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Assessment of cross-taxa utility of coconut microsatellite markers

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ABSTRACT

Micro-satellite or simple sequence repeats (SSRs) are abundant across eukaryotic genomes and show high levels of polymorphism. Micro-satellite DNA often has flanking regions that are highly conserved in related species and this renders the primer pairs designed in one species useful for the amplification of the same DNA region in related genomes, minimizing laborious cloning and screening steps. The present investigation explored the transferability of coconut SSRs to other palms, *viz.,* oil palm, arecanut, palmyrah and date palm. The annealing temperature of 86 coconut-specific SSR primers was standardized using gradient PCR. Of the 86 primers, 55 primers gave clear bands of expected size range (100 to 300 bp) and these were tested for their cross-taxa amplification. The percentage of cross-amplification of coconut SSR loci were 36.36% in oil palm, 29.09% in arecanut, 18.18% in palmyrah and 12.70% in date palm. The results suggest usefulness of coconut SSRs for phylogenetic and comparative genomic studies in other palms.

Key words: Coconut, microsatellites, cross-amplification, palms.

INTRODUCTION

Microsatellites or simple sequence repeats (SSRs) have been widely recognized as powerful and informative genetic marker in plants. They are widely employed for a large number of genetic projects compared to the other molecular markers available due to the highly reproducible and reliable identification of alleles when these markers are applied. The major drawback of using SSRs as molecular markers is the cost and effort required for their development, and this has restricted their use to only a few of the agriculturally important crops. Hence research on species relationship has increasingly focused on assessing the ability of SSR primers to amplify the same locus across different species and genera. Success in the cross-species amplification of any DNA sequence is inversely related to the evolutionary distance between the two species. Many studies have shown that micro-satellites cloned from one species can amplify homologous products in related species, minimizing laborious cloning and screening steps. Cross species conservation of SSR loci has been observed in *Citrus* (Kijas *et al*., 8), *Prunus* (Cipriani *et al*., 6), *Elaeis* (Bilotte *et al*., 2), *Pinus* (Chagne *et al*., 5; Shepherd *et al*., 13) and *Phoenix* (Bilotte *et al*., 4). Palms have 202 described genera with about 2779 species (Uhl and Dransfield, 14). Due to the limited amount of sequence data on palms available in databases compared to other economically important plant species, it is often necessary to construct genomic libraries and screen for specific SSRs for different palm species. Difficulties have often been encountered in isolating clean and reproducible single-locus microsatellite DNA markers in palms, probably because of the high proportions of highly repeated sequences in the nuclear DNA and the large size of the palm genomes. Several microsatellite loci have been identified in coconut (Rivera *et al.,* 11; Perera *et al.,* 9, 10; Baudouin and Lebrun, 1). Therefore, in the present study, the cross-transferability of coconut specific SSR primers in other palms *viz*., arecanut, oil palm, palmyrah and date palm were assessed.

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MATERIALS AND METHODS

The spindle leaves of coconut, arecanut, oil palm, palmyrah and date palm were used as the plant material for DNA extraction. DNA was extracted using the protocol reported by Upadhyay *et al*. (15) with slight modifications. Quantification of DNA in the preparation was done by reading the absorbance at 260 and 280 nm in a spectrophotometer. The working concentration of DNA was adjusted to 20 ng/µl according to the reading obtained from the quantification. It was then stored at 4°C. The stock DNA was stored at -20°C.The purity and intactness of the the genomic DNA isolated from different palm species was checked by running in 0.8% agarose gel stained with ethidium bromide (2 µl/ 100 ml from a stock having a concentration of 10 mg/ml) and was visualized in a gel documentation system. A total of 86 SSR primer pairs specific to coconut were used in the present study. The primers were synthesized from M/s Sigma Aldrich Inc, USA. Three sets of primers, namely, CNZ primers (Rivera *et al*., 11), CnCir primers (Baudouin and Lebrun, 1) and CAC primers (Perera *et al*., 9, 10) were used (Table 1). Initially, the use of the reported microsatellite

markers required optimization of the assay. The annealing temperatures were determined for each primer pair using gradient PCR. Once optimized, the PCR reaction was conducted in volumes of 20 µl containing 35 ng genomic DNA, 0.2 µM each of forward and reverse primers, 50 µM of each dNTPs, 1X buffer (10 mMTris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$) and 1 unit of *Taq* DNA polymerase. PCR amplifications were performed on an Eppendorf gradient thermal cycler with a PCR profile of 94°C for 5 min followed by 29 cycles of 1 min at 94°C, 2 min at the different annealing temperatures standardized for the individual SSR locus, and 2 min at 72°C with a final extension for 5 min at 72°C. After amplification, a volume of 3 µl of loading buffer (98 percent formamide, 10 mM EDTA, 0.005 percent each of xylene cyanol and bromophenol blue as tracking dyes) was added to each of the amplified product. The amplified products were run on 3.0 percent agarose gel, stained with ethidium bromide and visualized in a gel documentation system. Only clear and unambiguous bands were scored. The size of the amplicons was compared using a 100 bp ladder.

