Indian J. Plant Genet. Resour. 28(1): 153-160 (2015)
DOI 10.5958/0976-1926.2015.00019.4

Genetic Diversity Studies in Certain Indigenous Mango (*Mangifera indica* L) Varieties

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(Received: 14 January 2015; Revised: 12 February 2015; Accepted: 13 March 2015)

The large seedling diversity of mango has not been fully evaluated. The evaluation of seedling diversity was carried out for morphological traits in the Chittoor area of Andhra Pradesh in India. The statistical analysis carried out for fruit characteristics showed significant differences among the varieties for various fruit characteristics. Molecular characterization was carried out using microsatellite markers. Most of the indigenous varieties from Kalepalli region are grouped in the same cluster. It is interesting to note that the morphological characterization and molecular characterization follow almost the same pattern suggesting the genetic control of these fruit characters. The study shows that the diversity observed within a geographic region is derived from the varieties that are being grown in that region. The promising seedling varieties selected with desirable traits would help the farmer in benefit sharing as and when they are registered. In addition it would help in 'on farm conservation' and use of these varieties in crop improvement programmes.

Key Words: Characterization, Clusters, Conservation, Diversity, Landraces

Introduction

The mango (*Mangifera indica* L) originated in northeastern India, the Indo-Myanmar border region and Bangladesh (Chittagong Hill tract), where it is still found in feral state, with very small fruits and it is under cultivation in India for at least 4000 years with over 1000 varieties recognized (Mukherjee, 1953). Almost all of them are selections made from naturally occurring open-pollinated seedlings. Seven centers of diversity have been recognized in India (Yadav and Rajan, 1993). In the Peninsular Indian region, Chittoor is one of the main centers of mango growing, wherein several indigenous landraces were observed under cultivation. These indigenous varieties are known as 'naati' types in local language. Most of these seedling types are regular bearers having desirable traits, which were located by survey under the "UNEP/GEF project on "Conservation and Sustainable Use of Cultivated and Wild Tropical Fruit Diversity: Promoting Sustainable Livelihoods, Food Security and Ecosystem Services". The genetic variability studies were conducted utilizing these seedling originated naati types utilizing microsatellite markers. Molecular tools including both nuclear marker and cytoplasmic markers are currently playing major role in population studies, phytogeography, mapping, parentage analysis. Many different molecular markers have been used in mango for cultivar identification, such as RAPD (Rajwana *et al.*, 2008; Marcela *et al.*, 2009), ISSR (Sagar *et al*., 2007; Pandit *et al*., 2007), AFLP (Yamanaka *et al*., 2006), and SSR (Schnell *et al*., 2005, 2006) for clonal identification and for roughly estimating genetic relationships among genotypes. *Mangifera* germplasm has been collected and analyzed using simple sequence repeat (SSR) markers recently by Dillon *et al*. (2013, 2014). Microsatellites has greater importance due to their abundant occurrence throughout the genome of all the eukaryotes due to their co-dominant nature high level polymorphism because of it variation in repeat lengths (Ravishankar *et al*., 2011). Hence, a study was undertaken to assess genetic variability of naati (seedling) mango varieties in Chittoor, Andhra Pradesh of India for seedlings of desirable traits for further commercialization

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or for home garden conditions or for crop improvement could be selected.

Materials and Methods

The Polakala, Talupulapalle and Bangarupalyam regions of Chittoor district in Andhra Pradesh has a dry and hot climate with low temperatures of 12-18° C in winter and high temperatures of 38-46° C during summer and an average rainfall of 918.1 mm/year. The soil of the region is generally red sandy loam and in some places clayey soils are also seen. A total of 44 naati types spreading across the villages of three communities belonging to 21 farmers were identified through survey. The genetic diversity studies were conducted utilizing these 44 *naati* (indigenous) types.

