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Analysis of genetic profiling and diversity in mango cultivar using SSR

Muniyappan C[1], Rajangam J[1*], Saraswathy S[1], Venkatesan K[1], Rajesh S[2] & Madhan Mohan M[3]

[1]Horticultural College and Research Institute, Tamil Nadu Agricultural University, Periyakulam, Theni 625 604, Tamil Nadu, India

[2]Centre for Plant Molecular Biology and Biotechnology (CPMB), Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

[3]Agricultural Research Station, Tamil Nadu Agricultural University, Vaigai dam, Theni 625 562, Tamil Nadu, India

*Email: jrajangam2016@gmail.com

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Abstract

The present study was conducted to show genetic variation and investigate inter-relationships between 30 mango genotypes. We are selected 9 SSR markers with 30 genotypes cv. Kalepad, Neelum, Swarnarekha, Alphonso Rumani, Sendura, Banganapalli, Mulgoa and Bangalora etc. The genomic DNA was extracted from mature leaf samples with cetyltrimethylammonium bromide (CTAB) method. Samples were run by Polymerase chain reaction (PCR) they produced 350 amplified products, of which 258 were polymorphic and 132 monomorphic. The sizes of the alleles detected ranged from 120 to 290 bp. SSR markers were highly polymorphic with an average of 3.04 alleles per primers. SSRs gave moderate polymorphic information content (PIC) range value of 0.147 to 0.778. The amplified products varied between 2 (MSSR 152, MSSR 155 andMSSR173) to 3 and 4 (MSSR 106, MSSR140, MSSR146, MSSR156, MSSR160 and MSSR190) bands per primer. We obtained moderate genetic diversity, with Jaccard's similarity co-efficient values ranging from 0.065 between cluster I and II to 0.25 between clusters II and III. The dendrogram generated from the unweighted pair group arithmetic average (UPGMA) cluster analysis broadly placed 30 mango cultivars into three significant clusters at co-efficient similarity of 0.65. The cluster size varied from 1 to 27 and cluster I was the largest cluster comprising twenty-seven cultivars, followed by cluster II possessing two and clusters I possess one variety. Cluster I had the highest diverse cultivars namely, Mallika, Gundur pacharichi, Rumani, Sajahan, Dillpasanth, Senthuram, Amarapalli, Arka aruna, Banganapalli, Sundar langra, Sothuparai local etc. cluster II contain the cultivar Neelgoa, Arka puneet Cluster III contain the cultivars viz., Neelphonso. Unique fingerprints were identified in the cultivars. The tendency of clustering among mango cultivars revealed that they have a strong affinity towards further breeding programmes.

Keywords

cluster analysis; dendrogram; PIC; phenogram

Introduction

Mango (*Mangifera indica* L.),belonging to the family Anacardiaceae, occupies a paramount place among the fruit crops grown in India and is christened as the "King of fruits" owing to its delicious flavour and taste; there are 1000 varieties found in the country (1). However, there is a lot of confusion in the nomenclature of the mango cultivars, which is attributed to the lack of a systematic approach in nomenclature. Characterization of available cultivars is a prerequisite for their conservation and utilization in further breeding programmes. Genetic analysis, including assessment of genetic diversity, relatedness between or within species,

population and individuals and genotype characterization, are central tasks for many disciplines of biological sciences (2). Conventionally, genetic analysis was dependent on morphological and biochemical markers. During the past few decades, molecular techniques have increasingly complemented classical strategies of genetic analysis. The most fundamental of these molecular techniques are DNA markers, which portray genome sequence composition and enable of differences in the genetic information carried by different individuals. Therefore, these markers are potent tools for identifying, fingerprinting and estimating relatedness between genotypes (3). 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). Based on microsatellites, two types of DNA markers could be generated. 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 (4).

Traditionally, the genetic variation in mango was estimated using morphological markers and isozymes. Different molecular marker, such as randomly amplified polymorphic DNA (RAPDs) (5), amplified fragments length polymorphism (AFLP) (6), inter-simple sequence repeats (7) and simple sequence repeats have been employed for genetic diversity assessment in mango cultivars (8). Assessment of the genetic structure of closely related cultivars is also possible with SSRs. Based on information and robustness; SSRs have been preferred to determine the genetic relationships among the mango cultivars. Micro satellite markers were used to analyze the closely related mango cultivars.

