

Identification of molecular markers associated with mite resistance in coconut (*Cocos nucifera* L.)

K.V. Shalini, S. Manjunatha, P. Lebrun, A. Berger, L. Baudouin, N. Pirany, R.M. Ranganath, and D. Theertha Prasad

Abstract: Coconut mite (*Aceria guerreronis* 'Keifer') has become a major threat to Indian coconut (*Cocos nucifera* L.) cultivators and the processing industry. Chemical and biological control measures have proved to be costly, ineffective, and ecologically undesirable. Planting mite-resistant coconut cultivars is the most effective method of preventing yield loss and should form a major component of any integrated pest management stratagem. Coconut genotypes, and mite-resistant and -susceptible accessions were collected from different parts of South India. Thirty-two simple sequence repeat (SSR) and 7 RAPD primers were used for molecular analyses. In single-marker analysis, 9 SSR and 4 RAPD markers associated with mite resistance were identified. In stepwise multiple regression analysis of SSRs, a combination of 6 markers showed 100% association with mite infestation. Stepwise multiple regression analysis for RAPD data revealed that a combination of 3 markers accounted for 83.86% of mite resistance in the selected materials. Combined stepwise multiple regression analysis of RAPD and SSR data showed that a combination of 5 markers explained 100% of the association with mite resistance in coconut. Markers associated with mite resistance are important in coconut breeding programs and will facilitate the selection of mite-resistant plants at an early stage as well as mother plants for breeding programs.

Key words: coconut, *Aceria guerreronis*, mite resistance, SSR, RAPD.

Résumé : L'acarien du cocotier (*Aceria guerreronis* Keifer) constitue maintenant une menace importante pour les producteurs et transformateurs du cocotier (*Cocos nucifera* L.) en Inde. Les moyens de lutte chimique et biologique se sont avérés coûteux, inefficaces et indésirables sur le plan écologique. L'emploi de cultivars résistants à l'acarien est la méthode la plus efficace pour prévenir les pertes de rendement et constituerait l'assise principale de tout programme de lutte intégrée. Des génotypes du cocotier, tant des accessions résistantes que sensibles, ont été collectés dans diverses régions du Sud de l'Inde. Trente-deux paires d'amorces SSR et sept amorces RAPD ont été employées pour réaliser des études moléculaires. Sur la base d'analyses ponctuelles, neuf marqueurs SSR et 4 marqueurs RAPD associés à la résistance aux acariens ont été identifiés. Suite à des régressions multiples par paliers sur les marqueurs SSR, une combinaison de six marqueurs a montré une parfaite association (100 %) avec la réaction aux mites. Une analyse semblable sur les marqueurs RAPD a révélé que la combinaison de trois marqueurs expliquait 83,86 % de la résistance au sein des lignées à l'étude. Des régressions multiples combinées effectuées sur les marqueurs SSR et RAPD ont montré que la combinaison de cinq marqueurs expliquait 100 % de l'association avec la résistance aux acariens chez le cocotier. Les marqueurs associés à la résistance aux acariens sont importants dans les programmes de sélection chez le cocotier et faciliteront la sélection précoce de génotypes résistants et de géniteurs dans le cadre des efforts d'amélioration génétique.

Mots-clés : cocotier, *Aceria guerreronis*, résistance aux acariens, SSR, RAPD.

[Traduit par la Rédaction]

Received 4 February 2006. Accepted 13 November 2006.

Published on the NRC Research Press Web site at <http://genome.nrc.ca> on 15 March 2007.

Corresponding Editor: R. Appels.

K.V. Shalini, S. Manjunatha, and D.T. Prasad.¹ Plant Molecular Biology Laboratory, Department of Biotechnology, University of Agricultural Sciences, GKVK, Bangalore 560065, India.

P. Lebrun, A. Berger, and L. Baudouin. Centre de coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), TA 80/03, Avenue Agropolis, 34398 Montpellier CEDEX, France.

N. Pirany. Department of Animal Science, University of Tabriz, Tabriz, Iran.

R.M. Ranganath. Department of Botany, Bangalore University, Jnana Bharathi, Bangalore 560056, India.

¹Corresponding author (e-mail: prasaddt@yahoo.co.in).

Introduction

Coconut, *Cocos nucifera* L. (Arecaceae), is an important oil-yielding species across the tropical world. Coconut palms are host to at least 12 species of Eriophyidae in 9 genera. Three species of eriophyid mites have been found on Florida coconut palms. *Aceria guerreronis* 'Keifer' was described in 1965 from coconuts in Mexico (Keifer 1965) and confirmed in Florida (Howard et al. 1990). Its recent appearance in India and Sri Lanka is the beginning of a significant threat to major coconut-producing regions of the world, especially in Asia and Oceania (Moore 2000). Use of predators and pathogens to control coconut mites has failed because of their reduced effect under natural circumstances (Julia et al. 1979; Hall et al. 1980; Howard et al. 1990). Chemical methods to control mite infestation, which are dependent on community-based programs among farmers, have resulted in limited success. Further, reports suggest that chemical resi-

Table 1. Geographical description of agro-climatic parameters of the regions in which the coconut samples were collected.