The 44 indigenous varieties were evaluated for fruit characteristics *viz.,* fruit weight, fruit length, fruit width, skin weight, stone weight, TSS and pulp percentage using 'Bioversity International Descriptors (2006). Evaluation was carried out by taking fruit samples in five replicates. The variance analysis (ANOVA) was carried out using RCBD. The total carotenoids were determined colorimetrically as per the method described by AOAC (1995). The clustering was carried out using the UPGMA method.

The molecular characterization was carried out using SSR markers, which comprised of DNA isolation and polymorphism analysis. The genetic analysis was carried out based on the data generated. Total genomic DNA was extracted from the leaf material using the modified CTAB method (Ravishankar *et al.,* 2000). The concentration of DNA was determined by spectrophotometer at 260nm. The integrity was determined by agarose gel electrophoresis (0.8%).

PCR and Polymorphism Analysis

The fluorescence based PCR method proposed by Schuelke (2000) was used to amplify the microsatellites in a quick, accurate and efficient manner. PCR amplification was performed in a 20µl volume containing 75-100 ng pomegranate DNA, Taq Buffer 10X (Tris with 15mM $MgCl_2$, pH-9)-2.85µl, 1mM dNTPs- 3.5 µl, locus labeled forward specific primer (5µM)- 2.0µl, reverse primer (5µM)- 2.0µl, Nuclease free water-5.65µl and 1.0 unit of Taq DNA polymerase. PCR was performed on Life Pro Thermocycler with the following temperature profile: 94°C for 2 min followed by 35 cycles at 94°C for 30s, 30s at optimal annealing temperature of 55°C and 72°C for 1min. A final extension reaction was allowed to proceed at 72°C for 5min. Amplified products were initially separated on 3% agarose gel for confirmation of the amplification. These samples were separated on the automatic 96 capillary automated DNA Sequencer.

The raw data generated was analyzed and compiled using Peak Scanner V1.0 software for detecting the alleles**.** This produces a size curve based on the known size standard fragments, with the help of which the unknown fragment sizes are determined. The results obtained were used for genetic analysis using Cervus 3.0 software (Kalinowski *et al*., 2007) for determining the number of alleles, observed heterozygosity (Ho), expected heterozygosity (He) and Polymorphic Information Content (PIC). The genetic similarity and genetic distance between 44 genotypes was estimated by the Neighbour Joining method using Darwin software (Perrier *et al*., 2003; Perrier and Jacquemoud-Collet., 2006).

Results and Discussion

Chittoor in Andhra Pradesh is one the main centers of mango growing in the peninsular region of India. The survey carried out under the UNEP/GEF project "Conservation and Sustainable Use of Cultivated and Wild Tropical Fruit Diversity: Promoting Sustainable Livelihoods, Food Security and Ecosystem Services" resulted in the identification of 44 *naati* varieties (indigenous) having varied size, shape and color (Fig.1), which were utilized for conducting genetic diversity studies. The statistical analysis carried out showed significant differences among the varieties for fruit characteristics (Table 1) thereby pointing out that the varieties are different. The evaluation of the indigenous varieties showed that the variety Thumbavaripalli Munirathnam Reddy Manoranjitham has high total carotenoids (29.0 mg/100g) and high TSS (24^0Brix). Another naati variety P. Reddyvaripalli V. Ramamoorthy Reddy Naati 3 also showed high total carotenoids (26.44 mg/100g). The pulp recovery in most of the seedling types was observed to vary between 54 to 98%, with most of the varieties having > 65%. Selection of seedlings in Israel has resulted in new mango cultivars with excellent peel color *viz.*, 'Naomi' and Tango (Lavi *et al.,* 1997). The seedling (*naati*) types observed in this study *viz.*, TSAN4 and VRR3 were observed to have excellent peel color, indicating that good diversity exists for desirable traits in the seedlings. In a similar study carried out with seedling progenies in Goa,

