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 (*Coçcos 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 guerreonis*, 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 résitantes 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 palliers 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.

Corresponding 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-

 doi:10.1139/G06-136

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 sheeth 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 a 40 °C. Dried leaflets were finely powdered using th grinder, filtered through 40-mesh sieve and stored at roo temperature in clean dry plastic bags. DNA was extracte from these samples using the CTAB (cetyltrimethylammo nium bromide) method with minor modifications (additio of 2% polyvinylpyrrolidone and 1% β-mercaptoethanol (Callahan and Mehta 1991; Porebski et al. 1997; Shalini e al. 2004).

RAPD analysis

PCR (polymerase chain reaction) was performed at th Plant Molecular Biology Laboratory, Department of Biotec nology, University of Agricultural Sciences, Bangalore Indi in 0.2 mL, thin-walled PCR tubes using an MJ-Researc Thermocycler (Model PTC-100). The RAPD protocol de scribed by Williams et al. (1990) was used with modificatio (standardization of $MgCl_2$, deoxynucleoside triphosphate (dNTPs), template DNA, and primer concentration) to am plify coconut DNA. RAPD analysis was carried out in total volume of 20 µL containing 25–30 ng of templa DNA, 1× *Taq* polymerase assay buffer, 200 µmol/L eac of the dNTPs, 3 mmol/L $MgCl_2$, 10 pmol random decame primer (Operon Technologies Inc., Huntsville, Ala.), and 1 U *Taq* polymerase. PCR amplification was carried out wi an initial denaturation at 95 °C for 5 min followed by 4 cycles, each step consisting of denaturation at 95 °C fo 1 min, primer annealing at 36 °C for 1 min, and extensio at 72 °C for 2 min with a final extension step at 72 °C fo 10 min. The amplified samples were resolved on 1.4% agarose gel in 1× TBE (Tris–borate–EDTA) buffer contai ing ethidium bromide (0.5 µg/mL), and bands were captur using Hero Lab gel documentation facility. Further, bulke segregant analysis (BSA) was carried out with RAPD pr mers by generating 2 DNA pools from 9 mite-resistan (HUMPGR, HUDXT1, HUDXT2, GU2F1, BR59, BR3 GU10F1, GUMP16, and GUMP9) and 9 mite-susceptibl (GUMP3, GUMP12, GUHY1, BR80, GU9F1, GUHY GUF1, BR50, and AR6) trees.

SSR analysis

PCR was performed at the Centre de coopération Interna tionale en Recherche Agronomique pour le Développemen France, using an MJ-Research Thermocycler (Model PT 100) with SSR primers (Table 3). Reactions took place in total volume of 10 µL containing 12.5 ng of template DN 1× *Taq* polymerase assay buffer, 200 µmol/L each of th dNTPs, 0.2 µmol/L each of forward and reverse primer, an 0.5 U *Taq* polymerase; 2 mmol/L $MgCl_2$ concentration pro vided optimum results during amplification. The forwa

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

Damage category (PNSD*)	Range, mean ± 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±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±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±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±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 γP^{33}-ATP using T_4 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× 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× 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.
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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).

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

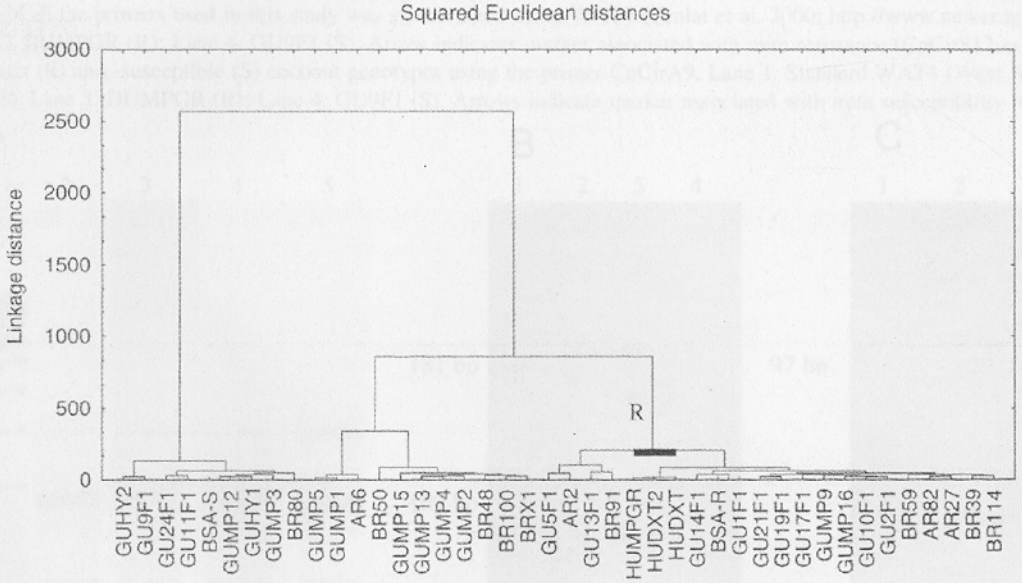


Table 4. Single-marker analysis (*a*) and stepwise multiple regression analysis (*b*) for mite resistance in coconut based on RAPD profiles generated using 7 selected random decamer primers.

