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# Molecular characterization of ten mango cultivars using simple sequences repeat (SSR) markers

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**Simple sequence repeats (SSRs) which is an efficient genetic markers for comparative genome mapping can be helpful in the classification of genotypes, germplasm resource utilization and breeding programmes. Therefore, the present study was conducted to show genetic variation and investigate inter-relationship between ten mango genotypes. Twenty (20) SSR markers were tested with 10 genotypes: Kalepad, Neelum, Swarnarekha, Alphonso Rumani, Sendura, Banganapalli, Himayuddin, Mulgoa and Bangalora. The genomic DNA was extracted from the leaf samples using cetyltrimethyl ammonium bromide (CTAB) method. Polymerase chain reaction (PCR) amplification of the DNA isolated from 10 mango genotypes with 20 SSR primers produced a total of 240 amplified products, of which 184 were polymorphic and 56 monomorphic. The sizes of the alleles detected ranged from 120 to 369 bp. SSR markers were highly polymorphic with an average of 2.70 alleles per primers. SSRs gave moderate values of polymorphic information content (PIC) range of 0.320 to 0.774. The amplified products varied between 2 (LMMA 1, 5, 7, 12, 16, MiSHRS-1 and MiSHRS-37) to 3 and 4 (LMMA 4, 6, 9, 10, 11, 13, 14, 15 MiSHRS-4, 48, 18, 39 and LMMA 8) bands per primer. We obtained moderate degree of genetic diversity, with Jaccard's similarity co-efficient values ranging from 0.075 between cluster I and II to 0.285 between clusters II and III. The dendrogram generated from the unweighted pair group arithmetic average (UPGMA) cluster analysis broadly placed 10 mango cultivars into three major clusters at co-efficient similarity of 0.65. The cluster size varied from 1 to 6 and cluster III was the largest cluster comprising of six cultivars followed by cluster II possessing three and cluster I possessing one variety. Cluster I had the highest diverse cultivars namely, Kalepad, Neelum and Swarnarekha. Cluster II included cultivar of Alphonso. Cluster III contain the cultivars viz., Rumani, Sendura, Bangnapalli, Himayuddin, Mulgoa and Bangalora. Unique fingerprints were identified in the cultivars. The unique fingerprints size ranged from LMMA-8 (257-270 bp), LMMA-11 (232- 245 bp) to MiSHRS 39 (340-369 bp). The tendency of clustering among mango cultivars revealed that they have strong affinity towards further breeding programme.**

**Key words:** Cultivars, genetic diversity, mango, simple sequence repeats (SSR).

## NTRODUCTION

Mango (*Mangifera indica* L.) belonging to the family Anacardiaceae occupies paramount place among the fruit crops grown in India and christened as the "King of fruits" owing to its delicious flavor and taste; there are 1000 varieties found in the country (Singh, 1996). However, there is a lot of confusion in nomenclature of

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the mango cultivars, which is attributed to the lack of systematic approach in nomenclature. Characterization of available cultivars is a prerequisite for their conservation as well as utilization in the further breeding programmes. Genetic analysis including assessment of genetic diversity, relatedness between or within species, population and individuals as well as genotype characterization, are central tasks for many disciplines of biological sciences. Conventionally, genetic analysis was dependent on morphological and/or biochemical markers. During the past few decades, classical strategies of genetic analysis have been increasingly complemented by molecular techniques. The most fundamental of these molecular techniques are DNA markers which portray genome sequence composition, thus, enabling the detection of differences in the genetic information carried by the different individuals. Therefore, these markers are powerful tools in genotype identification or fingerprinting and the estimation of relatedness between genotypes. Consequently, they provide the means to utilize our existing germplasm resources to understand fundamental plant processes and mechanisms. Furthermore, marker-mediated genetic analysis elucidates the genetic basis of agronomic characters and leads to their direct manipulation by plant breeders.

Microsatellites consist of highly variable tandem repeats of very short motifs (1-6 bp) (Litt and Lutty, 1989). Based on microsatellites, two types of DNA markers could be generated, that is, simple sequence repeats (SSRs) and inters simple sequence repeats (ISSRs). In SSRs, the polymorphism is detected by PCR amplification using primers complementary to unique flanking sequences. SSRs are becoming the markers of choice in genetic studies because they are transferable, multiallelic codominant markers, easily reproducible, randomly and widely distributed along the genome (Rafalski et al., 1995).

