Environment and Ecology 40 (3B) : 1578—1585, July—September 2022
ISSN 0970-0420

Molecular Characterization of Indigenous Mango Cultivars of Coastal Andhra Pradesh using SSR Markers

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Received 2 March 2022, Accepted 6 June 2022, Published on 11 August 2022

ABSTRACT

A study was conducted at Horticultural research station, Venkataramannagudem for evaluating the variability of mango cultivars to conserve the elite ones and to identify the superior genotypes using molecular markers for future crop improvement. Genetic variation and relationship among 34 indigenous mango cultivars were analyzed using Simple Sequence Repeats (SSRs) markers. Eighteen out of the twenty primers screened were informative and 65 amplified DNA bands with size ranging from 50 to 800 bp were selected as SSR markers. The number of amplified fragments varied from 1 (MngSSR 14 and MiIIHR 18) to 9 (MiIIHR 23) with an average of 3.61 alleles per locus. Specific SSR markers for some mango cultivars were identified which could be exploited for DNA finger printing of these cultivars. UPGMA cluster analysis grouped all the cultivars into two clusters with a genetic similarity coefficient range of 0.65 to 0.88. This study revealed that cultivars from coastal districts of Andhra Pradesh unveiled maximum diversity and indicated the potential of SSR markers for the identification and management of mango germplasm for breeding purposes.

Keywords Mango, SSR primers, Dendrogram, Polymorphic information content, Similarity coefficient.

INTRODUCTION

Mango (*Mangifera indica* L.) is an important member of the family Anacardiaceae in order Sapindales and is believed to have originated in the Indo-Burma (Myanmar) region. In India mango ranks first in terms of area with 2.20 million ha, second in respect of production with 18.64 million tonnes and with a productivity of 8.47 million tonnes/ha, while Andhra Pradesh ranks second in terms of area with 0.32 million ha, second in respect to production of 2.80 million tonnes and with a productivity of 8.75 million tonnes/ha (NHB 2015).

Enormous genetic diversity of mango exists in India, which is the primary center of domestication. Considerable genetic diversity of this fruit exists in Andhra Pradesh with several named local cultivars and unnamed local land races. Differentiation of cultivars through morphological features is inefficient and inaccurate. Confusion exists in the nomencla-

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ture of mangoes due to different local names for the same variety. This problem is further compounded as mango is highly heterozygous as performance varies with the climate which resulted in a high level of genetic diversity. Many of these complications of a phenotype-based assay can be overcome through direct identification of genotype with DNA- based genetic markers.

Polymerase Chain Reaction (PCR) technology had led to the development of several novel genetic assays based on selective DNA amplification. The protocol is also relatively quick and easy to perform. Because the SSR technique is an amplification-based assay, only nanogram quantities of DNA are required. One of the strengths of these new assays is that they are more amenable to automation than conventional techniques. It is simple to perform and is preferable to analyze where the genotypes of a large number of individuals are to be determined at a few genetic loci. With this idea the experiment was undertaken to evaluate genetic variation and relationships of indigenous mango cultivars in the Coastal Andhra Pradesh by using SSR markers.

MATERIALS AND METHODS

The present study was conducted at Horticultural Research Station, Venkataramannagudem during 2013-2015 to study the performance of indigenous mango cultivars of Coastal Andhra Pradesh. A well-planned germplasm collection survey based on diversity richness was conducted in coastal districts of Andhra Pradesh which includes Horticultural Research Station and private owned mango orchards. In order to carry out SSR analysis, mature leaves from each of the 34 cultivars were collected randomly. Total genomic DNA was extracted by using modified CTAB (Cetyl Trimethyl Ammonium Bromide) method (Murray and Thompson 1980). Finally, the DNA samples were stored at - 20ºC. The concentration and quality of DNA was estimated by using Nano Drop spectrophotometer at 260 nm and verified by running sample on 0.8% agarose gel stained with Ethidium bromide (1 µg/ml of gel).

