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Original Article

Development of di-nucleotide microsatellite markers and construction of genetic linkage map in mango (*Mangifera indica* L.)

Chataporn Chunwongse[1,3], Chalermpol Phumichai[4], Pumipat Tongyoo[1,3], Narisa Juejun[1,3], and Julapark Chunwongse [1,2,3]*

[1] *Center for Agricultural Biotechnology,*

[2] *Department of Horticulture, Faculty of Agriculture, Kasetsart University, Kamphaeng Saen Campus, Kamphaeng Saen, Nakhon Pathom, 73140 Thailand.*

[3] *Center of Excellence on Agricultural Biotechnology: (AG-BIO/PERDO-CHE), Bangkok, 10900, Thailand.*

[4] *Department of Agronomy, Faculty of Agriculture, Kasetsart University, Bang Khen Campus, Chatuchak, Bangkok, 10900 Thailand.*

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Abstract

Forty-two di-nucleotide microsatellite, or simple-sequence repeat (SSR), markers were developed using CA and CT-enriched genomic libraries of *Mangifera indica* L. Six cultivated mangoes and two wild species were tested for primer amplifications. Most loci could amplify *M. caloneura* Kruz and *M. foetida*. The average number of alleles per locus was 4.4. The average expected heterozygosity and the maximum polymorphism information content value were 0.57 and 0.53, respectively. The SSRs developed in this study together with 65 SSRs and 145 restriction fragment length polymorphism (RFLP) markers reported previously were used in the genetic linkage analysis. A partial genetic linkage map was constructed based on 31 F1 progenies from a cross between 'Alphonso' and 'Palmer'. The map spanned a distance of 529.9 centiMorgan (cM) and consisted of 9 microsatellite markers (6 from this study) and 67 RFLP markers. The new SSR markers and the present map will be useful for mango genetic studies and breeding applications in the future.

Keywords: di-nucleotide SSR, *Mangifera*, linkage map

1. Introduction

Mango *(Mangifera indica* L.*)*, a diploid fruit tree with 2n = 2x = 40 (Arumuganathan and Earle, 1991), is native to India and Southeast Asia. Later on, mango germplasm has been introduced to other continents, including Africa, America, and Australia. The species is now cultivated commercially in tropical and many subtropical regions (Mukherjee, 1997). Similar to other fruit trees, several problems exist in mango improvement programs. Long juvenile stage requires maintenance and care for an extensive period of time before any selection can be done. A large acreage of land is required to grow mangoes. In addition, breeders for the south-east Asian mango, the mild turpentine taste variant, are faced with another problem i.e. poly-embryony, the phenomenon of multiple seedlings (one zygotic seedling and several nucellar seedlings) arising from a single seed. This characteristic reduces the chance of recovering true hybrid seedlings (Schnell and Knight, 1992). The inconvenience of the multiple seedlings in breeding program can be overcome by using the mild taste mono-embryonic cultivars such as 'Keitt', 'Kent' or 'Shelly' instead;

* Corresponding author.
Email address: julapark.c@ku.ac.th

or a codominant DNA marker such as SSR marker can be used by breeders of Indochinese type mango in selection of the zygotic seedlings.

Molecular markers and marker-assisted selection (MAS) have proved to be useful tools for breeding of economically important crops. Molecular markers can be used to identify true hybrids in polyembryonic species such as Indochinese type mango and some citrus species (unpublished data, Oliveira *et al*., 2002). Furthermore, MAS allows early selections of major genes controling traits and thus reduces the time and space needed for growing out seedlings. Genome research and molecular technologies in temperate fruit trees such as Rosaceae crops have progressed significantly in the last decade (Dirlewanger *et al*., 2004). Unfortunately, the utilization of molecular markers in mango is still in its infancy. Only a limited number of highly informative markers such as microsatellite markers are available in mango (Viruel *et al*., 2005; Duval *et al*., 2005; Schnell *et al*., 2005; Honsho *et al*., 2005; Ukoskit, 2007; Ravishankar *et al*., 2011; and Suprapaneni *et al.,* 2013). Microsatellite, or simple-sequence repeat (SSR), markers are polymerase chain reaction (PCR) based markers which detect differences of the copy numbers of short stretch repetitive DNA sequences. This type of marker is co-dominant, reproducible, highly polymorphic and transferable from one population to another (Kalia *et al*., 2011). There were several reports on applications of mango microsatellite markers, including genetic diversity (Duval *et al*., 2005; Suprapaneni *et al.* 2013), cultivar identification (Eiadthong *et al*., 1999), and pedigree analysis (Olano *et al*., 2005). In order to utilize marker technology to its full potential, more markers are needed to construct a high density mango genetic linkage map. The saturated map with whole genome coverage will be the basis for many genetic analyses such as quantitative trait loci (QTL) analysis, map-based gene cloning, comparative genomics studies and genome-wide association studies (Liu *et al*., 1996; Milbourne *et al*., 1998; Fukino *et al*., 2008; Huang *et al*., 2010; Li *et al.*, 2013).