Genetic characterization serves the twin purpose of the identification of genotypes and estimation of their genetic relatedness (Ravishankar et al., 2000). Traditionally, the genetic variation in mango was estimated using morphological markers and isozymes. These techniques however do not provide an accurate estimation of variation and could lead to misidentification or duplication of genotypes. The incorrect labeling of cultivars and ambiguous identification of individual is a limitation that impedes progress in mango improvement programmes. Precise characterization of genetic variation at the molecular level is possible using DNA based markers. Different molecular marker, such as randomly amplified polymorphic DNA (RAPDs) (Bajpai et al., 2008), amplified fragments length polymorphism (AFLP) (Eiadthong et al., 2000), inter-simple sequence repeats (Pandit et al., 2007) and simple sequence repeats (Duval et al., 2005; Schnell et al., 2006; Viruel et al., 2005) have been employed for genetic diversity assessment in mango cultivars. SSR have gained considerable importance in genetic studies owing to their desirable attributes such as hyper-variability, multiallelic nature, co-dominant inheritance and reproducibility. Assessment of the genetic structure of closely related cultivars is also possible with SSRs. Based on informative and robustness; the use of SSRs has been preferred to determine the genetic relationships among the mango cultivars. Keeping in view these advantages, we analyzed the closely related mango cultivars with micro satellite markers.

## MATERIALS AND METHODS

A total of 10 mango genotypes used in this study were collected from the mango orchard of State Horticultural Farm, Kanyakumari, Tamil Nadu, India.

### DNA isolation

Total genomic DNA was isolated from fully expanded leaves using the hexadecyltrimethylammonium-bromide (CTAB) method (Murray and Thompson, 1980) with few modifications. Briefly, 2 g of leaves were ground in liquid nitrogen to a fine powder. The powder was added to 6 mL of extraction buffer (100 mM Tris-HCl pH 8.0, 20 mM EDTA, 1.4 M NaCl, 2% (wv-1) CTAB, 2–mercaptoethanol 2% and 1% (wv-1) PVP) and incubated at 65°C for 30 min. The DNA was extracted with chloroform – octanol (24: 1). The DNA was washed with 70% ethanol and dissolved in 100 – 400 µL of TE (10mM Tris–HCl pH 8.0, 1 mM EDTA and 0.2 mg $mL^{-1}$ RNase). The DNA concentration was determined spectrophotometrically at 260 nm. Stock DNA samples were stored at -20°C and diluted to 20 ng $uL^{-1}$ when in use.

### PCR procedure

The PCR reactions were performed on Perkin Elmer 9,600 thermocycler (USA). Each PCR reaction consisted of 2 µl of 10 x reaction buffers, 0.5 µl of 10 mM dNTPs, 2 µl of each forward and reverse primer, 0.3 µl of Tag DNA polymerase, 2 µl of DNA and 13.2 µl of sterile water in a final reaction volume of 20 µl. The PCR reaction profile was DNA denaturation at 95°C for 5 min followed by 40 cycle of 94°C for 1 min; primer annealing at 51°C for 1 min, 72°C for 1 min, and finally 72°C for a final extension of 5 min. Amplification products were separated by electrophoresis (8.3 V $cm^{-1}$) in 1.5% agarose gels and stained in ethidium bromide. A photographic record was taken under UV illumination.

### Data analysis

Only clear and repeatable amplification products were scored as 1 for present bands and 0 for absent ones. The specific bands useful for identifying species and cultivars were named with a primer number followed by the approximate size of the amplified fragment in base pairs. Polymorphism was calculated based on the presence or absence of bands. The 0 or 1 data matrix was created and used to calculate the genetic distance and similarity using “Simqual” a subprogram of the NTSYS-PC program (numerical taxonomy and multivariate analysis system program) (Rohlf, 2000). The genetic associations between accessions were evaluated by calculating the Jaccard’s similarity coefficient for pair wise comparisons based on the proportions of shared bands produced by the primers (Jaccard, 1908). The dendrogram was constructed by using a distance matrix using the unweighed pair group method with arithmetic average