DNA amplification was done using twenty arbitrary decamer primers SSR-8, SSR-15, SSR-16, SSR-20, SSR-39, SSR-46, SSR-52, SSR-61, SSR-82, MiIIHR- 13, MiIIHR- 15, MiIIHR- 17, MiIIHR- 18, MiIIHR- 19, MiIIHR- 23, MiIIHR- 24, MiIIHR- 30, MiIIHR- 31, MngSSR-14 and MngSSR-24. PCR reactions were performed on each DNA sample in a 20 µl reaction mix containing 5.0 ng of template DNA (2.0 µl), 1.5 unit of Taq polymerase (0.3 µl) (Bangalore Genei Pvt Ltd., Bangalore, India), dNTPs (Genei) - 0.2 mM (0.4µl) ; 1 p mole of Forward primer (2.0 µl) and 1 p mole of reverse primer (2.0 µl), Buffer (Genei) – 1X (2.0 µl) and rest amount with sterile deionized water (11.3 µl).

DNA amplification was performed in an oil-free thermal cycler (Thermal Cycler, Eppendorf). All the primers were initially evaluated for their annealing temperature using a gradient PCR technique. The annealing temperatures and PCR conditions for these SSRs were first standardized by using a PCR with temperature gradient technique, which indicated that annealing temperatures of 41 to 56°C was optimum for obtaining scorable bands. Stuttering of bands was common with SSRs if annealing temperatures were not optimized. The reaction mix was preheated at 94ºC for three minutes followed by 32 cycles of one min denaturation at 94ºC one min annealing at 41-56ºC and elongation or extension at 72ºC for one minute thirty seconds. After the last cycle, a final step of ten minutes at 72ºC was added to allow complete extension of all amplified fragments. After completion of cycling program, reactions were held at 4ºC. The amplified products were separated electrophoretically on a 2.0% agarose gel. TBE buffer 10 X (Tris-base-108 g, Boric acid – 55 g and 40 ml of 0.5 M EDTA, pH 8.0 per l) was prepared and 0.5X TBE buffer was used as running buffer for electrophoresis. 2 µl of gel loading dye (Bromophenol blue) was added to 20 µl of SSR PCR product and mixed well before loading into wells. 50 bp (Genei) ladder were electrophorised alongside the SSR reactions. Electrophoresis was carried out at 60-90 V for 2-3 hrs and the DNA bands were photographed under UV light by using Major science UVDI gel documentation system.

Since SSR markers are co-dominant, these markers could express different values of alleles per

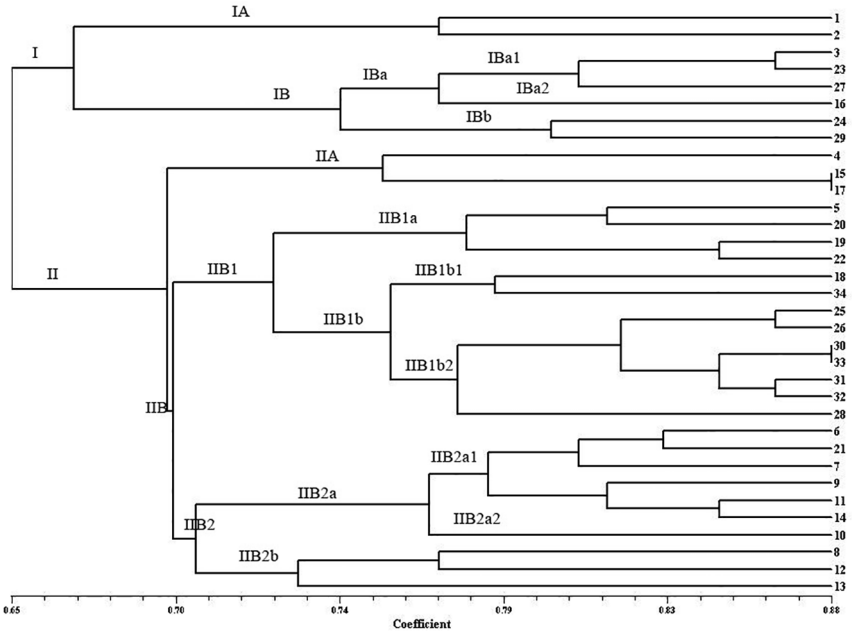

Fig. 1. Dendrogram of mango cultivars based on SSR markers.