Thus, the objectives of this study were to develop di-nucleotide microsatellite markers from mango genomic DNA, characterize the markers and construct a genetic linkage map using data from the new markers, 65 published SSR markers, and the restriction fragment length polymorphism (RFLP) markers from our previous study (Chunwongse *et al*., 2000).

2. Materials and Methods

2.1 Construction of enriched genomic library

Genomic DNA from leaves of 'Nang Klang Wan' mango cultivar was extracted as described by Doyle and Doyle (1991). The modified version of the enrichment protocol described by Watcharawongpaiboon and Chunwongse (2008) was used to isolate the mango microsatellites. The mango DNA was digested with *Tru*9I and ligated to *Mse*I adaptor (Vos *et al*., 1995). After ligation, mango DNA was preamplified with *Mse*I primer. Two 5' biotinylated oligonucleotides, B-$[CA]_{15}$ and B-$[CT]_{15}$ (BSU, Thailand) were used to bind repetitive DNA sequences. Streptavidin-coated Dynabeads–M280 (Life Technologies, USA) and the micro-concentrator (Promega, USA) were used to isolate the captured fragments which were then reamplified with *Tru*9I primer. The PCR products were purified using Wizard PCR Preparation kit (Promega, USA). Cleaned PCR products were ligated to pGEM-T Easy vector (Promega, USA) and transformed into the competent *Escherichia coli* strain DH5α by electroporation and plated on LB agar containing 0.1 g/ml ampicillin with 0.2 M 5-bromo-4-chloro-indolyl-β-D-galacto-pyranoside (X-gal) and 0.1 M Isopropyl-B-D-thiogalacto-pyranoside (IPTG).

2.2 Plasmid sequence identification

Single white colonies were picked and grown overnight for plasmid DNA extractions (Wizard plasmid DNA purification kit, Promega, USA). After confirming the present of SSRs by dot blot hybridization with $(CA)_{10}$ and $(CT)_{10}$ repeat probes, DNA of positive clones were diluted and subjected to cycle sequencing using ABI Big Dye version 3.0 sequencing kit (Applied Biosystem, USA). The sequencing reactions were done using T7 primer and SP6 primer. After sequencing, the products were precipitated and sent to the sequencing facility for separation (BSU, Thailand).

2.3 Analysis of sequencing data and primer design

DNA sequences were analyzed for microsatellite repeats using a software Sputnik (http://espressosoftware.com/sputnik/) of the University of Washington. DNA regions flanking the SSR were picked for primer designs using Prophet 5.0 DNA analysis software (National Computing Resource for Life Science Research, NCBI). Oligonucleotides were synthesized by Pacific Science Company, Thailand.

2.4 Testing of SSR primers

After testing for optimum annealing temperature using gradient block thermal cycler PTC200 (MJ Research, USA), the obtained PCR primer pairs were used to amplify genomic DNAs of three Florida mango cultivars ('Tommy Atkins', 'Irwin', and 'Keitt'), three Thai mango cultivars ('Nang Klang Wan', 'Nam Dok Mai', and 'Khew Savoy') and two wild species (*M. caloneura* Kurz and *M. foetida*). The PCR reactions were performed in a total volume of 20 µl reaction containing 20 ng of mango genomic DNA, 1xPCR buffer, 10 mM dNTPs, 1.5 mM $MgCl_2$, 10 µM each of forward and reverse primers, and 1 unit of *Taq* polymerase. The amplification profile was predenatured at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at selected temperature (50-55°C) for 30 s and extension at 72°C for 1 min, and a final extension at 72°C for 5 min. PCR

products were separated on 4.5% polyacrylamide gel in 1X TBE and visualized by silver staining (Bassam *et al*., 1991). PhiX174/*Hin*fI was used as a molecular weight standard marker.

