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SHORT RESEARCH ARTICLE

Molecular marker assisted selection of novel traits for quality seed production in komadan coconut palms

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Abstract

A study was conducted to identify the molecular marker linked to novel traits in Komadan mother palms. Twenty RAPD and 18 SSR markers were used in this study for characterization of ten Komadan palms and ten West Coast Tall (WCT). Six RAPD markers (OPC20, OPP3, OPP2, OPP5, OPA5 and OPD3) and five SSR markers (CNZ1, CNZ10, CNZ43, CAC10 and CnCirH4) produced amplicon specific to Komadan. These markers can be used to distinguish Komadan characters from other type and can be used for screening true to type of Komadan palms. In the bulk line analysis OPC 20 produced more reliable reproducible amplicon, and the product was eluted and sequenced for developing SCAR marker. A total of 161 Komadan segregants were screened by using SCAR marker specific to Komadan traits, the total of 86 genotypes showed expected product size.

Keywords: Komadan, West Coast Tall, RAPD, SSR, SCAR, true to type

Coconut (*Cocos nucifera* L.), is the only reported species under the genus *Cocos* belongs to the family Arecaceae. It is grown as both as a homestead as well as a plantation crop over large areas in the Asian countries. Coconut palms are found abundantly in coastal regions of most tropical islands, significantly contributing to the sustenance of fragile island ecosystems and the livelihood of people. The coconut palms shows diversity in terms of plant height (tall or dwarf), mode of pollination (autogamy or allogamy), inflorescence and fruit colour, copra and oil yield in fruits, etc.

Komadan is a local coconut off-type, popular in the erstwhile central Travancore area of Kerala associated with the family history of an old *Tharavadu* called *Komattu* house. Komadan is superior to West Coast Tall (WCT) in morphological characters of the palm including nut and copra characters and the superiority of Komadan coconut types in relation to the economically important characters. Each genotype has its own unique characters based on economic part of the plants. Now a day, there is high demand of Komadan seedlings and it is sold at a higher prize. Molecular markers are useful for locating the specific characters even in the seedling stage. Identification of specific markers for novel *Komadan* traits helps in early identification of the true types traits. The expression of characters in coconut is highly influenced by environment, so that phenotypic markers may not be enough to varietal identification of the coconut. The DNA based markers will be one of the methods to identify the true to type of Komadan palms at seedling stage because of its polymorphism, reproducibility and less influence of environment. Present study is aimed at to identify the molecular markers that can be used for characterization of true to type Komadan palms as well as Komadan segregants.

The study consists of ten *Komadan* oalms and ten WCT palms characterized by using 20 RAPD primers and 18 SSR primers. The Komadan segregants were analyzed by using SCAR marker which was eluted from high reproducible and reliable RAPD marker. Genomic DNA from these accessions was isolated by using the procedure of QIAGEN DNeasy plant mini kit and DNA stored at -20˚C in deep freezer. The quantity and quality of isolated DNA was checked by agarose gel electrophoresis in a BIO-SYS, horizontal gel electrophoresis

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Table 1. Details of the RAPD primers used for screening

S. no.	Sequence name	RAPD sequence	No of bands	No of polymorphic bands	Specific marker
1	OPC 20	5′-ACT TCG CCA C-3′	5	1	900bp
2	OPP 3	5′- CTG ATA CGC C-3′	6	1	950bp
3	OPA 5	5′- AGG GGT CTT G-3′	6	1	1800bp
4	OPD 3	5′-GTC GCC GTC A-3′	6	1	1400bp
5	OPP 2	5′-TCG GCA CGC A-3′	6	1	900bp
6	OPP 5	5′-CCC CGG TAA C-3′	6	1	250bp
7	OPB 5	5′-TGC GCC CTT C-3′	5	-	-
8	OPC 7	5′-GTC CCG ACG A-3′	4	-	-
9	OPD 18	5′-GAG AGC CAA C-3′	7	-	-
10	OPD 20	5′-ACC CGG TCA C-3′	6	-	-
11	OPE 7	5′-AGA TGC AGC C-3′	4	-	-
12	OPF 13	5′- GGC TGC AGA A-3′	4	-	-
13	OPF 18	5′-TTC CCG GGT T-3′	5	-	-
14	OPG 19	5′-GTC AGG GCA A-3′	6	-	-
15	OPH 19	5′-CTG ACC AGC C-3′	5	-	-
16	OPK 14	5′-CCC GCT ACA C-3′	3	-	-
17	OPK 19	5′-CAC AGG CGG A-3′	5	-	-
18	OPL 17	5′-AGC CTG AGC C-3′	5	-	-
19	OPP 1	5′-GTA GCA CTC C-3′	8	-	-
20	OPP 6	5′-GTG GGC TGA C-3′	6	-	-

