

L. Herselman · R. Thwaites · F. M. Kimmins ·
B. Courtois · P. J. A. van der Merwe · S. E. Seal

Identification and mapping of AFLP markers linked to peanut (*Arachis hypogaea* L.) resistance to the aphid vector of groundnut rosette disease

Received: 3 March 2004 / Accepted: 10 June 2004 / Published online: 29 July 2004
© Springer-Verlag 2004

Abstract Groundnut rosette disease is the most destructive viral disease of peanut in Africa and can cause serious yield losses under favourable conditions. The development

Communicated by C. Möllers

L. Herselman (✉)
Agricultural Research Council-Grain Crops Institute,
Private Bag X1251 Potchefstroom, 2520, South Africa
e-mail: HerselmanL.sci@mail.uovs.ac.za
Tel.: +27-51-4012715
Fax: +27-51-4305692

R. Thwaites · F. M. Kimmins · S. E. Seal
Natural Resources Institute, University of Greenwich at
Medway,
Central Avenue,
Chatham Maritime, Kent, ME4 4TB, UK

B. Courtois
UMR 1096, CIRAD-Biotrop,
TA40/03, Avenue Agropolis,
34398 Montpellier Cedex 5, France

P. J. A. van der Merwe
Chitedze Agricultural Research Station, ICRISAT-Malawi,
P.O. Box 1096 Lilongwe, Malawi

Present address:
L. Herselman
Department of Plant Sciences, University of the Free State,
P.O. Box 339 Bloemfontein, 9300, South Africa

Present address:
R. Thwaites
Department of Agricultural Sciences, Imperial College London,
Wye Campus,
Wye Ashford, Kent, TN25 5AH, UK

Present address:
F. M. Kimmins
NR International,
Park House, Bradbourne Lane,
Aylesford, Kent, ME20 65N, UK

Present address:
P. J. A. van der Merwe
Agricultural Research Council-Grain Crops Institute,
Private Bag X1251 Potchefstroom, 2520, South Africa

of disease-resistant cultivars is the most effective control strategy. Resistance to the aphid vector, *Aphis craccivora*, was identified in the breeding line ICG 12991 and is controlled by a single recessive gene. Bulked segregant analysis (BSA) and amplified fragment length polymorphism (AFLP) analysis were employed to identify DNA markers linked to aphid resistance and for the development of a partial genetic linkage map. A F_{2:3} population was developed from a cross using the aphid-resistant parent ICG 12991. Genotyping was carried out in the F₂ generation and phenotyping in the F₃ generation. Results were used to assign individual F₂ lines as homozygous-resistant, homozygous-susceptible or segregating. A total of 308 AFLP (20 *Eco*RI+3/*Mse*I+3, 144 *Mlu*I+3/*Mse*I+3 and 144 *Pst*I+3/*Mse*I+3) primer combinations were used to identify markers associated with aphid resistance in the F_{2:3} population. Twenty putative markers were identified, of which 12 mapped to five linkage groups covering a map distance of 139.4 cM. A single recessive gene was mapped on linkage group 1, 3.9 cM from a marker originating from the susceptible parent, that explained 76.1% of the phenotypic variation for aphid resistance. This study represents the first report on the identification of molecular markers closely linked to aphid resistance to groundnut rosette disease and the construction of the first partial genetic linkage map for cultivated peanut.

Introduction

Peanut (*Arachis hypogaea* L.) is cultivated in the semi-arid tropical and sub-tropical regions of approximately 100 countries on six continents between 40°N and 40°S (Naidu et al. 1999). In developing regions of Asia, Africa and South America, peanut is the principal source of digestible protein, cooking oil and vitamins (Savage and Keenan 1994) and contributes significantly to food security and alleviating poverty (Smartt 1994). Peanut is an important crop in sub-Saharan Africa (SSA) and is mostly grown by smallholder farmers as a subsistence crop under rainfed conditions (van der Merwe et al. 2001).

Groundnut rosette disease causes greater yield loss than any other viral disease affecting peanut in the semi-arid tropics of the world and is the most destructive viral disease of peanut in Africa (Naidu et al. 1999). It is assumed to be endemic to peanut-growing regions of SSA and Madagascar. The most serious yield losses were reported during 1975 when an epidemic in northern Nigeria destroyed approximately 0.7 million ha of peanuts, with an estimated loss of US\$250 million (Yayock et al. 1976).

Groundnut rosette disease is caused by a complex of three agents comprising *Groundnut rosette virus* (GRV) (Reddy et al. 1985), satellite RNA (sat RNA) (Murant et al. 1988) and *Groundnut rosette assistor virus* (GRAV) (Casper et al. 1983). GRAV, GRV and sat RNA are intricately dependent on each other, and all three must be present for severe disease symptoms. GRV is mechanically transmissible (Hull and Adams 1968) and replicates independently in plants. It supports replication of the sat RNA (Murant et al. 1988). GRAV and GRV do not cause obvious symptoms on their own or, at most, result in transient mottle in peanut; sat RNA is primarily responsible for disease symptoms. Variants of the sat RNA cause chlorotic and green forms of the disease (Murant and Kumar 1990). *Aphis craccivora* Koch is the principal aphid vector of groundnut rosette disease agents (Storey and Ryland 1955).

