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Molecular Analysis of Intracultivar Polymorphism of 'Panchadarakalasa' Mango by Microsatellite Markers

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Abstract

Juicy mangoes (sucking type/*rasaalu*) are one of the most significant mango *(Mangifera indica* L.) businesses in Andhra Pradesh state, India. The mango orchardists cultivate a great number of juicy cultivars for which genetic homogeneity has never been demonstrated.'Panchadarakalasa' is one of the choicest juicy cultivars of mango in the state, where certain level of intracultivar variability in fruit morphology has been observed among trees. In this study, fruit and leaf samples of 16 trees of 'Panchadarakalasa' (PK Acc-l to PK Acc-16) spread over the three eco-geographical regions (Coastal Andhra, Rayalaseema and Telangana) of the state were collected during summer 2009, which were subjected to *in-situ* morphological and *ex-situ* microsatellite analysis, respectively to identify whether there is variability in the plants grown in the state. Characterization and evaluation of fruit samples based on 9 quantitative and 7 qualitative traits revealed phenotypic variations among accessions under study. Twenty out of 109 mango-specific microsatellite markers validated, were amplified. Of the 20 microsatellites amplified, only 4 were polymorphic with a total of 11 alleles ranging from 130 bp to 245 bp. The polymorphic information content of the polymorphic alleles ranged from 0.25-0.56, whereas the Jaccard's similarity coefficient values ranged from 0.9-1.0. The pair-wise genetic dissimilarities ranged from 0.00-0.10 with a mean value of 0.05. Dendrogram based on unweighted pair group method of arithmetic means algorithm indicated that the accessions were not grouped as per geographic separation. Microsatellite analysis revealed smaller intracultivar variability of 10% in *in-situ* conditions and a genetic divergence between trees attesting that 'Panchadarakalasa' whatsoever cultivated throughout the state is not pure clone. The traditional nursery practices are likely to be responsible for the intracultivar polymorphism since the 'Panchadarakalasa' not propagated exclusively vegetatively. Highly polymorphic microsatellites like SSR-83, MngSSR-24 and MngSSR-26 were more useful in differentiating the 'Panchadarakalasa' accessions. The results generated with microsatellite markers will be helpful in intracultivar improvement as well as in the application of breeder rights in the country.

Keywords: Fruit Morphology, Fruit Quality, Intravarietal Diversity, Molecular Analysis, Simple Sequence Repeats

1. Introduction

Mango is botanically *Mangifera indica* L., belonging to the family Anacardiaceae. It is believed to have originated in Eastern India (Knight, 1980). It is one of the most economically grown fruits in the tropical and subtropical areas around the world (Rajwana *et al.*, 2011) both for fresh (table/juicy) and industrial consumption. The global consumption of mango has increased significantly because of its nutritional and bioactive properties (Poovarodom *et al.*, 2010). Since mango has been under cultivation in India since antiquity and is highly cross-pollinated, there are thousands of varieties of mango arising from natural cross-pollination or mutation (Karihaloo *et al.*, 2003). Juicy cultivars of mango (juicy mangoes/sucking type), popularly known as '*rasaalu*' are the specialty mangoes of Andhra Pradesh state, India. The juicy mango business is one of the most relevant in the state mango business with a leading role in the socio-economic situation of the Krishna-Godavari zone in the Coastal Andhra region, as well as in other production areas in Telangana and Rayalaseema regions. 'Panchadarakalasa' is one of the choicest juicy mangoes among millions of people in the state. Although 'Panchadarakalasa' originated in Krishna-Godavari zone, it is well adapted and produced in all the three eco-geographical regions (Coastal Andhra, Telangana and

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Rayalaseema) of the state. Although it is not distantly marketed and currently exported but an important demand exists in the local markets.

In India, most of the mango cultivars including juicy mangoes were selected from native mango populations 100 to 150 years ago. Since their initial selection, the identities and trueness-to-type of many cultivars have been obscured due to various biological and cultural factors. The mango peasants cultivate a great number of mango varieties including juicy mangoes for which genetic homogeneity has never been demonstrated. In India, a large proportion of the commercial mango orchards have been established asexually from grafts. In spite of this, there was a wide variation in fruit morphology, production and quality within and between the orchards of a cultivar. There were very few reports on the intracultivar variability of certain mango cultivars on the basis of morphological traits (Naik, 1948; Oppenheinmer, 1956; Naik, 1971) and molecular markers (Bally *et al.,* 1996; de Souza and Lima, 2004; Diaz-Matallana *et al.,* 2009; Singh *et al.,* 2009; Rocha *et al.,* 2012). This intracultivar variability may have serious economic ramifications for mango orchardists who unknowingly establish mango orchards with less productive genotypes of a cultivar. In addition, this intracultivar variability within cultivar mother blocks will further aggravate the variability within and between orchards of a cultivar. It has also been observed that there was locality dependent variation in the fruit appearance and quality of 'Panchadarakalasa' across the state (personal communication), which is somewhat troublesome for mango growers. Since fruit morphology and quality traits are quantitative in inheritance, this variation could probably be due to the influence of environment and/or genotype. To determine this both morphological and molecular analysis is necessary.

Several procedures for the identification and characterization of intracultivar variability in mango have been developed based on morphological or genetic traits. Traditionally, in India, intracultivar variability in mango has been characterized based particularly on fruit characteristics like size, shape and color (Naik, 1948; Oppenheinmer, 1956; Naik, 1971; Singh *et al.,* 2009). Morphological traits, being influenced by environmental parameters, are unreliable. Recently, intracultivar variability in mango has been characterized based on molecular markers like randomly amplified polymorphic DNA-RAPDs (Bally *et al.,* 1996; de Souza and Lima, 2004; Diaz-Matallana *et al.,* 2009) and inter simple sequence repeats-ISSRs (Singh *et al.,* 2009; Rocha *et al.,* 2012). From all these studies, different estimates for the degree of intracultivar genetic variation were obtained, reflecting the differences in the selected sets of accessions of cultivars of mango or marker systems. These researches suggest that there was considerable genetic variability within the cultivars of mango, which offer good scope for breeding within the cultivar for intracultivar improvement. Simple sequence repeats (SSRs) or microsatellites are widely used as a versatile tool in plant breeding programs because of their high ability for showing diversity among the genotypes. In mango, although microsatellites have been successfully used for intercultivar genetic diversity analysis (Duval *et al.,* 2005; Honsho *et al.,* 2005; Schnell *et al.,* 2005; Viruel *et al.,* 2005; Schnell *et al.,* 2006; Lopez *et al.,* 2009; Hirano *et al*., 2010; Wahdan *et al.,* 2011; Begum *et al*., 2012; Vasugi *et al*., 2012), no attempt has been made on intracultivar genetic diversity analysis. Morphological traits combined with molecular characterization are essential for better understanding of genetic diversity in mango (Singh *et al*., 2009; Begum *et al.,* 2012). Morphological descriptors and molecular markers were used to determine the intra-varietal diversity of oca (*Oxalis tuberosa* Mol.) varieties (Pissard *et al*., 2008).