Name of the cultivars :

1 : Banganapalli- 1
2 : Banganapalli- 2
3 : Banganapalli- 3
4 : Banglora- 1
5 : Banglora- 2
6 : Baramasi
7 : Cherukurasam
8 : Chinnnarasam
9 : Chinna Suvarnarekha
10: Elamandala
11: Hyder
12: Imam Pasand
13.: Jalal
14: Jehangir
15: Kolanka Goa
16: Kottapalli Kobbari
17: Kowsuri Pasand
18: Nalla Andrews
19: Nalla Rasalu
20: Navaneetham
21: Nuzividu Tiyya Mamidi
22: Nuzividu Rasalu
23: Panchadara Kalasa
24: Pandurivari Mamidi
25 : Paparao Goa
26: Peddarasam
27: Panukula Mamidi
28: Royal special
29: Rajamanu
30: Sora Mamidi
31: Suvarnarekha
32: Tella Gulabi
33: Tella Rasalu
34: Rajamamidi

locus. Data were entered using a matrix in which all observed bands or characters were listed. The SSRs pattern of each cultivar was evaluated assigning character state '1' to all the bands that could be reproducible and detected in the gel and '0' for the absence of band for each primer. The scores obtained using all the primers in the SSR analysis were then pooled to create a single data matrix and used to construct a UPGMA (Unweighted Pair Group Method of Arithmetic Means) dendrogram among cultivars using a computer program, the NTSYS-pc software package version 2.02 (Rohlf 2000).

RESULTS AND DISCUSSION

Molecular markers enable the assessment of genetic similarity among cultivars in the early stages of development and are advantageous for diversity studies (Nicolosi *et al.* 2000). The purity of DNA of mango cultivars used in the present study ranged from 1.61 to 1.80 indicated that the DNA obtained from this protocol was free of contaminants.

Out of 20 SSR markers, eighteen microsatellites were amplified and produced sixty five alleles. The

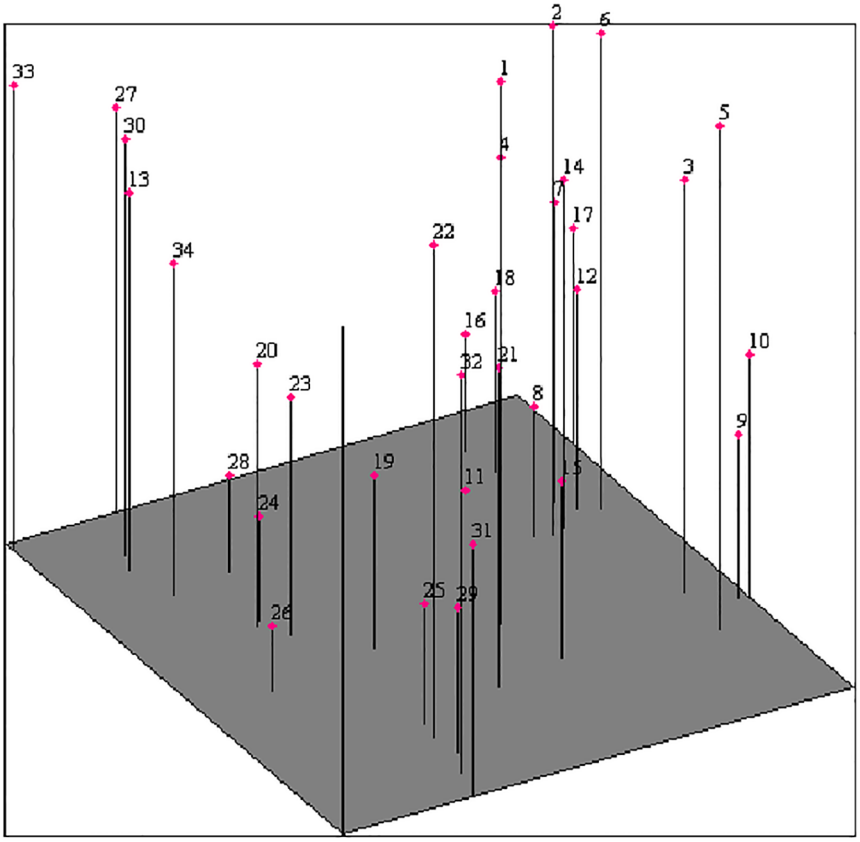

Fig. 2. Relative position of mango cultivars based on SSR markers (Three dimensional).