Host-plant resistance is considered to be the most cost-effective management measure for rosette disease because resource-poor farmers seldom adopt cultural or chemical control practices due to a lack of capital, labour constraints and differential crop priorities. Resistance to groundnut rosette disease was first identified in 1952 in peanut landraces from Burkina Faso and the Ivory Coast (Sauger and Catherinet 1954a, b). It was effective against GRV and its sat RNA and was governed by two recessive genes (de Berchoux 1960; Bock et al. 1990). Resistance to GRAV has not been identified (Chiyembekeza et al. 1997). Resistance to the aphid vector *A. craccivora* was first identified by Padgham et al. (1990) and found to be governed by a single recessive gene (van der Merwe 2001). Aphid-resistant sources are, however, susceptible to GRAV, GRV and sat RNA (Minja et al. 1999). On-farm evaluations in Malawi over a 3-year period identified agronomically acceptable genotypes showing resistance to either GRV and its sat RNA, or aphids (Chiyembekeza et al. 1997).

Breeding efforts should focus on developing genotypes with resistance to both the virus (GRV) and its vector, which would be expected to be more stable than those with only one of the components. Molecular markers linked to either GRV or aphid resistance genes would facilitate efficient pyramiding of these genes in a single variety. This would compliment classical breeding programmes and provide the basis for map-based cloning for groundnut rosette resistance genes. However, results throughout the world (Grieshammer and Wynne 1990; Halward et al. 1991; He and Prakash 1997; Hopkins et al. 1999; Subramanian et al. 2000; Herselman 2003) have

indicated that molecular work on cultivated peanut presents a significant challenge due to its inherent narrow genetic base.

Genetic variability in *A. hypogaea* is difficult to detect using molecular marker systems, i.e. isozymes (Grieshammer and Wynne 1990; Lacks and Stalker 1993), restriction fragment length polymorphisms (RFLPs) (Kochert et al. 1991; Halward et al. 1994), random amplified polymorphic DNAs (RAPDs) (Halward et al. 1991; Subramanian et al. 2000), amplified fragment length polymorphisms (AFLPs) (He and Prakash 1997; Herselman 2003) or microsatellite analysis (Hopkins et al. 1999; Ferguson et al. 2004). Herselman (2003), however, successfully used *MluI/MseI*-AFLP primer combinations to detect genetic variability in closely related cultivated peanut genotypes.

Garcia et al. (1996) used RAPD and sequence-characterized amplified region (SCAR) technology to map two dominant genes that conferred resistance to the root-knot nematode *Meloidogyne arenaria* (Neal) Chitwood Race 1 in a segregating F_2 population derived from a cross between wild *A. cardenasii* Krapov. and W.C. Greg. and cultivated *A. hypogaea* species. Two markers were identified that mapped 10 ± 2.5 and 14 ± 2.9 cM from the two resistance genes, respectively. Burow et al. (1996) reported three RAPD markers linked to a single dominant *M. arenaria* resistance gene that were derived from tetraploid plants of the hybrid Florunner with three wild peanut species. Stalker and Mazingo (2001) reported the identification of RAPD markers explaining up to 35% of the variation for early and late leaf spot resistance in a peanut population which contained the wild species *A. cardenasii* in its pedigree and 10% of the variation in a *A. hypogaea* \times *A. hypogaea* cross. The latter was the first report on molecular markers associated with resistance genes in an *A. hypogaea* \times *A. hypogaea* cross.

Although reports of linkage between various morphological traits exist (Patel et al. 1936; Coffelt and Hammons 1973; Murthy et al. 1988), no linkage map has been constructed for cultivated peanut, neither have any traits been mapped to a specific peanut chromosome (Stalker 1991). When complete, a peanut map should consist of ten linkage groups corresponding to the haploid chromosome complement of tetraploid peanut. Burow et al. (1999) constructed a genetic linkage map for wild peanut consisting of 22 linkage groups and using a backcross population developed from a cross between the cultivated peanut Florunner and [(*A. batizocoi* Krapov. and W.C. Greg. \times (*A. cardenasii* \times *A. diogeni* Hoehne)) 4x] using approximately 350 segregating RFLP loci. A total map distance of 2,700 cM was covered.

The investigation reported here was undertaken to identify AFLP markers linked to aphid resistance for groundnut rosette disease and to develop a basic genetic linkage map for cultivated peanut.

Materials and methods

Plant material

A segregating F_2 population derived from a cross between the aphid-resistant female parent (R parent) ICG 12991 and the aphid-susceptible male parent (S parent) ICGV-SM 93541 was obtained from Dr. P.J.A. van der Merwe based at ICRISAT-Malawi in 2001. ICG 12991 is a spanish-type landrace from India, while ICGV-SM 93541 was developed by ICRISAT-Malawi and is a red seeded variety with early leaf spot resistance. Parental and F_2 seed were treated with a protectant fungicide (thiram at 3 g kg seed⁻¹) and an inoculant for effective nitrogen fixation [*Bradyrhizobium* sp. (*Arachis*)] prior to sowing. A total of 200 F_2 seeds were planted in sterile soil in 4-l plastic pots (one seed per pot) and maintained in a glasshouse at 28 \pm 2 $^\circ$ C/21 \pm 2 $^\circ$ C (day/night) under a 14/10-h (day/night) photoperiod. Young leaves were sampled from individual plants for DNA analysis at approximately 21 days after planting. F_2 plants were self-pollinated and F_3 seed were harvested 131–146 days after planting.

