalem	Prakasam	Coastal
DM Acc-20	16°43' N	80°38' E	Gururajapalem	Prakasam	Coastal

DM Acc = Desi mango accession



**Fig. 1** DIVA-GIS mapping of collection sites of 11 indigenous landraces of mango in Andhra Pradesh.

cultivar development and provide a basis for future genetic studies. Therefore, the present study was undertaken to prepare an inventory based on the molecular characterization and *in-situ* characterization and evaluation for assessing the genetic distinctiveness and relationships of indigenous landraces of mango in AP.

## MATERIALS AND METHODS

### Exploration survey

An exploration survey was conducted during 2008. Following a selective sampling strategy, 20 indigenous landraces of mango, excluding landraces which are phenotypically distinct from the already named cultivars, were selected and earmarked for further reference from homesteads, avenues and commercial orchards covering two eco-geographical regions (Coastal and Rayalaseema) of AP according to their location and fruit morphology in order to cover the maximum observed variability. Collection sites of 11 indigenous landraces of mango in AP was depicted through DIVA-GIS mapping (Table 1, Fig. 1).

### Fruit sampling for morpho-physiological characterization

For *in-situ* morpho-physiological characterization and evaluation based on fruit characteristics, random sampling of 10 tree-ripe fruits were collected from all sides of selected trees by using mango harvesters from each genotype, and 33 morpho-physiological and organoleptic characters of sampled fruits were recorded following mango fruit descriptors (IPGRI 2006). The fruit samples were evaluated for 10 quantitative traits [fruit length (cm), fruit width (cm), fruit thickness (cm), fruit weight (g) fiber length (mm), peel (%), pulp (%), stone (%), total soluble solids (TSS) (°Brix) and shelf life (days)]. Percent peel, pulp and stone were calculated by the weight of the peel, pulp and stone, respectively divided by total weight of the fruit multiplied by 100. TSS was recorded with hand refractometer. In addition, the fruit samples were also characterized for 23 qualitative traits: fruit shape, skin colour of mature fruit, skin thickness, skin texture, quantity of fiber, pulp colour, adherence of skin to pulp, fibre in pulp, length of fibre, fruit stalk insertion, fruit attractiveness, basal cavity, beak, beak type, sinus, sinus type, groove, shoulders, slope of shoulders, apex, eating quality and utility. Eating quality was assessed qualitatively through organoleptic evaluation by a panel of mango tasters with combined assessment of flavour, acidity, sweetness, aroma and astringency and quantitative measurement of TSS of the pulp of the fruit samples when ripe. The utility i.e. whether table or juicy was determined based on fiber content and pulp texture.

### Leaf sampling for molecular characterization

Fresh leaves of sampled trees were collected for DNA extraction. Since six indigenous land races were found to be horticulturally superior with respect to fruit characteristics, the leaf samples of these accessions were used for molecular characterization along with 5 popular commercial juicy mango cultivars *viz.*, 'Chinnarasam', 'Peddarasam', 'Cherukurasam', 'Panchadarakalasa' and 'Suvarnarekha'. The leaves were wrapped in moist tissue paper and were refrigerated at -50°C when not subjected to immediate use.

### DNA extraction

The genomic DNA from leaf samples was extracted by a modified CTAB method (Porebski *et al.* 1997). From the sampled trees, 4-5 leaves were collected and cut into small bits after removing the midrib and ground to a fine powder with liquid nitrogen (-196°C) using a pestle and mortar. About 500 mg of ground leaf powder was placed in a 50-ml centrifuge tube to which 10 ml of 2% pre-heated CTAB buffer (98%, Himedia) was added and incubated for 1 h at 65°C with gentle mixing every 10-15 min. Tubes were removed and allowed to cool to room temperature. Equal volumes of chloroform (99%, SRL): iso-amyl alcohol (98%, Qualigens) (24: 1) were added and swirled gently for 5-10 min to mix the contents and centrifuged at 10,000 rpm for 10 min. The top aqueous phase was collected into another freshly labeled tube. Extraction with chloroform: iso-amyl alcohol (24: 1) was repeated once more. Equal volumes of ice-cold iso-propanol (99%, SRL) were added to the collected supernatant. Precipitated DNA was centrifuged at 10,000 rpm for 15 min and the pellet that formed was washed twice with 0.5 ml of 70% ethanol (99.9%, Merck) and air dried until no more traces of ethanol was observed. The pellet was dissolved in 300 µl of T<sub>10</sub>E<sub>1</sub> (10 mM Tris-HCl, pH 8 and 1 mM EDTA, pH 8 prepared using sterile double distilled water) and incubated at 50°C to ensure complete dissolution of DNA pellet. RNase (10 mg/ml) at 1 mg/ml was added and incubated at room temperature for about 1 h. The extraction and dissolution in TE steps were repeated in triplicate. Extracted DNA was preserved at -20°C until use. Qualitative and quantitative tests of DNA were done by using 1.0% low melting agarose gel (GeNei™, Bangalore) and spectrophotometry (A260/A280 ratio) with a Nano Drop 1000 Spectrophotometer (Thermo Scientific).

### SSR amplification

PCR amplification was performed in a Perkin Elmer Thermocycler (PCR-Gene Amp PCR System 9700) as per the protocol suggested by Williams *et al.* (1990) using 109 mango-specific microsatellite markers (Table 2). Amplified products were separated by electrophoresis in a 3% metaphor-agarose gel using Tris-acetate EDTA (TAE) buffer at pH 8.0. The amplified fragments were observed and photographed under UV light in Gel Doc System (Syngene). 198 microsatellite loci were chosen for their clear pattern and high allele numbers to study diversity within the whole sample. DNA for each sample was extracted 3-4 times while PCR/SSR was carried out 2-3 times.

### Data analysis

Bands on gels were scored as present (1) and absent (0) for each marker and data were entered in a binary matrix as discrete variables. The genetic distinctiveness and relationships of indigenous landraces of mango was calculated using Jaccard's similarity coefficient and a dendrogram showing the genetic relatedness of the six land races with five popular cultivars of mango was constructed using the unweighed pair group method with arithmetic mean (UPGMA) feature of the NTSYS PC Software (Version 2.1) statistical analysis package (Rohlf 2000). The polymorphic information content (PIC) of microsatellite markers was calculated according to the formula given by Powell *et al.* (1996).