The objective of this study was to assess the intracultivar genetic diversity of 'Panchadarakalasa' mango trees cultivated in the three eco-geographical regions of Andhra Pradesh, using morphological traits and SSR markers to identify whether there is variability in the plants grown in the state.

2. Materials and Methods

2.1. Eco-Geographic Survey

An eco-geographic survey was conducted by a team of scientists during May-June 2009. Following simple random sampling strategy, 3 mango mother blocks and 12 mango orchards possessing 'Panchadarakalasa' trees, spread over all the three eco-geographical regions and covering 7 districts, were selected. One 'Panchadarakalasa' tree was sampled from each of the 12 mango orchards and two mango mother blocks and two trees were sampled from one mango mother block (accession IDs starting with PK; PK Acc-1 to PK Acc-16) through simple random sampling (Table 1).

Table 1. Collection sites of 'Panchadarakalasa' mango accessions

Accession	Sampling unit	Collection site		
		Village	District	Region
PK Acc-1	Mother block, ARI	Rajendranagar	Rangareddy	Telangana
PK Acc-2	Mother block, FRS	Sangareddy	Medak	Telangana
PK Acc-3	Mother block, FRS	Sangareddy	Medak	Telangana
PK Acc-4	Orchard	Kathipudi	East Godavari	Coastal Andhra
PK Acc-5	Orchard	Kathipudi	East Godavari	Coastal Andhra
PK Acc-6	Orchard	Pithapuram	East Godavari	Coastal Andhra
PK Acc-7	Orchard	Pithapuram	East Godavari	Coastal Andhra
PK Acc-8	Orchard	Pithapuram	East Godavari	Coastal Andhra
PK Acc-9	Orchard	Bobbili	Vizainagaram	Coastal Andhra
PK Acc-10	Orchard	Bobbili	Vizainagaram	Coastal Andhra
PK Acc-11	Orchard	Bobbili	Vizainagaram	Coastal Andhra
PK Acc-12	Orchard	Anakapalli	VisakhapatnamCoastal Andhra	
PK Acc-13	Orchard	Anakapalli	VisakhapatnamCoastal Andhra	
PK Acc-14	Orchard	Errakoneru	East Godavari	Coastal Andhra
PK Acc-15	Orchard	Pondugala	Prakasam	Coastal Andhra
PK Acc-16	Mother block, HRS	AnantharajupetaKadapa		Rayalaseema

PK Acc = Panchadarakalasa accession; ARI= Agricultural Research Institute

FRS= Fruit Research Station; HRS= Horticultural Research Station

2.2. Fruit Sampling, Characterization, Evaluation and Analysis

Following simple random sampling strategy, ten tree-ripe fruits spread over all the sides of the tree canopy were collected from each of the 16 selected trees (accessions) of 'Panchadarakalasa'. Morpho-physiol-ogical characters of fruit samples were recorded following descriptors of mango (IPGRI, 2006). Fruit samples were evaluated for 9 quantitative traits like 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) and for 7 qualitative traits like fruit shape, skin colour of mature fruit, skin thickness, skin texture, quantity of fiber, pulp colour and eating quality. The mean data of each of the random fruit sample of 16 'Panchadarakalasa' accessions was analysed for 9 quantitative traits following 'Descriptive Statistics' for mean, standard error, standard deviation and coefficient of variation.

2.3. Leaf Sampling

Mature leaves of each of the sampled 'Panchadarakalasa' accession were collected for genetic characterization. The leaves were identified and maintained in styrofoam boxes with ice to be transported from the collection sites to the Institute of Biotechnology, Acharya N. G. Ranga Agricultural University (ANG RAU), Rajendranagar where they were frozen in liquid nitrogen. Then, they were stored in a freezer at -80 °C, until the time of the extraction of the genomic DNA.

2.4. DNA Extraction

The Genomic DNA from leaf samples was extracted by a modified Cetryl Trimethyl Ammonium Bromide (CTAB) method (Porebski *et.al.*, 1997).

2.5. Microsatellite Screening and Amplification

Polymerase chain reaction (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. 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, Cambridge, United Kingdom). Eleven alleles generated by the four polymorphic microsatellites (SSR-36, SSR-83, MngSSR-24 and MngSSR-26) were chosen for their clear pattern and high allele numbers to study diversity within the whole sample.

2.6. Data Analysis

The simple sequence repeat (SSR) bands were scored visually on the basis of their presence (1) or absence (0), separately for each accession of 'Panchadarakalasa' mango and each SSR primer. The sizes of fragments (molecular weight in base pairs) were estimated by using 100-bp ladder marker, which was run along with the amplified products. The scores obtained using all four polymorphic primers in the SSR analysis were then used for constructing a single matrix. Pair-wise difference matrix between accessions was determined using Jaccard's similarity coefficient. Data analysis was performed using the numerical taxonomy and multivariate analysis system (NTSYS)-pc version 2.1 computer program package (Rohlf, 2000). The coefficients were utilized to construct a dendrogram using the unweighted pair group of arithmetic means algorithm (UPGMA).

3. Results

3.1. Morphological Variability

From the results of mean, standard error, standard deviation and coefficient of variation (Table 2), it is evident that there was significant variation in 9 quantitative fruit traits among 16 accessions of 'Panchadarakalasa' under study.