**Table 1.** The sequence and details of the primer pairs.

| Primer name | Primer details (5'-3') |
|---|---|
| LMMA1 | f:atggagactagaatgtacagag |
| | r:attaaatctcgtccacaagt |
| LMMA 4 | F:AGATTTAAAGCTCAAGAAAAA |
| | R:AAAGACTAATGTGTTTCCTTC |
| LMMA 5 | F:AGAATAAGCTGATACTCACAC |
| | R:TAACAAATATCTAATTGACAGG |
| LMMA 6 | F:ATATCTCAGGCTTCGAATGA |
| | R:TATTAATTTTCACAGACTATGTTCA |
| LMMA 7 | F:ATTTAACTCTTCAACTTTCAAC |
| | R:AGATTTAGTTTTGATTATGGAG |
| LMMA 8 | F:CATGGAGTTGTGATACCTAC |
| | R:CAGAGTTAGCCATATAGAGTG |
| LMMA 9 | F:TTGCAACTGATAACAAATATAG |
| | R:TTCACATGACAGATATACACTT |
| LMMA 10 | F:TTCTTTAGACTAAGAGCACATT |
| | R:AGTTACAGATCTTCTCCAATT |
| LMMA 11 | F:ATTATTTACCCTACAGAGTGC |
| | R:GTATTATCGGTAATGTCTTCAT |
| LMMA 12 | F:AAAGATAGCATTTAATTAAGGA |
| | R:GTAAGTATCGCTGTTTGTTATT |
| LMMA 13 | F:CACAGCTCAATAAACTCTATG |
| | R:CATTATCCCTAATCTAATCATC |
| LMMA 14 | F:ATTATCCCTATAATGCCCTAT |
| | R:CTCGGTTAACCTTTGACTAC |
| LMMA 15 | F:AACTACTGTGGCTGACATAT |
| | R:CTGATTAACATAATGACCATCT |
| LMMA 16 | F:ATAGATTCATATCTTCTTGCAT |
| | R:TATAAATTATCATCTTCACTGC |
| MiSHRS-1 | F: TAACAGCTTTGCTTGCCTCC |
| | R: TCCGCCGATAAACATCAGAC |
| MiSHRS-4 | F: CCACGAATATCAACTGCTGCC |
| | R: TCTGACACTGCTCTTCCACC |
| MiSHRS-37 | F: CTCGCATTTCTCGCAGTC |
| | R: TCCCTCCATTTAACCCTCC |
| MiSHRS-48 | F: TTTACCAAGCTAGGGTCA |
| | R: CACTCTTAAACTATTCAACCA |
| MiSHRS-18* | F: AAACGAGGAAACAGAGCAC |
| | R: CAAGTACCTGCTGCAACTAG |
| MiSHRS-39† | F: GAACGAGAAATCGGGAAC |
| | R: GCAGCCATTGAATACAGAG |

(UPGMA) sub-program of NTSYS-PC.

## RESULTS AND DISCUSSION

Twenty (20) SSR primers were used for generating banding profile (Table 1). Out of which 17 primers gave consistent and discrete bands. Three typical SSR profiles are shown in Figure 1. The details with respect to band statistics are shown in Table 2. The 20 SSR primers produced a total of 240 amplified products, of which 184 were polymorphic and 56 monomorphic. The number of alleles detected varied form 2 (LMMA 1, 5, 7, 12, 16, MiSHRS-1 and MiSHRS-37) to 3 and 4 (LMMA 4, 6, 9, 10, 11,13,14,15 MiSHRS-4, 48, 18, 39 and LMMA 8). The average number of alleles per primer pair was 2.70. The allele size ranged from 120 (MiSHRS-4 and MiSHRS-37) to 369 bp (MiSHRS-39). Earlier, Shareefa (2008) and Nayak (2010) reported similar values of SSR polymorphism (71 to 81.8), number of alleles and allele size in mango cultivars. In the present study, most of the SSR primers detected multiple loci, which can be attributed to the allopolyploid nature of mango (Mukherjee, 1950).

The characteristics of PCR products namely, the polymorphism information content (PIC) is presented in Table 2. In the present experiment, SSR markers gave low PIC values ranging from 0.320 (MiSHRS-37) and 0.683 (MiSHRS-4). The average PIC value for MiSHRS primer series was 0.552 whereas it was 0.518 in LMMA primer series in our study which is similar to the findings of Shareefa (2008) and Nayak (2010). PIC values of these markers were also low to moderate in Florida mango cultivars (Schnell et al., 2006). 20 SSR primers in the ten mango cultivars detected a total of 240 scorable bands with an average of 2.70 bands/SSR, ranging from 2 to 4 bands/SSR. This is lower than those reported by Viruel et al. (2004) in their work with 16 primer pairs among 28 mango genotypes, probably due to the lower number of analyzed samples as well as due to the less diverse genotypes analyzed.