Phenotypic evaluation of F_3 populations

A non-viruliferous aphid (*A. craccivora*) colony was derived from approximately 20 aphids collected from cowpea (*Vigna unguiculata* L.) plants in a field trial at Potchefstroom, South Africa. The colony was maintained on two susceptible peanut genotypes, Sellie and ICGV-

SM 93555, in a glasshouse maintained at a 28 \pm 2 $^\circ$ C/25 \pm 2 $^\circ$ C (day/night) under a 14/10-h (day/night) photoperiod.

Ten seeds of 139 individual F_3 plants were selected for aphid resistance screening. Seed were treated similar to F_2 seeds and planted in 0.3-l plastic pots (one seed per pot) in sterile soil in a randomized complete block design. Each seed represented a repeat. Seed from the aphid-susceptible parent ICGV-SM 93541 and aphid-resistant parent ICG 12991 were included as positive and negative controls, respectively. Two wingless (apterae) aphids were transferred onto 7- to 14-day-old F_3 peanut seedlings. Plants were scored for presence or absence of aphid colonies (adults as well as nymphs) 7 days after infestation. Plants with no aphid colonies were re-infested with viable aphids 7 days after the first infestation. All plants were scored for the presence or absence of aphid colonies 7 days after the second infestation and repeated at 14 days. Homozygous resistant and susceptible F_2 plants were selected based on aphid colonization on each of the ten F_3 progenies. Lines considered to be resistant had a mean number of aphids of 0.8 or less per plant, and those considered to be susceptible had a mean number of at least 16.7 aphids per plant. The F_2 and F_3 population data on disease reaction were subjected to chi-square tests.

AFLP analysis

Total genomic DNA was isolated using the CTAB (hexadecyltrimethylammonium bromide) method (Saghai Maroof et al. 1984). Absorbencies were measured at 260 nm and 280 nm, respectively, to determine DNA quantity and quality. AFLP analysis (Vos et al. 1995), gel

Table 1 *EcoRI*, *MluI*, *PstI* and *MseI* adaptor, primer+1 and primer+3 sequences (5'–3') used for AFLP analyses to identify and map markers linked to aphid resistance genes in a F_2 segregating population

Enzyme	Type	Sequence (5'–3')
<i>EcoRI</i>	Adaptor-F	CTCGTAGACTGCGTACC
	Adaptor-R	AATTGGTACGCAGTCTAC
<i>MluI</i>	Adaptor-F	CTCGTAGACTGCGTAAC
	Adaptor-R	CGCGGTTACGCAGTC
<i>PstI</i>	Adaptor-F	GACTGCGTAGGTGCA
	Adaptor-R	CCTACGCAGTCTACGAG
<i>MseI</i>	Adaptor-F	GACGATGAGTCCTGAG
	Adaptor-R	TACTCAGGACTCAT
<i>EcoRI</i>	Primer +1	GACTGCGTACCAATTCA
	Primer +3	GACTGCGTACCAATTCANN
<i>MluI</i>	Primer +1	ANN = AAC, AAG, ACT, AGC, AGG
	Primer +3	GACTGCGTAACCGCGT
		GACTGCGTAACCGCGTNN
<i>PstI</i>	Primer +1	TNN = TAA, TAC, TCA, TGC, TTA, TCT, TGT, TTG
	Primer +3	GACTGCGTAGGTGCAGT
		GACTGCGTAGGTGCAGTNN
<i>MseI</i>	Primer +1	TNN = TAA, TAG, TCA, TCC, TGC, TGG, TTA, TTC
	Primer +3	GATGAGTCCTGAGTAAN
		N = A, C, G, T
		GATGAGTCCTGAGTAANN
		NNN = CAA, CAC, CAG, CAT, CTA, CTC, CTG, CTT, ACA, ACC, ACT, CGT, CCG, GAA, GAG, GGC, TAC, TTG

electrophoresis and visualization of AFLP fragments were performed using the method of Herselman (2003).

Bulked segregant analysis (BSA) (Michelmore et al. 1991) was performed by constructing two bulks of the extremes (resistant and susceptible). Equivalent amounts of DNA from ten randomly selected F_2 individuals identified as homozygous aphid-resistant and ten randomly selected F_2 individuals identified as homozygous aphid-susceptible, on the basis of F_3 phenotypic data, were pooled to represent the resistant and susceptible bulks, respectively. Primers and adaptors used for marker identification are shown in Table 1. A total of 308 primer combinations (20 *EcoRI*+3/*MseI*+3, 144 *MluI*+3/*MseI*+3 and 144 *PstI*+3/*MseI*+3) were tested on the two parental lines and two bulk samples. Enzyme combinations were selected based on results obtained by Herselman (2003). Primer combinations that generated informative polymorphisms between the parental lines and the two bulk samples were tested on the 20 individual plants comprising the bulk samples followed by screening of informative primer combinations on a further 40 F_2 individuals from the segregating population. Markers revealing polymorphisms across the entire F_2 segregating population were used for constructing a putative linkage map.

Statistical analysis of data

A binary matrix reflecting specific AFLP markers as present (1) or absent (0) was generated for each genotype. Only reliable and repeatable markers were considered. Data obtained from AFLP analyses were analysed using a general linear model of the STATGRAPHICS PLUS COMPUTER programme (Manugistics, Rockville, Md., 1998) with genetic marker data as the independent variable and rosette disease rating as the dependant variable. Association between the DNA marker and trait (aphid resistance) was considered to be significant if the probability was $P < 0.05$. A threshold of 0.05 at the genome level was chosen that corresponds to a threshold of 0.026 for each individual test using the Bonferonni correction. The coefficient of determination (R^2) was used as a measure of the magnitude of association.