Materials and Methods

A total of 30 mango genotypes used in this study were collected from the mango orchard of Horticultural College and Research Institute, Periyakulam, Tamil Nadu, India.

DNA isolation

Total genomic DNA was isolated from fully expanded leaves using the hexadecyltrimethylammonium-bromide (CTAB) method (9) with few modifications. Briefly, 2 g of leaves were ground a fine powder in liquid nitrogen. 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 when in use.

PCR procedure

The PCR reactions were performed on a 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 94°C for 3 min followed by 40 cycles of 94°C for 30 sec; primer annealing at 63°C for 30 sec, 72°C for 1 min and finally 72°C for a final extension of 10 min. Amplification products were separated by electrophoresis (8.3 V/cm) in 1.5% agarose gels and stained in ethidium bromide. A photographic record was taken under UV illumination.

Data analysis

Only precise and repeatable amplification products were scored as 1 for present bands and 0 for absent ones. The specific bands helpful in 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) (10). 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 (11). The dendrogram was constructed using a distance matrix using the unweighted pair group method with an arithmetic average.

Results and Discussion

Nine SSR primers were used for generating banding profiles (Table 1). Out of these 9 primers gave consistent and discrete bands. The details concerning band statistics are shown in (Table 2). The 9 SSR primers produced 350 amplified products, of which 258 were polymorphic and 132 monomorphic. The number of alleles detected varied form 2 (MSSR 152, MSSR 155 and MSSR173) to 3 and 4 (MSSR 106, MSSR140, MSSR146, MSSR156 MSSR160 and MSSR190) bands per primer (Fig. 2, 3, 4). The average number of alleles per primer pair was 3.04. The allele size ranged from 120 (MSSR106 and MSSR-190) to 562 bp (MSSR-160). Earlier it was reported similar values of SSR polymorphism

Table 1. The sequence and details of the primer pairs

S.No	Primer	Primers (5'–3') details	Allele
1	MSSR106	F: GTGAAGGACGTTGATTCATT R: GATCCTTTCGACTGTAATCCT	150- 170
2	MSSR140	F: ACTATAAAGGGCGCATACAA R: CTACCGTGGTATTGGAATGA	250- 290
3	MSSR146	F: CTCTCCTTCATCTCATCGTTA R: TCCTTAGATTGCAGAGAAACC	260- 300
4	MSSR152	F:GTAAATCGGAGAGGGATAATG R: CCCCCTGTAGTTTTATTCTGA	150- 180
5	MSSR155	F: TGGCTTGAGTCAGAAGTTTT R: AACCATCGTGAGTAATTTGG	180- 220
6	MSSR156	F:TCCTGATCTCTTTAGCTCCTT R: GAGTTCTCGAACACCATTTCT	230-270
7	MSSR160	F: CGGATCTTTCACTTACTCTCA R: CATTGGTCAAAGGAAGAAGA	140-170
8	MSSR173	F:TCAGTCTCTCATTTCACTTGC R: ACTCTGAGGTGATGAACAAGA	250- 290
9	MSSR190	F:GCCCTAAAGAATCTGTAAACC R: ATGCATGCAGACACAACTTA	160- 220