Region	Latitude range	Longitude range	Altitude above sea level (m)	Topography	Soil profile	Annual rainfall (mm)
Agricultural Research Station, Brahmavar	12°30', 15°00'	74°05', 76°00'	9.00–13.51	Coastal	Laterite	3893.00
Agricultural Research Station, Arasikere	12°50', 14°55'	75°30', 77°20'	240.24–270.27	Plain	Granite	794.40
Farmer's field, Gubbi	12°10', 14°00'	76°35', 78°45'	240.24–270.27	Plain	Laterite / Granite Gneiss	646.00
Farmer's field Hunsur	11°30', 13°05'	76°05', 77°45'	240.24–270.27	Plain	Laterite / Granite Gneiss	734.00

dues persist for over 45 days after application on fruits as well as on vegetative tissues, and this results in a health hazard.

Coconut varieties differing in susceptibility to coconut mite have been identified in Cuba and Costa Rica (Mariau 1977; Julia and Mariau 1979; Julia et al. 1979; Schliesske 1988; Suarez 1991). Varieties from West Africa and the Americas are found to be more susceptible to coconut mites than those from Asia or Oceania. Harnessing genetic variability by adopting conventional breeding strategies entails a huge investment of time and resources. To speed up progress in classical breeding programs, identification of DNA markers related to genomic regions for traits like quality and productivity seems to be very important. This would enable the breeders to make selections among seedlings grown in a non-target environment (Tanksley and Nelson 1996). Simple-sequence repeat (SSR)-linked markers associated with the genes for disease resistance to southern corn rust (Liu et al. 2003; Chen et al. 2004), rice sheath blight (Che et al. 2003), and rice brown planthopper (Yang et al. 2002) and RAPD markers closely linked to genes resistant to gall midge (Nair et al. 1995; Nair et al. 1996), bacterial blight (Zhang et al. 1996), and brown planthopper (Jena et al. 1998) have been successfully identified. Detection of healthy plants at sapling or at a very early stage of tree growth is not easy to accomplish by classical selection methods. However, molecular markers can help in identifying the resistant plants at an early stage. This study is aimed at identifying SSR and RAPD markers closely associated with mite resistance in naturally occurring genotypes of coconut that could be further used in coconut improvement programs.

Materials and methods

Plant sample collection and DNA isolation

An extensive survey of coconut orchards from different agroclimatic regions of India's southern peninsula was undertaken to assess the magnitude of damage caused by the coconut mite epidemic (Table 1). While more than 70% of the palms were highly infested with mite, about 30% of trees were almost free of mite infestation. Ten nuts/plant selected randomly were used to assess the extent of the surface area infected, and data were recorded as percentage nut-surface damage (PNSD). A standard scale was adopted to classify trees as highly resistant, moderately resistant, and highly susceptible to coconut mite infestation (Table 2). Phenotyping for mite resistance was conducted in 40 trees spread across the southern peninsular region of India. DNA extracted from all these trees was used to generate data for molecular marker analysis.

Leaflets from coconut trees exhibiting high, medium, and

low mite infestation were cleaned thoroughly and dried at 40 °C. Dried leaflets were finely powdered using the grinder, filtered through 40-mesh sieve and stored at room temperature in clean dry plastic bags. DNA was extracted from these samples using the CTAB (cetyltrimethylammonium bromide) method with minor modifications (addition of 2% polyvinylpyrrolidone and 1% β -mercaptoethanol) (Callahan and Mehta 1991; Porebski et al. 1997; Shalini et al. 2004).