Fruit length, width and thickness ranged from 7.20 to 10.00, 5.80 to 8.00 and 4.40 to 7.60 cm, respectively. Fruit weight and fiber length ranged from 154.00 to 350.00 g and 5.00 to 9.00 cm, respectively. The peel, pulp and stone contents ranged from 18.20 to 23.80%, 56.30 to 64.60% and 17.10 to 23.10%, respectively. Total soluble solids ranged from 15.00 to 19.80 °Brix. These ranges, refereed to evident morpho-physiological variation in fruit size and weight, fiber length, peel, pulp and stone contents and total soluble solids among 16 'Panchadarakalasa' accessions (Table 2).

There were some differences among 16 accessions of 'Panchadarakalasa' with respect to certain qualitative traits (Table 3) like fruit shape, color of skin of mature fruit, skin thickness, pulp color and eating quality.

3.2. Microsatellite Polymorphism

Of the 109 SSRs validated with total sample of 16 'Panchadarakalasa' accessions, only 20 SSRs could amplify and produce distinct and clear bands, while the remaining 89 SSRs could not amplify. Of these 20 amplified SSRs, 16 SSRs were monomorphic (Table 4), while the remaining 4 SSRs (SSR-36, SSR-83, MngSSR-24 and MngSSR-26) were polymorphic (Table 5). These four polymorphic SSR primers produced 11 bands in the total sample of 16 accessions. Four bands were common in all the cultivars, while the other 7 bands were polymorphic (63.63%). The allele size of polymorphic bands ranged from 130 to 245 bp. There was a wide variation in the range of polymorphic bands produced by the primers. The level of polymorphism present in the microsatellites was variable ranging from 2 to 4 alleles per SSR with an average of 2.75 alleles per SSR. Primers SSR-36 and SSR-83 produced the lowest number of polymorphic bands (2 bands), while primer MngSSR-26 produced the highest number of polymorphic bands (4 bands). In this study, the polymorphic information content (PIC) values ranged from 0.25 to 0.56 with moderate mean value of 0.42 for all loci indicating the moderate discriminatory power of the 4 polymorphic SSRs. Markers with high PIC values such as SSR-83 and SSR-36 could be effectively used in intracultivar genetic diversity studies of 'Panchadarakalasa'. Agarose gel showing SSR amplification profile of 'Panchadarakalasa' accessions by SSR-83 primer is depicted in Figure 1. The

Jaccard's similarity coefficient values calculated from SSR data ranged from 0.9-1.0. The pair-wise genetic dissimilarities ranged from 0.00 to 0.10 with a mean value of 0.05. The largest genetic distance calculated by the Jaccard's similarity coefficient was 0.10. Genetic similarity between accessions was in the range of 90-100%.

Table 2. Quantitative fruit characteristics of 'Panchadarakalasa' accessions

Accession	Fruit length (cm)	Fruit width (cm)	Fruit thickness (cm)	Fruit weight (g)	Fibre length (mm)	Peel (%)	Pulp (%)	Stone (%)	Total soluble solids(°Brix)
PK Acc-1	7.50	5.80	5.50	190.00	5.00	20.50	58.40	21.10	15.00
PK Acc-2	8.00	6.50	6.50	172.00	8.00	19.80	58.10	22.10	17.00
PK Acc-3	10.00	6.00	5.80	255.00	5.00	22.40	58.80	18.80	16.00
PK Acc-4	9.00	6.50	5.80	260.00	7.00	19.20	59.20	21.50	18.00
PK Acc-5	8.00	6.20	6.00	228.00	9.00	19.70	62.70	17.50	19.80
PK Acc-6	9.50	7.20	6.40	286.00	8.00	18.20	64.60	17.10	16.00
PK Acc-7	8.00	6.80	5.00	208.00	5.00	22.10	56.30	21.60	15.00
PK Acc-8	8.30	6.00	5.60	212.00	5.00	19.80	57.10	23.10	16.00
PK Acc-9	8.50	8.00	7.00	240.00	9.00	22.50	60.00	17.50	19.60
PK Acc-10	8.00	6.50	4.40	154.00	5.00	20.10	59.10	20.80	17.00
PK Acc-11	7.80	6.10	4.80	160.00	6.00	20.00	58.10	21.90	16.00
PK Acc-12	9.70	6.30	5.60	220.00	9.00	22.70	59.10	18.20	16.40
PK Acc-13	8.50	7.60	5.20	200.00	5.00	20.00	62.50	17.50	17.00
PK Acc-14	8.00	6.50	5.00	190.00	6.00	20.60	56.80	22.60	16.20
PK Acc-15	7.20	6.50	5.20	168.00	6.00	23.80	58.30	17.90	15.60
PK Acc-16	8.90	7.50	7.60	350.00	5.00	22.90	60.00	17.10	18.50
Mean	8.43	6.63	5.71	218.31	6.44	20.89	59.32	19.77	16.82
Standard Error	0.20	0.16	0.21	12.89	0.41	0.40	0.56	0.56	0.37
Standard Deviation	0.80	0.64	0.84	51.56	1.63	1.60	2.25	2.23	1.47
CV (%)	2.36	2.40	3.68	5.90	6.34	1.92	0.95	2.83	2.18