Name of the cultivars :

1 : Banganapalli- 1
2 : Banganapalli- 2
3 : Banganapalli- 3
4 : Banglora- 1
5 : Banglora- 2
6 : Baramasi
7 : Cherukurasam
8 : Chinnnarasam
9 : Chinna Suvarnarekha
10: Elamandala
11: Hyder
12: Imam Pasand
13 : Jalal113
14 : Jehangir
15 : Kolanka Goa
16 : Kottapalli Kobbari
17 : Kowsuri Pasand
18 : Nalla Andrews
19 : Nalla Rasalu
20 : Navaneetham
21 : Nuzividu Tiyya Mamidi
22 : Nuzividu Rasalu
23 : Panchadara Kalasa
24 : Pandurivari Mamidi
25 : Paparao Goa
26 : Peddarasam
27 : Panukula Mamidi
28 : Royal special
29 : Rajamanu
30 : Sora Mamidi
31 : Suvarnarekha
32 : Tella Gulabi
33 : Tella Rasalu
34 : Rajamamidi

total number of alleles varied from 1 (MngSSR 14 and MiIIHR 18) to 9 (MiIIHR 23) with an average number (3.61) of alleles per locus. This is in accordance with the findings of Viruel *et al.* (2005), Anshuman *et al.* (2012), Kumar *et al.* 2013 and Malathi *et al.* (2013) who reported 5.50, 3.47, 2.70 and 2.87 alleles per locus, respectively in mango. The eighteen selected SSR primers amplified alleles across the 34 cultivars with varied degrees of polymorphism. High level of polymorphism was observed with MiIIHR 19 primer i.e., 9 alleles per locus. Further, null alleles were also observed among the mango cultivars with SSR prim-

Table 1. List of SSR markers used for DNA amplification in mango cultivars.

Sl. No.	Primer	Nucleotide sequences (5' - 3')	Allele number	Allele size (bp)	PIC
1	SSR -8	F : TTGATGCAACTTTCTGCC	3	200-240	0.85
		R : ATGTGATTGTTAGAATGAACTT			
2	SSR -15	F : TTTACCAAGCTAGGGTCA			
		R : CACTCTTAAACTATTCAACCA	4	180-270	0.93
3	SSR -16	F : GCTTTATCCACATCAATATCC			
		R : TCCTACAATAACTTGCC	--	--	--
4	SSR -20	F : CGCTCTGTGAGAATCAAATGGT			
		R : GGACTCTTATTAGCCAATGGGATG	4	230-700	0.84
5	SSR - 39	F : TGTCTACCATCAAGTTCG			
		R : GCTGTTGTTGCTTTACTG	3	160-200	0.90
6	SSR - 46	F : TCATTGCTGTCCCTTTTC			
		R : ATCGCTCAAACAATCC	3	180-1200	0.98
7	SSR - 52	F : AAAAACCTTACATAAGTGAATC			
		R : CAGTTAACCTGTTACCTTTTT	4	100-225	0.95
8	SSR - 61	F : AAAGATAGCATTTAATTAAGGA			
		R : GTAAGTATCGCTGTTTGTTATT	2	200-220	0.71
9	SSR - 82	F : TCTGACCCAACAAAGAACCA			
		R : TCCTCCTCGTCCTCATCATC	5	80-150	0.93
10	MiIIHR-13	F : CCCAGTTCCAACATCATCAG			
		R : TTCCTCTGGAAGAGGGAAGA	2	150-200	0.80
11	MiIIHR -15	F : CTAACCATTCGGCATCCTCT			
		R : TCTGTGATAGAATGGCAAAAGAA	7	100-280	0.72
12	MiIIHR -17	F : GCTTGCTTCCAACTGAGACC			
		R : GCAAAATGCTCGGAGAAGAC	--	--	--
13	MiIIHR- 18	F : TCTGACGTCACCTCCTTTCA			
		R : ATACTCGTGCCTCGTCCTGT	1	160	0.58
14	MiIIHR -19	F : TGATATTTTCAGGGCCCAAG			
		R : AAATGGCACAAGTGGGAAAG	9	100-700	0.90
15	MiIIHR -23	F : TCTGACCCAACAAAGAACCA			
		R : TCCTCCTCGTCCTCATCATC	3	100-170	0.52
16	MiIIHR-24	F : GCTCAACGAACCCAACTGAT			
		R : TCCAGCATTCAATGAAGAAGTT	3	250-260	0.77
17	MiIIHR-30	F : AGCTATCGCCACAGCAAATC			
		R : GTCTTCTTCTGGCTGCCAAC	2	200-220	0.54
18	MiIIHR -31	F : TTCTGTTAGTGGCGGTGTTG			
		R : CACCTCCTCCTCCTCCTCTT	3	100-150	0.69
19	MngSSR -14	F : TCATTAAGCTGTGGCAACCA			
		R : CATTGCATAGATGTGGTCATT	1	180	0.55
20	MngSSR - 24	F : CGATGGACTTCATAAGAAGAG			
		R : GCTAGCAGAATCACCTTGGTC	6	50-800	0.86
	Total		65		0.78
	Average		3.61		