Fig. 1. Variation for shape, size and colour in the *Naati* types of Chittoor

Nagabhushan and Mathew (1994) reported excellent peel colour in certian seedling type's *viz*., Mankurad, Salcete Mussarat, Malgesh, Xavier, Udgo, Saccrchi, Colaco, Furtado and others. Uttar Pradesh region of India is one of the diversity centres for seedling mango types. The evaluation of seedling types carried out by Teaotia and Srivastava (1979) showed that the variety Gaurjit is a good sucking type and the variety Kare-Ka-Fazli is a very high yielder having good fruit size. In the Chittoor seedling types also it was noticed that they are regular and heavy bearers. Hence, the evaluation and conservation of '*naati'* seedlings can be a very viable method in the improvement of a heterozygous crop like mango. The diversity analysis carried out using morphological characteristics showed two main clusters (Fig. 2). In the first cluster the commercial varieties Kalapadi, Sindhura, Khuddus, Athi Madhuram and Alphonso are grouped with the naati varieties of Talupulapalli. The varieties TBRBR, and TBRLB, both of which have good peel colour is grouped with the commercial variety Khuddus, which has attractive peel colour. The other coloured variety Totapuri is grouped with the coloured variety RVRRN3, which also is similar to Totapuri in shape. The interesting feature is that probably the coloured varietis are descendants of today's commercial variety Khuddus. The clustering to a large extent is based on the location of the seedling in a particular region. The seedlings of Talupulapalli are grouped in the same cluster to a large extent and that of Palamakulapalli and Reddyvaripalli are also grouped in the same cluster. In a study on morphological characterization vis-à-vis molecular characterization, Vasugi *et al.* (2013) have opined that morphological characterization of quantitative characteristics would follow the pattern of molecular characterization in mango.

The diversity analysis carried out from the molecular data showed 3 main clusters (Fig. 2). It can be seen from the diagram that varieties from Talupulapalli and Gandlapalli, which are from nearby places are grouped in the same cluster to a large extent. Similarly majority of the indigenous varieties from Kalepalli are grouped in the same cluster. It also shows that some of the commercial varieties like Neelum are grouped with these varieties, which shows that they may be open pollinated seedlings of Neelum. The variety Khuddus, a colored variety is grouped with Talupulapalli varieties, which also have good peel color. This gives an indication that these seedling varieties would have developed from the same pedigree as Khuddus. The phylogenetic tree shows Gandlapalli Sreeramulu Reddy Naati 5, Talapulapalli Sreeramulu Pillai Naati 2 are closely related varieties. The two indigenous colored varieties RVRRN3 and