RAPD analysis

PCR (polymerase chain reaction) was performed at the Plant Molecular Biology Laboratory, Department of Biotechnology, University of Agricultural Sciences, Bangalore India, in 0.2 mL, thin-walled PCR tubes using an MJ-Research Thermocycler (Model PTC-100). The RAPD protocol described by Williams et al. (1990) was used with modifications (standardization of MgCl₂, deoxynucleoside triphosphates (dNTPs), template DNA, and primer concentration) to amplify coconut DNA. RAPD analysis was carried out in a total volume of 20 μ L containing 25–30 ng of template DNA, 1 \times *Taq* polymerase assay buffer, 200 μ mol/L each of the dNTPs, 3 mmol/L MgCl₂, 10 pmol random decamer primer (Operon Technologies Inc., Huntsville, Ala.), and 1 U *Taq* polymerase. PCR amplification was carried out with an initial denaturation at 95 °C for 5 min followed by 45 cycles, each step consisting of denaturation at 95 °C for 1 min, primer annealing at 36 °C for 1 min, and extension at 72 °C for 2 min with a final extension step at 72 °C for 10 min. The amplified samples were resolved on 1.4% agarose gel in 1 \times TBE (Tris–borate–EDTA) buffer containing ethidium bromide (0.5 μ g/mL), and bands were captured using Hero Lab gel documentation facility. Further, bulked segregant analysis (BSA) was carried out with RAPD primers by generating 2 DNA pools from 9 mite-resistant (HUMPGR, HUDXT1, HUDXT2, GU2F1, BR59, BR39, GU10F1, GUMP16, and GUMP9) and 9 mite-susceptible (GUMP3, GUMP12, GUHY1, BR80, GU9F1, GUHY2, GUF1, BR50, and AR6) trees.

SSR analysis

PCR was performed at the Centre de coopération Internationale en Recherche Agronomique pour le Développement, France, using an MJ-Research Thermocycler (Model PTC-100) with SSR primers (Table 3). Reactions took place in a total volume of 10 μ L containing 12.5 ng of template DNA, 1 \times *Taq* polymerase assay buffer, 200 μ mol/L each of the dNTPs, 0.2 μ mol/L each of forward and reverse primer, and 0.5 U *Taq* polymerase; 2 mmol/L MgCl₂ concentration provided optimum results during amplification. The forward

Table 2. PNSD scale and genotypes of coconut according to level of mite infestation.

Damage category (PNSD*)	Range, mean \pm SE	Genotypes (PNSD*)
0 (no damage)	0	Nil
1–10 (superficial damage with just a triangular white patch near perianth and only up to 10% of the surface area with brown scar)	2.00–9.10, 6.04 \pm 0.68	HUDXT2 (2.0), HUMPGR (2.5), HUDXT1 (3.3), GU2F1 (7.0), BR59 (7.0), BR39 (7.0), GU10F1(7.0), GUMP16 (7.0), GUMP9 (8.8), AR27 (9.1), AR82 (9.1), BR114 (9.1), GU17F1 (9.1), GU14F1 (9.1).
11–25 (damage symptom significant; brownish scarring covers 11%–25% of the surface area; nut size not reduced.)	11.20–24.50, 16.25 \pm 1.76	GU21F1(11.2), GU19F1(11.2), GU1F1(12.5), AR2 (14.5), GU5F1(17.9), BR91(22.0), GU13F1(24.5).
26–50 (extensive brownish discoloration covering 26%–50% of the surface area with longitudinal cracks on the nuts; nut size is reduced)	28.70–50.00, 37.26 \pm 2.55	GUMP15 (28.7), BR100(29.5), GUMP2 (31.6), GUMP13 (32.0), BRX1 (32.5), BR48(32.9), GUMP4 (34.1), BR50(38.3), GUMP1 (49.5), AR6(50.0), GUMP5 (50.0).
51–100 (browning is extensive covering 51%–100% of surface area and malformed nuts with longitudinal cracks)	62.50–75.00, 67.90 \pm 1.75	GUMP12 (62.5), GUHY1 (62.5), BR80 (65.4), GUMP3 (65.4), GU11F1(68.7), GU24F1 (68.7), GUHY2 (75.0), GU9F1 (75.0)

*PNSD, percentage nut-surface damage.

SSR primer in each reaction was radiolabeled with γ P³³-ATP using T₄ DNA kinase. PCR amplification was carried out with an initial denaturation at 94 °C for 5 min followed by 35 cycles, each consisting of denaturation at 94 °C for 30 s, primer annealing at 51 °C for 1 min, and extension at 72 °C for 1 min with a final extension step at 72 °C for 8 min. Each of the PCR amplified products was mixed with an equal volume of 10 \times DNA loading buffer (100 mmol/L Tris (pH 8.0), 100 mmol/L EDTA, 0.25% xylene cyanol, 0.25% bromophenol blue, and 50% sucrose), and denatured at 94 °C for 3 min. Four microlitres of the reaction mixture was applied on 5% denaturing polyacrylamide gels and subjected to electrophoresis at 55 W constant power for 2 h using 1 \times TBE buffer. The gels were dried using a gel drier (Bio-Rad, Hercules, Calif.) at 80 °C for 30 min and were exposed to Kodak Biomax MR-2 film for 18 h.

Data analysis

Phenotypic data (from 40 trees) on mite resistance (PNSD) and genotypic data (32 SSR and 7 RAPD primers) derived by molecular marker analysis were analyzed independently and in combination.