**Table 2** List of microsatellite primers used in this study.

	<b>Primer</b>	<b>Sequence (5'-3')</b>	<b>Annealing temperature (°C)</b>	<b>Allele size range (bp)</b>
1	SSR1F SSR1R	TAACAGCTTTGCTTGCCCTCC TCCGCCGATAAACATCAGAC	57	191-207
2	SSR2F SSR2R	CCACGAATATCAACTGCTGCC TCTGACACTGCTCTCCACC	57	121-131
3	SSR3F SSR3R	AAACGAGGAAACAGAGCAC CAAGTACCTGCTGCAACTAG	50	90-111
4	SSR4F SSR4R	AGGTCCTTTATCTTCGGCCC AAACGAAAAAGCAGCCCA	55	199-203
5	SSR5F SSR5R	TGTAGTCTCTGTTTGCTTC TTCTGTGTCGTCAAACCTC	55	260-275
6	SSR6F SSR6R	CAACTTGGAACATAGAC ATACAGGAATCCAGCTTC	51	174-182
7	SSR7F SSR7R	AGAATAAAGGGGACACCAGAC CCATCATCGCCCACTCAG	52	222
8	SSR8F SSR8R	TTGATGCAACTTTCTGCC ATGTGATTGTTAGAATGAACTT	53	200-224
9	SSR9F SSR9R	CGAGGAAGAGGAAGATTATGAC CGAATACCATCCAGCAAATAAC	56	245
10	SSR10F SSR10R	TGTGAAATGGAAGTTGAG ACAGCAATCGTTGCATTC	52	220-235
11	SSR11F SSR11R	GTTTTCACTCTCAAAATGTGTG CTTTCATGTTATAGATGCAA	52	174-190
12	SSR12F SSR12R	CTCGCATTTCTCGCAGTC TCCCTCCATTTAACCCTCC	56	127-132
13	SSR13F SSR13R	GAACGAGAAATCGGGAAC GCAGCCATTGAATACAGAG	53	348-370
14	SSR14F SSR14R	AACCCATCTAGCCAACCC TTGACAGTTACCAAACCAGAC	-	-
15	SSR15F SSR15R	TTACCAAGCTAGGGTCA CACTCTTAAACTATTCAACCA	52	201-226
16	SSR16F SSR16R	GCTTATCCACATCAATATCC TCCTACAATAACTTGCC	54	160-170
17	SSR17F SSR17R	TAAGCTAAAAAGGTTATAG CCATAGGTGAATGTAGAGAG	52	190
18	SSR18F SSR18R	CGTCATCCTTACAGCGAACT CATCTTTGATCATCCGAAAC	56	100-115
19	SSR19F SSR19R	AATTATCCTATCCCTCGTATC AGAAACATGATGTGAACC	54	135-145
20	SSR20F SSR20R	CGCTCTGTGAGAATCAATGGT GGACTCTTATTAGCCAATGGGATG	58	295-310
21	SSR21F SSR21R	GTGCGAGGAGATATCTGT CTGGTTCTTCATTGTTGAGATG	56	110
22	SSR22F SSR22R	TGAGTTGTTGCTCCTGCT GGTGCTTGTTTCTCGT	52	190-200
23	SSR23F SSR23R	AAACAAAGAATGGAGCA TGGACTGAATGTGGATAG	50	240-270
24	SSR24F SSR24R	GATGAAACCAAAGAAGTCA CCAATAAGAACTCCAACC	53	310
25	SSR25F SSR25R	CTTGAAAAGAGATTGAGATTG AGAAGGCAGAAGGTTTAG	53	200-212
26	SSR26F SSR26R	GCCCTTGATAAGTTG TAAGTGATGCTGCTGGT	52	170-182
27	SSR27F SSR27R	TCTAAGGAGTTCTAAAATGC CTCAAGTCCAACATACAATAC	52	158-180
28	SSR28F SSR28R	GACCCAACAAATCCAA ACTGTGCAAACCAAAAG	52	160
29	SSR29F SSR29R	AAAGATAAGATTGGGAAGAG CGTAAGAAGAGCAAAGGT	52	156-170
30	SSR30F SSR30R	TAGGGATATAGCTGGAGG ACGCAGTAGAACCTGTG	54	290
31	SSR31F SSR31R	CAGCCTTATGTGTTGAAG AAACTAAACAAGCTGAACC	55	190
32	SSR32F SSR32R	CTTCATTTCTCCACTTTTG ATGAAATACTGGCTGGTT	54	230-234
33	SSR33F SSR33R	GCGTAAAGCTGTTGACTA TCATCTCCCTCAGAACA	52	148-168
34	SSR34F SSR34R	GAGGAACATAAAGATGGTG GACAAGATAAACAACCTGGAA	53	144-164
35	SSR35F SSR35R	TAGCTGTTTTGGCCTT ATGTGGTTTGTGCTTC	53	240
36	SSR36F SSR36R	CCTCAATCTCACTCAACA ACCCACAATCAAACCTAC	55	215-245

**Table 2 (Cont.)**

	<b>Primer</b>	<b>Sequence (5'-3')</b>	<b>Annealing temperature (°C)</b>	<b>Allele size range (bp)</b>
37	SSR37F	GACTTGCAGTTTCCTTT	50	150-175
	SSR37R	TCAAGAACCCCATTTG		
38	SSR38F	CCATTCTCCATCCAAA	52	165
	SSR38R	TGCATAGCAGAAAGAAGA		
39	SSR39F	TGCTACCATCAAGTTCG	53	150-190
	SSR39R	GCTGTTGTTGCTTTACTG		
40	SSR40F	ATTTTGATCCCGTTCT	52	225-240
	SSR40R	ATTCGATCATGGTTTTG		
41	SSR41F	ATCCCAGTAGCTTTGT	53	210-244
	SSR41R	TGAGAGTTGGCAGTGT		
42	SSR42F	ACGGTTTGAAGGTTTAC	50	165-170
	SSR42R	ATCCAAGTTTCTACTCCT		
43	SSR43F	AAGAGGGAATCTTAATCAAC	53	184-194
	SSR43R	GTCGTTTTGCGTTAGTG		
44	SSR44F	GCGTGCAATCTAGTGG	52	176-204
	SSR44R	GCTTGGTAAAAGGATAAG		
45	SSR45F	GCTCTTTCCTTGACCTT	52	174-194
	SSR45R	TCAAAAATCGTGTCATTTT		
46	SSR46F	TCATTGCTGTCCCTTTTC	54	154-210
	SSR46R	ATCGCTCAAACAATCC		
47	SSR47F	GTATAAATCGCGTGCAT	50	232-234
	SSR47R	AGTTCCCTCCTGTATCT		
48	SSR48F	TCGGTCATTTACACCTCT	53	192-212
	SSR48R	TTATTGAGCTTCTTTGTGTT		
49	SSR49F	ACCACGAAAAGACAACCTC	53	252-268
	SSR49R	TCATCTTTGTAAATAGGTTAAT		
50	SSR50F	ATGGAGACTAGAATGTACAGAG	52	202
	SSR50R	ATTAAATCTCGTCCACAAGT		
51	SSR51F	AAATAAGATGAAGCAACTAAAG	52	287
	SSR51R	TTAGTGATTTGTATGTTCTTG		
52	SSR52F	AAAAACCTTACATAAGTGAATC	52	207
	SSR52R	CAGTTAACCTGTTACCTTTTT		
53	SSR53F	AGATTTAAAGCTCAAGAAAAA	52	241
	SSR53R	AAAGACTAATGTGTTTCCTTC		
54	SSR54F	AGAATAAGCTGATACTCACAC	52	283
	SSR54R	TAACAAATATCTAATTGACAGG		
55	SSR55F	ATATCTCAGGCTTCGAATGA	54	118
	SSR55R	TATTAATTTTACAGACTATGTTCA		
56	SSR56F	ATTTAACTCTCAACTTTCAAC	51	212
	SSR56R	AGATTTAGTTTTGATTATGGAG		
57	SSR57F	CATGGAGTTGTGATACCTAC	56	271
	SSR57R	CAGAGTTAGCCATATAGAGTG		
58	SSR58F	TTGCAACTGATAACAAATATAG	52	185
	SSR58R	TTCACATGACAGATATACTACTT		
59	SSR59F	TTCTTTAGACTAAGAGCACATT	56	191
	SSR59R	AGTTACAGATCTTCTCCAATT		
60	SSR60F	ATTATTTACCCTACAGAGTGC	52	244
	SSR60R	GTATTATCGGTAATGTCTTCAT		
61	SSR61F	AAAGATAGCATTTAATTAAGGA	52	206
	SSR61R	GTAAGTATCGCTGTTTGTATT		
62	SSR62F	CACAGCTCAATAAACTCTATG	53	172
	SSR62R	CATTATCCCTAATCTAATCATC		
63	SSR63F	ATTATCCCTATAATGCCCTAT	54	170
	SSR63R	CTCGGTTAACCTTTGACTAC		
64	SSR64F	AACTACTGTGGCTGACATAT	52	215
	SSR64R	CTGATTAACATAATGACCATCT		
65	SSR65F	ATAGATTCATATCTTCTGTCAT	53	233
	SSR65R	TATAAATTATCATCTTCACTGC		
66	SSR66F	TGCGTCTTCTGTGTGTCTGT	52	175
	SSR66R	GGAATGCTGTGTGTGTGTG		
67	SSR67F	AGAAATGGTTGGTGGTGGTC	55	167
	SSR67R	ACCGTGTGTGTGTGTGTGTC		
68	SSR68F	GGTCAGCTGTGTGTGTGTG	56	158
	SSR68R	CAATTCAATGCTTTGGATGCT		
69	SSR69F	TGTTTCGATTTGCAAACCTTTTT	55	299
	SSR69R	GGCCTAATGTGTGTGTGTG		
70	SSR70F	TGGAAGGACCATGCTTGAAT	55	161
	SSR70R	GGTCACACACACACACA		
71	SSR71F	CGGCACACACACACACA	55	150
	SSR71R	AAGGTCATTGGGTTCAATCC		
72	SSR72F	TGTCACACACACACACAC	56	163
	SSR72R	AATGGAAGGACCATGCTTGA		

Table 2 (Cont.)