MAPMAKER/EXP 3.0 (Lander et al. 1987) was used for linkage analysis and the drawing of a linkage map and MAPMAKER/QTL 1.1 (Paterson et al. 1988) for the localization of resistance genes on the linkage map. Linkage data were used to assign markers to linkage groups if the minimum log-likelihood scores (LOD) were 3.0 and maximum recombination frequencies (θ) 0.5, using the Haldane mapping function. The resistance gene position on the map was determined by either treating the trait as a marker and mapping it with the other markers using MAPMAKER/EXP 3.0 or by using the scan command of MAPMAKER/QTL 1.1 to identify the position of the resistance gene. All linkage groups were scanned for presence of a quantitative trait loci (QTL) effect at a LOD threshold of 3.0 in every 2.0-cM interval using MAPMAKER/QTL at a recessive model (Lincoln et al. 1992).

Results

Evaluation of the F_3 population segregating for aphid resistance

Results from the F_3 seedling infestations showed a good fit to the expected 1:2:1 (homozygous-resistant:segregating:homozygous-susceptible) ratio for a single recessive gene, and this was confirmed by chi-squared tests (Table 2). The F_3 data were extrapolated to confirm the expected 1:3 (resistant:susceptible) ratio for the F_2 population (Table 2) indicating a single recessive gene governing resistance to the aphid vector of groundnut rosette disease. Based on the F_3 aphid colony data, ten homozygous resistant and ten homozygous susceptible F_2 plants were selected for the construction of the two bulk samples as well as another 40 F_2 plants for further AFLP analysis.

AFLP analysis

The 308 AFLP primer combinations—20 *EcoRI*/*MseI* combinations, 144 *MluI*/*MseI* combinations and 144 *PstI*/*MseI* combinations—used to screen the parental and bulk DNA amplified a total of 12,315 fragments, of which 986 (8.0%) were polymorphic between the parental lines. Only 118 (0.96%), however, were polymorphic between the bulk samples. *EcoRI*/*MseI*, *MluI*/*MseI* and *PstI*/*MseI* primer combinations amplified an average of 76.1, 40.6 and 34.4 fragments per primer combination, respectively. Although *MluI*/*MseI* primer combinations detected fewer fragments per primer combination than *EcoRI*/*MseI* primer combinations, the former detected the highest percentage of polymorphic fragments in the parental lines (11.31%) and bulk samples (1.35%), compared to the 6.20% and 0.61% with *PstI*/*MseI* primer combinations and the 1.18% and 0.59% using *EcoRI*/*MseI* primer combinations. Furthermore, the *MluI*/*MseI* approach detected an average of 4.59 and 0.55 polymorphisms per primer combination between the parental lines and between the two bulk samples, respectively, compared to the 2.13 and 0.21 detected by the *PstI*/*MseI* primer combinations and the 0.90 and 0.45 detected by the *EcoRI*/*MseI* primer combinations (Table 3).

Table 2 Segregation for groundnut rosette aphid resistance in F_2 plants and F_3 families developed from a cross between ICG 12991 (resistant parent) and ICGV-SM 93541 (susceptible parent)

	Number	Expected ratio	χ^2	P-value
F_2 plants				
Resistant	33	1:3	0.12	0.729
Susceptible	106			
F_3 families				
Homozygous-resistant	33	1:2:1	1.35	0.509
Segregating	76			
Homozygous-susceptible	30			

Table 3 Comparison between data obtained using *EcoRI*, *MluI* and *PstI* as rare-cutting restriction enzymes in combination with *MseI* as the frequent-cutter

	<i>EcoRI/MseI</i> reactions	<i>MluI/MseI</i> reactions	<i>PstI/MseI</i> reactions
Primers tested	20	144	144
Total number of fragments detected	1,522	5,842	4,951
Total polymorphisms	18	661	307
Informative primers between parents	60.0%	95.1%	82.6%
Informative primers between bulks	30.0%	26.4%	13.9%
Average fragments/primer combination	76.1	40.6	34.4
Maximum fragments/primer combination	115	87	65
Minimum fragments/primer combination	40	13	13
Maximum polymorphisms/primer combination	3	9	7
Polymorphic fragments in parents	1.18%	11.31%	6.20%
Polymorphic fragments in bulks	0.59%	1.35%	0.61%
Average polymorphisms/primer (parents)	0.90	4.59	2.13
Average polymorphisms/primer (bulks)	0.45	0.55	0.21

Thirty-two primer combinations (three *EcoRI/MseI*, 21 *MluI/MseI* and eight *PstI/MseI* primer combinations) amplified informative polymorphisms between the two bulk samples and the parental DNA. Based on informative polymorphisms between the bulk and parental samples, four putative *EcoRI/MseI*, 34 *MluI/MseI* and 13 *PstI/MseI* markers were tested on the 20 individuals from the bulk samples that revealed 24 polymorphic fragments (four *EcoRI/MseI*, 15 *MluI/MseI* and five *PstI/MseI*) co-segregating with the resistance trait.