2.5 Statistical analysis

The number of alleles, the observed (H_0) and expected (H_e) heterozygosities (Nei, 1978), and the polymorphic information content (PIC) values (Botstein *et al*., 1980) for each marker loci were estimated using PowerMarker V3.25 software (Liu and Muse, 2005).

2.6 Linkage map construction

The mapping population consisted of 31 F1 individuals obtained from a cross between 'Alphonso', an Indian mango, as the female parent and 'Palmer', a Florida mango, as the male parent. Both parents and the progenies are monoembryonic. The seedlings were all tested using at least 10 codominant RFLP markers to be certain that all progenies were derived from the cross between these two parents (data not published).

All amplifiable primer pairs designed in this study together with 65 published SSR primers (Duval *et al*., 2005; Schnell *et al*., 2005; Honsho *et al*., 2005; and Ukoskit, 2007) were screened for polymorphism between 'Alphonso' and 'Palmer' and a subset of six progenies from the mapping population. The segregated polymorphic markers were subsequently tested on the entire population. Cleared segregation of DNA bands was scored for mapping. A genetic linkage map was constructed using the cross-pollinator (CP) algorithm of Joinmap 3.0 (Van Ooijen and Voorrips, 2001). Scorable SSR data from this study together with 145 RFLP data from the previous study (Chunwongse *et al.*, 2000) were used to construct the map. Markers were assigned to linkage groups with the minimum logarithm of odds (LOD) score of 3.0 and the distances between markers were calculated using Haldane map function.

3. Results and Discussion

3.1 Development of SSR markers

The numbers of positive clones and primer designs are shown in Table 1. Since this technique used PCR amplification to enrich the fragments, a number of clones were duplicates. Therefore, it might not be suitable for estimating the frequency of di-nucleotide microsatellite in mango or other organisms. This technique, however, could give an overview of the selected DNA repeats in a certain genome. We found that both CA- and CT-enriched libraries contained considerable numbers of repetitive sequences, 37.7% and 42.7% respectively. Some of the clones contained no SSR due to the nonspecific binding of non-SSR fragments to the streptavidin coated magnetic beads. Most of the clones constructed using *Tru* 9I (T. âTAA) contained very low numbers of SSR located close to the cloning sites, consequently, we could design primers specific to each locus effectively. Fifty-five and 73 primer pairs were designed from CA- and CT-repeat sequences, respectively. A total of 42 primer pairs (17 CA-repeat markers and 25 CT-repeat markers) could amplify genomic DNA of mango cultivars tested (Table 2). The enrichment process described is rather inefficient as many false positive clones were detected. The enzyme used in this study frequently cut close to the repetitive sequences resulting in an inability to design several flanking primers (Table 1).

The whole genome sequencing and transcriptome analysis have become a viable choice for the research communities (Zhang *et al.*, 2011; Haas and Zody, 2010). DNA sequences for *Mangifera* species have been accumulating in the public database in recent years, the SSR mining would be accomplished efficiently from these information with much lower effort and cost. The expressed sequence tag (EST)-SSR has become a tool for studying diversity of several plant species, including potato (Milbourne *et al.*, 1998), grape (Scott *et al.*, 2000), barley (Thiel *et al.*, 2003), cassava (Zou *et al.*, 2011), and *Dendrobium* orchid (Juejun *et al.*, 2013). These EST-SSR of the expressed gene would help in increasing number of DNA marker available for mango research. The SSR markers are not expanding the whole genome to anchor all genes and alleles that can be used in association with phenotypes. Single nucleotide polymorphism (SNP) derived from the genome sequences of mango will be useful in genetic studies and breeding of mango. SNP marker is highly abundant in the genome of higher plants and can be incorporated into the automated genotype screening instruments. The vast amount of SNP genotypes could then be used to associate with horticultural traits of mango and be used efficiently in complex traits analysis such as yield and quality.