	Primer	Sequence (5'-3')	Annealing temperature (°C)	Allele size range (bp)
73	SSR73F	CCTGAGAGAGAGAGAGAGA	55	176
	SSR73R	GAGAGAGAGAGAGAGGTGG		
74	SSR74F	TGAAGGATAGGTCTGGTG	54	158
	SSR74R	CATGAGAGAGAGAGAGAGA		
75	SSR75F	CACGAGAGAGAGAGAGAGA	55	187
	SSR75R	GGGTCTCAGAGGGAGGATT		
76	SSR76F	CATGAGAGAGAGAGAGAGAGA	55	153
	SSR76R	AAAGG AJAAGGCAGGGAAATG		
77	SSR77F	GACAGACAAAGCCAGCAGAA	60	297
	SSR77R	CCCAGAGACAGAGAGAGAGA		
78	SSR78F	CCTTGGGTTCATTTCGCTAAA	55	165
	SSR78R	GGACGCCACACACACACAC		
79	SSR79F	TGGCGCTACACACACACAC	60	229
	SSR79R	CACACACACACACACACACG		
80	SSR80F	TGGTATTCAAGCATGGTCCTC	57	244
	SSR80R	TCCATCACACACACACAC		
81	SSR81F	TCTCCCTTCATCGATTGTCC	55	122
	SSR81R	GGAGCGTCTCTCTCTCCA		

SSR-82 to SSR-100 sequences not known (unpublished data)

Source: eurofins mwg/operon (www.Eurofinsdna.com)

## RESULTS AND DISCUSSION

In India, large areas of sexually originated (seedling trees) mangos are cultivated. They show considerable genetic variability as a result of intra-species breeding. Its development and culture in the country is mainly contributed by the Mughal Emperors especially Akbar who planted Lakh Bagh, amateur gardeners, nurserymen and farmers by means of selection and subsequent cloning. Now, it is an integral part of history and culture of the country. Local landraces are popular and widely grown in AP and they are important resources. In AP, actually juicy mangoes are considered one of the largest mango businesses for the internal market. Juicy cultivar development having high fruit quality is a fundamental step to obtain excellent results in this business.

The improvement of mango rather any crop needs to explore new recombinants primarily by means of exploiting the breeding methodologies. Diversity or heterogeneity is the main character desired for breeding either natural or manmade. It is required to have vast genetic pool to get new combinations of desired nature and developing new varieties. It has been mentioned earlier that the development of mango in the country is result of selections from the amateur gardeners. Most accessions of mango have arisen through a selection of desirable types among naturally produced seedlings (Mukherjee *et al.* 1968; Karihaloo *et al.* 2003). Understanding the genetic diversity among the varieties is important in mango production, improvement, and breeding; knowledge on this field can supply useful information for further scientific progress in developing new accessions (Ravishankar *et al.* 2000; Rajwana *et al.* 2011). Breeding has yet to play its role in the development of this crop as it has not been effectively manipulated in the distant past.

In mango, genetic resources for potential crop improvement are invaluable, hence their collection, evaluation, characterization and documentation is important. The management of mango genetic resources would be effective and efficient if the characterization is accurate, so that it results in clear grouping which can be used as reference for the breeders, farmers, traders, certification bodies, in intellectual property rights and trade agreements. Furthermore, it is important to protect the Indian mango cultivars from defraud and guaranty the originality. Traditionally, in India, mango varieties have been differentiated based particularly on fruit characteristics like size, shape and color, which being influenced by environmental parameters, are unreliable. Molecular markers, by virtue of their determinate number and protection against environmental influence, can solve problems posed by morphological markers. Identification of genotypes using molecular markers can help in

identification of trees for sale, minimizing the risk of mix-ups in orchards (Struss *et al.* 2001). A mango breeding programme based on exploration survey for *in-situ* morpho-physiological characterization of fruits and molecular analysis of genotype of the sampled trees may help in developing new improved cultivars for mango growers.

### Morpho-physiological characterization and evaluation of fruits

Morpho-physiological characterization and their evaluation are the prerequisites for proper conservation and efficient utilization in improving the better varieties. The major disadvantage of morphological characterization is that they are influenced by the environmental factors or the developmental stage of the plant. However, phenotypically indistinguishable trees can differ genotypically.

There was considerable variation in the fruit size (length, width and thickness) and weight, percent peel, pulp and stone by weight, fiber length and TSS (Table 3). Fruit length varied from 8.00 cm (DM Acc-8) to 14.80 cm (DM Acc-13). Fruit width ranged from 5.00 cm (DM Acc-16) to 10.50 cm (DM Acc-5). Fruit thickness ranged between 4.70 cm (DM Acc-11) and 7.50 cm (DM Acc-5, 6, 8). Fruit weight ranged from 164.00 g (DM Acc-16) to 552.00 g (DM Acc-5). Fibre length ranged from 6.00 mm (DM Acc-13) to 41.00 cm (DM Acc-15). The percent peel, pulp and stone by weight of the fruits also showed considerable variation. The percent peel varied from 8.80 (DM Acc-12) to 30.50 (DM Acc-16). The percent pulp varied from 48.30 (DM Acc-1) to 74.40 (DM Acc-17). The percent stone varied from 8.90 (DM Acc-17) to 22.30 (DM Acc-10). TSS varied from 11.00 cm (DM Acc-1) to 22.00 cm (DM Acc-9). On the whole, a remarkable variation among the desi mango accessions across the surveyed districts was revealed in the means of fruit characters considered (Table 3).

The fruits of DM Acc-3 was oblique, DM Acc-4 was ovate, DM Acc-17 was ovate reniform, DM Acc-15 and 18 were oblong and DM Acc-17 was oblong oval in shape (Table 4). Skin colour of mature fruit of DM Acc-15 was yellow, DM Acc-7 and 18 was golden yellow, DM Acc-3 was yellowish green, DM Acc-4 had red blush over yellow, DM Acc-17 had red blush all over. DM Acc-3, 4 and 18 had medium skin, DM Acc-7 had thick skin and DM Acc-15 and 17 had thin skin. All six elite accessions had smooth skin texture. DM Acc-3, 7, 17 and 18 had abundant fiber, while DM Acc-4 and 15 had scarce fiber. Pulp colour was yellow in DM Acc-3, 4 and 17, light yellow in DM Acc-15, dark yellow in DM Acc-18 and golden yellow in DM Acc-7.