Statistical analysis on 18 of these putative markers tested on the 20 individuals and an additional 40 individuals is presented in Table 4. A standardized notation of naming the *EcoRI*+3 (E+3), *MluI*+3 (MI+3) or *PstI*+3 (P+3) selective nucleotides first and the *MseI*+3 (M+3) selective nucleotides secondly, was used throughout. Twelve of these markers originated from the S parent ICGV-SM 93541 and six originated from the R parent

ICG 12991. The most significant association ($R^2=76.1\%$, $P=0.000$) with aphid resistance in 60 individual F_2 plants was obtained with marker MI-TTG/M-GAA1 originating from the S parent, followed by P-TCA/M-ACT1 ($R^2=31.2\%$; $P=0.000$). The marker originating from the R parent with the best association was MI-TTG/M-ACC2 ($R^2=11.7\%$; $P=0.004$).

Linkage analysis and gene mapping

Data obtained from 12 primer combinations that detected polymorphisms within the 60 individuals from homozygous selections from the segregating F_2 population were used for constructing a putative genetic linkage map for cultivated peanut using MAPMAKER/EXP and the information from 19 AFLP markers (three *EcoRI/MseI*, 12 *MluI/MseI* and four *PstI/MseI*). Five linkage groups were identified

Table 4 Statistical analyses of *EcoRI/MseI*, *MluI/MseI* and *PstI/MseI* marker data on individual plants from homozygous selections from the F_2 segregating population

Primer combination fragment	Linkage	Twenty individuals		Sixty Individuals		Linkage group
		R^2 (%) ^a	P ^b	R^2 (%)	P	
MI-TTG/M-GAA1	R ^c	63.42	0.000	76.06	0.000	1
P-TCA/M-ACT1	R	24.44	0.025	31.17	0.000	1
MI-TTG/M-ACC2	C ^d	20.83	0.025	11.71	0.004	Unlinked
MI-TAA/M-CTC1	R	0.00	0.660	10.05	0.008	3
MI-TAC/M-ACC1	R	13.07	0.065	9.58	0.009	2
MI-TAC/M-ACC2	R	14.55	0.054	8.39	0.014	2
MI-TAA/M-CTC2	C	14.55	0.054	6.06	0.032	4
MI-TAA/M-CAT1	C	4.04	0.196	5.09	0.046	5
P-TAA/M-ACC2	C	1.04	0.288	1.18	0.199	5
MI-TAA/M-CTC3	R	7.11	0.135	1.10	0.203	Unlinked
P-TCA/M-ACT2	R	11.13	0.104	0.76	0.237	Unlinked
E-AAG/M-CAG2	C	47.84	0.001	0.00	0.949	4
MI-TAC/M-CTG1	R	21.10	0.024	– ^e	–	–
P-TAA/M-ACC1	R	18.66	0.042	–	–	–
MI-TTG/M-GAA4	R	12.59	0.075	–	–	–
P-TCA/M-ACT3	C	11.13	0.104	–	–	–
MI-TAC/M-ACC2	R	6.17	0.151	–	–	–
E-AAG/M-CTA2	R	2.87	0.233	–	–	–

^a R^2 , Coefficient of determination

^b P , Probability

^cR Repulsion phase with R allele

^dC Coupling phase with R allele

^e–, Not repeatable

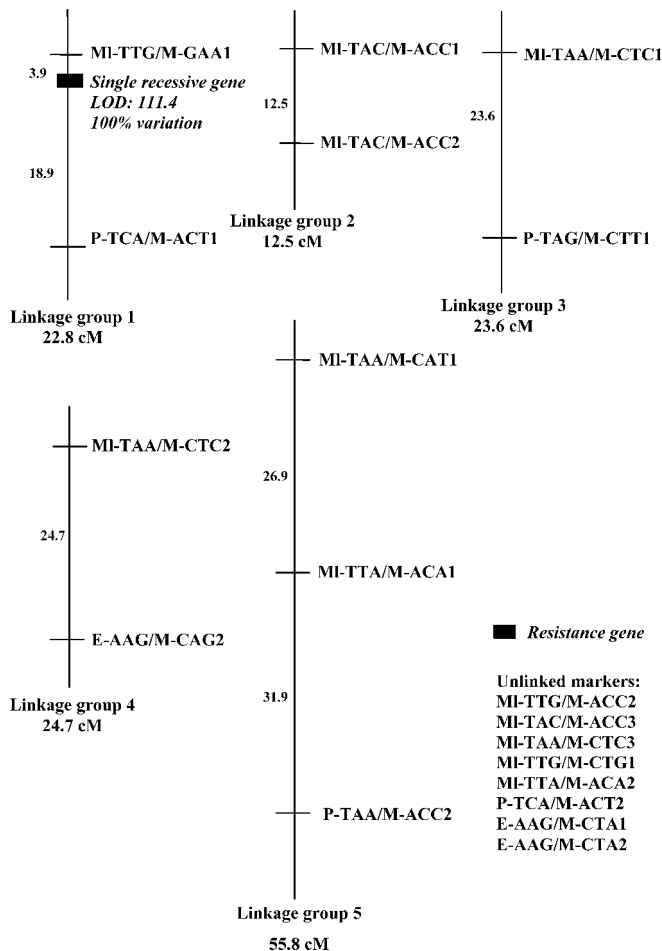


Fig. 1 AFLP-based genetic linkage map for cultivated groundnut developed using homozygous selections from a F_2 population derived from a cross between a resistant (ICG 12991) and susceptible (ICGV-SM 93541) parent for groundnut rosette vector resistance. Loci were ordered using *MAPMAKER/EXP*, and positions for putative QTLs for vector resistance were determined using *MAPMAKER/QTL*.

with two markers each mapping to linkage groups 1–4, and three markers mapped to linkage group 5. Eight putative markers were unlinked, and a total of 139.4 cM of the genome was covered in the construction of the map (Fig. 1).

