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Microsatellite marker based characterization of mango cultivars

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ABSTRACT

Simple sequence repeats (SSRs) or microsatellites are highly efficient in the classification of genotypes, genetic resources utilization and breeding programmes. In the present investigation, seventeen (17) SSR primers were used for the molecular characterisation of twenty mango cultivars of Bihar. Out of 58 scorable bands, 45 were found to be polymorphic. The number of alleles detected ranged from 2 (MIAC 5, MiIIHR 12, MiIIHR 20a, MiSHRS 37) to 6 (MIAC 11, MiIIHR06,mMiCIR 027). The polymorphic information content (PIC) ranged from 0.05 (MiIIHR 20a) to 0.38 (MIAC 2). The genetic relationship among twenty mango cultivars based on Jaccard's similarity coefficient was found to be ranging from 0.46 (between Fazli and Langra) to 0.90 (between Krishnabhog and SB Chausa and Krishnabhog and Bangalora).The dendrogram based on UPGMA cluster analysis grouped twenty mango cultivars into two major clusters while the cultivars Neelum and Fazli were found to be distinct from other cultivars. SSR markers are reliable and reproducible and have been proved to be useful for varietal identification and mango breeding programmes to maximize genetic variability among the mango cultivars. The results of this study have showed the potential of SSR markers in deciphering the existing genetic diversity among the Indian mango cultivars.

Key words: *Mangifera indica,* SSR markers, characterization, genetic diversity.

INTRODUCTION

Mango (*Mangifera indica* L.), known as the 'King of fruits' is the most popular fruit of tropics and subtropics and the choicest fruit of millions of people in the country. The major states in India producing mango are Uttar Pradesh, Andhra Pradesh, Maharashtra, Bihar, Orissa, Karnataka, west Bengal and Gujarat. Mango tree performs well both under tropical and subtropical conditions. Traditionally, mango characterisation has been done using phenological and morphological traits of flowers, leaves, fruits and seeds (IPGRI, 5).Markers are the particular plant features that can be noted and documented with confidence, comparative affluence and ease. Morphological markers are limited in number, have complex inheritance pattern and are affected by environmental conditions (Karihaloo *et al.,* 7). In addition, identification of the cultivars using morphological features is inefficient and inaccurate. For cultivar identification, the molecular markers are more efficient than the morphological markers (Azam *et al*., 2). A number of different classes of DNA markers have been used in fruits such as RAPD (Ravishankar *et al*., 12), ISSR (Sagar *et al.,* 11 and Pandit *et al.*, 11), AFLP (Yamanaka *et al*., 17) and SSR (Schnell *et al*., 14).Among all, SSR markers are more advantageous over other markers. Microsatellites or SSRs have become the marker of choice for fingerprinting and genetic diversity analysis in many plant species (Gupta and Varshney, 4) due to their high polymorphism, codominant nature and reproducibility. SSRs or microsatellites, are a class of molecular markers based on tandem repeats of short (2–6 bp) DNA sequences (Litt and Luty, 9). Microsatellite markers in mango have been developed by several research groups recently, (Viruel *et al*., 16). Many of the vegetative characteristics represent continuous variation and a high degree of plasticity which many times do not reflect the real diversity of the mango germplasm. Biochemical markers such as isozymes and protein patterns though minimally influenced by the environment, offer limited polymorphism and often do not allow discrimination between closely related genotypes. DNA markers overcome most of these disadvantages of morphological and biochemical markers. Therefore, for determination of genetic relationships among different mango cultivars and association of molecular markers with important traits, SSRs markers have been preferred based on their information content and robustness. In the present study, 17 SSR primers were used to investigate the genetic relationship among 20 mango cultivars of Bihar.

