Theor Appl Genet (2001) 103:592–600

C. Djian-Caporalino · L. Pijarowski · A. Fazari
M. Samson · L. Gaveau · C. O'Byrne · V. Lefebvre
C. Caranta · A. Palloix · P. Abad

High-resolution genetic mapping of the pepper (*Capsicum annuum* L.) resistance loci *Me*$_3$ and *Me*$_4$ conferring heat-stable resistance to root-knot nematodes (*Meloidogyne* spp.)

Received: 23 March 2000 / Accepted: 2 January 2001

Abstract The PM687 line of *Capsicum annuum* L. has a single dominant gene, *Me*$_3$, that confers heat-stable resistance to root-knot nematodes (RKN). *Me*$_3$ was mapped using doubled-haploid (DH) lines and F_2 progeny from a cross between the susceptible cultivar 'Yolo Wonder' ('YW') and the highly resistant line 'PM687'. Bulked-segregant analysis with DNA pools, from susceptible or resistant DH lines, was performed to identify RAPD and AFLP markers linked to *Me*$_3$. There was no polymorphism between bulks of ten DH lines using over 800 RADP primers (4,000 amplified fragments analysed). Using 512 AFLP primers (74,000 amplified fragments analysed), and bulked DNA templates from 20 resistant and 20 susceptible plants, we identified eight repulsion-phase and four coupling-phase markers linked to *Me*$_3$. Analysed in 103 DH progeny, they defined a 56.1-cM interval containing the target gene. The nearest were located 0.5, 1.0, 1.5 and 3.0 centimorgans (cM) on both sides of the gene. Analysis of the F_2 progeny (162 plants) with the nearest coupling-phase marker confirmed its close position. Another resistance gene to RKN, present in 'PM687' (*Me*$_4$), was shown to be linked to *Me*$_3$, 10 cM from it. In order to localize *Me*$_3$ and *Me*$_4$ on our reference intraspecific pepper linkage map, two AFLP markers were mapped. The *Me*$_3$ nearest marker was 10.1 cM from a RAPD marker named Q04_0.3 and 2.7 cM from a RFLP marker named CT135. We investigated map-position orthologies between *Me*$_3$ and two other nematode resistance genes, the tomato *Mi-3* and the potato *Gpa*$_2$ genes, which mapped in the telomeric region of the short arm of the tomato and potato chromosome 12 (or XII for potato).

Keywords *Capsicum annuum* L. · *Meloidogyne* · *Me*$_3$ resistance gene · AFLP · Bulked segregant analysis

Communicated by H.C. Becker

C. Djian-Caporalino (✉) · L. Pijarowski · A. Fazari · M. Samson
L. Gaveau · C. O'Byrne · P. Abad
Unité Santé Végétale et Environnement,
Institut National de la Recherche Agronomique, B.P. 2078,
06600 Antibes Cedex, France
e-mail: caporali@antibes.inra.fr
Fax:+33-4-93-67-89-55

V. Lefebvre · C. Caranta · A. Palloix
Unité de Génétique et Amélioration des Fruits de Légumes,
Institut National de la Recherche Agronomique,
Domaine Saint-Maurice, B.P. 94, 84143 Montfavet Cedex, France

Introduction

Solanaceae, especially peppers (*Capsicum* spp.) and tomatoes which are the most-widely cultivated vegetables in the world (FAO 1996), are susceptible to root-knot nematodes (RKN). Resistance to these widespread obligate plant endoparasites is of great economic importance because they severely decrease the yields of susceptible cultivars (Lindsey and Clayshutte 1982; Fery and Dukes 1984; Di Vito et al. 1985; Thies et al. 1997).

Two RKN-resistant *Capsicum annuum* accessions, 'PM687' derived from the Indian local population PI 322719 and 'PM217' derived from the PI 201234 accession from Central America, were identified in 1985 (Hendy et al. 1985b). Both are resistant to a wide range of RKN populations (Djian-Caporalino et al. 1999). Major resistant genes were described, all dominant and acting individually in a gene-for-gene interaction (Hendy et al. 1985b). Among them, the *Me*$_3$ gene has a broad spectrum against the major *Meloidogyne* species, *Meloidogyne incognita*, *Meloidogyne arenaria*, *Meloidogyne javanica* and some *Meloidogyne hapla* populations, and is not heat sensitive (Djian-Caporalino et al. 1999).