The analysis of molecular data showed high level of genetic similarity within the analyzed cultivars, while different levels of genetic diversity were detected among ten mango genotypes determined based on the Jaccard's pair wise similarity co-efficient. We obtained moderate degree of genetic diversity, with Jaccard's similarity co-efficient values ranging from 0.075 between cluster I and II to 0.285 between clusters II and III. The dendrogram generated from the unweighted pair group arithmetic average (UPGMA) cluster analysis broadly placed 10 mango cultivars into three major cluster at co-efficient of 0.65 (Figure 2). The cluster size varied from 1 to 6 and cluster III was the largest cluster comprising of six cultivars followed by cluster II possessing 3 and cluster I possessing one variety. Cluster I has the most diverse cultivars namely, Kalepad, Neelum and Swarnarekha. Cluster II included cultivar of Alphonso. Cluster III has the cultivars viz., Rumani, Sendura, Bangnapalli, Himayuddin, Mulgoa and Bangalora.

The Jaccard's similarity values (65%) clearly depicted rich genetic variability in the cultivars studied. Our findings are supported by the earlier studies on genetic diversity analysis in mango using different marker system (RAPD) (Bajpai et al., 2008; Singh, 2005). The rich genetic variation found in cultivar progeny could be attributed to the cross pollinated nature of mango crop,

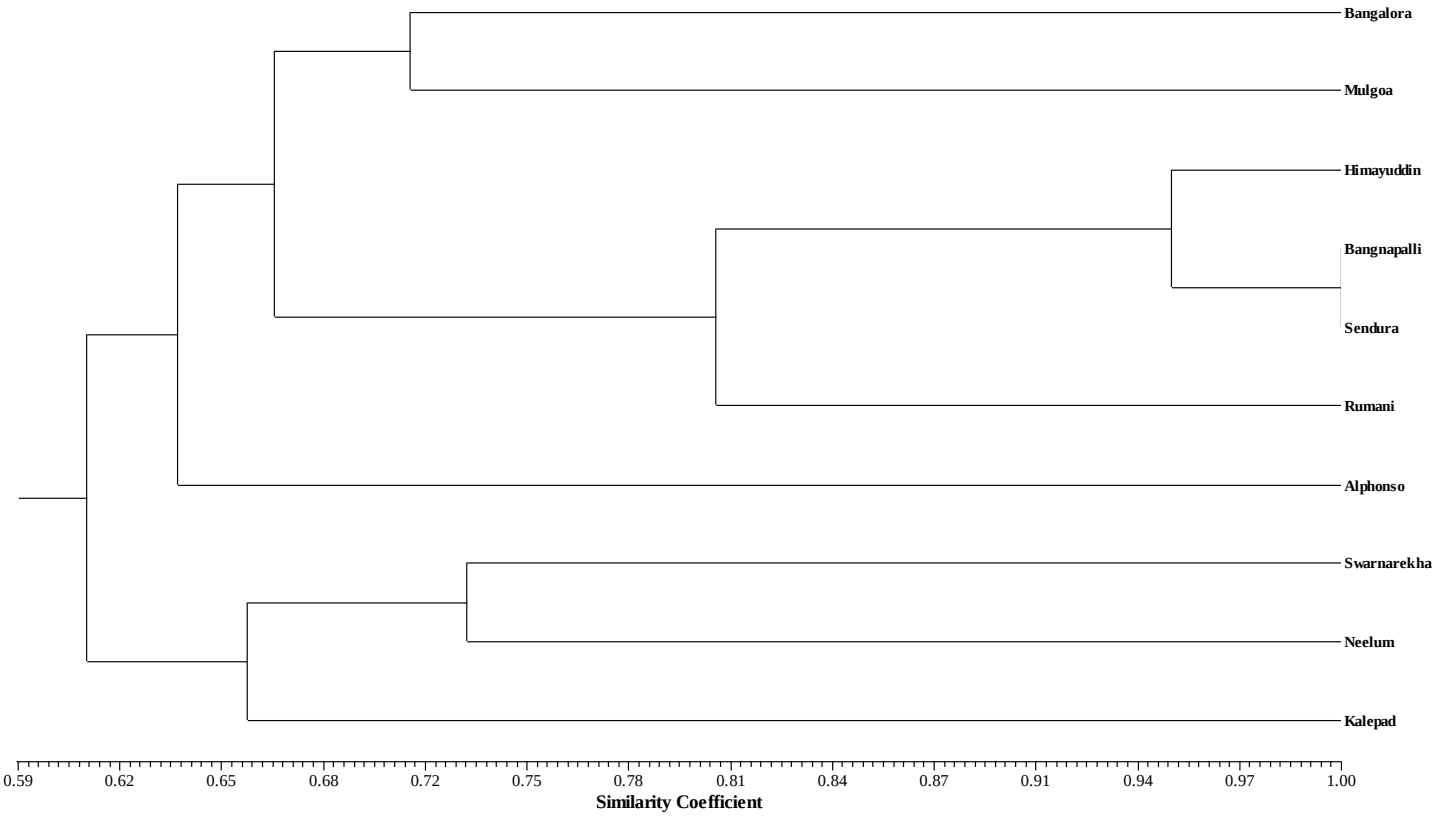
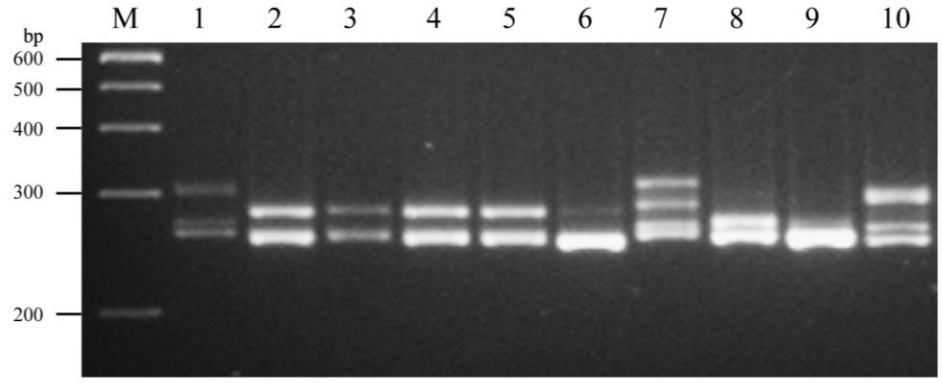