Mite resistance

The range and mean values of PNSD due to mite infestation are presented in Table 2.

Single-marker analysis (SMA)

The association of molecular markers with PNSD values was computed using SAS software package v. 6.12 (SAS Institute Inc. 1989). Phylogenetic relationships among the lines were assessed by adopting cluster analysis using STATISTICA software (SAS Institute, N.C.). The dendrogram (Fig. 1) was computed according to Ward's method of clustering, using a minimum variance algorithm (Ward 1963).

Analysis of variance using a general linear model was adopted to identify putative markers (SSR and RAPD) associated with PNSD (SAS Institute Inc. 1989). Association of SSR and RAPD markers with mite resistance was performed independently as well as in combination. The R^2 values were determined to find the extent of variability explained by these markers; R^2 values assumed positive or negative val-

Table 3. Characteristics of SSR primers.

No.	SSR	No. of alleles scored*	Size range	Chromosome No.
1	CnCir A3	4	224–240	NM
2	CnCir A4	4	196–204	12 [†]
3	CnCir A8	5	274–282	NM
4	CnCir A9	4	89–103	14*
5	CnCir B5	4	270–278	NM
6	CnCir B6	6	196–208	NM
7	CnCir B12	9	153–177	8 [†]
8	CnCir C3	10	174–206	NM
9	CnCir C7	5	157–167	NM
10	CnCir C11	3	217–225	15 [‡]
11	CnCir C12	4	167–183	NM
12	CnCir D8	9	241–259	8 [†]
13	CnCir E2	15	115–175	NM
14	CnCir E4	5	224–238	1 [‡]
15	CnCir E7	4	214–220	13 [‡]
16	CnCir E10	5	226–246	NM
17	CnCir E11	7	180–218	11 [‡]
18	CnCir E12	2	164–174	NM
19	CnCir F2	4	193–205	NM
20	CnCir G4	3	166–171	13 [‡]
21	CnCir G11	6	188–208	NM
22	CnCir H4	5	218–236	3 [‡]
23	CnCir H7	5	131–139	NM
24	CnCir R11	3	136–150	NM
25	CnCir S1	4	242–262	NM
26	CnCir S5	3	224–228	NM
27	CnCir S7	9	189–219	NM
28	CnCir S8	3	84–102	NM
29	CnCir S12	9	149–183	NM
30	mCnCir 47	8	122–172	13 [‡]
31	mCnCir86	9	172–200	9 [‡]
32	mCnCir119	9	187–217	1 [‡]

Note: NM, not mapped. Rest of the primer information can be obtained from P. Lebrun (personal communication).

*In the present study.

[†]Lebrun et al. 2001.

[‡]www.neiker.net/Link2palm/.

Fig. 1. Dendrogram of coconut samples based on RAPD analysis (as described in Materials and method) using STATISTICA program. The first 2 letters in the label represent the place where the samples were collected: Hunsur (HU), Gubbi (GU), Arasikere (AR), and Brahmavar (BR). Genotypes that were highly resistant are marked (R) with a bar.