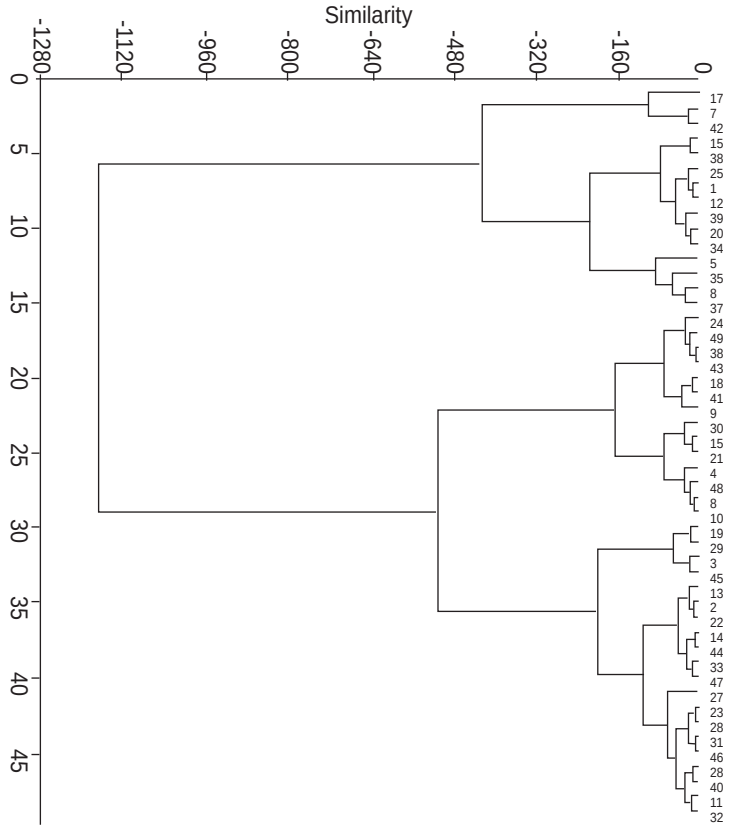

Fig. 2. Cluster diagram based on morphological characteristics

TBRT7 are grouped together. Similar to the evaluation of naati seedlings the 'Appemidi' types from the Western Ghats regions were studied by Vasugi *et al.* (2012), who concluded that genotypes belonging to different geographic region might have evolved from the existing mango gene pool from which they were selected by local people to domesticate them in different areas for cultivation. Karibasappa *et al.* (1999) observed by canonical analysis and cluster analysis eleven clusters in sixty-nine genotypes of mango. They concluded that geographic diversity might not necessarily be related with genetic diversity. Ravishankar *et al.* (2000), studied the genetic diversity in eighteen commercial varieties of mango grown in India using RAPD analysis, they observed two major groups; one consisting of northern, eastern and western varieties, another consisting of southern cultivars, their study also indicated that the variety Kesar from western region of India was associated with Neelum and Rumani. This study showed that genetic diversity within a particular region. Karihaloo *et al.* (2003) in a similar study by RAPD analysis in 29 Indian mango cultivars found that the varieties irrespective of the number of embryos they possessed were grouped together based on the region and genetic diversity existed within the regions. The population in one region need not follow the same pattern as that of the other.

The success of molecular markers in characterizing and analyzing genetic diversity in mango has been demonstrated by a number of studies. Valdomiro and Paulo Sarmanho (2004) used RAPD markers to detect genetic polymorphism in the mango germplasm, suggesting that this technique may be useful tool for germplasm evaluation and management and also for directing crossings in the breeding programmes. SSR analysis has great potential for mango improvement and can be performed for variety identification, validation of parentages, and estimation of genetic variation in existing populations and characterization of intra-cultivar diversity. Ravishankar *et al.* (2011) observed that microsatellites have greater importance due to the abundant occurrence throughout the genome of all the eukaryotes, their co-dominant nature, high level polymorphism as a result of the variation in repeat lengths. Begum *et al*. (2013) observed that the PIC value provides approximation by taking into account the number of alleles at a locus and the relative frequencies