PK Acc = Panchadarakalasa accession

Table 3. Qualitative fruit characteristics of 'Panchadarakalasa' accessions

Genotype	Shape	Color of skin of matured fruit	Skin thickness	Skin texture	Quantity of fibre	Pulp color	Eating quality
PK Acc-1	Ovate	Yellowish green	Thick	Smooth	Abundant	Yellow	Good
PK Acc-2	Ovate	Yellowish green	Thin	Smooth	Abundant	Yellow	Good
PK Acc-3	Ovate	Yellowish green	Thin	Smooth	Abundant	Yellow	Good
PK Acc-4	Ovate	Yellowish green	Thin	Smooth	Abundant	Yellow	Good
PK Acc-5	Ovate	Yellowish green	Thin	Smooth	Abundant	Yellow	Good
PK Acc-6	Round	Yellowish green	Thin	Smooth	Abundant	Yellow	Good
PK Acc-7	Ovate	Yellowish green	Thin	Smooth	Abundant	Yellow	Good
PK Acc-8	Ovate	Dark yellow	Thin	Smooth	Abundant	Yellow	Good
PK Acc-9	Ovate	Dark yellow	Thin	Smooth	Abundant	Yellow	Good
PK Acc-10	Round	Yellow	Thin	Smooth	Abundant	Yellow	Good
PK Acc-11	Ovate	Yellow	Thin	Smooth	Abundant	Yellow	Good
PK Acc-12	Ovate	Yellow	Thin	Smooth	Abundant	Yellow	Good
PK Acc-13	Round	Yellow	Thin	Smooth	Abundant	Yellow	Good
PK Acc-14	Ovate	Yellow	Thin	Smooth	Abundant	Golden yellow	Good
PK Acc-15	Ovate	Yellowish green	Thin	Smooth	Abundant	Yellow	Good
PK Acc-16	Ovate	Green	Thin	Smooth	Abundant	Yellow	Excellent

PK Acc = Panchadarakalasa accession

Table 4. Characteristics of monomorphic microsatellite markers used in this study

Primer	Sequence 5'-3'	Annealing temperature (°C)	Size range of alleles (bp)
SSR-8	F: TTGATGCAACTTTCTGCC	53	200-224
	R: ATGTGATTGTTAGAATGAACTT		
SSR-15	F: TTTACCAAGCTAGGGTCA	52	201-226
	R: CACTCTTAAACTATTCAACCA		
SSR-16	F: GCTTTATCCACATCAATATCC	54	160-170
	R: TCCTACAATAACTTGCC		
SSR-20	F: CGCTCTGTGAGAATCAAATGGT	58	295-310
	R: GGACTCTTATTAGCCAATGGGATG		
SSR-39	F: TGTCTACCATCAAGTTCG	53	150-190
	R: GCTGTTGTTGCTTTACTG		
SSR-46	F: TCATTGCTGTCCCTTTTC	54	154–210
	R: ATCGCTCAAACAATCC		
SSR-52	F: AAAAACCTTACATAAGTGAATC	52	207
	R: CAGTTAACCTGTTACCTTTTT		
SSR-59	F: TTCTTTAGACTAAGAGCACATT	56	191
	R: AGTTACAGATCTTCTCCAATT		
SSR-61	F: AAAGATAGCATTTAATTAAGGA	52	206
	R: GTAAGTATCGCTGTTTGTTATT		
SSR-65	F: ATAGATTCATATCTTCTTGCAT	53	233
	R: TATAAATTATCATCTTCACTGC		
SSR-82	F: TCTGACCCAACAAAGAACCA	57	108-155
	R: TCCTCCTCGTCCTCATCATC		
SSR-84	F: TCTATAAGTGCCCCCTCACG	58	210-250
	R: ACTGCCACCGTGGAAAGTAG		
SSR-85	F: GCTTGCTTCCAACTGAGACC	58	229-269
	R: GCAAAATGCTCGGAGAAGAC		
SSR-88	F: CTGAGTTTGGCAAGGGAGAG	55	222-244
	R:TTGATCCTTCACCACCATCA		
MngSSR-14	F: TCATTAAGCTGTGGCAACCA	59	160-192
	R: CATTGCATAGATGTGGTCATT		
MngSSR-27	F: CGAAACCGACTGCCTATTTT	57	158-172
	R: CCATTAATAAAGTTGTGGCCA		

bp= Base pair

Table 5. Characteristics of polymorphic microsatellite markers used in molecular analysis of intracultivar variability in 'Panchadarakalasa' mango

Primer	Sequence (5'-3')	Annealing temperature(°C)	Size range of alleles(bp)	No. of alleles	PICvalues
SSR-36	F: CCTCAATCTCACTCAACA	55	215-245	2	0.43
	R: ACCCCACAATCAAACTAC				
SSR-83	F: AGCTATCGCCACAGCAAATC	57	190-213	2	0.56
	R: GTCTTCTTCTGGCTGCCAAC				
MngSSR-24	F: CGATGGACTTCATAAGAAGAG	58	150	3	0.45
	R: GCTAGCAGAATCACCTTGGTC				
MngSSR-26	F: ACCTTGGTCAGGACAAAATCC	60	135-150	4	0.25
	R: GACTTCATAAGAAGAGGCGTC				

PIC= Polymorphic information content ; bp= Base pair
Source: eurofins mwg/operon (www.Eurofinsdna.com)

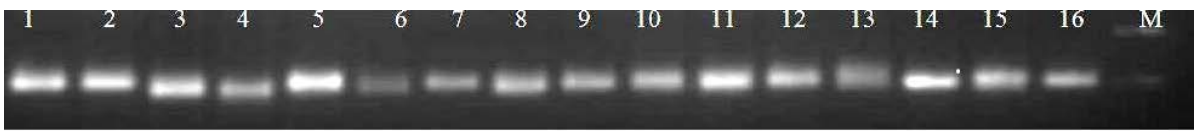

Figure 1. SSR profile of 'Panchadarakalasa' accessions by primer SSR-83. Lane 1 to16 denote 'Panchadarakalasa' accessions PK Acc-1to PK Acc-16 serially; M= 100 bp standard marker