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MATERIALS AND METHODS

The present study was carried outin the Department of Horticulture (Fruit and Fruit Technology), Bihar Agricultural University (BAU), Sabour, Bhagalpur during 2016-2017. Twenty mango cultivars which are popular in different parts of the country namely (Langra, Neelum, Zardalu, Fernandin, Bombay Green, Mulgoa, Alphonso, Vanraj, Himsagar, Fazli, Bangalora, Mallika, Mankurad, Suvarnrekha, Dashehari, Krishnabhog, SB Chausa, Baneshan, Kesar and Bombai) were used for the present investigation. These cultivars are being maintained at AICRP (Fruits) orchard of BAU Sabour.

Leaf samples of twenty mango cultivars were collected and DNA extraction was done following CTAB method. After optimising the concentration of components, PCR amplification was carried out with 25 ng of genomic DNA, 5 µl premix Taq ver, 0.50 µl each of forward and reverse primer for 10 µl volume, the details of PCR protocol followed is presented here under. The PCR reaction profile comprised of initial DNA denaturation at 94°C for 4 minutes followed by 45 cycles of denaturation at 94°C for 30 sec; primer annealing at (46-55°C) for 1 min, extension at 72°C for 2 min and finalextension at 72°C for 5 min. Amplification products were separated by electrophoresis in 1.5% agarose gel and stained in ethidium bromide followed by a photography record under UV illumination.

The scoring of the band was done by observing the photograph carefully. Only clear and repeatable amplification products were scored as 1 for present bands and 0 for absent ones. Each band was treated as one SSR primer. Polymorphism was calculated based on the presence or absence of bands. Zero 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, 13). The Jaccard's similarity coefficient (J) was used to calculate the similarity between pair's accessions (Jaccard, 6). The dendrogram was constructed by using a distance matrix using the unweighed pair group method with arithmetic average (UPGMA) sub-program of NTSYS-PC.

RESULTS AND DISCUSSION

Out of 17 SSR primers used in the present investigation, 16 SSR primers gave consistent and discrete bands. Sixteen SSR primers produced 58 scorable bands of which 45 were polymorphic (77.6%) (Table 1). The numbers of alleles recorded were 2 for primers MIAC 5, MiIIHR12, MiIIHR 20a, MiSHRS 37, 3 for primers MIAC 2, MIAC 3, MIAC 6, mMiCIR 005 and MiSHRS 36, 4 for primers MICA-231-1, mMiCIR 016, mMiCIR030, 5 for primers mMiCIR 008 and 6 for primers MIAC 11, MiIIHR06 and mMiCIR 027. Similar percentage polymorphism was reported in previous studies on mango characterization (Shareefa, 15 and Nayak, 10). In our research programme, some of the primers amplified one or two bands in each genotype, suggesting the detection of a single locus, whereas in some primers (e.g. MIAC 11, mMicir030), multiple bands were amplified suggesting the allopolyploid nature of the mango. The size of amplicons ranged from 50 bp (mMiCIR 027) to 1000bp in MIAC 11.

PIC (Polymorphism information content) parameter is indicative of the degree of in formativeness of the marker. In the present experiment, SSR primers gave PIC values ranging from 0.05 (MiIIHR20a) to 0.38 (MIAC 2) (Table 1). Earlier, Shareefa (15) and Nayak (10) also reported very low to moderate PIC values for SSR markers in mango. PIC values of SSR markers were also low to moderate in Florida mango cultivars (Schnell *et al*., 14). The genetic relationship among mango hybrids, based on Jaccard's Similarity Coefficient, ranged from 0.46 to 0.90. Earlier reports in this regard are in tune with our finding. Shareefa (15) working on mango with SSR markers, reported similarity values in the range of 0.45 to 0.88. Earlier studies on genetic diversity among 50 Indian mango cultivars using RAPD techniques revealed that similarity was in the range of 61% to 95% (Kumar *et al*., 8). Karihaloo *et al*. (7) while using RAPD markers in 29 Indian mango cultivars comprising popular landraces and some advanced cultivars reported that Jaccard's similarity values among Indian mangos ranged from 0.318 and 0.75 with a mean of 0.565. The genetic similarity between different mango genotypes based on the Jaccard's similarity coefficient ranged from 0.79 (between Chausa and Dashehari and between Langra and Mallika) and 0.41 (between Olour and Sensation depicting a good degree of genetic diversity among different genotypes (Abirami *et al*., 1). Jaccard's similarity coefficient of 46 mango germplasm collected from eastern and northern regions of India varied from 0.88 (between Papia and Safeda Calcutta) to 0.378 (Lucknow Safeda and Anopan) when analyzed using RAPD markers, whereas varied from 0.83 (between Amin Ibrahimpur and Amin Prince) to 0.467 (between Krishna bhog and Amin Khurd) with ISSR markers (Bajpai *et al*., 3). Out-crossing behaviour of mango crop, high degree of heterozygosity, diverse genetic backgrounds of cultivars and high discriminatory power of the SSR markers seem to have contributed to the rich genetic variability in mango cultivars studied (Fig. 1 and Fig. 2).