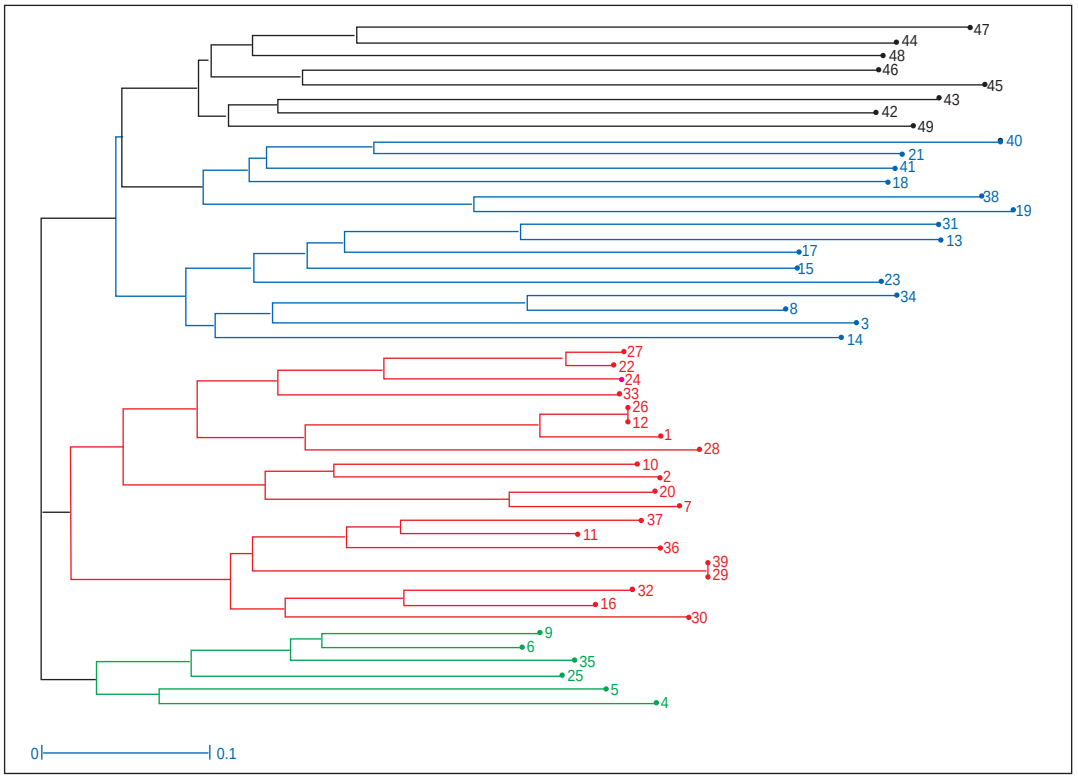

Fig. 3. A dendrogram based on NJ method, summarizing the data on differentiation between 44 naati varieties according to microsatellite analysis

1. TSAN4	2.TSAN6	3.TSAN 8	4.TBRLB	5.TBRN1	6.TBRD	7.TBRT	8.TBRN2	9.TBRBR
10.TBRC	11.TSPN1	12.TSPN2	13.KRRN2	14.KSCN	15.KPSN1	16.KGCN1	17.KRRN3	18.RKRRN1
19.RKRRN2	20.RVRRN 3	21.RVRRN2	22.GGNK	23.GPJN 2	24.GSRN3	25.GSRN4	26.GSRRN5	27.GSRRN1
28.GSRRN2	29.THKSN 3	30.THMRM	31.THMRP	32.THKSRN2	33.THKSRN4	34.PKRN1	35.PKRN2	36.PKRNGB
37.BVRRN1	38.BVRRN2	39.BVRRG	40.GKSRN1	41.GKSRN2	42.Totapuri	43.Neelum	44.Sindhura	
45.Chakkaraguthulu	46. Kalapadi	47.Khuddus	48.Atimadhuram	49.Alphonso				

of these alleles. In this study the polymorphic information content (Table 2) ranged from 0.667(MiIIHR23) to 0.869 (MiIIHR26), average PIC value of 0.76975 for all loci indicated the moderate discriminatory power of the 8 polymorphic SSRs. Marker with high PIC value such as MiIIHR26 and MiIIHR17 could be effectively used in intra-cultivar genetic diversity studies. The observations made here are in agreement with the studies conducted in mango (Duval *et al*., 2005; Honsho *et al*., 2005).

Microsatellite markers have proven useful in assessing intra-cultivar genetic diversity and identifying accessions of 'Chittoor' cultivar. Various mechanisms are responsible for genomic polymorphism between the accessions when two or more different phenotypes exist in the same population of a species and the occurrence of more than one form might be due to single nucleotide changes, deletions, insertions and variable numbers of simple sequence are several different mechanisms responsible for genomic polymorphism between accessions (Begum *et al*., 2013). The 8-labeled SSR markers employed in this study detected 97 alleles in 44 genotypes. The number of alleles for each locus ranged from 9 to 19, with a mean number of alleles per locus being 12.13. Observed heterozygosity (Ho) and expected heterozygosity (He) for each locus ranged from 0.364 to 0.674 and 0.693 to 0.889 respectively (Table 2). The data obtained is useful for working out the diversity and would help in choosing the parents for a hybridization programme. The high heterozygosity that is present in mango comes in the way of clearly pointing out the lineage of the existing naati varieties (indigenous).