**Table 3** Morpho-physiological evaluation of fruits of indigenous land races of mango.

Accession	Fruit length (cm)	Fruit width (cm)	Fruit thickness (cm)	Fruit weight (g)	Peel (%)	Pulp (%)	Stone (%)	Fiber length (mm)	TSS (°Brix)	Shelf life (days)
DM Acc-1	10.00	6.90	6.40	300.00	30.00	48.30	21.70	15.00	11.00	6
DM Acc-2	9.00	6.00	7.00	190.00	15.80	68.40	15.80	30.00	19.00	6
DM Acc-3	13.00	5.80	5.40	284.00	22.90	60.00	21.10	17.00	18.50	7
DM Acc-4	8.50	6.00	5.50	202.00	22.30	62.90	14.90	20.00	16.60	7
DM Acc-5	13.00	10.50	7.50	552.00	19.90	71.00	9.10	26.00	13.00	6
DM Acc-6	11.00	7.30	7.50	470.00	13.80	73.00	13.20	40.00	15.00	6
DM Acc-7	12.00	8.50	6.50	370.00	24.30	59.50	16.20	25.00	15.00	6
DM Acc-8	8.00	7.20	7.50	360.00	16.70	71.60	11.70	13.00	19.00	7
DM Acc-9	8.00	6.20	6.00	228.00	24.10	58.30	17.60	30.00	22.00	7
DM Acc-10	9.00	6.50	5.80	260.00	19.20	58.50	22.30	22.00	18.00	7
DM Acc-11	12.50	6.20	4.70	226.00	17.70	60.20	22.10	30.00	13.20	7
DM Acc-12	12.80	6.30	6.70	360.00	8.80	80.00	11.10	30.00	12.00	7
DM Acc-13	14.80	6.50	6.60	380.00	18.40	61.80	19.70	6.00	14.50	6
DM Acc-14	12.00	6.00	6.40	290.00	10.30	72.40	17.20	30.00	13.00	7
DM Acc-15	12.00	8.00	7.00	380.00	10.00	71.50	14.50	41.00	16.30	6
DM Acc-16	7.50	5.00	5.00	164.00	30.50	57.30	12.20	30.00	21.00	5
DM Acc-17	12.20	7.40	6.50	360.00	16.70	74.40	8.90	15.00	18.00	7
DM Acc-18	12.70	5.80	5.50	258.00	19.50	64.30	16.20	8.00	21.20	4
DM Acc-19	9.50	6.80	6.80	308.00	21.90	63.90	14.90	20.00	18.30	6
DM Acc-20	10.50	7.50	6.20	340.00	20.50	61.80	17.70	22.00	18.10	6

DM Acc = Desi mango accession

**Table 4** Morpho-physiological characterization of fruits of indigenous land races of mango.

Accession	Fruit shape	Colour of skin of mature fruit	Skin thickness	Skin texture	Pulp colour	Quantity of fiber	Adherence of skin to pulp	Fibre in pulp
DM Acc-1	Oblique Reniform	Red blush over greenish yellow	Thick	Rough	Light yellow	Abundant	Adherent	Present
DM Acc-2	Ovate Oblong	Yellow	Medium	Smooth	Yellowish green	Abundant	Adherent	Present
DM Acc-3	Oblique	Yellowish green	Medium	Smooth	Yellow	Abundant	Adherent	Present
DM Acc-4	Ovate	Red blush over yellow	Medium	Smooth	Yellow	Scarce	Adherent	Present
DM Acc-5	Ovate Reniform	Greenish yellow	Medium	Smooth	Yellow	Scarce	Adherent	Present
DM Acc-6	Ovate Reniform	Golden yellow	Thin	Smooth	Yellow	Scarce	Adherent	Present
DM Acc-7	Ovate Reniform	Golden yellow	Thick	Smooth	Golden yellow	Abundant	Adherent	Present
DM Acc-8	Roundish	Dark yellow	Medium	Smooth	Yellow	Scarce	Non-adherent	Absent
DM Acc-9	Ovate	Yellow	Thin	Smooth	Golden yellow	Scarce	Adherent	Present
DM Acc-10	Oblong Oval	Greenish yellow	Thin	Smooth	Yellow	Abundant	Adherent	Present
DM Acc-11	Oblong Elliptic	Greenish yellow	Thick	Rough	Dark yellow	Scarce	Adherent	Present
DM Acc-12	Oblong	Yellowish green	Thin	Smooth	Dark yellow	Scarce	Non-adherent	Present
DM Acc-13	Oblong	Yellowish green	Thick	Smooth	Dark yellow	Abundant	Adherent	Present
DM Acc-14	Oblong	Golden yellow	Thin	Smooth	Dark yellow	Abundant	Adherent	Present
DM Acc-15	Oblong	Yellow	Thin	Smooth	Light yellow	Scarce	Adherent	Present
DM Acc-16	Oval	Golden yellow	Thin	Smooth	Dark yellow	Scarce	Adherent	Present
DM Acc-17	Oblong Oval	Red blush all over	Thin	Smooth	Yellow	Abundant	Adherent	Present
DM Acc-18	Oblong	Golden yellow	Medium	Smooth	Dark yellow	Abundant	Adherent	Present
DM Acc-19	Ovate Reniform	Yellowish green	Medium	Smooth	Yellow	Abundant	Adherent	Present
DM Acc-20	Ovate Reniform	Reddish yellow	Thick	Smooth	Golden yellow	Abundant	Adherent	Present

**Table 4 (Cont.)**

Accession	Fibre in pulp	Length of fiber	Fruit stalk insertion	Fruit attractiveness	Basal cavity	Beak	Beak type	Sinus	Sinus type
DM Acc-1	Present	Long	Oblique	Good	Absent	Absent	Perceptible	Present	Deep
DM Acc-2	Present	Medium	Vertical	Good	Absent	Present	Pointed	Absent	Absent
DM Acc-3	Present	Long	Vertical	Good	Absent	Present	Pointed	Present	Slight
DM Acc-4	Present	Medium	Vertical	Excellent	Absent	Absent	Perceptible	Present	Slight
DM Acc-5	Present	Short	Oblique	Average	Absent	Present	Pointed	Present	Deep
DM Acc-6	Present	Short	Oblique	Excellent	Present	Present	Pointed	Present	Slight
DM Acc-7	Present	Long	Vertical	Excellent	Absent	Present	Pointed	Present	Deep
DM Acc-8	Absent	Short	Oblique	Excellent	Present	Absent	Perceptible	Absent	Absent
DM Acc-9	Present	Short	Oblique	Excellent	Present	Absent	Perceptible	Absent	Absent
DM Acc-10	Present	Short	Oblique	Good	Absent	Present	Pointed	Absent	Absent
DM Acc-11	Present	Short	Vertical	Average	Absent	Absent	Perceptible	Present	Slight
DM Acc-12	Present	Short	Vertical	Good	Absent	Absent	Perceptible	Absent	Deep
DM Acc-13	Present	Medium	Vertical	Good	Absent	Present	Pointed	Present	Deep
DM Acc-14	Present	Medium	Vertical	Excellent	Absent	Absent	Perceptible	Present	Slight
DM Acc-15	Present	Medium	Vertical	Good	Absent	Present	Pointed	Absent	Absent
DM Acc-16	Present	Short	Oblique	Excellent	Absent	Absent	Perceptible	Absent	Absent
DM Acc-17	Present	Medium	Vertical	Excellent	Absent	Present	Pointed	Absent	Absent
DM Acc-18	Present	Long	Oblique	Excellent	Absent	Absent	Perceptible	Present	Slight
DM Acc-19	Present	Medium	Vertical	Excellent	Present	Present	Prominent	Present	Deep
DM Acc-20	Present	Long	Oblique	Excellent	Present	Present	Pointed	Present	Slight

DM Acc = Desi mango accession

**Table 4 (Cont.)**

Accession	Groove	Shoulders	Slope of shoulders	Apex	Eating quality	Utility
DM Acc-1	Absent	Ventral broader and higher than dorsal	Rising and then rounded	Round	Poor	Juicy
DM Acc-2	Present	Ventral broader and higher than dorsal	Ending in a long curve	Round	Good	Juicy
DM Acc-3	Present	Ventral broader and higher than dorsal	Rising and then rounded	Round	Excellent	Juicy
DM Acc-4	Present	Equal	Rising and then rounded	Round	Good	Juicy/Table
DM Acc-5	Absent	Equal	Rising and then rounded	Round	Intermediate	Juicy
DM Acc-6	Absent	Ventral broader and higher than dorsal	Rising and then rounded	Round	Intermediate	Juicy
DM Acc-7	Present	Equal	Sloping abruptly	Round	Excellent	Juicy
DM Acc-8	Absent	Equal	Rising and then rounded	Round	Excellent	Table
DM Acc-9	Absent	Ventral broader and higher than dorsal	Ending in a long curve	Round	Excellent	Juicy
DM Acc-10	Present	Ventral broader and higher than dorsal	Ending in a long curve	Round	Excellent	Juicy
DM Acc-11	Present	Ventral broader and higher than dorsal	Ending in a long curve	Round	Good	Juicy
DM Acc-12	Absent	Ventral broader and higher than dorsal	Ending in a long curve	Round	Intermediate	Juicy
DM Acc-13	Present	Equal	Ending in a long curve	Round	Intermediate	Juicy
DM Acc-14	Present	Dorsal shoulder sloping to ending in a long curve	Ending in a long curve	Round	Good	Juicy
DM Acc-15	Present	Ventral broader and higher than dorsal	Rising and then rounded	Round	Excellent	Juicy
DM Acc-16	Absent	Equal	Ending in a long curve	Round	Excellent	Juicy
DM Acc-17	Absent	Ventral broader and higher than dorsal	Ending in a long curve	Round	Good	Juicy/Table
DM Acc-18	Absent	Equal	Rising and then rounded	Round	Excellent	Juicy
DM Acc-19	Absent	Ventral broader and higher than dorsal	Rising and then rounded	Round	Good	Juicy/Table
DM Acc-20	Present	Equal	Rising and then rounded	Round	Excellent	Juicy/Table