3.2 Cross-amplification, polymorphism, and heterozygosity

According to Kostermans and Bompard (1993), the common mango, *M. indica* L., and its close relative, *M. caloneura* Kruzs, are both in the subgenus *Mangifera* (Ding Hou) Kosterm. The species *M. indica* L. belongs to the section *Mangifera*, *whereas M. caloneura* Kruzs belongs to

Table 1. Numbers of positive colonies, primers designed, and amplifiable primers

	CA-repeat	CT-repeat
Colonies picked	439	510
Positive colonies after dot blot	257	201
SSR containing sequences	97	86
Primers designed	55	73
Amplifiable primers	17	25

Table 2. SSR primer sequences and associated information: SSR length based on SSR sequences of cultivar 'Nang Klang Wan', Na = No. of allele, He = Expected heterozygosity, Ho = Observed heterozygosity, PIC = Polymorphism information content, mapping = used in linkage analysis and gr = mapped in linkage group

	primer	Forward (5'-> 3')	Reverse (5'-> 3')	repeat motif	SSR length (bp)	size range (bp)	Na	He	Ho	PIC	mapping	gr
1	MMCA2	AGGCGGATGTTCATTATCCA	GCATGCTGACATGCTGTTTT	CA	20	265-288	4	0.72	0.50	0.67	✓	
2	MMCA5	CAAAAAGGAGCTTCATCCAA	CGTTTGGTTGCAGAGAGAATG	TG	12	300-604	3	0.55	1.00	0.46		
3	MMCA10	GGCCTCCGTCATAGATTTCA	CTTCCACCTCATCCAGCATT	GT	8	191	1	0.00	0.00	0.00		
4	MMCA33	CTTGGGTGAAGGGTGTTTGT	CTTCACAGCCTGAAGTGACCT	GT	18	276-286	3	0.63	0.83	0.55		
5	MMCA34	GCCACGGGAGTATGGATTTA	CCTATCATTGGCTTCCCTCA	CA	40	227-289	2	0.43	0.63	0.34		
6	MMCA49	TTCTCACCAACACCACCAAA	TGCTGATAAAAACAAAGGGTCA	AC	30	220-311	6	0.80	1.00	0.78	✓	
7	MMCA68	GCATGAAGAGGAACCAAAACA	ATAAGGTGACAGCCCTCCAAT	AT	10	260	1	0.00	0.00	0.00	✓	
8	MMCA83	GAGAAGTTCAAACACACGCAA	TTTTAGACTCCTCCTCTTTGTCTTTT	CA	8	280-320	3	0.32	0.13	0.29	✓	
9	MMCA92	GCAAAAAGACATATCTAGAAGCTGG	ATTGGCCATGGTGATGCTAT	GT	38	181-224	8	0.76	0.50	0.74	✓	
10	MMCA107	AGGGCCGGTTACCTCTCAT	AGACCAAACCAACAACGCTT	TA	8	196-218	2	0.22	0.25	0.19		
11	MMCA110	CCCTCAACTTTGTTTTCCATTC	TCCTTGGGAGTTGCTTTTGT	CA	12	202	1	0.00	0.00	0.00		
12	MMCA150	TTGGCTTTATACGGGTGAGC	GGGCCTCGTAGCAGTTAGGT	TG	12	246-278	3	0.52	0.43	0.46	✓	
13	MMCA178	CACTAACTTACACATTTCCATGCT	TGTATATTTCTAAGGCATGAAGATC	GT	10	188-238	4	0.65	0.13	0.58	✓	
14	MMCA198	ACCCTTCTTAGCTAATTACGTCACCTT	TATGCACCCATATGCACTTGCCTA	GT	8	178	1	0.00	0.00	0.00		
15	MMCA268	TATCACTGTGAATGTTTTGGCTTT	ATACCTTTCCTTCAAACAAGCAAC	AC	40	147-191	8	0.85	0.71	0.83		
16	MMCA288	AAAGTAAGTGACACCCCAAATGTT	TTGTGGATATGCCCAATTATGTAG	AT	10	314-356	4	0.69	0.25	0.63	✓	