Table 2. Details of the SSR primers used for screening

S. no	SSR primers	Sequence (5 ´-3´)	Size of the product	Annealing temperature
1.	CAC50	FPTTACTCACCCCATAACAAAGRPTTGTAGTTGCCCATATCTCTT	153	58
2	CAC10	FPGATGGAAGGTGGTAATGCTGRPGGAACCTCTTTTGGGTCATT	156-163	56
3	CAC68	FPATTATTTTCTTGTTACATGCATCRPAACAGCCTCTAGCAATCATAG	142	52.5
4	CAC08	FPATCACCCCAATACAAGGACARPAATTCTATGGTCCACCCACA	190	54.3
5	CAC65	FPAAAAGGATGTAATAAGCTGGRPTTTGTCCCCAAATATAGGTAG	151	54
6	CAC77	FPCAGAGGTCACAACCATATTGRPCTTTAGCTATTTGTTCCAAGG	131	54.3
7	CAC20	FPCTCATGAACCAAACGTTAGARPATCATATACATACATGCAACA	124-132	53
8	CAC71	FPATAGCTCAAGTTGTTGCTAGGRPATATTGTCATGATTGAGCCTC	172-283	57.3
9	CAC72	FPTCACATTATCAAATAAGTCTCACARPGCTCTCTTTCTCATGCACA	124-132	57.3
10	CAC84	FPTTGGTTTTTGTATGGAACTCTRP AAATGCTAACATCTCAACAGC	150-163	57.4
11	CNZ01	FPATGATGATCTCTGGTTAGGCTRP AAATGAGGGTTTGGAAGGATT	109-140	52
12	CNZ10	FPCCTATTGCACCTAAGCAATTARPAATGATTTTCGAAGAGAGGTC	148	52.5
13	CNZ43	FPTCTTCATTTGATGAGAATGCTRPACCGTATTCACCATTCTAACA	197	55.1
14	CNZ44	FPCATCAGTTCCACTCTCATTTCRPCAACAAAAGACATAGGTGGTC	165	54.3
15	CNZO6	FPTACTCATCATCATACGACGCRPCTCCCACAAAATCATGTTATT	85	53
16	CNZ29	FPTAAATGGGTAAGTGTTTGTGCRPCTGTCCTATTTCCCTTTCATT	135	58.7
17	CNZ46	FPTTGGTTAGTATAGCCATGCATRPAACCATTTGTAGTATACCCCC	116	54.3
18	CnCirH4	FPTTAGATCTCCTCCCAAAGRPATCGAAAGAACAGTCACG	200-250	52

unit and documented using UVP gel documentation system. The quality and quantity of DNA present in sample was ensured by using spectrophotometer. The 5µL of DNA dissolved in 0.1x TAE was added to 3 mL of distilled water and absorbance at 260 nm and 280 nm was read against water as blank.

Table 3. Details of pollination and fruitset

Cross combination	No of palms	No. of bunches	No. of fruits set	Fruits sown
1. Komadan x WCT	10	12	119	48
2. WCT x Komadan	10	20	287	41
3. Komadan x CGD	10	15	75	48
4. Komadan selfing	10	14	156	49
Total			637	186

*Eventhough fruitset was obtained in 637 female flowers pollinated, only 186 fruits in the four different combinations were ready for harvest.

The concentration of DNA in sample was calculated using the formula:

$$\text{Amount of DNA (µg/mL)} = \frac{\text{A260 x 50 x Dilution factor}}{1000}$$

Where, A260 = Absorbance at 260 nm

The quality of DNA judged from the ratio of absorbance values at 260 nm and 280 nm. A ratio of 1.8–2.0 indicates best quality of DNA. The DNA of twenty plants was isolated and amplified using RAPD and SSR markers for the identification of true to type characters of Komadan Palms. The details of 20 RAPD markers (Table 1) and 18 SSR markers (Table 2) are listed below.