DM Acc = Desi mango accession

Eating quality was excellent in DM Acc-3, 7, 15 and 18 and good in DM Acc-4 and 17. A great range of variation was encountered in the qualitative traits among the accessions within and between ecogeographically surveyed districts. In fruit tree species, qualitative traits have been found useful in identification and assessment of varieties for fruit production on a large scale as these traits help in developing the ideotypes (Leakey *et al.* 2000).

In the present study, the quantitative (Table 3) and qualitative (Table 4) data of fruits revealed sufficient variability for different morpho-physiological characteristics of fruits among the twenty land races of mango under study. The study highlights the diversity of mango fruit forms that exists in Andhra Pradesh, which was also observed in wild and cultivated Indian mangos (Karihaloo *et al.* 2003). On the whole, morphologically, all the twenty landraces of mango studied are very distinct with respect to the size, shape, morphology and the taste of the fruits. While prospecting various landraces appeared to share morphological traits but were collected separately because they occurred in different locations. The huge variability in mango genotypes may also have resulted from a long period of cultivation, thereby giving ample opportunity for hybridization and forces of selection to operate. Earlier studies on diversity of crop species within centers of diversity also have demonstrated with examples the importance of quantitative traits outside the centre of diversity and qualitative traits within the centre of diversity (Tolbert *et al.* 1979; Witcombe and Gilani 1979). Historically mango genotypes have been characterized by several researchers using morphological traits. Several studies have been made on morphological characterization of many different cultivars of mango all over the world (Ascenso *et al.* 1981; Mukherjee *et al.* 1983; Subramanyam and Iyer 1989; Illoh and Olorode 1991; Jintanawong *et al.* 1992; Subedi *et al.* 2009). Mukherjee *et al.* (1983) based on detailed surveys of mango orchards in eastern India have identified many superior clones. Based on their survey, 23 elite clones were identified. Some of the clones which ranked excellent grade by them are: 'Dawadi', 'Emrat Bhog', 'Shah Pasand', 'Sadik Pasand', 'Kali Bhog' (all from Murshidabad) and 'Misrikanta' (from Maldah). They also suggested that it would be worth putting these clonal selections in a varietal trial for comparison with the standard commercial variety for releasing as new superior selections for commercial exploitation and for utilization in the hybridization programme. An assessment of various vegetative and fruit characters was made by Subramanyam and Iyer (1989) in mango germplasm collected from different regions of India, to select suitable parents for hybridization.

Accordingly they identified two dwarf plants from collections made in Kerala state, which they named as 'Kerala Local' and 'Local Kalapady'. A preliminary selection of 19 mango accessions and cultivars from a collection at the Umbeluzi Research Station in Mozambique was done. The study focused on colour, size, shape, weight and volume of fruit, number of embryos per seed, peel thickness, adherence, flavour, texture, fibre content, juice, soluble solids, sugars, acidity, pH and ratio of soluble solids to acidity. As a result, the five most desirable varieties were selected (Ascensão *et al.* 1981).

Preferences for mango varieties often differ among countries, regions, ethnicities, and cuisines of markets in which they are consumed. The characteristics by which mangos are typically selected are a mix of eating quality, keeping, and fruit characteristics. Fruit quality characteristics such as flavor, aroma, flesh texture, and fiber are generally of high importance, as are fruit size, external appearance. Of the 20 indigenous landraces of mango subjected to morpho-physiological characterization and evaluation, only six accessions *viz.*, DM Acc-3, 4, 7, 15, 17 and 18 were found to be horticulturally superior with respect to fruit characteristics. Of the six horticulturally superior indigenous landraces, five accessions *viz.*, DM Acc-3, 4, 7, 15 and 18 were of juicy type, while DM Acc-17 was of table type. Some of these could present marked morphological differences issuing from punctual mutations or epigenetic variation and clonally propagated.

### Marker characteristics and SSRs polymorphism

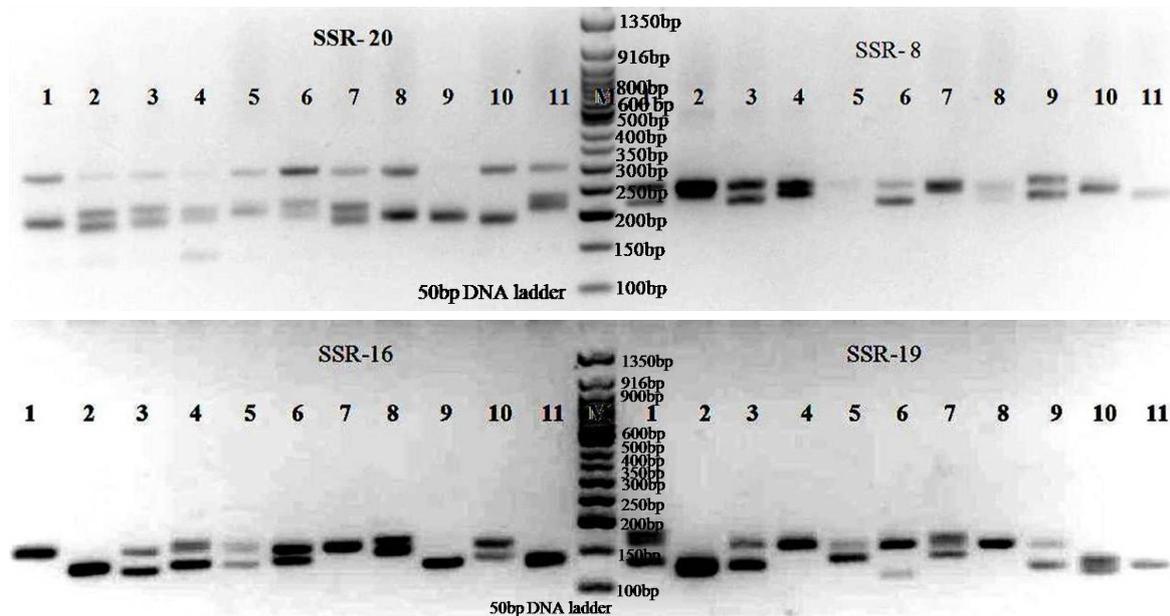
SSRs are widely used as a versatile tool in plant breeding programs as well as in evolutionary studies because of their ability for showing diversity among cultivars (Adato *et al.* 1995). Of the 109 mango-specific microsatellite markers used, 57 were polymorphic displaying 198 alleles. This is in line with the findings of Wahdan *et al.* (2011) who found that 36 out of 42 primers gave reproducible polymorphic DNA amplification patterns among two Egyptian mango strains 'Hania' and 'Aml' and Hirano *et al.* (2010) who observed that 11 out of 24 SSR markers were found to be highly variable among 113 mango accessions they examined. The number and percentage of polymorphism in SSR markers depend on the number and variability of the cultivars and/or accessions analyzed. Characteristics of ten highly polymorphic microsatellite markers in mango including microsatellite motif, primer sequence, annealing temperature, allele size, number of alleles and polymorphic information content are given in Table 5. A representative of

**Table 5** Characteristics of highly polymorphic microsatellite markers in mango including microsatellite motifs, primer sequences, annealing temperature, allele size, number of alleles and polymorphic information content.