17	MMCA289	CATAGACTCTTGCATGATCCAACT	TTGATACAACAGATAGAGCATTTT	GT	18	332-371	4	0.66	0.75	0.62	✓	✓
18	MMCT5	GTGTTGTGTGCATAAGGTTGAAGT	AGGTTCAGATCCCAACAGTAAAAG	CT	14	102-146	9	0.84	0.75	0.83	✓	
19	MMCT24	ATTTGCTTCCCACATGTTCACCTG	ATTTTGTCTTCCTCCACTGTCCAC	CT	12	227-262	3	0.64	0.43	0.57		
20	MMCT30	CCAGATGCTCAGGAAAGATTAGAT	CTCGAGCTGCAGCATTTACTTTAC	GA	14	288-314	2	0.43	0.63	0.34		
21	MMCT35	CAGAAAGTGATTACATACCGACAT	TATAACTCTCAGCTTTCCACTTCA	GA	28	141-182	8	0.80	0.75	0.78	✓	
22	MMCT36	TGGAAACTGCGACTAAGCGACACT	TGAGCAAAATATTCTTCTGACTGG	TC	20	120-155	7	0.79	0.88	0.76	✓	
23	MMCT39	TTTCTTCTTTTCATCGCCCATTCA	GCAGGAGGTCGCATTTCTACAGTC	TC	36	240-282	4	0.67	0.38	0.61	✓	
24	MMCT43	ATGGACGGTGACTTAGGTGC	CATGGGTTTGATGACGACAG	AG	14	252-258	3	0.23	0.25	0.21		
25	MMCT53	TTGGACTTGAGGGATTTTGC	CTCCTCGCCAACTTCAACTC	GA	10	205	1	0.00	0.00	0.00		
26	MMCT56	CAACGATGGTGATGGCTAGA	GGGCTTTGTTTTGGTTGTGT	AG	14	241-250	4	0.54	0.50	0.48	✓	
27	MMCT70	ACGGTTTTTAGAGGTGACATC	AGTATAATTGGCAAGGAAAGAGGT	CT	22	263-275	6	0.73	0.75	0.69	✓	✓
28	MMCT72	TCATGGTAACTATCAAATCACTCA	TGCAAATCTCACCAGACAACA	AG	36	165-185	4	0.65	0.38	0.58		
29	MMCT75	TTGGCAACTTGGTGGATGTAG	GCAAAATTGTGGCTTAGGTAGTG	AG	30	249-270	6	0.69	0.63	0.65	✓	
30	MMCT89	CCATTTGGCAATGGCTAGTT	ACAATGCAGCCCTAGTTTGG	TA	18	157-183	5	0.61	0.50	0.56		
31	MMCT103	TAGGACTGAATGGAGGTGGC	GGCATTCATAACATCTTTGGC	GA	14	260-275	4	0.61	0.50	0.56	✓	
32	MMCT121	CTAGCTCATGAAATGAATCTCTAGG	TCCTCACTACTCCGCTGCTT	AG	10	278-300	3	0.63	0.63	0.56	✓	
33	MMCT128	CTAATCTCAGGGACGGAAAGC	CACCAATGCAAGATTCAGAAC	TC	18	259-305	5	0.72	0.75	0.67	✓	✓
34	MMCT134	GGTGTGCTGTTGCACAAAAT	CGTTCTTCATACAAAACTACAGCA	AG	12	320-410	7	0.83	0.75	0.81	✓	✓
35	MMCT138	TGGCAACAAAAATGGAAACTT	ACCTAACATCCGCTCCTTCC	GA	28	274-372	6	0.77	0.75	0.73	✓	✓
36	MMCT139	GGAATCGCAGTTCAAAGCATA	GATCACGCTCCCGATCTCTAT	GA	10	283-288	3	0.59	0.88	0.52		
37	MMCT197	TACCCTACTTCTCCAGAGATGACC	ATTGTGAGATTACTGGGGTTTGAT	TC	42	260-289	7	0.77	0.63	0.74	✓	
38	MMCT224	ACAGAGAATAACGGCTAGAATTGG	TCGTCCCTCTACTATCATCATCAA	GA	42	266-321	5	0.71	0.63	0.67		
39	MMCT257	ATCTTCTTGCATTAGCTGTAGCCT	GTACACTAATGGCCATGATACCAA	TG	10	226-311	6	0.75	0.75	0.71	✓	✓
40	MMCT260	ATGAAGAAAAATTGAAGAGCCAAC	TCTAGTGATTGTAATCCTACTGAC	GA	40	178-207	5	0.73	0.71	0.70		
41	MMCT288	AGAAGAAGTCTACCGGTTTGAATG	GCCGATATTTGTGTGGCCTTGCCC	CT	44	322-378	7	0.77	0.63	0.74	✓	
42	MMCT505	CGTCAACTGAGTCCTACTTTGAGA	ATACTTTTCCACAGTCCAAAAAGC	GA	38	141-173	5	0.76	0.63	0.72		