Diversity Studies and Core Collection

In a highly heterozygous crop like mango wherein large variability is observed for several traits, core collection becomes extremely important. The wider the range of choice a breeder will have in selecting the appropriate kind of diversity, the better will be the chances for his

Table 1. Fruit characteristics of the Naati mango varieties

S. No.	Local Name	Code Name	Fruit weight (g)	Fruit length (cm)	Fruit width (cm)	Fruit thickness (cm)	Skin weight (g)	Stone weight (g)	TSS (°Brix)	Pulp (%)	Carotenoids mg/100 g
1	Talapulapalle Sreeramulu Achari Naati 4	TSAN4	370.68	9.44	8.30	7.30	32.57	33.83	18.3	82.09	11.91
2	Talapulapalle Sreeramulu Achari Naati 6	TSAN6	201.82	8.84	6.90	5.96	34.68	38.28	18.98	63.85	-
3	Talapulapalle Sreeramulu Achari Naati 8	TSAN 8	84.00	5.76	5.26	4.74	14.88	23.63	17.78	54.15	-
4	Talapulapalle Babi Reddy Lalbaba	TBRLB	268.09	8.90	7.66	6.90	63.05	32.57	16.8	64.33	6.99
5	Talapulapalle Babi Reddy Naati 1	TBRN1	542.00	14.83	9.80	7.68	33.73	52.7	26.08	84.05	-
6	Talapulapalle Babi Reddy Dilpasand	TBRD	302.70	11.28	7.66	6.36	42.48	41.65	18.46	72.21	6.85
7	Talapulapalle Babi Reddy Thorappadi Variant 2	TBRTV2	662.05	14.08	9.16	8.36	91.71	46.82	19.04	79.08	21.04
8	Talapulapalle Babi Reddy Naati 2	TBRN2	589.07	13.70	9.08	7.80	81.39	51.89	20.33	77.38	9.75
9	Talapulapalle Babi Reddy Bogam Rangasani	TBRBR	369.32	10.34	7.82	7.10	50.85	52.07	20.32	72.13	4.57
10	Talapulapalle Babi Reddy Chittithotha	TBRC	327.68	11.10	7.84	6.94	46.54	44.53	17.83	72.21	5.87
11	Talapulapalle Sreeramulu Pillai Naati 1	TSPN1	129.76	9.00	5.10	4.32	28.86	19.78	17.96	62.51	5.46
12	Talapulapalle Sreeramulu Pillai Naati -2	TSPN2	396.98	10.52	8.56	7.62	44.25	43.08	16.1	78.00	4.98
13	Kalepalle Rajendra Reddy Naati 2	KRRN2	238.43	8.52	7.00	6.48	42.43	25.07	18.82	71.69	15.1
14	Kalepalle Subramanyam Chetty Najoka	KSCN	195.87	9.34	6.48	5.64	23.83	32.16	18.32	71.41	-
15	Kalepalli P Subramanyam Naati 1	KPSN1	243.17	9.83	7.20	6.13	35.10	29.97	15.9	73.24	-
16	Kalepalli P Govinda Chetty Naati 1	KGCN1	444.86	12.84	8.74	7.68	81.80	55.26	20.16	69.19	4.54
17	Kalepalli Rajendra Reddy Naati 3	KRRN3	715.08	13.80	9.84	8.48	107.25	41.38	19.54	79.22	-
18	P. Reddyvaripalle K Rajasekar Reddy Naati 1	RKRRN1	362.09	10.73	7.98	7.25	61.11	32.27	18.9	74.21	18.51
19	P. Reddyvaripalle K Rajasekara Reddy Naati 2	RKRRN2	114.26	7.53	5.23	4.87	19.07	24.67	16.33	61.72	14.78
20	P. Reddyvaripalli V Ramamoorthy Reddy Naati 3	RVRRN 3	421.37	12.46	8.56	7.32	69.15	38.36	13.64	74.49	26.44