Primer ID	Primer sequence (5'-3')	Tm	Allele size	SSR motif	No. of alleles <sup>†</sup>	PIC values <sup>†</sup>
SSR-16	F: GCTTTATCCACATCAATATCC R: TCCTACAATAACTTGCC	54	150-180	(TA) <sub>2</sub> (CA) <sub>10</sub> TA(CA) <sub>3</sub> TA(CA) <sub>4</sub>	4	0.85
SSR-19	F: AATTATCCTATCCCTCGTATC R: AGAAACATGATGTGAACC	54	140-180	(ACACACAT) <sub>3</sub> (ACACACACAT) <sub>3</sub>	5	0.88
SSR-20	F: CGCTCTGTGAGAATCAAATGGT R: GGACTCTTATTAGCCAATGGGATG	58	250-310	(AT) <sub>14</sub> (GT) <sub>18</sub>	6	0.63
SSR-24	F: GATGAAACCAAAGAAGTCA R: CCAATAAGAACTCCAACC	53	300-320	(TG) <sub>10</sub>	3	0.39
SSR-26	F: GCCCTTGCATAAGTTG R: TAAAGTGATGCTGCTGGT	52	180-230	(TG) <sub>14</sub>	4	0.67
SSR-52	F: AAAAACTTACATAAGTGAATC R: CAGTTAACCTGTTACCTTTTT	52	100-210	(GA) <sub>16</sub>	4	0.72
SSR-84	F: TCTATAAGTGCCCCCTCACG R: ACTGCCACCGTGGAAAAGTAG	58	200-260	NA	6	0.77
SSR-85	F: GCTTGCTTCCAACCTGAGACC R: GCAAAAATGCTCGGAGAAGAC	58	250-310	NA	5	0.64
SSR-89	F: CGCCGAGCCTATAACCTCTA R: ATCATGCCCTAAACGACGAC	55	110-140	NA	4	0.86
MNGSSR-14	F: TCATTAAGCTGTGGCAACCA R: CATTGCATAGATGTGGTCATT	59	165-185	(GA) <sub>116</sub>	3	0.64

<sup>†</sup>Based on the complete set of 11 genotypes of mango as described in "Material and Methods".

NA = Not available (unpublished).



**Fig. 2** (A) Microsatellite profiles of eleven genotypes of mango with SSR-20 (left panel) and SSR-8 (right panel) or (B) with SSR-16 (left panel) and SSR-19 (right panel).

the PCR amplification product of 11 genotypes of mango with SSR-20 and SSR-8 and SSR-16 and SSR-19 is shown in **Fig. 2A** and **2B**, respectively, which yielded sufficient polymorphism to distinguish between popular named cultivars and local land races of mango under study.

Information regarding genetic variability at molecular level could be used to help identify and develop genetically unique germplasm that complements existing cultivars. A high PIC value was observed in microsatellites ranging from 0.39 to 0.88 with an average of 0.66 (**Table 5**). This is higher than that reported by Wahdan *et al.* (2011) in their work with 42 SSR primer pairs ranging from 0.25 to 0.75, with a mean value 0.51 for all loci among two Egyptian mango strains 'Hania' and 'Aml' and Schnell *et al.* (2005) in their work 15 microsatellite loci isolated from mango ranged from 0.21 to 0.63 for the polymorphic loci among 59 Florida cultivars and four related species from the USDA germplasm collection for mango. This may probably be due to the higher number of analyzed samples, as well as due to the more diverse genotypes analyzed. The level of poly-

morphism present in the microsatellites was variable ranging from 2 to 6 alleles per marker. The level of polymorphism in the present study is in line with that of the 16 SSRs in 28 mango genotypes with a total of 88 bands and an average of 5.5 bands/SSR, ranging from 3 to 9 bands/SSR (Viruel *et al.* 2005). Diversity at molecular level within the sample was high with a total number of 198 alleles displayed. Duval *et al.* (2009) also reported high diversity within the sample with a total number of 140 alleles displayed wherein nineteen microsatellite markers were utilized to analyse a total of 307 accessions from India, South-East Asia, Florida, Africa and the Caribbean. Kumar *et al.* (2001), working mainly on south Indian cultivars observed moderate level of RAPD diversity among them. The high level of SSR polymorphism observed in the present material is consistent with the extensive morphological diversity reported in Indian mango cultivars (Mukherjee 1948, 1953; Naik and Gangolly 1950; Rajan *et al.* 1999). However, it is obvious that from the variation in band frequencies of all the SSR primers validated, the results indicate that mango

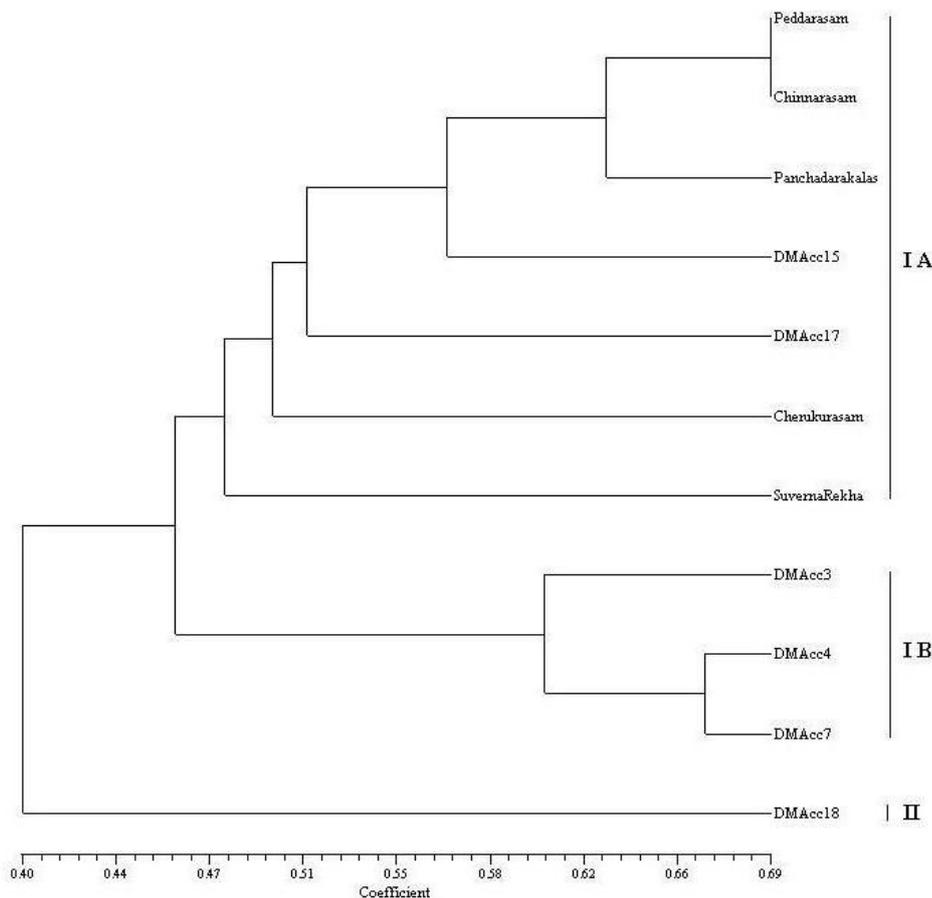


Fig. 3 Dendrogram of 11 genotypes of mango based on UPGMA cluster analysis.

too represents very rich reserves of genetic variability which in turn will provide a wide spectrum for the selection of desirable types from the germplasm.