(UPGMA) sub - program of NYSYS-PC

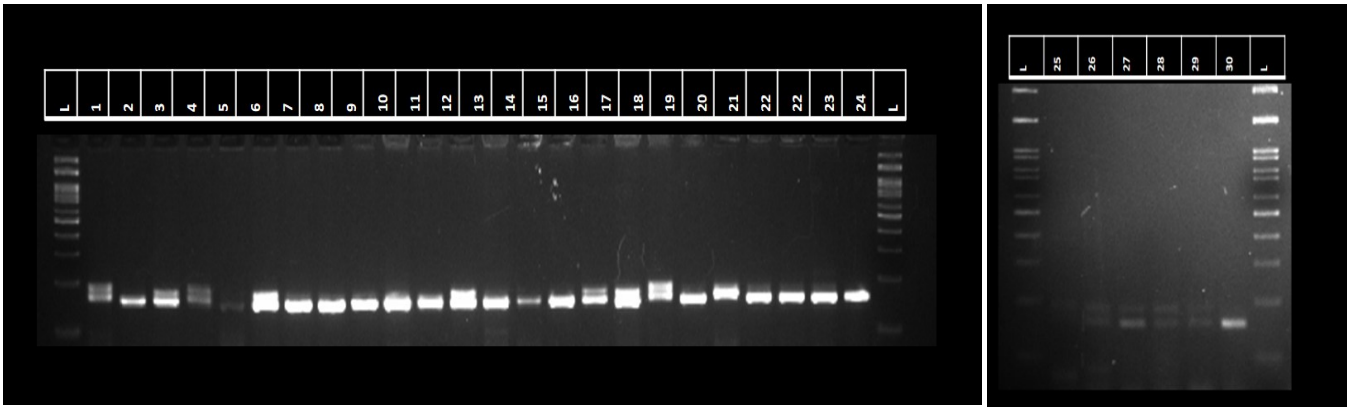

Fig. 1. SSR Profile of mango cultivars generated by the primer MSSR- 155.

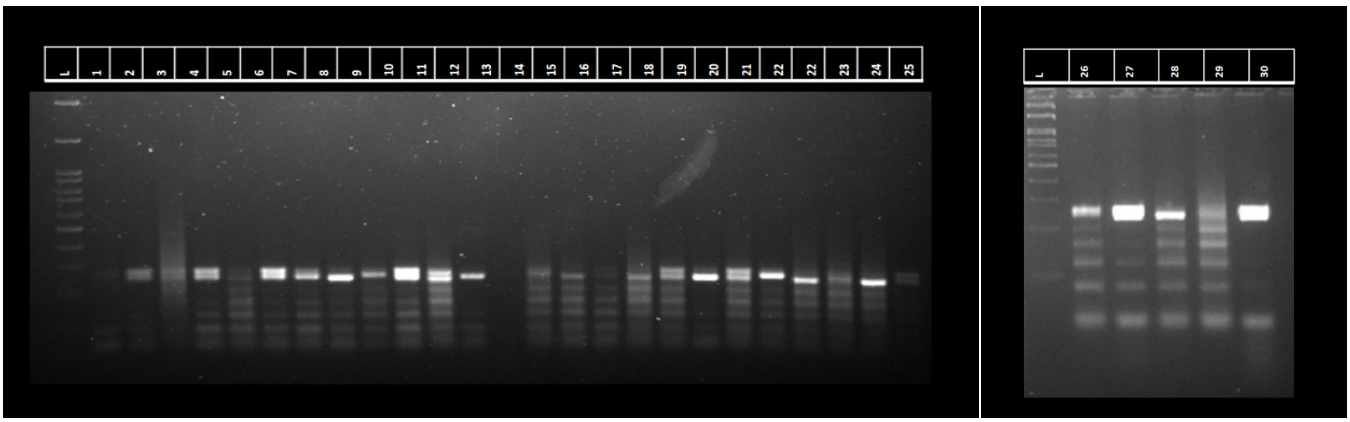

Fig. 2. SSR Profile of mango cultivars generated by the primer MSSR- 156.