RESULTS AND DISCUSSION

Initially, the annealing temperatures of 86 coconut-specific SSR primers were standardized by running gradient PCR. PCR was then performed at the standardized annealing temperature for each primer pair. Out of these 86 primers, 55 primers, which gave clear, unambiguous bands of expected size range (100 to 300 bp) when run in 3% agarose gel along with a 100 bp ladder were selected for further analysis. The annealing temperatures of these 55 primers are furnished in table 2. The 55 screened SSR coconut primers were checked for their cross-taxa utility in other palms *viz*., arecanut, oil palm, palmyrah and date palm. Cross amplification was scored as positive only when sharp bands in the size range 100 to 300 bp were obtained (Fig. 1). Out of the 30 CNZ SSR primers, nine primers amplified in arecanut, ten in oil palm, six in palmyrah and three in date palm (Table 3). Of the 13 CnCir primer pairs, four primers amplified in arecanut, seven in oil palm, one in palmyrah and two in date palm. Of the 12 CAC primer pairs, three primers each amplified in arecanut, oil palm and palmyrah and one in date palm (Table 3).

The present work was carried out to determine the extent to which pairs of primers designed for amplification of SSR loci in coconut could be used for amplification in other members of Arecaceae family *viz.,* arecanut, oil palm, date palm and palmyrah. Although microsatellite markers have been reported in oil palm (Billotte *et al*., 2), peach palm (Billotte *et al*., 3) and date palm (Billotte *et al*., 4), no microsatellites

Table 1. Coconut-specific SSR primers used in the study.

Sl. No.	Primer	Sl. No.	Primer	Sl. No.	Primer	Sl. No.	Primer
1.	CNZ 12	23.	CNZ 46	45.	CnCir D1	67.	CAC 72
2.	CNZ 31	24.	CNZ 21	46.	CnCir F3	68.	CAC 77
3.	CNZ 10	25.	CNZ 34	47.	CnCir B11	69.	CAC 50
4.	CNZ 04	26.	CNZ 23	48.	CnCir B3	70.	CAC 65
5.	CNZ 06	27.	CNZ 24	49.	CnCir D8	71.	CAC 68
5.	CNZ 02	28.	CNZ 26	50.	CnCir E1	72.	CAC 56
7.	CNZ 19	29.	CNZ 17	51.	CnCir C9	73.	CAC 38
8.	CNZ 01	30.	CNZ 37	52.	CnCir A3	74.	CAC 52
9.	CNZ 20	31.	CNZ 05	53.	CnCir C5	75.	CAC 23
10.	CNZ 03	32.	CNZ 29	54.	CnCir A9	76.	CAC 21
11.	CNZ 05	33.	CNZ 32	55.	CnCir B6	77.	CAC 20
12.	CNZ F6	34.	CnCir E2	56.	CnCir 09	78.	CAC 8
13.	CNZ F9	35.	CnCir E10	57.	CnCir A4	79.	CN11 A10
14.	CNZ 18	36.	CnCir C3	58.	CnCir C12	80.	CN11 E10
15.	CNZ E32	37.	CnCir E12	59.	CnCir D3	81.	CN11 E6
16.	CNZ 09	38.	CnCir F2	60.	CAC 2	82.	CN1 C6
17.	CNZ 16	39.	CnCir C7	61.	CAC 3	83.	CN1 H2
18.	CNZ 13F	40.	CnCir H4	62.	CAC 10	84.	CN1 G4
19.	CNZ 43	41.	CnCir H7	63.	CAC 13	85.	CN2 A5
20.	CNZ 44	42.	CnCir G11	64.	CAC 6	86.	CN2 A4
21.	CNZ 40	43.	CnCir B12	65.	CAC 84		
22.	CNZ 42	44.	CnCir 11	66.	CAC 71		

Table 2. Annealing temperature standardized for the selected primers.

Sl. No.	Primer	Annealing temperature (°C)	Sl. No.	Primer	Annealing temperature (°C)
1.	CNZ 02	58	29.	CnCir 09	60
2.	CNZ 12	50	30.	CnCir A9	57
3.	CNZ 16	51	31	CnCir B6	60
4.	CNZ 43	58	32.	CnCir A4	60
5.	CNZ 34	60	33.	CnCir C9	60
6.	CNZ 44	58	34.	CnCir F3	60
7.	CNZ 37	60	35.	CnCir B3	54
8.	CNZ 24	60	36.	CnCir C12	57
9.	CNZ 21	60	37.	CnCir 11	58
10.	CNZ 29	60	38.	CAC 3	56
11.	CNZ 32	60	39.	CAC 10	56
12.	CNZ 17	60	40.	CAC 77	58
13.	CNZ 05	50	41.	CAC 72	58
14.	CNZ 26	54	42.	CAC 52	56
15.	CNZ 40	57	43.	CAC 8	60
16.	CNZ 18	60	44.	CAC 21	50
17.	CNZ 13F	60	45.	CAC 38	51
18.	CNZ F6	57	46.	CAC 71	60
19.	CNZ F9	58	47.	CAC 84	56
20.	CNZ 01	60	48.	CAC 02	51
21.	CNZ 10	54	49.	CAC 13	56
22.	CNZ 03	54	50.	CN1 G4	57
23.	CNZ 31	56	51.	CN1 H2	54
24.	CNZ 42	56	52.	CN2A4	60
25.	CnCir B12	57	53.	CN2A5	58
26.	CnCir H4	59	54.	CN11 E6	58
27.	CnCir H7	59	55.	CN1C6	56
28.	CnCir E1	60			