21	P. Reddyvaripalle V Ramamurthy Reddy Naati -2	RVRRN2	245.18	8.24	7.12	6.44	33.99	35.37	21.52	71.71	9.52
22	Gandlapalle K Gurappa Chetty Naati Khader	GGCNK	214.01	8.66	6.44	5.76	33.73	34.07	18.74	68.32	10.71
23	Gandlepalle P Jayachandra Reddy Naati 2	GPJN 2	187.13	8.74	6.32	5.60	23.94	26.05	20.24	73.28	-
24	Gandlapalle Sreeramulu Reddy Naati 3	GSRN3	280.07	9.04	7.24	6.94	54.72	34.16	17.76	68.27	4.89
25	Gandlepalle Sreeramulu Reddy Naati 4	GSRN4	393.25	13.00	7.64	6.98	37.00	27.37	18.58	83.63	-
26	Gandlapalle Sreeramulu Reddy Naati 5	GSRRN5	148.08	10.10	5.74	4.16	23.99	23.1	17.26	68.2	8.15
27	Gandlapalle Sreeramulu Reddy Naati 1	GSRRN1	157.84	7.86	6.44	5.34	45.68	37.48	15.28	47.32	2.73
28	Gandlapalle Sreeramulu Reddy Naati 2	GSRRN2	178.23	10.00	5.83	5.23	26.84	27.18	13.9	69.69	7.5
29	Thumbavaripalle K. Subramanyam Reddy Naati 3	THKSRN 3	128.24	8.42	5.52	4.60	15.24	14.21	21.32	77.04	5.5
30	Thumbavaripalle Munirathnam Reddy Manoranjitham	THMRM	254.92	9.00	7.64	6.38	65.74	40.36	24.04	58.38	29.4
31	Thumbaripalle Munirathnam Reddy Punasa	THMRP	188.69	7.70	6.65	6.20	40.30	21.66	20.68	67.17	11.24
32	Thumbavaripalle K Subramanyam Reddy Naati 1	THKSRN1	490.49	12.00	8.60	7.60	45.67	37.47	19.85	98.32	3.74
33	Thumbavaripalle K Subramanyam Reddy Naati 2	THKSRN2	148.72	8.35	6.00	4.90	38.00	27.06	21.15	56.25	4.44
34	Thumbavaripalle K Subramanyam Reddy Naati 4	THKSRN4	186.86	9.08	6.44	5.98	37.51	25.17	21.54	66.46	39.5
35	Palamakulapalle K Ravindranath Naati 1	PKRN1	414.04	9.93	9.00	7.70	66.39	52.62	12.88	71.26	23.16
36	Palamakulapalle K Ravindranath Naati 2	PKRN2	549.51	14.87	8.40	7.80	117.22	82.19	13.73	63.71	12.93
37	Palamakulapalle K Ravindranath Naati 3	PKRN3	274.19	8.25	7.85	7.00	35.92	43.72	18.05	70.96	8.65
38	Palamakulapalle K Ravindranath Naati 4	PKRN4	168.86	7.55	6.45	5.95	31.45	27.17	18.6	65.29	-
39	Palamakulapalle K Ravindranath Green Baneshan	PKRNGB	464.41	12.97	8.43	7.50	77.57	70.75	8.77	68.06	8.99
40	Baitpalle V Ranga Reddy Naati 1	BVRRN1	530.73	11.63	9.10	7.98	80.57	47.01	14.08	75.96	5.67
41	Baitpalle V Ranga Reddy Naati 2	BVRRN2	324.34	9.55	8.25	6.63	44.81	41.54	16.3	73.38	10.51
42	Baitpalle V Ranga Reddy Gadiyaram	BVRRG	422.51	12.08	7.88	7.20	52.95	56.75	13.93	74.2	-
43	Gudipalle K Surendra Reddy Naati 1	GKSRN1	177.88	9.44	5.88	5.28	23.35	36.54	19.6	66.34	11.63
44	Gudipalle K Surendra Reddy Naati 2	GKSRN2	344.38	10.72	8.18	7.06	65.24	44.7	20.66	68.08	6.3