ers. The variation in alleles might be due to template DNA concentration and purity, primer selection, primer design, amplicon size, PCR conditions and sensitivity of product detection.

Out of twenty SSR primers used in analysis, only eighteen primers produced the polymorphic bands. All the SSR primers used in the present study produced discrete, scorable and unambiguous bands. The PCR product size obtained by amplification of SSR primers ranged from 50 to 800 bp (Table 1). It was comparable with the results generated by polymorphic bands ranging from 90 bp to 370 bp (Hammedunnisa Begum *et al.* 2012), 100 bp to 480 bp (Anshuman *et al.* 2012), 130 bp to 245 bp (Hammedunnisa Begum *et al.* 2013) in mango.

Table 2. Details of unique SSR fragments associated with specific mango cultivars.

Sl. No.	Name of the cultivar	Primer revealing unique SSR	Size of the fragment (bp)
1	Banglora -1	MiIIHR 24	260
2	Imam Pasand	SSR 20	280
3	Panchadara Kalasa	MiIIHR 19	100
4	Chinnarasam	MiIIHR 31	200
5	Royal Special	SSR 15	270

The Polymorphic Information Content values (PIC) were calculated for the SSR markers used in this study (Table 1). The highest PIC was recorded by the marker SSR 46 (0.98), while it was found to be lowest for the marker MiIIHR 23 (0.52). High PIC value of SSR 46 indicated that the primer was highly informative.

When the identification of cultivars is the purpose of the finger prints, the most important function of a primer is to discriminate as many cultivars as possible. The polymorphic fragments present in only one cultivar were considered as unique fragments (Table 2). Out of the total 65 polymorphic fragments, five fragments were unique to particular cultivars viz., Banglora -1 (MiIIHR 24), Imam Pasand (SSR 20), Panchadara Kalasa (MiIIHR 19), Chinnarasam (MiIIHR 31) and Royal Special (SSR 15) which could be exploited for DNA fingerprinting of these cultivars.

The similarity values obtained for each pair wise comparison of SSR markers in all the 34 cultivars of mango were used to construct a dendrogram based on hierarchical clustering and the results are presented in Fig. 1. The description of the data by using three dimensional pictorial graph is presented in Fig. 2.

The similarity coefficient based on 18 SSR markers ranged from 0.65 to 0.88. Among the 34 cultivars studied, the highest similarity index (0.88) was recorded between Sora Mamidi, Tella Rasalu, Kolanka Goa and Kowsuri Pasand. The dendrogram demonstrated the distribution of cultivars into two main clusters at similarity coefficient value of 0.65, on the basis of their similarity calculated as proportion of shared alleles. The dendrogram derived from the UPGMA cluster analysis revealed one major cluster with 26 cultivars grouped into several sub clusters and one minor cluster with 8 cultivars (Fig. 1).

Cluster I was further divided into sub cluster IA with 2 cultivars and IB with 6 cultivars, at similarity coefficient value of 0.67 as shown in the dendrogram. Cluster IA consists of 2 cultivars (Banganapalli-1 and Banganapalli-2) grouped as a cluster at similarity coefficient value of 0.75 as in the dendrogram. The cluster IB was further divided into sub cluster IB-a containing 4 cultivars and IBb with 2 cultivars (Pandurivari Mamidi and Rajamanu). The sub cluster IBa was further divided into two clusters. Cluster IBa1 with Banganapalli-3 and Panchadara Kalasa grouped as a cluster and Panukula Mamidi as a solitary cluster at a similarity coefficient value of 0.81 as shown in the dendrogram. Cluster IBa2 formed as a solitary cluster with Kottapalli Kobbari at similarity coefficient value of 0.75 as shown in the dendrogram.