3.3. Cluster analysis, genetic diversity, genetic similarity and geographical diversity

Pair-wise comparison was performed among all the accessions included in this study. The genetic relationship achieved by applying SSR markers is shown in Figure 2. The first major bifurcation in the dendrogram (Figure 2) separated the 16 accessions into four major clusters (cluster-I to cluster-IV). Clusters III and IV could be further divided into two sub clusters. Clusters I and II were solitary; consisting of only one accession each. PKAcc-14 from Errakoneru, PKAcc-11 from Bobbili branched out from the base, were found the most unique and divergent. Of these two divergent accessions, the accession PKAcc-14 occupied a unique position and was most diverse from rest of the accessions. 'Panchadarakalasa' accessions from one site of collection were more or less clustered together with few exceptions. The multiple accessions collected from certain collection sites like Sangareddy (PKAcc-2 and PKAcc-3), Pithapuram (PKAcc-7 and PKAcc-8) and Bobbili (PKAcc-9, PKAcc-10 and PKAcc-11) did not form separate groups or subgroups. Of the 2 accessions (PKAcc-2 and PKAcc-3) from the Fruit Research Station, Sangareddy, the first one (PKAcc-2) was grouped in sub cluster IVB along with three accessions PKAcc-1 from Rajendranagar, PKAcc-15 from Pondugala and PKAcc-16 from Anantharajupeta, while the later (PKAcc-3) was grouped solitarily in sub cluster IVA. Of the 3 accessions (PKAcc-6, PKAcc-7 and PKAcc-8) from Pithapuram, PKAcc-6 and PKAcc-8 grouped in subcluster IIIB, while PKAcc-8 grouped in a distinct solitary subcluster IIIA. Further, certain 'Panchadarakalasa' accessions from different collection sites were clustered together. The multiple accessions collected from different collection sites like Kathipudi (PKAcc-4 and PKAcc-5) and Anakapalli (PKAcc-12 and PKAcc-13) grouped together in the same subcluster IIIB.

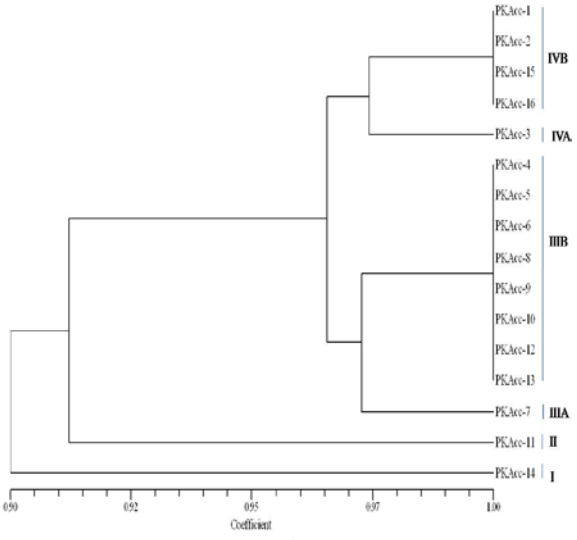

Figure 2. UPGMA dendrogram of 16 'Panchadarakalasa' accessions based on 4 SSR markers

A narrow range (0.9-1.0) of Jaccard's similarity coefficient was observed between the pairs of 16 accessions. The pair-wise genetic similarity between accessions was in the range of 90-100%; the lowest (90%) being observed between PKAcc-11 and PKAcc-14. Highest (100%) similarity was found between accessions PKAcc-1 (Rajendranagar), PKAcc-2 (Sangareddy), PKAcc-15 (Pondugala) and PKAcc-16 (Anantharajupeta). There was a pair-wise dissimilarity of only 2.85% between the two accessions (PKAcc-2 and PKAcc-3) collected from the same mother block, Fruit Research Station, Sangareddy. Of the 3 accessions collected from Pithapuram (PKAcc-6, PKAcc-7 and PKAcc-8), PKAcc-6 and PKAcc-8 had 100% similarity, while PKAcc-7 had only 2.7% dissimilarity with the other two accessions (PKAcc-6 and PKAcc-8). PKAcc-1, PKAcc-2, PKAcc-15 and PKAcc-16 were the accessions from different collection sites (Rajendranagar, Sangareddy, Pondugala and Anantharajupeta, respectively), which had 100% similarity. Two accessions each from Kathipudi (PKAcc-4 and PKAcc-5), Pithapuram (PKAcc-6 and PKAcc-8), Bobbili (PKAcc-9 and PKAcc-10) and Anakapalli (PKAcc-12 and PKAcc-13) had 100% similarity with the 4 highly polymorphic SSRs. There was a dissimilarity of 3.8% between the two distinct sub groups IVB (PKAcc-,1 PKAcc-2, PKAcc-15 and PKAcc-16) and IIIB (PKAcc-4, PKAcc-5, PKAcc-6, PKAcc-8, PKAcc-9, PKAcc-10, PKAcc-12 and PKAcc-13), exhibiting 100% pair-wise similarity within the groups.

3.4. Identification and differentiation of accessions

There were some loci which were present only in one accession; such loci may also be of use in the 'Panchadarakalasa' accessions differentiation. Here, comparison of the 7 alleles which were detected using 4 polymorphic SSRs revealed distinct banding patterns (alleles) to discriminate and identify the 'Panchadarakalasa' accessions. Microsatellites producing unique alleles for specific accessions of 'Panchadarakalasa' are given in Table 6. SSR-83 generated a unique allele of 200 bp for PKAcc-14. MngSSR-24 generated a unique allele of 150 bp for PKAcc-11. MngSSR-26 generated a unique allele of 140 bp for PKAcc-14.

Table 6. Microsatellites producing unique alleles for specific accessions of 'Panchadarakalasa'

Accession	SSR producing specific band	Size of the specific bands (bp)
PK Acc-11	MngSSR-24	150
PK Acc-14	SSR-83	200
PK Acc-14	MngSSR-26	140

4. Discussion

The peasants cultivate a great number of juicy cultivars and the juicy mangoes are one of the most significant mango businesses in Andhra Pradesh state, India. Juicy mango business in this state is based largely on 'Panchadarakalasa', one of the choicest juicy cultivars of mango with the great appreciation for fresh consumption (sucking type) due to its superior characteristics, such as sweet taste, pulp color and flavor. It has been under cultivation for more than a century in this state, which stands out as a major producer and supplier of this cultivar. In spite of possessing so many

virtues, this cultivar is troublesome for peasants because of its locality dependent variation in the fruit size, shape and quality resulting in heterogeneity in production and quality. Data on the regional polymorphism of this cultivar is scarce or non-existent. In this study, the genetic diversity of 'Panchadarakalasa' mango trees cultivated in all the three eco-geographical regions of the state was assessed based on morphological traits and microsatellite markers, to identify whether there is variability in the plants grown in the state.