(*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

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

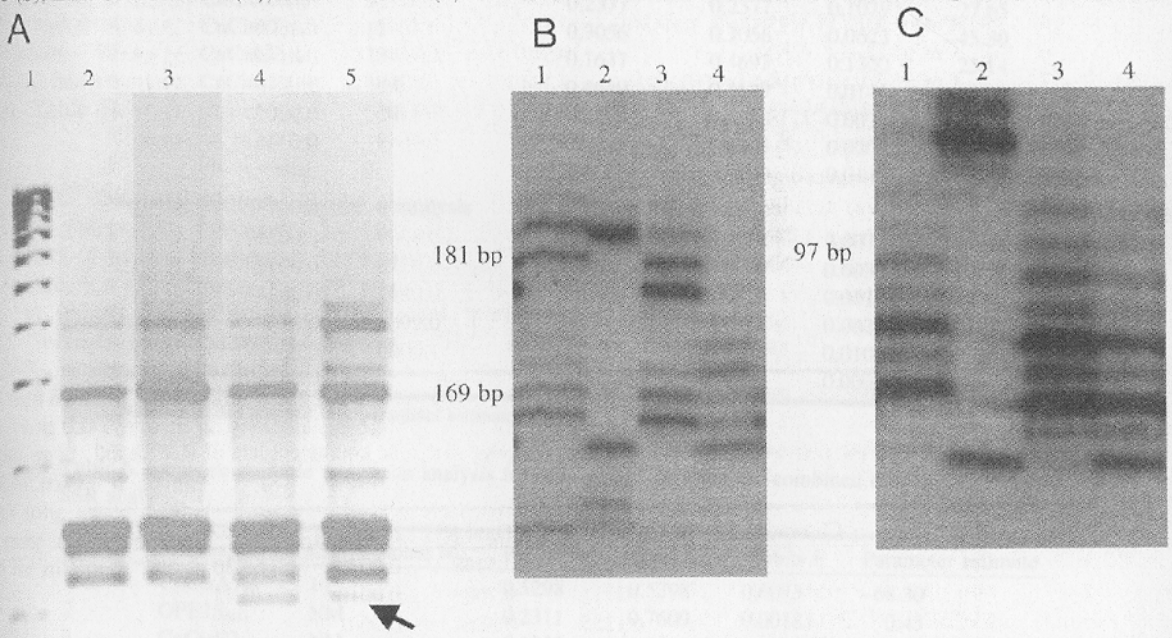
where Y represents PNSD, m_j the RAPD and SSR markers, b_j the partial regression coefficients that specify the empirical relationship between Y and m_j, and e is the random error of Y, including environmental variation. This allows us to ascertain the maximum likelihood solutions of relationship between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

Results

RAPD

Of the 160 random decamer primers used, 132 generated band each. Seven primers, namely OPE4, OPE6, OPE1 OPF2, OPG7, OPP15, and OPP16, produced 5 to 17 band and were selected for further analysis. Ninety-one band ranging in size from 200 to 3000 bp, which were consisten unambiguous and reproducible, were used in the statistic analyses. The number of bands per primer varied from 8 t

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 ($CnCirS12_{171}$). (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 ($CnCirA9_{99}$).



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

SMA established the association of mite resistance with markers $OPE4_{900}$ (36.46%), $OPE6_{800}$ (10.95%), $OPG7_{2000}$ (11.11%), and $OPP15_{425}$ (13.19%) (Table 4). SMRA revealed that the markers $OPE18_{600}$, $OPP16_{1200}$, and $OPP16_{1650}$ 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 $OPE18_{600}$ 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 $CnCirA9_{99}$ and $CnCirS7_{215}$ (52.98%). In SMRA, a combination of 6 SSR markers accounted for 100% of mite resistance (Table 5). $CnCirA9_{99}$ showed a negative association with mite resistance (Fig. 2C) in both SMA and SMRA (Table 5, PE: –68.30). $CnCirS12_{171}$ 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

$$[2]\quad PNSD = 34.7 + [(-68.30)\,CnCirA9_{99}] + [(0.43)\,OPE18_{600}] + [(11.5)\,CnCirE2_{151}] + [(-9.2)\,CnCirS7_{219}] + [(4.70)\,OPP16_{1200}] + 0.00083$$

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, $OPE18_{600}$, $CnCirE2_{151}$, and $OPP16_{1200}$ contributed to enhanced resistance, and $CnCirA9_{99}$ and $CnCirS7_{219}$ 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 interspaces 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 re duced economic value for the husk in the fiber industr (Howard and Rodriguez 1991). It has become a major prob lem in coconut-producing regions of Asia, especially Indi and Sri Lanka (Sathiamma et al. 1998). In India, the averag percentage of infestation ranges from 3.60% (Lakshadwee islands) to 54.60% (Kerala), resulting in significant reduc tion of yield (Sathiamma et al. 1998; Nampoothiri et a 2000).

During the survey, we identified coconut trees that wer 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 al most nil. It therefore seemed to us that coconut plants d 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 coco nuts for linkage analysis is a difficult task. Currently, f mapping populations, which have been used for constructin molecular linkage maps, are available. Molecular maps hav been used to assess genetic diversity among coconut gen types and also to establish their identity (Duran et al. 199 Perera et al. 1998; Lebrun et al. 1999). Attempts have be made to tag quantitative trait loci (QTLs) associated wi

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 $OPE18_{600}$ 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 $CnCirA9_{99}$ 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 $CnCirA9_{99}$ band appears to be a strong locus contributing to mite susceptibility. Further, $OPE4_{900}$ 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.

$OPE18_{600}$ 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%. $OPE18_{600}$ 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, $CnCirA9_{99}$ and $CnCirE2_{151}$, were found to be associated with mite susceptibility. The marker $CnCirA9_{99}$ 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 negative value of PE indicated susceptibility to mite infestation, the numerical value indicating the strength of the association. Combined analysis suggests that $OPE18_{600}$, $CnCirE2_{151}$, and $OPP16_{1200}$ can be used for selecting enhanced resistance to mites, and $CnCirA9_{99}$ and $CnCirS7_{219}$ 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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