C. annuum is diploid (2n=2*x*=24) (Pickersgill 1977) with a large genome [2,702–3,420 megabases (Mb)/1 C], about 3–4 times the size of the tomato genome (Arumuganathan and Earle 1991). Several linkage maps of the pepper genome have been published (Tanksley 1984; Prince et al. 1993; Lefebvre et al. 1995, 1997; Livingstone et al. 1999). Two intraspecific maps were generated using doubled-haploid (DH) populations obtained from F_1 hybrids of *C. annuum*, and contained

mainly RFLP and RAPD markers (Lefebvre et al. 1997). They spanned a total length of 1,582 cM with 227 different markers distributed on a total of 18 linkage groups (LGs) and were estimated to cover at least 67% of the pepper genome. According to these mapping data, an interval of 1 cM should correspond to a DNA segment of 1.2 to 2.2 Mb. A high marker density is therefore required for positional-cloning of genes by 'chromosome landing' (Tanksley et al. 1995). Because the cloning capacity of bacterial and yeast artificial chromosomes (BACs, Shizuya et al. 1992, and YACs, Burke et al. 1987) is 200–400 kb (Eun-Young et al. 1999) and 270–1,200 kb (Tai and Staskawicz 1999) for pepper, respectively, a mapping precision of at least 0.1–0.3 cM or 0.1–1.0 cM is necessary to be able to land on one BAC or YAC clone, respectively.

We have used 'bulked segregant analysis' (BSA) (Giovannoni et al. 1991; Michelmore et al. 1991) to identify molecular markers tightly linked to the dominant gene Me_3. BSA has been already used to map several genes of agronomic interest in pepper (Caranta et al. 1999a; Moury et al. 2000; Pierre et al. 2000). Two PCR-based markers, RAPD (random amplified polymorphic DNA, Williams et al. 1990) and AFLP (amplified fragment length polymorphism, Zabeau and Vos 1993; Vos et al. 1995), are particularly efficient for BSA because several to a large number of loci can be quickly screened with one primer or one primer combination.

AFLP markers are generated using the polymerase chain reaction (PCR) on small genomic restriction fragments (80–400 bp). Similar to RAPDs, AFLPs can be produced with a generic set of primers and do not depend on sequence information or probe availability, but detect 10-times more loci (from 20 to 100 in pepper, Lefebvre et al. 2000) than RAPDs. The restriction site-specific adapters, the adapter-specific primers with a variable number of selective nucleotides, and the high-stringency PCR conditions used, allow good reproducibility of AFLP amplification products (Vos et al. 1995). AFLP analysis has already been used in the *Solanaceae*, for tomato (Thomas et al. 1995), potato (Van Eck et al. 1995) and pepper (Caranta et al. 1999a, Lefebvre et al. 2000). Several resistance genes to nematodes: the Gpa_2 gene and the QTL for resistance to *Globodera pallida* (Rouppe Van der Voort et al. 1997; Bradshaw et al. 1998), and the genes involved in resistance to RKN in peach rootstocks (Lu et al. 1998), for example, have been mapped using AFLPs.

We have therefore assessed the effectiveness of AFLP for mapping the Me_3 gene in pepper. One coupling-phase AFLP marker was located 0.5 cM from the Me_3 gene in the DH progeny. This gene could reside in a cluster with other genes conferring resistance to nematodes in pepper, tomato and potato.

Material and methods

Plant material

The genetically very distant *C. annuum* parents 'Yolo Wonder' ('YW') and 'PM687' (Lefebvre et al. 1997) were used in the crosses. Resistant tests to *Meloidogyne* were previously described by Djian-Caporalino et al. (1999). The resistant line 'PM687' originating from India was obtained from the PI 322719 population. Two RKN resistance genes have been identified in 'PM687': Me_3 is effective against the three main RKN species *(M. arenaria, M. incognita, and M. javanica)* and some populations of *M. hapla* (Djian-Caporalino et al. 1999). It is only susceptible to one *M. arenaria* population, *M. arenaria* Ain Taoujdate (Hendy et al. 1985b). The second gene, Me_4, controls only *M. arenaria* Ain Taoujdate (Table 1). Me_5 was identified in 'YW' used in the crosses as the susceptible parent. It confers partial resistance to *M. javanica* Abou Dhabi (Table 1).