After scanning the linkage map using recessive genetics, we identified one putative QTL for aphid resistance on linkage group 1, 3.9 cM from marker MI-TTG/M-GAA1 (Fig. 1), which explained 100% of the variation in groundnut rosette disease in the segregating population (LOD: 111.4). Treating the trait as a marker and mapping it with the other markers using *MAPMAKER/EXP* confirmed the position of the resistance gene on the map.

Discussion

In this study, molecular markers linked to aphid resistance in peanut were successfully identified and mapped using a F_2 population segregating for aphid resistance in combina-

tion with BSA and AFLP analysis. BSA, combined with selective genotyping, reduced the amount of mapping needed to localize the targeted gene. AFLP analysis has the advantage of being reproducible and having a high multiplex ratio. However, AFLP markers are dominant and in most cases need to be converted into sequence-tagged site markers to generate breeder-friendly markers for marker-assisted selection (MAS). Three AFLP enzyme combinations (*EcoRI/MseI*, *MluI/MseI* and *PstI/MseI*) were used to screen the F_2 population. *MluI/MseI* primer combinations proved to be the most efficient for detecting polymorphisms in cultivated peanut. *PstI/MseI* primer combinations were used for the first time and selected based on the methylation sensitivity of *PstI* and the successful application of this approach in plant species with large genomes (Vuylsteke et al. 1999; Young et al. 1999). *PstI/MseI* primer combinations detected the lowest number of loci (average of 34.4 loci per primer combination) as was expected due the methylation sensitivity of *PstI*.

Evaluation of polymorphic markers across 60 individuals of the segregating population revealed eight putative AFLP markers (five in repulsion phase and three in coupling phase with the R allele) closely linked ($P < 0.05$) to aphid resistance in the F_2 cultivated peanut population. Markers MI-TTG/M-GAA1 and P-TCA/M-ACT1 (originating from the S parent) accounted for the greatest variation in aphid resistance (76.1%; $P = 0.000$ and 31.2%; $P = 0.000$, respectively) and are linked to the aphid-susceptible allele of parent ICGV-SM 93541. These markers may be useful in MAS and would serve to select against the aphid-resistant allele of parent ICG 12991, thereby providing a greater proportion of homozygous-resistant selections and help in the identification of both segregating and homozygous susceptible selections (Haley et al. 1994). However, the applicability of these markers will be limited to populations with an identical aphid-susceptible allele as parent ICGV-SM 93541. Ideally, MAS for a single recessive gene should employ markers originating from both the R and S parents. The marker in coupling with the S allele would assist in the identification of heterozygous individuals, while the marker in coupling with the R allele would assist in the selection of individuals containing the resistant allele. An even better solution would be to have two co-dominant markers flanking the gene on both sides. The present study is the first report on the identification of molecular markers closely linked to a resistance gene for cultivated peanut that explains more than 75% of the phenotypic variation.

Information from 19 polymorphic AFLP markers (three *EcoRI/MseI*, 12 *MluI/MseI* and four *PstI/MseI*) was used to construct a partial genetic linkage map. Eleven markers were distributed among five linkage groups covering 139.4 cM of the genome. Eight of the polymorphic markers were unlinked. The single recessive gene for aphid resistance was mapped between markers MI-TTG/M-GAA1 and P-TCA/M-ACT1 on linkage group 1. These markers showed the best associations (76.1% and 31.2%) with aphid resistance and mapped 22.8 cM apart. The gene

mapped 3.9 cM from marker MI-TTG/M-GAA1 and 18.9 cM from marker P-TCA/M-ACT1. This is consistent with a single recessive gene for aphid resistance (van der Merwe 2001).

We report here the first application of the rare-cutting restriction enzyme *Pst*I in combination with the frequent cutter *Mse*I in AFLP analysis on cultivated peanut. *Mlu*I/*Mse*I primer combinations were identified as the best AFLP enzyme combination for the detection of polymorphisms in cultivated peanut. The application of *Mlu*I/*Mse*I primer combinations should facilitate the efficient detection of polymorphisms in wild species of peanut, since higher variability exists among diploid wild species than among cultivated peanut (Kochert et al. 1991; Halward et al. 1991). The efficient ability of this enzyme combination to detect polymorphisms in cultivated peanut should enable researchers to develop a more saturated genetic linkage map for cultivated peanut or at least enable them to add AFLP markers to the existing RFLP maps of peanut, constructed using a combination of cultivated and wild species (Halward et al. 1994; Burow et al. 1999). Since the germplasm base of cultivated peanut is extremely narrow, breeders might have to evaluate and utilize related wild species in order to enhance the genetic variability available for the development of improved cultivars. Extensive screening of wild *Arachis* species has revealed these genetic resources to be valuable as sources of disease and insect resistance, tolerance to environmental stresses (ICRISAT 1982) and variation for protein and oil quality (Cherry 1977). The development of genetic linkage maps for peanut will enable breeders to tag and follow the introgression of specific chromosome segments linked to desirable traits from wild species into breeding lines of cultivated peanut. However, it is desirable to develop a genetic map of cultivated peanut as alien species have not been used in the breeding of modern peanut cultivars (Isleib and Wynne 1992).

Recent studies have demonstrated the potential of single-nucleotide polymorphisms (SNPs) as the next generation of genetic markers in plants (Hayashi et al. 2004). Because SNPs are highly abundant, occur frequently throughout genomes and tend to be relatively stable genetically (Batley et al. 2003), their potential use as the next generation of genetic markers in a species lacking polymorphisms (e.g. peanut) should be explored in future.