Cluster II was further divided into sub cluster IIA with 3 cultivars and sub cluster IIB with 23 cultivars at similarity coefficient value of 0.70 as shown in the dendrogram. Kolanka Goa and Kowsuri Pasand were identical having common alleles among them and Banglora-1 formed a solitary sub cluster at a similarity coefficient value of 0.75 as shown in the dendrogram. Cluster IIB was further divided into 2 clusters i.e., IIB1 with 13 cultivars and IIB2 with 10 cultivars. IIB1 was further divided into 2 sub clusters at a similarity coefficient value of 0.73 as shown in the dendrogram. The sub cluster IIB1a consists of Banglora-2 and Navaneetham as a cluster and Nalla Rasalu and Nuzividu Rasalu as a cluster at a similarity coefficient value of 0.77 as shown in the dendrogram.

At a similarity coefficient value of 0.75 the cluster IIB1b was again divided into 2 sub clusters. Nalla Andrews and Rajamamidi formed sub cluster IIB1b1 at a similarity coefficient value of 0.78 as shown in the dendrogram. In sub cluster IIB1b2, the Sora Mamidi and Tella Rasalu were identical having common alleles among them with extremes in the dendrogram at a similarity coefficient value of 0.88, the cultivars Paparao Goa and Peddarasam formed a cluster at a similarity coefficient value of 0.86, Suvarnarekha and Tella Gulabi formed a cluster at a similarity coeffi-

cient value of 0.85 and Royal Special was formed as a solitary cluster at a similarity coefficient value of 0.78 as shown in the dendrogram.

At a similarity coefficient value of 0.70, cluster IIB2 was divided into 2 clusters with cluster IIB2a consisting of 7 cultivars and cluster IIB2b consisting of 3 cultivars. Sub cluster IIB2a was again further divided into 2 sub clusters. The sub cluster IIB2a1 consists of Baramasi and Nuzividu Tiyya Mamidi as a cluster at a similarity coefficient value of 0.83, the cultivars Hyder and Jehangir formed as a cluster at a similarity coefficient value of 0.84 and the cultivars Cherukurasam and Chinna Suvarnarekha each formed solitary clusters at a similarity coefficient value of 0.81 as shown in the dendrogram. The sub cluster IIB2a2 consists of Elamandala at a similarity coefficient value of 0.78.

Cluster IIB2b consists of Chinnarasam and Imam Pasand as a sub cluster at a similarity coefficient value of 0.78 and Jalal formed as a solitary sub cluster at a similarity coefficient value of 0.73 as shown in the dendrogram.

The cultivars Banganapalli -1, Banganapalli -2 and Banginapalli-3 were closely clustered suggested that the cultivars were derived from a single ancestor. Selections from same geographic location were grouped separately into different sub clusters. Thus, the tendency of cultivars grouping in clusters was irrespective of geographic boundaries, demonstrated that geographical isolation was not the only factor for causing genetic diversity. This suggested that there are forces other than geographical seperation such as natural or artificial selection, exchange of breeding material, genetic drift and environmental variation. The existence of duplicates might be due to multiple donations of the same seed source but from different collections. Therefore, diverse geographic origins among cultivars might not always be a reliable indicator for sampling of genetically diverse materials. From the conservation point of view, identification and elimination of duplicates in a collection could save time and resources both financial and human in germplasm maintenance due to reduced number of cultivars.

Although the development of microsatellites was considered as laborious and expensive, they were extremely efficient, reproducible and highly informative genetic markers (Eiadthong *et al*. 1999). The present study of use of microsatellite markers in characterization of mango cultivars revealed that 18 polymorphic microsatellite markers were found efficient to distinguish cultivars and would certainly be useful for purposes such as the certification of varieties, for identification of pest and disease resistant lines and for development of superior plants from the crosses of these mango cultivars. The few amplification products generated could be effectively used as markers for differentiation of a part of the mango cultivars studied. A finer molecular analysis of mango cultivars is required with more number of SSR markers as far as possible in order to detect and identify unique as well as fine resolution of molecular polymorphism between different identical cultivars. The use of large number of SSRs with greater genome coverage could help to reveal genetic diversity more accurately and also help to differentiate the cultivars unambiguously with identical allelic patterns as revealed by the set of primers used in this study.

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