the section *Euantherae* Pierre. A more distant relative, *M. foetida*, is a member of section *Perennes* in subgenus *Limus* (Marchand) Kosterm.

M. indica L. used in this study are from two sources. The three Florida cultivars are hybrids between Indian and south-east Asian types but are more closely related to Indian types (Olano *et al.*, 2005). They all produce monoembryonic seeds. The three Thai cultivars are south-east Asian types with polyembryonic seeds.

When markers on the six cultivars and two wild species were assessed, 41 of 42 primer pairs from *M. indica* L. were cross-amplified with *M. caloneura* Kurz, and *M. foetida* (Figure 1). Cross amplification of genomic SSR markers to related species was also reported by Schnell *et al.* (2005) and Duval *et al.* (2005). This characteristic can be of use in studying the relationship among species in the genus *Mangifera*.

The average level of polymorphism of markers found in tested mango was relatively high (0.53). The maximum PIC value was 0.83. Thirty markers (71%) show a PIC value more than 0.5 and are considered informative markers (Botstein *et al.*, 1980). Five markers (11.3%) did not show any polymorphism among the mango cultivars and species. This might be due to the area of the genome in which markers were selected, i.e., conserved genic region or homozygosity.

Among the SSR markers used, we found that MMCT5 has the largest number of alleles (9). The average number of alleles for all markers with this set of mango was 4.4. Seventy-one percent of the markers showed a higher level of expected heterozygosity (He) than the observed heterozygosity (Ho) demonstrating the inbreeding due to the breeding and selection processes (Templeton and Read, 1994).

Two markers, MMCA178 and MMCA289, could distinguish Florida mango cultivars used in this study from Thai cultivars. One SSR marker, MMCA68, could amplify only Thai cultivars (Indochinese type mango specific) (Figure 1b). These results, however, need to be verified in a larger number of mango genotypes from both the Indian and Indochinese groups. Nonetheless, these SSR markers could be used in genetic diversity studies, cultivar identifications and mango improvement programs. SSR markers can be used effectively to genotype the zygotic seedlings of the polyembryony mangoes. In the breeding program of Indochinese type mango, which predominantly have polyembryony, breeders need to sort the zygotic seedlings from the somatic seedlings (Schnell and Knight, 1992), SSR markers can be used to characterize the maternal genotypes out of the hybrid genotypes. This would save time and expense in maintaining all the seedlings during juvenile stage to the stage when phenotype of hybrids can be identified visually by breeders. On the other hand, the identified somatic seedlings could also be used as the genetically uniform root stocks that can be used in the experiments that require the uniformity of rootstock such as fertilizer trial.

3.3 Segregation of SSR markers in mango hybrid population

Thirty new SSR markers (18 CT-repeat and 12 CA-repeat primers) and forty-six published SSR markers showed polymorphisms (76.8%) among 'Alphonso', 'Palmer', and a subset of the progenies. The high level of polymorphism was typical for out-crossing species (Sharon *et al.*, 1997). When tested on the entire population, 69 markers (90%) of the seventy-six polymorphic SSR markers showed Mendelian segregation ratio at the p-value less than 0.05. The non-Mendelian markers might be due to the selections against certain progenies or some chromosomal aberrations such as duplications. (Kashkush *et al*., 2001). The polymorphic loci that segregated in Mendelian fashion were used for the linkage analysis.