### Genetic diversity

The use of genetic distance among cultivars is important for plant breeding programmes. In addition, the understanding of intra-specific genetic variation patterns is important for genetic resource management and conservation. It is very clear from UPGMA cluster analysis, which identified two clusters (cluster I and II) in the eleven mango genotypes in this study (Fig. 3). A dissimilarity coefficient of 0.06 was observed between the two clusters (cluster I and II). Cluster I could be further divided into two sub clusters (cluster IA and IB). A dissimilarity coefficient of 0.14 was observed between the two sub clusters (cluster IA and IB). Subcluster IA contains all the five popular named cultivars used for comparison in addition to the two (DM Acc-15 and 17) of the six horticulturally superior landraces. Subcluster IB contains exclusively three (DM Acc-3, 4 and 7) of the six landraces. Cluster II was solitary; consisting of only one landrace DM Acc-18 from Yaganti-palli, branched out from the base, was found the most unique and divergent. Rahman *et al.* (2007) evaluated molecular diversity in 28 mango germplasm of Bangladesh using RAPDs and observed that the UPGMA dendrogram based on genetic distance segregated the germplasm into two main clusters.

The six landraces collected from different eco-geographical regions were distributed across the dendrogram (Fig. 3) and displayed a high diversity. Only one sub cluster (cluster IA) constituted by all popular cultivars used for comparison is linked by consequently less high dissimilarities. The first sub-cluster (cluster IA) groups all the popular cultivars including DM Acc-15 and DM Acc-17 from Reddigudem and Konampet, respectively. Three landraces *viz.*, DM Acc-3, 4 and 7 from Pondugala grouped together in one sub-cluster (cluster IB), while DM Acc-18 from Yaganti-

palli formed a distinct cluster. From these results, it is evident that the landraces clustered according to their geographical origin. This clearly indicates that the effect of geographic origin influenced clustering. Lopez-Venzuela *et al.* (1997), based on RAPD profiles, grouped mango cultivars into four categories, which coincided with their geographical origin. Ravishankar *et al.* (2000) also concluded that the mango cultivars in different parts of the Indian sub-continent have originated from germplasm existing in the particular geographical area.

UPGMA cluster analysis revealed magnificent genetic diversity as evident from the Jaccard's similarity coefficient ranging from 0.40 to 0.69 (Fig. 3). Kumar *et al.* (2001) also reported Jaccard's similarity in the range of 61 to 95% in 50 Indian cultivars and Fitmawati *et al.* (2010) observed 69 to 98% similarity in 82 cultivars of mango from Indonesia, where as similarity in present material was 40 to 69%. Obviously, the present material was genetically more diverse than that used by the above researchers. Jaccard's similarity coefficient uses only the presence of bands among genotypes as similarity and not the absence of bands (Jaccard 1908), as the absence of bands could be due to several reasons like duplication, deletion, point mutation, inverse etc., resulting in abolition of the site. The molecular analysis of 11 mango genotypes suggested that diversity is moderate to high and has shown differences, if the similarity matrices were used, which indicated that the reasons for absence of bands are not the same and hence they are more diverse. Autopolyploidy, out breeding, wide range of agro-climatic conditions prevailing in different mango growing regions, wide spread hybridization and recombination of characters have contributed immensely to the existing variability in mango (Ravishankar *et al.* 2000). Moreover, the high genetic diversity within populations is explained by the breeding system since mango is an allogamous species (Ward *et al.* 2005).

## Genetic relatedness and distinctiveness

The dendrogram (Fig. 3) revealed a maximum similarity of 69% and a minimum of 40%. Peddarasam of the five popular named cultivars and DDM Acc-18 of the six horticulturally superior land races were the two extremes of the tree. Surprisingly, all the landraces except DM Acc-15 and 17 were distinctly separated from the popular cultivars with which they were compared. A dissimilarity coefficient of 0.056 was observed between DM Acc-15 and DM Acc-17. Further, DM Acc-15 with 0.068 dissimilarity coefficient was placed nearer to 'Cherukurasam'. Similarly, DM Acc-17 with 0.012 dissimilarity coefficient was placed nearer to Cherukurasam.

Of the six horticulturally superior indigenous landraces, two namely DM Acc-4 and 7 from Pondugala with only 67% pair-wise similarity were found to be the least divergent followed by DM Acc-3, while DM Acc-18 was found to be the most divergent exhibiting only 29% similarity with rest of the genotypes followed by DM Acc-17 and DM Acc-15. DM Acc-17, the horticulturally superior colored table genotype from Konampeta had only 68% similarity with colored dual purpose Suvarnarekha.

The produced tree was consistent with known genetic relationships. The dendrogram clusters are in agreement with the geographical origin of the mango landraces. Similarly, Ravishankar *et al.* (2000) concluded that cultivars in India might have arisen from germplasm in a particular geographical area. Schnell *et al.* (1995) in their study of 25 accessions also obtained a wide distribution of Indian cultivars in RAPD based dendrogram, which is indicating how cultivars may vary in a specific region. The dendrogram generated based on Jaccard's similarity coefficient (Fig. 3), which considered presence of bands between genotypes, revealed that 'Peddarasam' and 'Chinnarasam' formed a distinct group with a maximum pair-wise similarity of 69% are the popular juicy types in the Nuzvidu region of Andhra Pradesh. All the five popular cultivars *viz.*, 'Chinnarasam', 'Peddarasam', 'Cherukurasam', 'Panchadarakalasa' and 'Suvarnarekha' used for comparison, clustered together distinctly along with two horticulturally superior landrace DM Acc-15 and DM Acc-17. Further, these popular cultivars had a similar usage *i.e.* juicy type. Of the six horticulturally superior landraces of mango, almost all except DM Acc-15, DM Acc-17 and DM Acc-18, clustered together. Jaccard's similarity coefficient revealed that DM Acc-18 clearly forms a separate solitary cluster and occupies the extreme position in the dendrogram. It can be seen that landraces DM Acc-3, 4 and 7 which originated from the same node are indigenous to Pondugala. Thus there seem to be a clear segregation of landraces mainly depending upon their geographical location. Since the landraces used here are seedling selections, the result from this study show that the landraces might have evolved from the genotypes in that geographical location. The dendrogram can also be used to select the most appropriate parental material to improve horticultural traits. The selection and hybridization programmes in mango can be affected based on the clustering.

Mango cultivars and species from India and other countries have been characterized using different molecular markers. Lopez-Valenzuela *et al.* (1997) and Schnell *et al.* (1995) mentioned the geographical clustering of cultivars with RAPDs. Isolation within Indian cultivars as North and South Indian, has also been reported by Karihaloo *et al.* (2003) and Ravishankar *et al.* (2004) using RAPDs. This grouping was observed to be dissolved by an increase in the sample size (Ravishankar *et al.* 2000) and by change in the selected cultivar set (Chunwongse *et al.* 2000). Adato *et al.* (1995) used RFLP analysis based on Jeffrey's minisatellite probes identified and analyzed genetic variations among mango genotypes and came to the conclusion that Indian cultivars represented a varied group. On the contrary SSR-anchored primers were used to identify and to validate genetic variation among Thai cultivars (Eiadthong *et al.* 1999b), but could not separate the cultivars according to

their embryonic types, nor the types eaten as ripened fruit or unripe fruits. Kashkush *et al.* (2001) used AFLP technique to identify mango cultivars, for studying the genetic relationship among 16 mango cultivars and seven mango rootstocks and they finally conclude that AFLP markers are suitable for cultivar identification and estimating genetic relationships. Based upon more recent genetic analysis involving microsatellite markers, it is now estimated that the majority of Florida cultivars are descended from only four monoembryonic Indian cultivars *i.e.*, 'Mulgoba', 'Sanderasha', 'Amini' and 'Bombay' together with the polyembryonic 'Turpentine' from the West Indies (Schnell *et al.* 2005). Viruel *et al.* (2005) found that microsatellite markers can distinguish monoembryonic from polyembryonic accessions. Hirano *et al.* (2010) compared the genetic structure of mango accessions from Myanmar with that of mango accessions from Florida, India and Southeast Asia with 11 SSRs and analysed genetic diversity which revealed high genetic diversity at the national level and a pattern of mixed geographic origin. Similar separations among mango accessions based on geographical origins have been found (Karihaloo *et al.* 2003; Viruel *et al.* 2005; Pandit *et al.* 2007; Rajwana *et al.* 2008). Mango cultivars showed genetic differences based on geographical origin and their known history (Duval *et al.* 2009; Hirano *et al.* 2010). On the whole, earlier studies indicate clearly the differentiation of mango accessions regardless the marker system use to fingerprint based on type of embryony (mono- or polyembryonic), geographical origin or genetic status (cultivars, landraces, species). In the present study, we have also obtained clearly and consistently with SSRs. Our results also suggest that indigenous landraces show genetic differences based on geographical origin and their known history and that genetic exchange persists.