Table 1. Primer sequences, Number of alleles and PIC values for 16 SSR loci found in twenty mango cultivars.

S. No.	Locus	Primer Sequence (5'-3')	Size of alleles	No. of alleles	PIC values
1.	MIAC-2F	GCTTTATCCACATCAATATCC	140-160	3	0.38
	MIAC2-R	TCCTACAATAACTTGCC			
2.	MIAC3-F	TAAGCTAAAAAGGTTATAG	180-210	3	0.19
	MIAC-3R	CCATAGGTGAATGTAGAGAG			
3.	MIAC-5F	AATTATCCTATCCCTCGTATC	100-120	2	0.37
	MIAC-5R	AGAAACATGATGTGAACC			
4.	MIAC-6F	CGCTCTGTGAGAATCAAATGGT	180-270	3	0.12
	MIAC-6R	GGACTCTTATTAGCCAATGGGATG			
5.	MIAC-11F	GTGCGAGGAGATATCTGT	100-1000	6	0.15
	MIAC-11R	CTGGTTCTTCATTGTTGAGATG			
6.	MICA231-1F	TGGAAGGACCATGCTTGAAT	160-300	4	0.26
	MICA231-1R	GGTCACACACACACACACA			
7.	mMiCIR005F	GCCCTTGCATAAGTTG	160-300	3	0.18
	mMiCIR005R	TAAGTGATGCTGCTGGT			
8.	mMiCIR008F	GACCCAACAAATCCAA	140-600	5	0.24
	mMiCIR008R	ACTGTGCAAACCAAAAG			
9.	mMiCIR016F	TAGCTGTTTTGGCCTT	200-400	4	0.17
	mMiCIR016R	ATGTGGTTTGTTGCTTC			
10.	mMiCIR027F	ACGGTTTGAAGGTTTTAC	50-200	6	0.16
	mMiCIR027R	ATCCAAGTTTCCTACTCCT			
11.	MMiCIR030F	GCTCTTTCCTTGACCTT	200-700	4	0.17
	mMiCIR030R	TCAAAATCGTGTCATTTC			
12.	MiIIHR06F	CGCCGAGCCTATAACCTCTA	140-750	6	0.06
	MiIIHR06R	ATCATGCCCTAAACGACGAC			
13.	MiIIHR20aF	CCTAACGCGCAAGAAACATA	210-270	2	0.05
	MiIIHR20aR	ACCCACCTTCCCAATCTTTT			
14.	MiIIHR12F	GCCCCATCAATACGATTGTC	90-200	2	0.21
	MiIIHR12R	ATTTCCCACCATTATTGTCGTTG			
15.	MiSHRS-36F	GTTTTCATTCTCAAAATGTGTG	130-240	3	0.123
	MiSHRS-36R	CTTTCATGTTCATAGATGCAA			
16.	MiSHRS-37F	CTCGCATTTCTCGCAGTC	200-250	2	0.13
	MiSHRS-37R	TCCCTCCATTTAACCCTCC			