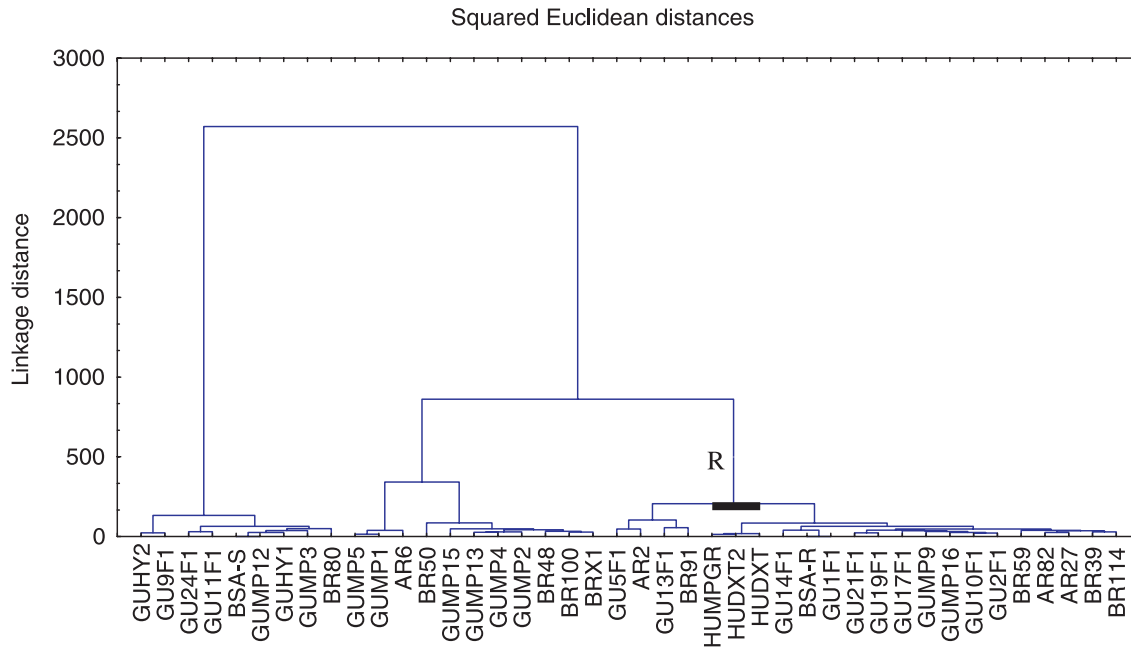


Table 4. Single-marker analysis (a) and stepwise multiple regression analysis (b) for mite resistance in coconut based on RAPD profiles generated using 7 selected random decamer primers.

(a) Single-marker analysis					
SI No.	Marker _{size}	Partial R^2	Prob > F	PE	
1	OPE4 ₉₀₀	0.3646	0.0001	40.27	
2	OPP15 ₄₂₅	0.1319	0.0295	30.31	
3	OPG7 ₂₀₀₀	0.1111	0.0470	15.47	
4	OPE6 ₈₀₀	0.1095	0.0487	33.32	
(b) Stepwise multiple regression analysis					
SI No.	Marker _{size}	Partial R^2	Total R^2	Prob > F	PE
1	OPE18 ₆₀₀	0.7227	0.7227	0.0001	0.33
2	OPP16 ₁₂₀₀	0.0852	0.8079	0.0005	-45.46
3	OPP16 ₁₆₅₀	0.0307	0.8386	0.0191	46.54

Note: PE, parameter estimate; SI, serial number.

ues (parameter estimate) indicating the association of the marker (bands of appropriate size) with increased or decreased resistance to mite infestation.

Stepwise multiple regression analysis (SMRA)

Stepwise regression of molecular markers (SSR and RAPD) against mite resistance was performed to identify suitable markers that would account for a progressive quantum increase in mite resistance (R^2). Further, a multiple regression approach was adopted using mite resistance as an independent variable and genotypic data as the dependent variable. The analysis model was

$$[1] \quad Y = a + b_1m_1 + b_2m_2 + b_3m_3 + \dots b_jm_j \dots b_nm_n + e$$

where Y represents PNSD, m_j the RAPD and SSR markers,

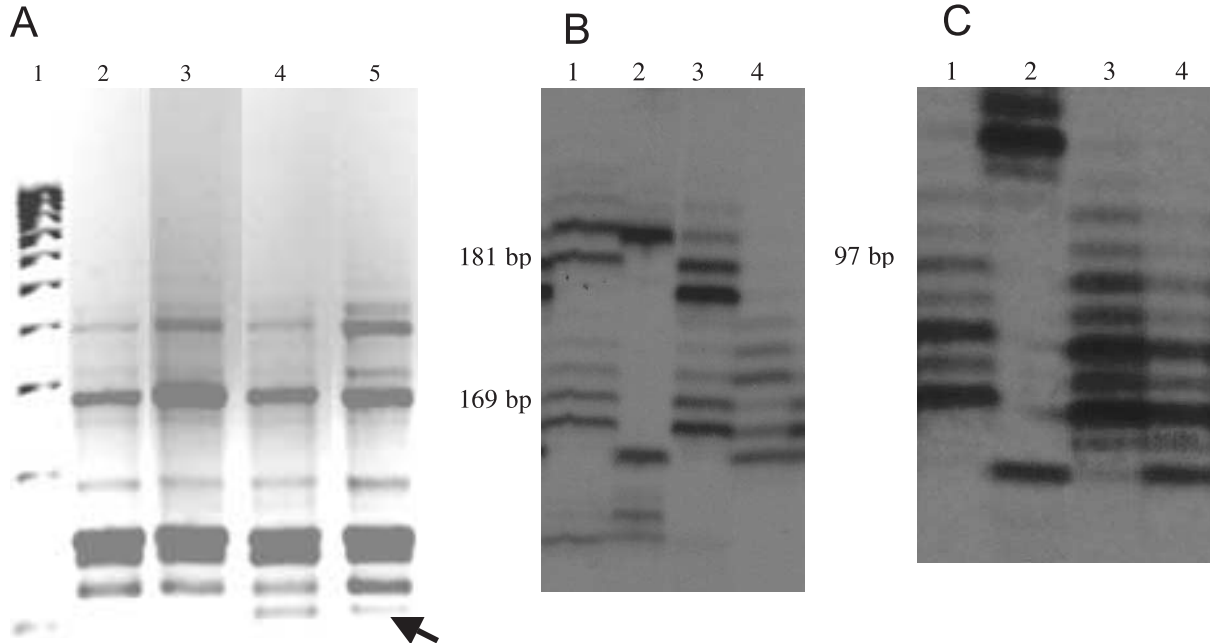
b_j the partial regression coefficients that specify the empirical relationship between Y and m_j , and e is the random error of Y , including environmental variation. This allows us to ascertain the maximum likelihood solutions of relationships between individual quantitative traits and various markers.