This study has elucidated a number of issues regarding the use of molecular markers in an analysis of cultivated peanut. AFLP analysis data were successfully used to construct the first partial AFLP-based genetic linkage map for cultivated peanut. The resistance gene was successfully mapped onto this linkage map. Future studies should focus on the conversion of the AFLP markers to SCAR markers as well as the identification of a marker originating from the R parent for aphid resistance and markers linked to the two recessive genes governing virus (GRV) resistance. The availability of more than one marker would be more useful in peanut breeding programmes where aphid and virus resistance are pyramided into the same cultivars or lines.

Acknowledgements This publication is an output from a research project funded by the Department for International Development of the United Kingdom. However, the Department for International Development can accept no responsibility for any information provided or views expressed (DFID project code R7445, Crop Protection Programme).

References

- Batley J, Mogg R, Edwards D, O'Sullivan H, Edwards KJ (2003) A high-throughput SNUPE assay for genotyping SNPs in the flanking regions of *Zea mays* sequence tagged simple sequence repeats. *Mol Breed* 11:111–120
- Bock K, Murrant A, Rajeshwari R (1990) The nature of the resistance in groundnut to rosette disease. *Ann Appl Biol* 117:379–384
- Burow MD, Simpson CE, Paterson AH, Starr JL (1996) Identification of peanut (*Arachis hypogaea* L.) RAPD markers diagnostic of root-knot nematode (*Meloidogyne arenaria* (Neal) Chitwood) resistance. *Mol Breed* 2:369–379
- Burow MD, Simpson CE, Starr JL, Paterson AH (1999) Generation of a molecular map of the cultivated peanut, *Arachis hypogaea*L. In: Plant Anim Genome VII Conf. San Diego, p 235
- Casper R, Meyer S, Lesemann D-E, Reddy DVR, Rajeshwari R, Misari SM, Subbarayudu SS (1983) Detection of a luteovirus in groundnut rosette diseased groundnuts (*Arachis hypogaea*) by enzyme-linked immunosorbent assay and immunoelectron microscopy. *Phytopathol Z* 108:12–17
- Cherry JP (1977) Potential sources of peanut seed proteins and oil in the genus *Arachis*. *J Agric Food Chem* 25:186–193
- Chiyembekeza AJ, Subrahmanyam P, Hildebrand GL (1997) Identification and farm evaluation of rosette-resistant groundnut genotypes in Malawi. In: Reddy DVR, Delfosse P, Lenne' JM, Subrahmanyam P (eds) Groundnut virus diseases in Africa: summary and recommendations. Sixth meeting of the international working group, Agricultural Research Council, Plant Protection Research Institute, Pretoria, South Africa. International Crops Research Institute for the Semi-Arid Tropics, Andhra Pradesh, India and Belgian Administration for Development Co-operation, Belgium, pp 20–21
- Coffelt TA, Hammons RO (1973) Influence of sizing peanut seed on two phenotypic ratios. *J Hered* 64:39–42
- de Berchoux C (1960) La rosette de l'arachide en Haute-Volta. *Comportement des lignées résistantes. Oléagineux* 15:229–233
- Ferguson ME, Burow MD, Schulze SR, Bramel PJ, Paterson AH, Kresovich S, Mitchell S (2004) Microsatellite identification and characterization in peanut (*A. hypogaea* L.) *Theor Appl Genet* 108:1064–1070
- Garcia GM, Stalker HT, Shroeder E, Kochert GA (1996) Identification of RAPD, SCAR and RFLP markers tightly linked to nematode resistance genes introgressed from *Arachis cardenassi* to *A. hypogaea*. *Genome* 39:836–845
- Grieshammer U, Wynne JC (1990) Isozyme variability in mature seeds of U.S. peanut cultivars and collections. *Peanut Sci* 18:72–75
- Haley SD, Afanador L, Kelly JD (1994) Selection for monogenic pest resistance traits with coupling- and repulsion-phase RAPD markers. *Crop Sci* 34:1061–1066
- Halward TM, Stalker HT, LaRue EA, Kochert G (1991) Genetic variation detectable with molecular markers among unadapted germplasm resources of cultivated peanut and related wild species. *Genome* 34:1013–1020
- Halward T, Stalker HT, Kochert G (1994) RFLP map of peanut. In: Phillips RL, Vasil IK (eds) DNA-based markers in plants. Kluwer, Dordrecht, pp 246–260
- Hayashi K, Hashimoto N, Diagen M, Ashikawa I (2004) Development of PCR-based SNP markers for rice blast resistance genes at the *Piz* locus. *Theor Appl Genet* 108:1212–1220