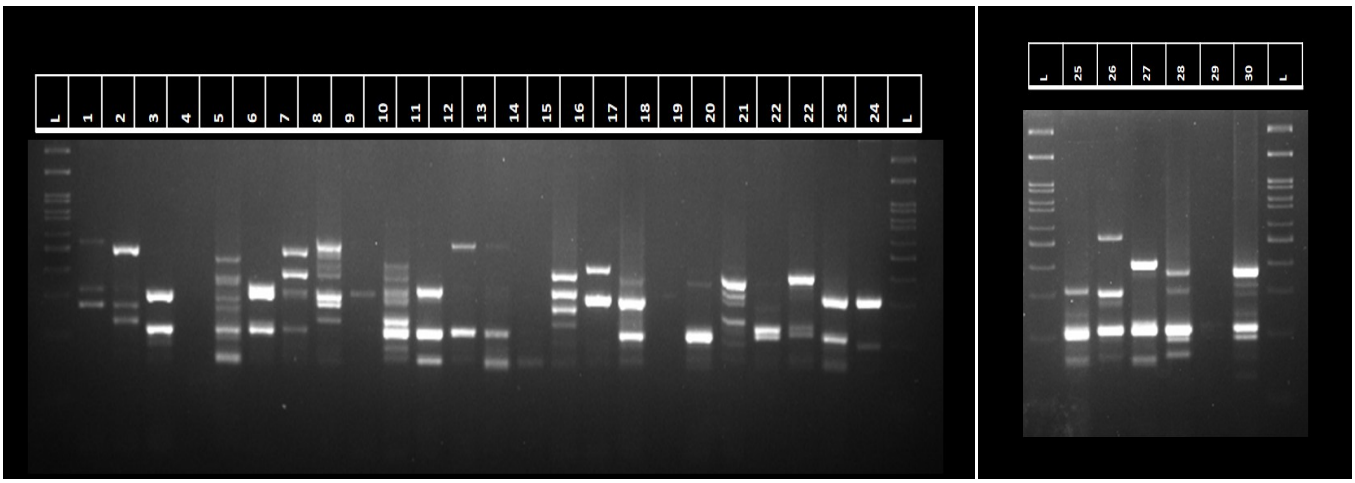

Fig. 3. SSR Profile of mango cultivars generated by the primer MSSR- 160.

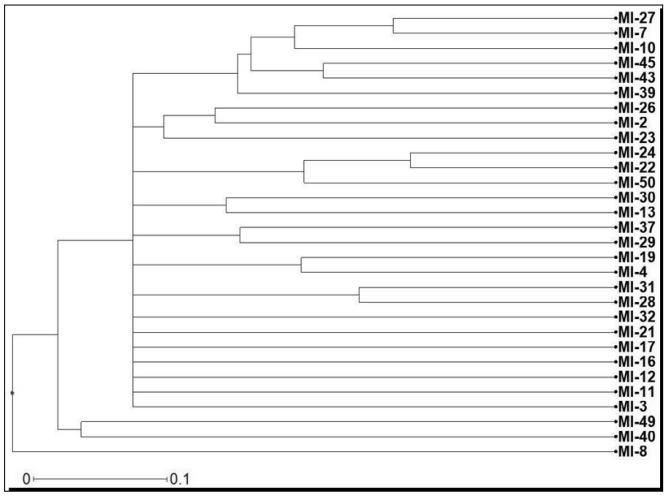

Fig. 4. Phenogram of thirty mango genotypes based on SSR data follow this pattern.

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

Locus	Number of alleles	Product size	PIC values
MSSR 106	3	120-140	0.451
MSSR 140	3	180-200	0.267
MSSR 146	4	180-210	0.230
MSSR 152	2	170-220	0.335
MSSR 155	4	170-200	0.388
MSSR 156	3	280-290	0.354
MSSR 160	5	158-562	0.778
MSSR 173	2	240-250	0.147
MSSR 190	3	120-140	0.337

(71 to 81.8), number of alleles and allele size in mango cultivars (12, 13). In the present study, most of the SSR primers detected multiple loci, which can be attributed to the allopolyploid nature of mango (14).

PIC Values

The characteristics of PCR products namely, the polymorphism information content (PIC) is presented in Table 2. In the present experiment, 9 SSR markers with 30 genotypes gave low PIC values ranging from 0.147 (MSSR-173) to 0.778 (MSSR-160) (Table 2). The average PIC value for MSSR primer series was 0.425. In our study which is like the previous findings (12, 13). PIC values of these markers were also low to moderate in Florida mango cultivars (15). 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. Similar finding have been reported where sample occur at 16 primer pairs among 28 mango genotypes have a low level of variation, probably due to the lower number of analyzed samples as well as due to the less diverse genotypes analyzed (16).