Results

RAPD

Of the 160 random decamer primers used, 132 generated 1 band each. Seven primers, namely OPE4, OPE6, OPE18, OPF2, OPG7, OPP15, and OPP16, produced 5 to 17 bands and were selected for further analysis. Ninety-one bands ranging in size from 200 to 3000 bp, which were consistent, unambiguous and reproducible, were used in the statistical analyses. The number of bands per primer varied from 8 to

Fig. 2. (A) RAPD profile of mite-resistant (R) and susceptible (S) coconut genotypes using OPE18 primer. Lane 1. DNA ladder markers (500-5000 bp); Lane 2. GUMP3 (S); Lane 3. Bulked DNA sample from susceptible group (GUMP3, GUMP12, GUHY1, BR80, GU9F1, GUHY2, GUF1, BR50, and AR6); Lane 4. HUMPGR (R); Lane 5. Bulked DNA sample from resistant group (HUMPGR, HUDXT1, HUDXT2, GU2F1, BR59, BR39, GU10F1, GUMP16, and GUMP9). Arrow indicates marker associated with mite resistance. (B) SSR profile of mite-resistant (R) and susceptible (S) coconut genotypes using the primer CnCirS12. Lane 1. Standard WAT4 (West African Tall). The allele size of all the primers used in this study was standardized using WAT4 (Teulat et al. 2000; <http://www.neiker.net>); Lane 2. GUMP3 (S); Lane 3. HUMPGR (R); Lane 4. GU9F1 (S). Arrow indicates marker associated with mite resistance (CnCirS12₁₇₁). (C) SSR profile of mite-resistant (R) and -susceptible (S) coconut genotypes using the primer CnCirA9. Lane 1. Standard WAT4 (West African Tall); Lane 2. GUMP3 (S); Lane 3. HUMPGR (R); Lane 4. GU9F1 (S). Arrows indicate marker associated with mite susceptibility (CnCirA9₉₉).



17 with an average of 13 bands. Of these, 68 bands were polymorphic (74.7%).

SMA established the association of mite resistance with markers OPE4₉₀₀ (36.46%), OPE6₈₀₀ (10.95%), OPG7₂₀₀₀ (11.11%), and OPP15₄₂₅ (13.19%) (Table 4). SMRA revealed that the markers OPE18₆₀₀, OPP16₁₂₀₀, and OPP16₁₆₅₀ showed strong association with mite resistance, accounting together for 83.86% (Table 4). BSA of mite-resistant and -susceptible coconut plants with selected primers strongly supports the association of OPE18₆₀₀ with mite resistance (Fig. 2A).

SSR

Thirty-two SSR primers produced a total of 185 polymorphic bands with sizes ranging from 84 to 282 bp (Table 3) and an average of 5.78 bp. Of these, 13 SSRs were mapped on the coconut genome using 6 different mapping populations (Herran et al. 2000; Ritter et al. 2000; Lebrun et al. 2001). In this study, SSR primer CnCirE2 amplified the maximum number of bands (15), ranging from 115 to 175 bp, whereas CnCirE12 produced only 2 bands, of 164 and 174 bp.

The proportion of phenotypic variation accounted for by each primer pair (R^2) was computed. SMA established an association of 9 SSR markers with mite resistance (Table 5). The maximum association was found with CnCirA9₉₉ and CnCirS7₂₁₅ (52.98%). In SMRA, a combination of 6 SSR markers accounted for 100% of mite resistance (Table 5).

CnCirA9₉₉ showed a negative association with mite resistance (Fig. 2C) in both SMA and SMRA (Table 5, PE: -68.30). CnCirS12₁₇₁ was found to be positively associated with mite resistance (Fig. 2B; Table 5, PE: 42.50).

Cluster analysis

Cluster analysis with RAPD profiles formed 3 major groups. Genotypes from the first major cluster (at 150 linkage distance) were susceptible to coconut mites (Table 2). Genotypes from the second major cluster had moderate susceptibility, and genotypes from the third major cluster were resistant to mite infestation. Among them, HuTXD1, HuTXD2, and HuMpGr, which clustered closely, were highly resistant to mite infestation.