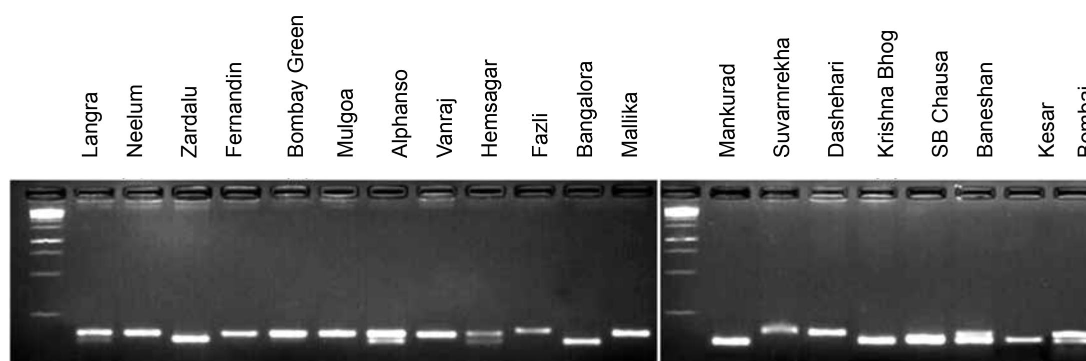

Fig. 1. SSR agarose gel profile of mango cultivars using primer MIAC-2.

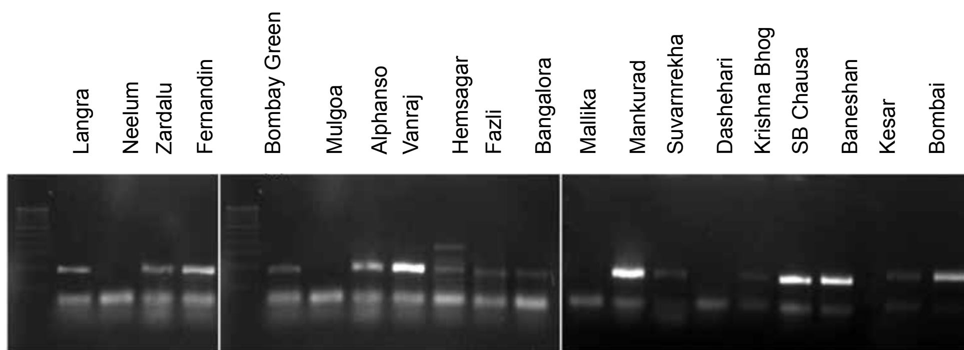

Fig. 2. SSR agarose gel profile of mango cultivars using primer mMiIIHR12.

Unweighted Pair Group Method with Arithmetic mean (UPGMA) clustering reflects the history of breeding and selection of the cultivars studied, grouping the cultivars according to parentage, geographical origin and type. The dendrogram generated from the UPGMA cluster analysis broadly placed 20 mango cultivars into two major clusters (Fig. 3). Cluster 'A' comprised of Langra, Vanraj, Hemsagar, while cluster 'B' bifurcated into two sub-clusters namely, cluster 'B'1 and cluster 'B'2. Cluster 'B' 1 consisted of further sub clusters, 'B'IA and cluster 'B'IB. Cluster B1A consists of zardalu, Mankurad and Kesar while cluster B1B comprised of Fernandin, Bombay green, Mallika, Dashehari and Bombai. Cluster B2 again divided into two sub clusters, cluster B2A and cluster B2B. Cluster B2A consisted of Mulgoa, Alphonso, Bangalora, Krishnabhog, SB Chausa and Beneshan. Clustering of cultivars belonging to different geographic region suggests that they might have evolved from the existing mango gene pool from which they were further selected to domesticate them in different areas for cultivation. While the cultivar Neelum and Fazli were found to be distinct from the other cultivars. The cultivar Neelum and Fazli were also distinct from other cultivars in morphological and biochemical characters.