Assessment of intracultivar diversity of mango has traditionally been made through morphological traits by several researchers (Naik, 1948; Oppenheinmer, 1956; Naik, 1971; Singh *et al*., 2009), where in intracultivar variability was found. Here also, analysis of 9 quantitative fruit traits following descriptive statistics indicated significant variability in fruit morpho-physiology among 16 accessions of 'Panchadarakalasa' under study. In addition, the data on 7 qualitative fruit traits also revealed considerable variation among total sample under study. On the whole, morphological analysis indicated considerable variability among the 'Panchadarakalasa' trees grown across the state. However, assessment of genetic variability based on phenotype has certain limitations, since most of the morphological characters of economic importance are often limited in number; have complex inheritance and dramatically influenced by environmental factors (Bernatzky and Tanksley, 1989). These results are suggesting both to focus our attention on the effects of the environment on the genotype and to consider, as a practical consequence, the importance of preserving these accessions found in different areas to truly preserve the richness of the germplasm of a cultivar.

In contrast, molecular markers based on DNA sequence polymorphisms are independent of environmental conditions and show a higher level of polymorphism. To confirm whether the phenotypic variability in this study is due to the influence of environment or genotype, molecular analysis with microsatellites was undertaken. In the present study, relatively small number of SSR bands (11) was amplified in a set of 16 'Panchadarakalasa' accessions using 4 primers. The pair-wise genetic dissimilarities ranged from 0.00 to 0.10 with a mean value of 0.05, thus showing a small degree of intercultivar genetic diversity at the DNA level. This dissimilarity value of 0.10 is much higher to that calculated from RAPD (10 primers) data among 15 accessions of 'Kensington Pride' cultivar of mango (0.05) by Bally *et al*. (1996) but much lower than that calculated from RAPD data (32 primers) among 25 accessions of 'Rosa' cultivar of mango (0.45) by de Souza and Lima (2004), indicating that the level of intracultivar genetic diversity in different cultivars of mango is dependent on the total sample size and number of molecular markers used. Amplified fragment length polymorphism (AFLP) analysis of the 160 phenotypically divergent 'Plavac Mali' vines (*Vitis vinifera* L.) has also revealed significantly lower polymorphism (Zdunic *et al*., 2009). In the present study, the genetic dissimilarity of 0.10 among accessions although small, the genetic differences among the sampled materials may affect some phenotypic character that is useful for the culture. This intracultivar variability in 'Panchadarakalasa' as evident from both the morphological and microsatellite analysis could probably be due to poor diffusion of uniform quality planting material to mango growers in the state. In many rural areas, where mango cultivation has a high potential, no fruit tree nurseries are available. Even the surveyed governmental nurseries could not meet the high demand of farmers for grafts. Although the use of homogenous, well documented plant material for mother blocks is highly recommended, some of the surveyed nurseries still use heterogeneous scions from commercial orchards for the development of grafts. This will definitely result in variable quality planting material.

In this study, the extent of diversity among accessions was studied in relation to their location and set of accessions with narrow genetic base developed from particular location were identified. From the perusal of the geographical locality of each accession (Table 1) and their clustering pattern (Figure 2), it could be inferred that the grouping of the accessions is not associated with their geographical location. The four groups formed do not present clear cut separation from the accessions related to the sampling locations, which also indicates low variability. In the present study, from the highest value of Jaccard's similarity coefficient of 1.00, it is evident that accessions with 100% of similarity were found, indicating the presence of duplicates. The presence of duplicates among the accessions studied explains the utilization of same scions to the formation of grafted trees. The accessions with a common ancestry and/or multiplied clonally from a single mother plant exhibited highest (100%) genetic similarity. The presence of 50% of the accessions in the same group (IIIB) may be explained by the prevalence of this accession in the Coastal Andhra region of the state and by the fact that this cultivar has been almost exclusively propagated clonally, which may be favoring multiplication of genetically similar plants, thus reducing genetic variability.

'Panchadarakalasa' mango is a clonally propagated fruit crop in Andhra Pradesh. Although the pair-wise genetic similarity between the accessions within the two distinct sub groups IIIB and IVB is 100% (Figure 2), there was a genetic dissimilarity of 3.8% between the two distinct sub groups IIIB and IVB, indicating the distinctness of two sub groups. The accessions PKAcc-1, PKAcc-2, PKAcc-15 and PKAcc-16 of sub group IVB could have been multiplied clonally from one mother tree and could be regarded as one distinct clone. Similarly, the accessions PKAcc-4, PKAcc-5, PKAcc-6, PKAcc-8, PKAcc-9, PKAcc-10, PKAcc-12 and PKAcc-13 of sub group IIIB might have been multiplied clonally from another mother tree and could be regarded as another distinct clone. Bally *et al*. (1996) observed absolutely low level of genetic dissimilarity coefficient of 0.05 among 15 accessions of 'Kensington Pride', a polyembryonic cultivar of mango using 10 RAPD markers and concluded that they are pure clones. de Souza and Lima (2004) also observed a genetic dissimilarity coefficient of 0.45 among 25 accessions of 'Rosa' cultivar of mango using RAPD markers and concluded that they are not pure clones. Rocha *et al*. (2012) while studying intravarietal heterogeneity in 'Uba', a polyembryonic cultivar of

mango using ISSR markers, no duplicates (clones) were found among the 102 accessions at the Zona da Mata of Minas Gerais state, Brazil. Molecular analysis with ISSR markers also revealed an intra-varietal genetic diversity attesting that oca (*Oxalis tuberosa* Mol.) varieties are not pure clones (Pissard *et al*., 2008). Our microsatellite data here also strongly suggest that the 'Panchadarakalasa' samples collected in Andhra Pradesh state do not belong to the same clone. Hence, it is proposed to use the term 'variety' instead of 'clone'.