Combined SMRA of SSR and RAPD data

$$\begin{aligned}
 [2] \quad \text{PNSD} = & 34.7 \\
 & + [(-68.30) \text{CnCirA9}_{99}] + [(0.43) \text{OPE18}_{600}] \\
 & + [(11.5) \text{CnCirE2}_{151}] + [(-9.2) \text{CnCirS7}_{219}] \\
 & + [(4.70) \text{OPP16}_{1200}] + 0.00083
 \end{aligned}$$

Of the SSR and RAPD markers used in the study, 5 markers (Table 6) showed a strong association with PNSD. As indicated in the above equation, OPE18₆₀₀, CnCirE2₁₅₁, and OPP16₁₂₀₀ contributed to enhanced resistance, and CnCirA9₉₉ and CnCirS7₂₁₉ were the actual alleles identifying susceptibility to mites. The PEs of each marker indicate

Table 5. Single-marker analysis and stepwise multiple regression analysis for mite resistance using 32 SSR primers of coconut.

SI No.	SSR _{size}	Chromosome No.	Partial R^2	Total R^2	Prob > F	PE
Single-marker analysis						
1	CnCirA9 ₉₉	14	0.5298	0.5298	0.0073	-68.30
2	CnCirE2 ₁₆₃	NM	0.3619	0.3619	0.0385	-43.16
3	CnCirH7 ₁₃₃	9	0.2377	0.2377	0.1079	27.65
4	CnCirE7 ₂₁₄	13	0.3056	0.3056	0.0623	-45.30
5	CnCirE7 ₂₁₈	13	0.1637	0.4693	0.1300	25.14
6	CnCirS12 ₁₇₇	NM	0.4649	0.4649	0.0146	-55.83
7	CnCirS7 ₂₁₅	NM	0.5298	0.5298	0.0073	39.47
8	mCnCir47 ₁₅₀	13	0.4305	0.4305	0.0205	37.21
9	mCnCir86 ₁₉₂	9	0.4649	0.4649	0.0146	-48.92
Stepwise multiple regression analysis						
1	CnCirA9 ₉₉	14	0.5298	0.5298	0.0073	-68.30
2	CnCirS12 ₁₇₁	NM	0.3011	0.8309	0.0031	42.50
3	CnCirE2 ₁₅₁	NM	0.1269	0.9578	0.0012	-25.90
4	mCnCir86 ₁₉₄	9	0.0319	0.9897	0.0024	21.00
5	CnCirF2 ₂₀₅	NM	0.0072	0.9968	0.0103	5.00
6	CnCirG4 ₂₀₇	13	0.0032	1.0000	0.0001	4.50

Note: NM, not mapped; PE, parameter estimate; SI, serial number.

Table 6. Stepwise multiple regression analysis for mite resistance using the combined data of RAPD and SSR analysis.

SI No.	Marker _{size}	Chromosome No.	Partial R^2	Total R^2	Prob > F	Parameter estimate
1	CnCirA9 ₉₉	14	0.5298	0.5298	0.0073	-68.30
2	OPE18 ₆₀₀	NM	0.2311	0.7609	0.0018	0.43
3	CnCirE2 ₁₅₁	NM	0.1255	0.8864	0.0001	11.50
4	CnCirS7 ₂₁₉	NM	0.0830	0.9694	0.0010	-9.20
5	OPP16 ₁₂₀₀	NM	0.0306	1.0000	0.0001	4.70

Note: NM, not mapped; SI, serial number.

the strength of the association and direction of impact on mite resistance. In rice, association analysis has been used to establish 2 qualitative traits, days to 50% flowering and culm number (Virk et al. 1995). Adopting a similar strategy, Mishra and Mandi (2004) identified a suite of markers linked to drought tolerance in tea. This strategy is a convenient tool for perennial crops and a "short cut" method for establishing marker-trait association. This association analysis also circumvents the need for mapping populations.

Discussion

The Eriophyid mite *A. guerreronis* is taking a heavy toll in the coconut gardens of South India. It infests the young buttons at the postfertilization period and settles at the inter-spaces between the perianth and button. The population increases many fold within a short span of time because of the mites' short life cycle (8–10 days). The appearance of symptoms of infestation is usually seen 3–4 weeks after the initial colonization. In mite-infested plants, the buttons show browning followed by necrosis on the periphery of the perianth. This results in warty shrinking of husk and cracks, cuts, gummosis, and freckles developing at later stages. The nuts, which remain in bunches, are found to be undersized and malformed. The degree of malformation varies with the infestation. At maturity, the husk of the nut is very tight and

shrunken, causing difficulty in dehusking and resulting in reduced economic value for the husk in the fiber industry (Howard and Rodriguez 1991). It has become a major problem in coconut-producing regions of Asia, especially India and Sri Lanka (Sathiamma et al. 1998). In India, the average percentage of infestation ranges from 3.60% (Lakshadweep islands) to 54.60% (Kerala), resulting in significant reduction of yield (Sathiamma et al. 1998; Nampoothiri et al. 2000).