It is clear from the results that SSR analysis is efficient to prove the genetic distances between mango accessions at the genomic level. India has

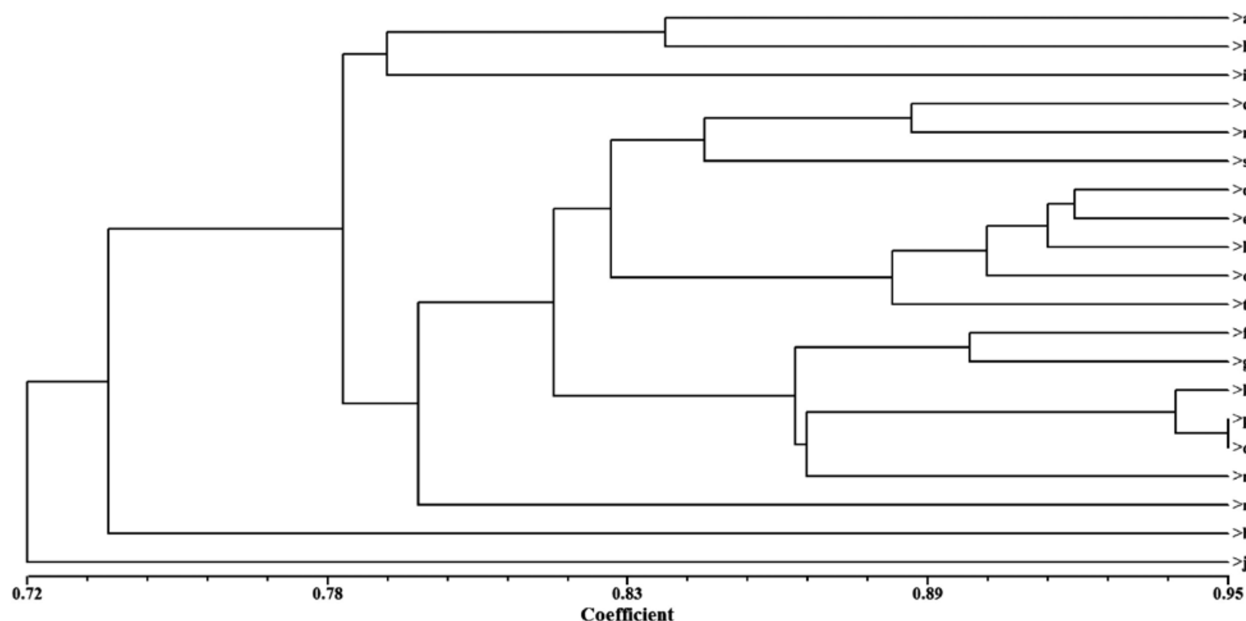

Fig. 3. Phenogram of twenty mango cultivars based on SSR data. The phylogenetic tree obtained from unweighted pair-group (UPGMA) cluster method of Nei's genetic distances option in the NTSYS-PC 2.1 programme.

*a=Langra; b=Neelum; c=Zardalu; d=Fernandin; e=Bombay Green; f=Mulgoa; g=Alphonso; h=Vanraj; i=Himsagar; j=Fazli; k=Bangalora; l=Mallika; m=Mankurad; n=Suvarnrekha; o=Dashehari; p=Krishnabhog; q=SB Chausa; r=Baneshan; s=Kesar; t=Bombai

a wide genetic diversity of mango germplasm. The results of this study have proven the potential of SSR loci in deciphering the existing genetic diversity among the Indian mango cultivars. Our study revealed that SSR markers are useful not only for varietal identification, but also in selecting parents for mango breeding programmes to maximize genetic variability among the mango cultivars. The present study will provide information about available genetic repository of mango in Bihar which can be utilized in mango improvement programme and also lays a foundation for further research involving construction of genetic linkage maps, association studies and population studies.

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