Table 2. Characteristics of the 8 microsatellite markers with repeat motif, number of alleles, Observed Heterozygosity (Ho), Expected Heterozygosity (He) and Polymorphic Information Content (PIC)

Locus	Primer (5'-3')	Repeat motif	Number of alleles	Allele size range (bp)	He	Ho	PIC
MiIIHR17	F: GCTTGCTTCCAACTGAGACC R: GCAAAATGCTCGGAGAAGAC	$(GT)_{13}$ GAGT$(GA)_{10}$	10	230-269	0.867	0.477	0.841
MiIIHR18	F: TCTGACGTCACCTCCTTTCA R: ATACTCGTGCCTCGTCCTGT	$(GT)_{12}$	11	148-193	0.724	0.023	0.693
MiIIHR23	F: TCTGACCCAACAAAGAACCA R: TCCTCCTCGTCCTCATCATC	$(GA)_{17}$GG$(GA)_{6}$	13	117-156	0.693	0.409	0.667
MiIIHR26	F: GCGAAAGAGGAGAGTGCAAG R: TCTATAAGTGCCCCCTCACG	$(GA)_{14}$GGA$(GAA)_{2}$	19	127-171	0.889	0.523	0.869
MiIIHR30	F: AGCTATCGCCACAGCAAATC R: GTCTTCTTCTGGCTGCCAAC	$(CT)_{13}$	11	190-213	0.857	0.674	0.831
MiIIHR31	F: TTCTGTTAGTGGCGGTGTTG R: CACCTCCTCCTCCTCCTCTT	$(GAC)_{6}$	10	207-260	0.752	0.523	0.718
MiIIHR34	F: CTGAGTTTGGCAAGGGAGAG R: TTGATCCTTCACCACCATCA	$(GGT)_{9}(GAT)_{5}$	09	203-245	0.771	0.364	0.734
MiIIHR36	F: TCTATAAGTGCCCCCTCACG R: ACTGCCACCGTGGAAAGTAG	$(TC)_{17}$	14	210-250	0.834	0.545	0.805

success for any particular goal. Earlier the breeders were content to go not much further for their material than the old landraces and varieties that were then available in their own countries or from neighbouring ones, but in the last 50 years or so, breeders are requiring a much wider range of genetic diversity (Paroda and Arora, 1991). As per Frankel and Soulé (1981), one of the methods of conservation is in the region of cultivation. The indigenous varieties are observed to have large diversity in most of the mango growing regions in India. However, maintaining everything in *ex situ* collection is extremely difficult due to the large area that is needed for maintenance. The diversity studies would help in identifying core collections that can represent the diversity of a particular region. This in turn help in identifying varieties for crossing and development of recombinants with desirable traits.

The study very clearly points out that there is excellent scope for locating useful seedling diversity in mango and the varietal variability in a geographic region is the resultant of the present day commercial varieties in that particular region. The assessment of such diversity in the seedling population would help in locating genotypes with useful traits and also some of them can directly be popularized as a commercial variety. The real benefit sharing by the farmers would happen when these varieties are registered and further propagated. Further these varieties would also be conserved 'on farm' by the farmers *in situ.*

Acknowledgements

This paper is the output of UNEP/GEF project "Conservation and Sustainable Use of Cultivated and Wild Tropical Fruit Diversity: Promoting Sustainable Livelihoods, Food Security and Ecosystem Services" implemented in 36 rural communities in India, Indonesia, Malaysia and Thailand. The project is coordinated regionally by Bioversity International with financing from the Global Environment Facility (GEF), and implementation support from United Nations Environment Programme (UNEP).

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