When considering intracultivar breeding programs of 'Panchadarakalasa' mango, SSR profiling studies dealing with the structure of intracultivar diversity may give some insights about the selection of genetically distinct and elite accessions of this cultivar. Here, the pair-wise genetic dissimilarity of 0.00-0.10 among accessions revealed that some of the accessions like PK Acc-3, PK Acc-7, PK Acc-11 and PK Acc-14 could be considered as distinct genotypes exhibiting varied level of pair-wise dissimilarity with the rest of the accessions under study (Figure 2). It could be possible to select these accessions of 'Panchadarakalasa' adapted to the agro-climatic conditions of the cultivation region after further yield trials. Hence, it is necessary to test these genetically distinct new accessions *viz*., PK Acc-3, PK Acc-7, PK Acc-11 and PK Acc-14 under replicated yield trials to compare them against standard commercial juicy varieties (Peddarasam, Chinnarasam, Cherukurasam and Panchadarakalasa) to confirm their distinctiveness and superiority. There were no reports on the utilization of intracultivar selection for improvement of this 'Panchadarakalasa' mango. However, exploitation of natural variability through selection of superior clones of other commercial mango cultivars has been undertaken by several workers. Singh and Chadha (1981) located four superior clones from orchards of 'Dashehri', while Singh *et al*. (1985) isolated high yielding clones from 'Langra' orchards. Pandey (1998) studied different clones of this cultivar, *viz*., 'Alphonso of Behat' in Saharanpur (Uttar Pradesh), 'Alphonso Batli' of Kirkee, Pune (Maharashtra), 'Alphonso Punjab', 'Alphonso White' of North Kanara district of Karnataka, and observed that they vary from one another in more than one character. Therefore, it is also possible to select accession of 'Panchadarakalasa' mango tree adapted to the conditions of soil and climate of the cultivation region. Rocha *et al*. (2012) also studied intravarietal heterogeneity among the 102 accessions of 'Uba' mango using ISSR markers at the Zona da Mata of Minas Gerais state, Brazil and concluded that it is possible to select elite accession of 'Uba' mango tree adapted to the conditions of soil and climate of the cultivation region.

The identification of accessions of a cultivar is extremely important both for cultivation and intracultivar improvement of fruit crops. Accession identification based on morphological characteristics can be difficult and complicated. PCR technologies, such as microsatellite analysis, can readily and quickly identify accessions of a cultivar using young leaves. In this study, the markers SSR-83 and SSR-36 with high PIC values (Table 5) provide an opportunity for direct comparison and identification of different accessions independent of any influences. Some specific bands/loci observed in the 'Panchadarakalasa' accessions studied (Table 6) may be useful for accession identification. Further, the presence of specific loci indicates the genetic distinctness of the accessions under study. For example MngSSR-24 and MngSSR-26 produced a unique allele of 150 bp and 140 bp, respectively with PKAcc-11, while SSR-83 generated a unique allele of 200 bp with PKAcc-14. Presence of one particular band is possibly due to genomic recombination and may be of use in accession discrimination. This study has shown that even though the genome of mango is allotetraploid and relatively large, the microsatellite allelic patterns generated through PCR are capable of individualizing accessions.

A great proportion of the commercial orchards of mango in the state are raised asexually through grafts. The development and supply of mango grafts in the state is undertaken through clonal propagagation by the Private, Government and University nurseries. The variability (heterogeneity) and purity (homogeneity) of mother blocks of nurseries and commercial orchards can be analyzed through the utilization of fingerprints based on microsatellite markers. In the present study, microsatellite analysis revealed a pair-wise dissimilarity of only 2.85% between the two accessions PKAcc-2 and PKAcc-3 collected from the mother block, Fruit Research Station, Sangareddy indicating heterogeneity in mother block itself. Of the 12 individual accessions (PKAcc-4 to PKAcc-15), collected from the 12 individual orchards, the accessions PKAcc-4 to PKAcc-6, PKAcc-8 to PKAcc-10 and PKAcc-12 to PKAcc-13 of sub group IIIB, PKAcc-15 of sub group IVB, PKAcc-7 of sub group IIIA, PKAcc-11 of group II and PKAcc-14 of group I, indicate that there was heterogeneity among the orchards sampled. However, this study could not give any insight about the homogeneity and/or heterogeneity within the orchards sampled because of the fact that only one tree (accession) per orchard was sampled. For having better insight about the homogeneity and/or heterogeneity of orchards, multiple trees must be sampled from the individual orchards to be sampled. Further, more accurate DNA studies involving multiple samples from the individual mother blocks of nurseries across the state give correct information about the homogeneity of mother blocks. This information can be extremely useful to the mango nurseries for the correct choice (i.e., supported by more accurate intravarietal variability analysis) of the mango multiplication materials. This process can harmonize both quantity and quality of fruit production across the state. Development of an efficient and sustainable system for supplying interested farmers with high quality uniform planting material of the most elite form of the variety together with information on good management practices is urgently needed to harmonize 'Panchadarakalasa' mango production and quality in the state.

5. Conclusion

Classical analysis based on morphological fruit traits revealed considerable phenotypic variations among 16 accessions of 'Panchadarakalasa'. PCR-based SSR analysis confirmed genomic polymorphism in this

cultivar. Morphological markers combined with molecular characterisation are essential for better understanding of intravarietal heterogeneity of 'Panchadarakalasa' mango. Our results here strongly suggest that the 'Panchadarakalasa' samples collected in Andhra Pradesh state do not belong to the same clone. Hence, it is proposed to use the term 'variety' instead of 'clone'. Microsatellite markers have proven to be a valuable tool for identifying intracultivar heterogeneity in mango. If SSR diversity is combined with fruit and other important agronomic characteristics, performing the similar studies on the other 'Panchadarakalasa' accessions may lead to planning of a better intracultivar breeding program in the state.

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References

Bally ISE, Graham GC and Henry RJ. 1996. Genetic diversity of Kensington mango in Australia. *Aust J Exp Agric*, **36**: 243-247.

Begum H, Reddy MT, Malathi S, Reddy BP, Arcahk S, Nagaraju J and Siddiq EA. 2012. Molecular analysis for genetic distinctiveness and relationships of indigenous landraces with popular cultivars of mango (*Mangifera indica* L) in Andhra Pradesh, India. *Asian Aust J Plant Sci Biotechnol*, **6 (1):** 24-37.

Bernatzky R and Tanksley SD. 1989. Restriction fragments as molecular markers for germplasm evaluation and utilization. In: Brown AHD, Frankel OH, Marshel DR and Williams JT (Eds.), **The Use of Plant Genetic Resources**, Cambridge University Press, New York, pp. 353-362.

de Souza VAB and Lima PSC. 2004. Genetic variability in mango genotypes detected by RAPD markers. *Acta Hort*, **645**: 303-310.