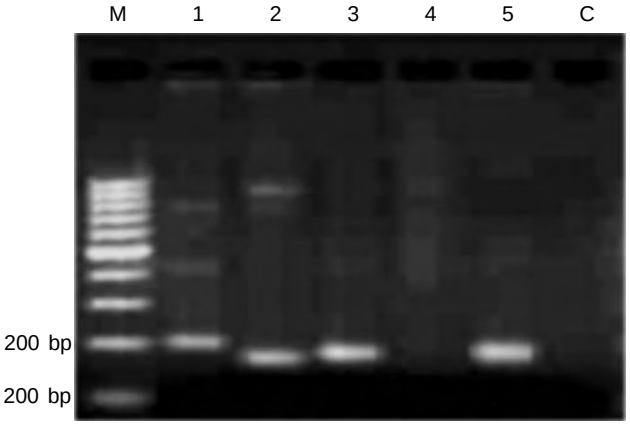

Fig. 1. Cross amplification of coconut-specific SSR primer (CnCir C9) in palms [M: 100 bp ladder, 1: Coconut, 2: Arecanut, 3: Oil palm, 4: Palmyrah, 5: Date palm, C: Negative control].

have been isolated from arecanut and palmyrah. The percentage of cross-amplification of coconut SSR primers in other palms ranged from 12.70 to 36.36%. The highest percentage was obtained in oil palm (36.36%) followed by arecanut (29.09%), palmyrah (18.18%) and date palm (12.70%) (Table 4). There have been earlier reports of cross-species conservation of SSR loci in tree species like *Citrus* (Kijas *et al*., 8) and *Pinus* (Shepherd *et al*., 13). Ten of

Table 4. Percentage of cross-amplification of coconut SSR primers in other palms.

Sample	Cross-amplification (%)
Arecanut	29.09
Oil palm	36.36
Palmyrah	18.18
Date palm	12.70

Table 3. Coconut-specific SSR primers showing cross-amplification in other palms.

Sl. No.	CNZ primers	CnCir primers	CAC primers
Arecanut	CNZ 01, CNZ 03, CNZ 05, CNZ 10, CNZ 18, CNZ 31, CNZ 42, CN2A4, CN1G4	CnCir 09, CnCir C9, CnCir B3, CnCir F3	CAC 38, CAC 84, CAC 13
Oil palm	CNZ 12, CNZ 43, CNZ 44, CNZ 24, CNZ 40, CNZ 18, CNZ 10, CNZ 31, CN2A4, CN1G4	CnCir 09, CnCir 11, CnCir B12, CnCir H7, CnCir C9, CnCir B3, CnCir F3	CAC 38, CAC 71, CAC 84
Palmyrah	CNZ 12, CNZ 24, CNZ 18, CNZ 42, CN2A4, CN1H2	CnCir C12	CAC 21, CAC 71, CAC 84
Date palm	CNZ 44, CN2A4, CN1H2	CnCir 09, CnCir B3	CAC 84

the 17 peach microsatellites (59%) gave correct amplification in all the *Prunus* species surveyed with three of them also being amplified in apple (Cipriani *et al*., 6). PCR amplification tests on a subset of 16 other palm species and allele-sequence data showed that *E. guineensis* SSRs are putative transferable markers across palm taxa (Billotte *et al*., 2). 29.3% of the peach microsatellites amplified in all the Rosaceae species (apple, strawberry) tested. The percentage of amplification in the six *Prunus* species tested was 75.6. 80.5, 43.9 and 31.7% amplification was obtained in the case of chestnut tree, grapevine and walnut tree respectively (Dirlewanger *et al*., 7). Ten microsatellite loci were isolated from *Araucaria cunninghamii* of which successful amplification were shown by eight loci in *Eutacta*, five loci in *Bunya* and three loci in *Agathis* (Scott *et al*., 12). Eighteen nuclear simple sequence repeat loci isolated from peach palm *Bactris gasipaes* var. *gasipaes* were found to be readily transferable to related *Bactris* species as well as to the *Astrocaryum* and *Elaeis* genera of the same *Cocoeae* tribe (Billotte *et al*., 3).

Thus, coconut SSR primer pairs tested in the present study amplified characteristic bands in a set of genetically diverse palm species. These findings suggest a certain level of sequence conservation among the palm species. Transferability of coconut SSRs may also circumvent the costly and laborious SSR development procedures for other commercial palms, aid in phylogenetic studies of other palms, in integration of genetic linkage maps and prove useful in palm synteny studies.

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