Dendrogram

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 genetic diversity, with Jaccards' similarity coefficient value ranging from 0.068 between cluster I and II to 0.32 between clusters II and III. The dendrogram generated from the unweighted pair group arithmetic average (UPGMA) cluster analysis broadly placed 30 mango cultivars into three major cluster at co-efficient of 0.65 (Fig.1). The cluster size varied from 1 to 27 and Cluster I was the largest cluster comprising twenty-seven cultivars, followed by cluster II, which possessed 2 and cluster III, which possessed one variety (Table 3). Cluster I has the most diverse cultivars namely, Selambhu naddu, Mallika, Gundur pacharichi, Rumani, Sajahan, Dill pasanth, Senthuram, Amarapalli, Arka aruna, Banganapalli, Sundar langra, Sothuparai local, Selambhu alaghupathai naddu, Sindhu , Samba kooja, Bangalora, Pedhrasam, Pkm-2, Mulgoa, Imam pasand, Neelashan, Ratna, Mohandhas, Arka anmol, Malpacharichi, Kuruvi neelum, Pkm-1. Cluster II included cultivar of Neelgoa, Arka puneet. Cluster III has the cultivars viz., Neelphonso (Fig.1).

Jaccard similarity

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) (2, 5). The rich genetic variation found in cultivar progeny could be attributed to the cross-pollinated nature of mango crop. 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 (17). In Kalepad and Neelum south Indian, especially Tamil Nadu, flowering cultivar of mango has regular bearing throughout 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, Arka Aruna, PKM-1, PKM-2, Mulgoa and Bangalora are popular varieties of South India. Thus, these diverse cultivars could have resulted in high genetic variability among the cultivars (18).

As with other vegetative propagated clonal crops, the differences among mango cultivars can result from epigenetic modifications in response to the environment (19). Somatic and bud mutations also play a minor role in clonal polymorphism in woody plants. Clonal variation occurs at two stages mainly fruit morphology and tree performance have been reported (20). 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 to identify of cultivars or rootstock for different horticultural purposes, such as breeder's right, identification of pollen parents and determination of genetic relatedness (21). The potential of SSR markers in fingerprinting is well established in mango (22). Unique fingerprints are genotype and marker-specific alleles that may serve as indicators of a particular region of the genome that is specific to a particular trait of horticultural importance.

Conclusion

Based on the result cluster I had unique and most diversified cultivar compare to other two cluster it will help to identified the best utility to introduce diversity in future mango breeding programmes. DNA fingerprinting technology has the potential of significantly improving mango breeding projects in terms of cost, time and efficiency by enabling the eventual use of marker-assisted selection (MAS) and reduction like backcross generations needed for gene introgression. Our study revealed that SSR markers are helpful for varietal identification and future mango breeding programmes to maximize genetic variability among the mango cultivars.

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

Cluster	Number of genotypes	Name of the genotypes
Cluster I	27	Selambhu naddu; Mallika; Gundur pacharichi; Rumani; Sajahan; Dill pasanth; Senthuram; Amarapalli; Arka aruna; Banganapalli; Sundar langra; Sothuparai local; Selambhu alaghupathai naddu; Sindhu; Samba kooja ;Bangalora; Pedhrasam; Pkm-2; Mulgoa; Imam pasand; Neelashan; Ratna; Mohandhas; Arka anmol; Malpacharichi; Kuruvi neelum;Pkm-1.
Cluster II	2	Neelgoa;Arka puneet
Cluster III	1	Neelphonso

Authors' contributions

The manuscript was developed through a collaborative effort among the authors. MR contributed to the original draft and conceptualization of the study. SV played a significant role in revising the draft, including preparing tables and figures, and conducting thorough proofreading. RM further contributed to the revision, ensured proper formatting, and provided overall supervision. All authors reviewed and approved the final version of the manuscript, ensuring its readiness for submission.

Compliance with ethical standards

Competing interests: Authors do not have any conflict of interests to declare.

Ethical issues: None

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