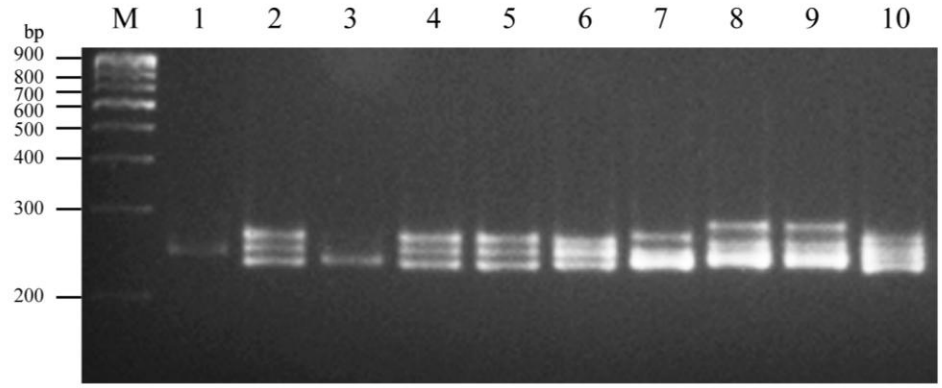

**Figure 1.** Phenogram of ten mango cultivars based on SSR data. The phylogenetic tree obtained from unweighted pair-group (UPGMA) cluster method of Nei's genetic distances option in the NTSYS-PC1.2 program.