## Landrace identification

Currently, most of the mango cultivars are being identified on the basis of leaf, fruit and stone characteristics. These traits may be affected by environmental conditions (Lakshminarayana 1980). DNA markers have the potential to be used in mango for marker assisted selection (MAS) and for cultivar identification (Lavi *et al.* 1993). Of these, microsatellites, in addition to their usefulness in mapping and breeding (McCouch *et al.* 1997), have become the markers of choice for fingerprinting purposes in most plant species (Gupta and Varshney 2000) due to their high polymorphism, codominance and reproducibility. In species such as mango and most fruit trees, where no or few sequences are available in public sequence databases or in related species, the major drawback for the application of microsatellite analyses is the difficulty and cost of the isolation process that usually involves the construction and screening of genomic libraries to find the sequences flanking the repeat regions. The low yield obtained using conventional library screening methods has been greatly improved by the use of enrichment protocols for specific microsatellite repeats (Zane *et al.* 2002; Viruel and Hormaza 2004). Therefore, SSR markers seem to be useful for identification of landraces.

The results revealed that 57 out of 109 SSRs showed district polymorphism among 11 genotypes studied indicating the robust nature of microsatellites in revealing polymorphism. In the present study microsatellite profiling was found efficient enough to reveal usable level of DNA polymorphism among 11 mango genotypes (Table 6). Of the 10 highly polymorphic microsatellite markers used, one microsatellite SSR-84 was sufficient to discriminate among the 11 genotypes in this study. Further, with just marker SSR-20 it was possible to discriminate among the ten genotypes. SSR-20 was able to distinguish all the six superior land races. Of the 9 highly polymorphic SSRs validated, SSR-84 could distinguish all of the six land races, while 8 SSRs *viz.*, SSR-16, 19, 24, 26, 52, 85, 89 and MNGSSR-14 could distinguish 5 land races of mango. The primers SSR 84 and 20 used in this study produced unique banding patterns that

**Table 6** Cultivar or landrace-specific bands obtained with various SSR primers.

Cultivar /Landrace	Primer producing specific band(s)	Size of the specific band (bp)
Peddarasam	SSR-26	180, 190, 220, 230
Cherukurasam	SSR-19	130
	SSR-26	190, 230
	SSR-52	100, 210
	SSR-84	200, 215, 235
	SSR-85	250, 265, 310
Chinnarasam	SSR-19	190, 200, 220, 230
	SSR-84	200, 215, 260
Panchadarakalasa	SSR-20	200, 210, 300
	SSR-19	180, 230
Suvarnarekha	SSR-19	190, 200, 220, 230
	SSR-85	250, 265, 275
	SSR-84	225, 235, 250
DM Acc-3	SSR-20	200, 220, 300
DM Acc-4	SSR-19	150, 180
	SSR-26	190, 220
	SSR-52	180
	SSR-85	250, 275, 310
DM Acc-7	SSR-19	130, 150
	SSR-84	200, 225
	SSR-89	110, 120
	MNGSSR-14	175, 185
DM Acc-15	SSR-20	200
	SSR-26	220, 230
	SSR-89	130, 140
DM Acc-17	SSR-20	190, 300
	SSR-26	190
	MNGSSR-14	165, 175
DM Acc-18	SSR-8	240
	SSR-19	140, 160
	SSR-20	220, 230, 300
	SSR-26	190, 230, 330
	SSR-52	100
	SSR-84	215, 235
	MNGSSR-14	165

could differentiate all 11 genotypes of mango. The results demonstrate the usefulness of microsatellites for studies on identification, variability, germplasm conservation and domestication of germplasm in mango. It is highly recommended to use molecular markers such as SSRs for the purpose of finding similarities or differences among mango cultivars. RAPDs have been successfully used to identify 25 accessions of mango and to validate their genetic relationships (Schnell *et al.* 1995). Genetic relatedness of traditional Indian mango cultivars grown in commercial scale was studied using RAPD makers (Ravishankar *et al.* 2000). Results of the study indicated that cultivars from a particular geographical region were closely related. Lopez-Valenzuela *et al.* (1997) reported that RAPD marker can distinguish mangoes based on embryonic types and their geographical origin. Microsatellites are becoming the marker of choice for fingerprinting and genetic diversity studies in a wide range of living organisms (Shiran *et al.* 2007). Viruel *et al.* (2005) studied polymorphism with 16 microsatellite in a collection of 28 mango cultivars of different origins. The SSRs studied allowed unambiguous identification of all the mango genotypes studied. In Thailand, a study was done to identify mango cultivars and evaluate their genetic variation using SSR anchored primers. Results indicated that two Thai mango cultivars were found to be far distant of the genetic relationship from the other cultivars. Seven cultivars were in the same group as two Florida cultivars, one Philippine cultivar and one Indonesian cultivar. Four other Thai cultivars were divided into two groups: each group enclosed Indian cultivars. The analysis did not present evident distinction between the polyembryonic and monoembryonic seed races (Eiadthong *et al.* 1999b). Honsho *et al.* (2004) isolated and characterized new SSRs in mango to identify 36 cultivars from different places, namely Thailand, Australia,

USA and Taiwan. An AC genomic library was created using the mango variety 'Irwin'. SSR alleles indicated high frequencies and tended to be shared by Thailand cultivars, whereas rare alleles were found in cultivars from other regions. This could have been due to the similar genetic background in 29 of the 36 cultivars from Thailand. Wahdan *et al.* (2011) reported that the banding patterns obtained from the 36 primers were highly distinct and each of the two Egyptian mango strains 'Hania' and 'Aml' utilized in the study could be distinguished from the other, indicating that, PCR by using SSR primers was an efficient method for genotype identification. Consequently, the approach described in this work shows that microsatellite analysis is a powerful tool also for the characterization and identification of six promising landraces of mango by comparing the alleles, which were detected using the 10 highly polymorphic microsatellite markers.

The five single-plant elite landraces *viz.*, DM Acc-3, 4, 7, 15, 17 and 18 with genotypes not matching those of five popular cultivars used for comparison were found to be genetically distinct. Genetic distance clearly separates all the landraces except two from all popular cultivars. A substantial amount of genetic diversity exists in the landraces of AP under study; however, the landraces were more genetically diverse than the popular cultivars. These results indicate that it is possible to identify informative microsatellites and these markers are capable of discriminating the closely related mango genotypes. Moreover, our findings suggest that microsatellite markers can play an important role as a source of additional information to supplement the morphological descriptors recommended by IPGRI. The existence of many landraces indicates that there is great possibility to increase fruit quality through improvement in genetic and agronomic practices.

## CONCLUSIONS

Although the present study has examined only a few quantitative and many qualitative characters of mango fruits, it has found subjectively the existence of a marked variation among 20 desi mango accessions collected from the two eco-geographical regions of AP. Morphologically, all the twenty landraces of mango studied are very distinct with respect to the size, shape, morphology and the taste of the fruits. Our results here strongly suggest that the samples studied do not belong to the same clone. In addition, there is a wide genetic variability available in mango among and between landraces and popular cultivars leaving a wide scope for improvement. Further, the large range of similarity coefficient varied from landraces using microsatellites provides greater confidence for the assessment of genetic diversity and relationships. The practical approach developed in this study is useful in the finger printing also. Among the 10 highly polymorphic SSRs studied, SSR-84 was found to be useful in fingerprinting of 11 genotypes. This fingerprinting makes identification and characterization of genotypes very easy and further it will be of greater help in background selection during back cross breeding programmes. Since all the six horticulturally superior indigenous landraces collected from different regions of AP were found to be genetically distinct from the popular cultivars compared indicating their potentiality and distinctiveness for commercial cultivation in AP after thorough testing.

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