During the survey, we identified coconut trees that were completely free from mite infestation (resistance reaction) in spite of being surrounded by highly susceptible plants, suggesting that the possibilities of escape are very low or almost nil. It therefore seemed to us that coconut plants do have built-in genetic resistance to mite infestation.

Earlier studies indicate that tall coconuts are cross-pollinated, whereas dwarfs are predominantly self-pollinated. Hence, developing mapping populations in coconuts for linkage analysis is a difficult task. Currently, 6 mapping populations, which have been used for constructing molecular linkage maps, are available. Molecular maps have been used to assess genetic diversity among coconut genotypes and also to establish their identity (Duran et al. 1997; Perera et al. 1998; Lebrun et al. 1999). Attempts have been made to tag quantitative trait loci (QTLs) associated with

nut yield and other related traits by members of the Coconut Genetic Resources Network (COGENT) (Herran et al. 2000; Ritter et al. 2000; Lebrun et al. 2001). However, markers associated with mite resistance in coconut are yet to be developed.

The data generated by SSR and RAPD yielded 276 polymorphic bands, which were used to establish the association with mite resistance by SMA and SMRA. We attempted to understand the genetics of marker–trait association by treating the marker data of SSR and RAPD independently and in combination. BSA using RAPD primers revealed that OPE18₆₀₀ has significant association with mite resistance in SMRA (Table 4).

SMA

Of the 32 SSR markers used in this study, 13 have already been mapped on the coconut genome. The magnitude of the effect of marker on the phenotype, as revealed by R^2 , and the direction of influence (increase or decrease in resistance), as indicated by PE of the individual marker on PNSD, varied significantly (Table 5). The marker CnCirA9₉₉ is associated with mite resistance, as revealed by SMA and SMRA. This marker has been mapped to chromosome 14 at 0.0 cM with an R^2 value of 52.98% in 2 analyses (Herran et al. 2000; Ritter et al. 2000; Lebrun et al. 2001). The CnCirA9₉₉ band appears to be a strong locus contributing to mite susceptibility. Further, OPE4₉₀₀ was associated with mite resistance with a R^2 value of 36.46%, and it is yet to be mapped.

SMRA

The phenotypic effects of each marker on the trait were increased by the addition of each new marker to a string of markers, as revealed by total R^2 . The suite of markers thus identified could be used in selection strategies for enhancing mite resistance in coconut.

OPE18₆₀₀ in RAPD analysis showed maximum association with mite infestation, along with 2 other RAPD markers (Table 4). All 3 markers accounted for increased mite resistance up to 83%. OPE18₆₀₀ was shown to be associated with mite resistance by BSA (Fig. 2A and Table 4).

In SSR analysis, a combination of 6 markers accounted for up to 100% of the variation in mite infestation. The location of 3 of these is known on the molecular linkage map of coconut. The R^2 values ranged from 0.32% to 52.98%. Two markers, CnCirA9₉₉ and CnCirE2₁₅₁, were found to be associated with mite susceptibility. The marker CnCirA9₉₉ had been shown earlier to be associated with mite susceptibility in SMA (Table 5).

An attempt was made to assemble a suite of markers associated with reaction to mite infestation for their possible use in marker-assisted selection. SMRA using mite resistance as the dependent variable and molecular markers as independent variables (combined data of RAPD and SSR) revealed 5 markers that were strongly associated with PNSD (Table 6), 3 SSRs and 2 RAPD (earlier equation). The SSR markers associated with mite resistance were located on different chromosomes (Table 5), and thus it seems that mite resistance has multiple QTLs. Two of the SSRs were associated with decreased resistance to mite. The PE indicated the strength of their influence on the trait. A nega-

tive value of PE indicated susceptibility to mite infestation, the numerical value indicating the strength of the association. Combined analysis suggests that OPE18₆₀₀, CnCirE2₁₅₁, and OPE16₁₂₀₀ can be used for selecting enhanced resistance to mites, and CnCirA9₉₉ and CnCirS7₂₁₉ for identifying mite-susceptible plants (Table 6). The present study has identified a combination of suitable molecular markers linked to mite resistance, which can be used for evaluating coconut germplasm at early stages as well as mother plants for breeding purposes.

Acknowledgments

We acknowledge the financial support from the Department of Biotechnology, Government of India, to DTP (BT/PR1722AGR/08/115/99) and Senior Research Fellowship to KVS; and financial support from Kirkhouse Trust, UK, for KVS to conduct part of the research work at CIRAD, France.

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