Hybridization to develop segregants with Komadan traits

The each selected Komadan palms were controlled pollinated with selected male parents in the four different combinations with Komadan as one of the parent. The mid phase of inflorescence from the selected mother palms were used for pollination. Emasculation was done by scrapping of all the male flowers and bagged by using muslin cloth. The formation of gummy exudates from the female flowers indicates the receptiveness of stigma, were chosen for pollination by collected pollen grains form the male flowers. The flowers were repeatedly pollinated for 2–3 days with pollen collected from chosen male parent. Usually pollination was continued till anthesis in all the female flowers was over. The cover was retained for a more days and then removed after labelling. After setting seeds, 60 nuts selected from each combination were sown and the segregation of Komadan based characters were studied using SCAR markers eluted from highly polymorphic RAPD marker. The four different controlled pollinations includes, Komdan x CGD, Komadan x WCT, WCT x Komadan and Komadan x Komadan. The experimental material for SCAR marker analysis was consisted of 161 segregants of one-year- old hybrid seedlings.

Designing of SCAR primers and PCR analysis

The RAPD fragment which showing more reliability towards Komadan traits was eluted from the gel for sequencing. Gel elution was done using the MinElute GelExtraction kit (QIAGEN). Based on the sequence information of the cloned fragments, SCAR primer sets were designed using Primer3 (http://bioinfo.ut.ee/primer3-0.4.0/primer3/) and synthesized commercially (Sigma). The designed SCAR primers were employed to amplify the genomic DNAs of 161 segregants.

Molecular characterization applying RAPD and SSR analysis

Ten Komadan Palms and ten West Coast Tall (WCT) Palms were selected and the good quality DNA was obtained from those twenty palms. The genomic DNA is amplified by using 20 random primers. DNA-based molecular markers represent an attractive, alternative tool for genetic diversity analyses since their expression is not affected by environmental variations, and for their greater capacity to reveal a wider number of markers (Wadt et al. 1999). From the amplicon produced by RAPD primers, out of which twenty primers screened primers OPC20, OPP3, OPP2, OPP5, OPA5 and OPD3 produced amplicons which are specific to Komadan palms (Fig. 1-6). The total of six primers can be used for screening Komadan true type palms. The different type of RAPD markers are used for genetic variability analysis as well as identification of true to types (Rajesh et al. 2014). The issue of reproducibility and reliability can be overcome by designing the SCAR marker from most polymorphic and variable RAPD marker (Rajesh et al. 2014). These markers can also be used for developing SCAR marker which has more reliability. The co-dominant SCAR marker amplifies the unique sequences than RAPD marker (Rajesh et al. 2013).

The amplified genomic DNA 18 SSR primers, CNZ1, CNZ10, CNZ43, CAC10 and CnCirH4 produced specific marker to distinguish Komadan from other types. These primers can be used for screening of true to type Komadan palms. The four combination of controlled pollination with Komadan palms as one of the parent was done to produce Komadan segregants. From the crosses a total of 186 fruits were sown for further evaluation and characterization. (Table 3). The polymorphic RAPD marker specific for Komadan was eluted from the gel and cloned for sequencing. Cloning of the polymorphic bands from RAPD primers OPC 20 and OPP3 were done. The cloned DNA is subjected to electrophoresis for conformation.

Screening of coconut hybrid with developed SCAR

The issues of the reproducibility and reliability of RAPD assays can be significantly improved by the conversion

of RAPD into SCAR markers, by developing longer and, consequently, more specific primers from RAPD sequences. The primers OPC20, OPP3, OPP2, OPP5, OPA5 and OPD3 produced amplicons specific to Komadan. Among them OPC 20 give more reliable reproducible band, this primer was selected for developing SCAR marker. To generate stable and longen- specific SCAR marker from RAPD markers one pair of primer was designed and synthesized based on cloned sequence. The designed SCAR primer pairs of size ~190 bp then used to amplify the DNA isolated from the 161 hybrids DNA with the pooled parental DNA sample to test the segregation of Komadan specific character.

The designed SCAR primer pairs were then used to amplify the genomic DNA from 161 genotype of collected DNA samples from progeny to test the amplification Komadan specificity. Out of 161 genotypes screened total 83 genotypes were showed expected product size, of these 86 genotypes 17 from Komadan x CGD cross, 27 from Komadan x WCT cross, 15 from WCT x Komadan cross and 27 from Komadan selfing.

Authors' contribution

Conceptualization of research (JVG); Designing of the experiments (JVG); Contribution of experimental materials (JVG); Execution of field/lab experiments and data collection (RRB, RGR); Analysis of data and interpretation (RRB, RGR); Preparation of the manuscript (SAM, AC).

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