**Table 2.** Allele variation and PIC values for SSR marker identification in mango genotypes.

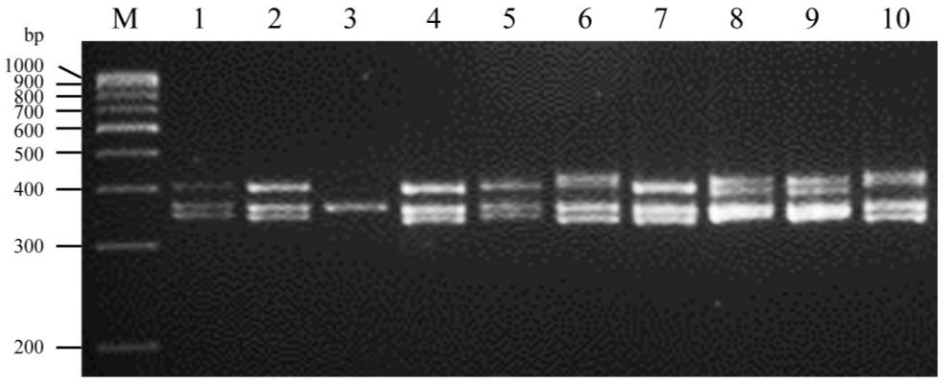
| Locus | Number of alleles | Product size (bp) | PIC values |
|---|---|---|---|
| LMMA1 | 2 | 200-210 | 0.560 |
| LMMA 4 | 3 | 235-240 | 0.520 |
| LMMA 5 | 2 | 280-285 | 0.395 |
| LMMA 6 | 3 | 220-235 | 0.382 |
| LMMA 7 | 2 | 198-215 | 0.690 |
| LMMA 8 | 4 | 257-270 | 0.774 |
| LMMA 9 | 3 | 170-180 | 0.380 |
| LMMA 10 | 3 | 145-172 | 0.642 |
| LMMA 11 | 3 | 232-245 | 0.720 |
| LMMA 12 | 2 | 195-205 | 0.430 |
| LMMA 13 | 3 | 180-200 | 0.620 |
| LMMA 14 | 3 | 165-170 | 0.320 |
| LMMA 15 | 3 | 195-225 | 0.364 |
| LMMA 16 | 2 | 220-245 | 0.465 |
| MiSHRS-1 | 2 | 200-210 | 0.524 |
| MiSHRS-4 | 3 | 120-130 | 0.683 |
| MiSHRS-37 | 2 | 120-150 | 0.320 |
| MiSHRS-48 | 3 | 205-225 | 0.520 |
| MiSHRS-18* | 3 | 210-240 | 0.615 |
| MiSHRS-39† | 3 | 340-369 | 0.650 |




A. SSR profile of mango cultivars generated by the primer LMMA8




B SSR profile of mango cultivars generated by the primer LMMA11




C. SSR profile of mango cultivars generated by the primer MiSHRS39

M - 100 bp ladder; 1 - Bangalora; 2 - Himayuddin; 3 - Rumani; 4 - Bangnapalli; 5 - Sendura; 6 - Alphonso; 7 - Swarnarekha; 8 - Neelum; 9 - Kalepad; 10 - Mulgoa

**Figure 2.** Simple sequence repeats (SSR) profiles of ten different mango cultivars.

high degree of heterozygosity and high discriminatory power of the SSR markers. The diverse genetic backgrounds of cultivars seem to have contributed to rich genetic variation observed in mango cultivars. In Kalepad and Neelum south Indian, especially Tamil Nadu, flowering cultivar of mango has regular bearing throughout

**Table 3.** Distribution of genotypes to different clusters based on UPGMA methods.

| Cluster | Number of genotype | Name of genotype |
|---|---|---|
| Cluster I | 3 | Kalepad, Neelum and Swarnarekha |
| Cluster II | 1 | Alphonso |
| Cluster III | 6 | Rumani, Sendura, Banganapalli, Himayuddin, Mulgoa and Bangalora |

the year. Swarnarekha cultivar prominently in Andra Pradesh has coloured fruit and is an early bearing cultivar. Alphonso has alternate bearing habit, which might be due to environmental, genetic and physiological factors. Other cultivars viz., Rumani, Sendura, Banganapalli, Himayuddin, Mulgoa and Bangalora, are popular varieties of South India. Thus, these diverse cultivars could have resulted in high genetic variability among the cultivars.

As with other vegetative propagated clonal crops, the differences among mango cultivars can result from epigenic modifications in response to the environment (Kaeppler et al., 2000). Somatic and bud mutations also play a minor role in clonal polymorphism in woody plants. Naik (1948) observed significant variation among the trees of some clones in mango with respect to fruit cha-racteristics and tree performance. It could be expected that most of the somatic mutations that occur during plant growth would have no effect on phenotype, although they could be identified at the molecular level.

DNA fingerprinting can be employed for individual identification of cultivars or rootstock for different horticultural purpose, such as breeder's right, identification of pollen parents and determination of genetic relatedness (Lavi et al., 1993). The potential of SSR markers in fingerprinting is well established in mango (Viruel et al., 2005; Shareefa, 2008). Unique fingerprints are genotype and marker specific alleles that may serve as indicators of a particular region of the genome specific to a parti-cular trait of horticultural importance. The genotypes carrying the unique alleles may prove useful for intro-ducing diversity in the future mango breeding programmes. The application of DNA fingerprinting technology has the potential of significantly improving mango breeding pro-jects in terms of cost, time and efficiency by enabling eventual use of marker-assisted selection (MAS) and reduction in the nature of backcross generations needed for gene introgression. Our study revealed that SSR markers are useful not only for varietal identification, but also in future mango breeding progrommes to maximize genetic variability among the mango cultivars.

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