3.4 Construction of genetic linkage map

To conduct QTL analysis, comparative mapping and whole genome association analysis, it is necessary to have a high density genetic linkage map which covers the entire genome of the organism. The amount of transferable markers in mango reported so far is still insufficient for a saturated map. In addition, a substantial number of individuals in the mapping population is required in order to detect the effects of the QTLs responsible for the traits of interest. Generating a large mapping population is quite a challenge for mango. Low fruit set and high fruit drop were the major causes of very low number of hybrids gained from massive pollinations. Small number of progenies in the mapping population will affect the calculated distance between markers and the quality of the map, resulting in the underestimations of QTL to be detected (Beavis, 1998). However, there are several mango genetic linkage maps reported and markers on these maps could be collectively used in QTL mapping in mango.

Chunwongse *et al*. (2000) constructed one maternal linkage map and one paternal linkage map of mango with AFLP and RFLP markers using the same set of population as

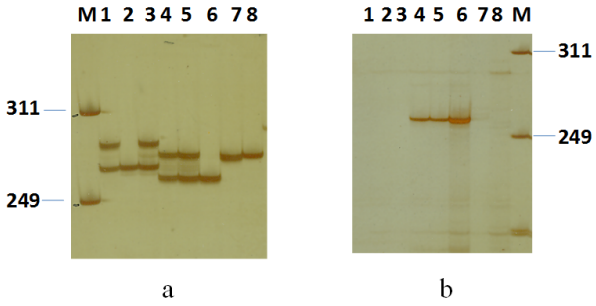

Figure 1. Mango genomic SSR markers a) MMCA2 and b) MMCA68 : M = PhiX174/*Hinf*I, 1 = 'Tommy Atkins', 2 = 'Irwin', 3 = 'Keitt, 4 = 'Nang Klang Wan', 5 = 'Nam Dok Mai', 6 = 'Khew Savoy', 7 = *Mangifera caloneura* Kurz and 8 = *M. foetida* . Size of DNA band is in bp.