Diaz-Matallana M, Schuler-Garcia I, Ruiz-Garcia M and Hodson-de-Jaramillo E. 2009. Analysis of diversity among six populations of Colombian mango (*Mangifera indica* L cv. Hilacha) using RAPDs markers. *Electron J Biotechnol*, **12**: 1-8.

Duval MF, Bunel J, Sitbon C and Risterucci AM. 2005. Development of microsatellite markers for mango (*Mangifera indica* L). *Mol Ecol Notes*, **4**: 824-826.

Hirano R, Htun-Oo T and Watanabe KN. 2010. Myanmar mango landraces reveal genetic uniqueness over common cultivars from Florida, India, and Southeast Asia. *Genome*, **53**: 321-330.

Honsho C, Hishiyama K, Eiadthong S and Yonemori K. 2005. Isolation and characterization of new microsatellite markers in mango (Mangifera indica). *Mol Ecol Notes*, **5**: 152-154.

IPGRI. 2006. Descriptors for Mango (Mangifera indica L). International Plant Genetic Resources Institute, Rome, Italy.

Karihaloo JL, Dwivedi YK, Archak S and Gaikwad AB. 2003. Analysis of genetic diversity of Indian mango cultivars using RAPD markers. *J Hortic Sci Biotech*, **78**: 285-289.

Knight RJR. 1980. Origin and world importance of tropical and subtropical fruit crops. In: Nagy S and Shaw PE (Eds.), **Tropical and Subtropical Fruits,** AVI, Westport, CT, USA.

Lopez DG, Delgado SH, Paz MG, Leor ENZ, Figueroa MS and Perez NM. 2009. Genetic analysis of mango landraces from Mexico based on molecular markers. *Plant Gen Res*, **7**: 244-251.

Naik KC. 1948. Improvement of mango (Mangifera indica L) by selection and hybridization. *Indian J Agric Sci*, **18 (1):** 35-41.

Naik KC. 1971. Mango improvement. *Andhra Agric J*, **18 (6):** 221-222.

Oppenheimer C. 1956. Study tour report on subtropical fruit growing and research in India and Ceylon Special Bulletin No. 3, State of Israel, Ministry of Agriculture, Agicultural Research Station, Rehovat, Israel, October.

Pandey SN. 1998. Mango cultivars. In: Srivastav RP (Ed.), **Mango Cultivation**, International Book Distributing Company, Lucknow, India, pp. 39-99.

Pissard A, Rojas-Beltran JA, Faux A-M, Paulet S and Bertin P. 2008. Evidence of intra-varietal genetic variability in the vegetatively propagated crop oca (*Oxalis tuberosa* Mol.) in the Andean traditional farming system. *Plant Syst Evol*, **270 (1-2):** 59-74

Poovarodom S, Haruenkit R, Vearasilp S, Namiesnik J, Cvikrova M, Martinincova O, Ezra A, Suhaj M, Ruamsuke P and Gorinstein S. 2010. Comparative characterization of durian, mango and avocado. *Food Sci Technol*, **45**: 921-929.

Porebski S, Bailey G and Baum BR. 1997. Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant Mol Biol Rep*, **15**: 8-15.

Rajwana IA, Khan IA, Malik AU, Saleem BA, Khan AS, Ziaf K, Anwar R and Amin M. 2011. Morphological and biochemical markers for varietal characterization and quality assessment of potential indigenous mango (Mangifera indica L) germplasm. *International J Agric Biol*, **13**: 151-158.

Rocha A, Salomao LCC, Salomao TMF, Cruz CD and de Siqueira DL. 2012. Genetic diversity of 'Uba' mango tree using ISSR markers. *Mol Biotechnol*, **50 (2):** 108-113.

Rohlf FJ. 2000. NTSYS-pc: Numerical taxonomy and multivariate analysis system, version 2.1 Exeter Software, Setauket, New York, USA.

Schnell RJ, Brown SJ, Olano CT, Meerow AW, Campbell RJ and Kuhn DN. 2006. Mango genetic diversity analysiand pedigree inferences for Florida cultivars using microsatellite markers. *J Am Soc Hortic Sci*, **13**: 214-224.

Schnell RJ, Olano CT, Quintanilla E and Meerow AW. 2005. Isolation and characterization of 15 microsatellite loci from mango (Mangifera indica L) and cross-species amplification in closely related taxa. *Mol Ecol Notes*, **5**: 625-627.

Singh H and Chadha KL. 1981. Improvement of Dashehari by clonal selection National Symposium on Tropical and Sub-tropical Fruit crops. Horticultural Society of India, Bangalore, p 5 (Abstr).

Singh RN, Gorakh S, Rao OP and Mishra JS. 1985. Improvement of Banarsi Langra through clonal selection. *Prog Hort*, **17**: 273-277.

Singh S, Gaikwad AB and Karihaloo JL. 2009. Morphological and molecular analysis of intracultivar variation in Indian mango (Mangifera indica L) cultivars. *Acta Hortic*, **829**: 205-212.

Vasugi C, Dinesh MR, Sekar K, Shivashankara KS, Padmakar B and Ravishankar KV. 2012. Genetic diversity in unique

indigenous mango accessions (Appemidi) of the Western Ghats for certain fruit characteristics. *Curr Sci*, **103 (2):** 199-207.

Viruel MA, Escribano P, Barbieri M, Ferri M and Hormaza JI. 2005. Fingerprinting, embryo type and geographic differentiation in mango (*Mangifera indica* L, Anacardiaceae) with microsatellites. *Mol Breed*, **15**: 383-393.

Wahdan MT, Abdelsalam AZ, El Naggar AA and Hussein MA. 2011. Preliminary horticultural studies to describe and identify two new Egyptian mango strains using DNA fingerprint. *J Am Sci*, **7 (2):** 641-650.

Williams JGK, Kubbelik A, Livak KJ, Rafiski JA and Tinjey SV. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res*, **18**: 6531-6535.

Zdunic G, Maletic E, Vokurka A, Kontic JK, Pezo I and Pejic I. 2009. Intravarietal variability of the cultivar 'Plavac Mali' (V*itis vinifera* L.). *Acta Hort*, **827**: 203-206.