- He G, Prakash CS (1997) Identification of polymorphic DNA markers in cultivated peanut (*Arachis hypogaea* L.). *Euphytica* 97:143–149
- Herselman L (2003) Genetic variation among Southern African cultivated peanut (*Arachis hypogaea* L.) genotypes as revealed by AFLP analysis. *Euphytica* 133:319–327
- Hopkins MS, Casa AM, Wang T, Mitchell SE, Dean RE, Kochert GD, Kresovich S (1999) Discovery and characterization of polymorphic simple sequence repeats (SSRs) in peanut. *Crop Sci* 39:1243–1247
- Hull R, Adams AN (1968) Groundnut disease and its assistor virus. *Ann Appl Biol* 62:139–145
- ICRISAT (1982) Annual report. Patancheru, India
- Isleib TG, Wynne JC (1992) Use of plant introductions in peanut improvement. In: Shands HL, Weisner LE (eds) Use of plant introductions in cultivar development, part 2. Crop Science Society of America, Madison, pp 75–116
- Kochert G, Halward T, Branch WD, Simpson CE (1991) RFLP variability in peanut (*Arachis hypogaea* L.) cultivars and wild species. *Theor Appl Genet* 81:565–570
- Lacks GD, Stalker HT (1993) Isozyme analysis of *Arachis* species and interspecific hybrids. *Peanut Sci* 20:76–81
- Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* 1:174–181
- Lincoln S, Daly MJ, Lander E (1992) Constructing genetic linkage maps with MAPMAKER/EXP 3.0. Whitehead Institute technical report, 3rd edn. Cambridge, Mass.
- Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulk segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. *Proc Natl Acad Sci USA* 88:745–749
- Minja EM, van der Merwe PJA, Kimmins FM, Subrahmanyam P (1999) Screening groundnut breeding lines for resistance to aphids, *Aphis craccivora* Koch. *Int Arachis Newsl* 19:21–23
- Murant AF, Kumar IK (1990) Different variants of the satellite RNA of groundnut rosette virus are responsible for the chlorotic and green forms of groundnut rosette disease. *Ann Appl Biol* 117:85–92
- Murant AF, Rajeshwari R, Robinson DJ, Raschke JH (1988) A satellite RNA of groundnut rosette virus that is largely responsible for symptoms of groundnut rosette disease. *J Gen Virol* 69:1479–1486
- Murthy TGK, Tiwari SP, Reddy PS (1988) A linkage group for genes governing pod characters in peanut. *Euphytica* 39:43–46
- Naidu RA, Kimmins FM, Deom CM, Subrahmanyam P, Chiyembekeza AJ, van der Merwe PJA (1999) Groundnut rosette. A virus disease affecting groundnut production in sub-Saharan Africa. *Plant Dis* 83:700–709
- Padgham DE, Kimmins FM, Ranga Rao GV (1990) Resistance in groundnut (*Arachis hypogaea* L.) to *Aphis craccivora* (Koch). *Ann Appl Biol* 117:285–294
- Patel JS, John CM, Seshadri CR (1936) The inheritance of characters in the groundnut. *Proc Indian Acad Sci* 3:214–233
- Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. *Nature* 335:721–726
- Reddy DVR, Murant AF, Duncan GH, Ansa OA, Demski JW, Kuhn CW (1985) Viruses associated with chlorotic rosette and green rosette disease in groundnut in Nigeria. *Ann Appl Biol* 107:57–64
- Saghai Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphism in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proc Natl Acad Sci USA* 81:8014–8018
- Sauger L, Catherinet M (1954a) La rosette chlorotique de l'arachide et les lignées sélectionnées. *Agron Trop* 9:28–36
- Sauger L, Catherinet M (1954b) Nouvelles observations sur la rosette chlorotique de l'arachide et les lignées sélectionnées. *Bull Agron Ministère Fr Outremer* 11:204–216
- Savage GP, Keenan JI (1994) The composition and nutritive value of groundnut kernels. In: Smartt J (ed) The groundnut crop: a scientific basis for improvement. Chapman and Hall, London, pp 173–213
- Smartt J (1994) The groundnut farming systems and the rural economy—a global view. In: Smartt J (ed) The groundnut crop: a scientific basis for improvement. Chapman and Hall, London, pp 664–699
- Stalker HT (1991) A morphological appraisal of wild species in section *Arachis* of peanuts. *Peanut Sci* 17:117–122
- Stalker HT, Mzingo LG (2001) Molecular markers of *Arachis* and marker-assisted selection. *Peanut Sci* 28:117–123
- Storey HH, Ryland AK (1955) Transmission of groundnut rosette virus. *Ann Appl Biol* 43:423–432
- Subramanian V, Gurtu S, Nageswara Rae RC, Nigam SN (2000) Identification of DNA polymorphism in cultivated groundnut using random amplified polymorphic DNA (RAPD) assay. *Genome* 43:656–660
- Van der Merwe PJA (2001) Project groundnut rosette disease management. Progress report: July 2000 to June 2001. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in partnership with Natural Resources Institute (NRI) and Serere Agricultural and Animal Research Institute (SAARI) funded by Department For International Development (DFID)
- van der Merwe PJA, Subrahmanyam P, Kimmins FM, Willekens J (2001) Mechanisms of resistance to groundnut rosette. *Int Arachis Newsl* 21:43–46
- Vos P, Hogers R, Bleeker M, Reijans M, Van De Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res* 21:4407–4414
- Vuyksteke M, Mank R, Antonise R, Bastiaans E, Senior ML, Stuber CW, Melchinger AE, Lübberstedt T, Xia XC, Stam P, Zabeau M, Kuiper M (1999) Two high-density AFLP linkage maps of *Zea mays* L.: analysis of distribution of AFLP markers. *Theor Appl Genet* 99:921–935
- Yayock JY, Rossel HW, Harkness C (1976) A review of the 1975 groundnut rosette epidemic in Nigeria. In: Institute of Agricultural Research (ed) Samaru Conf Paper No.9. Institute of Agricultural Research, Samaru
- Young WP, Schupp JM, Keim P (1999) DNA methylation and AFLP marker distribution in the soybean genome. *Theor Appl Genet* 99:785–792