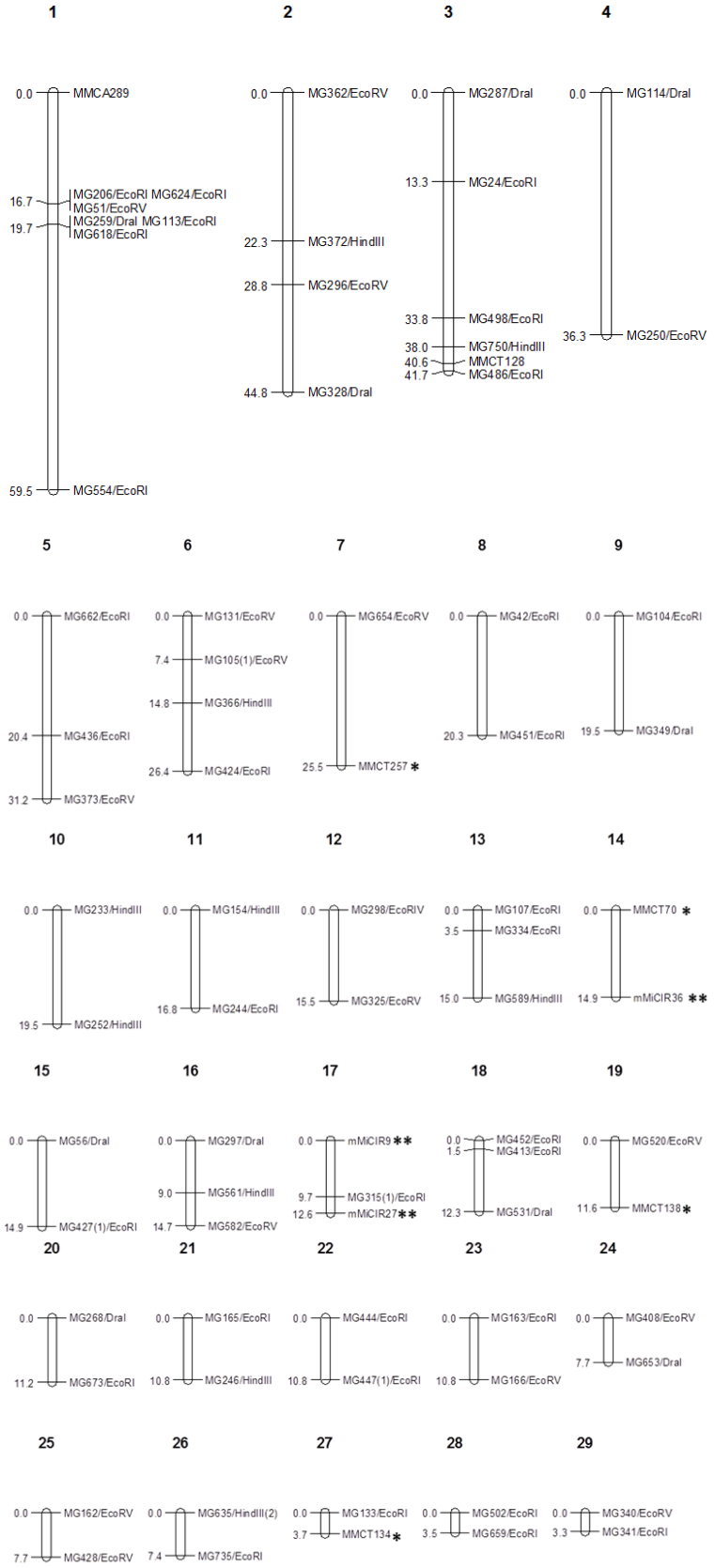

Figure 2. Linkage map of mango consists of 29 groups expanding 529.9 cM with 9 SSR and 67 RFLP markers. * = SSR markers from this study ** = SSR markers from Duval *et al.* (2005)

in this study. The two maps were generated according to double pseudo-testcross strategy (Weeden, 1994; Grattapaglia and Sederoff, 1994). The 'Alphonso' (maternal) map comprised 205 linked markers, covering 1,437.7 cM and the 'Palmer' (paternal) map comprised 246 linked markers, covering 2,561.9 cM. Kashkush *et al*. (2001) also constructed a linkage map based on AFLP markers segregating in 29 F1 progenies from a cross 'Keitt' x 'Tommy-Atkins'. The map consisted of 34 markers, covering 161.5 cM.

In the present study, linkage analysis was carried out using a total of 214 co-dominant marker data (69 SSR and 145 RFLP data). CP algorithm of JoinMap 3.0 enabled the unification of two parental maps, thus generated a single map construction for both parents. Seventy-six markers (9 SSRs and 67 RFLPs) were assigned to 29 linkage groups (LGs), with a total distance of 529.9 cM (Figure 2). The current map was aligned with the previous ones by Chunwongse *et al.* (2000). Even though genome coverage of the map was diminished when the AFLP data were excluded from the analysis, we intended to use only the SSR and the RFLP data because co-dominant type of markers are transferable among populations and the information between laboratories can be compared. Moreover, these markers can be used as anchors for further genetic linkage mapping and genome sequencing.

It is unlikely that the current SSR marker technology and the linkage map would be applied directly in the mango breeding program especially when dealing with the complex traits such as quality. With the advances in whole genome sequencing technology and EST sequencing projects in several crop species such as barley (Pasam *et al.,* 2012), maize (Li *et al.*, 2013) and rice (Huang *et al*. 2010), genome-wide association studies of mango become feasible. With the vast germplasm in the Indian sub-continent and Southeast Asia (Bompard and Schnell, 1997), this would open up the possibility of using the linkage disequilibrium analysis on the mango germplasm at the highest resolution and lead to a better understanding and utilization of genetics controlling agronomic and quality traits.

4. Conclusions

We developed 42 SSR markers from CA- and CT-enriched libraries. They were mostly amplifiable across species and highly polymorphic. A partial genetic linkage map was constructed comprising 29 LGs of 78 SSR and RFLP markers. The new SSR markers and the genetic linkage map from this study will be useful for mango research and breeding applications in the future.

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