A total of 103 DH lines from the F_1 hybrid 'PM687'×'YW', plus 163 plants from the F_2 progeny, were used to detect markers linked to the RKN resistance gene Me_3. DNA was also obtained from 'Perennial', another inbred line of *C. annuum* (see Table 1) used to generate the DH200 pepper map (Lefebvre et al. 1997), and from tomato plants (*Lycopersicon esculentum*) with *Mi-1* and *Mi-3* genes (cv 'Piersol' and PI 126443) that are resistant to RKN.

Table 1 List of *C. annuum* accessions and progenies used for mapping the Me_3 RKN resistance gene

Parental material	Origin	RKN resistance genes (spectrum of action)	Reference
'YW' ('Yolo Wonder')	Inbred line from University of California (USA)	Me_5 (*M. javanica* Abou Dhabi)	Hendy et al. 1985b
'PM687'	Inbred line derived from the local population PI 322719 (India)	Me_3 (Meloidogyne spp.)	Hendy et al. 1985b; Djian-Caporalino et al. 1999
		Me_4 (*M. arenaria* Ain Taoujdate)	Hendy et al. 1985b
'Per' ('Perennial')	Inbred line from University of Punjab (India)	Susceptible to all *Meloidogyne* populations tested	Unpublished data

Hybrid material	Name of the progeny	Type	Number of individuals	Reference
'PM687'×'YW'	DH_{100}	DH lines	103	Hendy et al. 1985b; Djian-Caporalino et al. 1999
	F_2	F_2 individuals	163	Unpublished data
'Per'×'YW'	DH_{200}	DH lines	94	Lefebvre et al. 1995, 1997

DNA extraction from plants and preparation of DNA pools

Total genomic DNA was extracted from fruits and purified by the method of Bernatzky and Tanksley (1986). DNA was precipitated with ethanol and suspended in 1×TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) at 500 ng/µl. Equal amounts of DNA from susceptible and resistant DH lines ('S' and 'R', respectively) were pooled to obtain the different bulks: A, B contained 7 'S'; C, D contained 7 'R'; E, F contained 10 'S'; G, H contained 10 'R'; I and J contained 20 'S' and 20 'R', respectively.

RAPD

RAPD amplifications were carried out basically as described by Williams et al. (1990) with a modified reaction mix [10 mM Tris, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 0.1 mM dNTPs (Appligene France), 0.1% Triton X-100 (v/v)], 0.2 µM of primer, 20 ng of template DNA and 2.0 U of *Taq* polymerase (Appligene France) in a PTC_{200} thermocycler (MJ research Inc.) (total reaction volume=25 µl). Decamer primers for RAPD markers (70% CG) were obtained from Bioprobe System (France). PCR conditions were: 92°C for 3 min followed by 45 cycles of 1 min at 92°C, 1 min at 36°C and 2 min at 72°C, followed by 10 min at 72°C. Amplification products were resolved by electrophoresis on 1.5% agarose gels stained with ethidium bromide. Generally, 4–8 amplicons were visualized and polymorphic loci were scored as either present (1) or absent (0).

A total of 800 RAPD primers, from which 144 gave markers located on our reference intraspecific pepper map 'Per'×'YW', were screened for polymorphism between 'YW', 'PM687' and DNA bulks. The primers revealing polymorphism between parents and between bulks were then analysed in the DH progeny to determine the recombination rates and hence the distance with the *Me*$_3$ gene.

AFLP

AFLP analysis (Vos et al. 1995) was carried out using the hexacutters *Hind*III or *Pst*I in combination with the tetracutter *Mse*I. In initial experiments, the restriction fragments were biotinylated, collected by binding to streptavidin-coated beads (Dynal), following the Zabeau and Vos method (1993), and the DNA was dissolved in 200 µl of $1\times TE_{0.1}$ (10 mM Tris-HCl plus 0.1 mM EDTA, pH 8.0). In later experiments, the bead purification step was omitted, since comparisons revealed that there was no benefit from this treatment. In this case, the primary template (resulting from double digestion and adapter ligation) was diluted 10-fold in $1\times TE_{0.1}$ buffer.

A two-step amplification procedure was then used (Vos et al. 1995) with minor modifications. Pre-amplification was carried out with 1-bp extension primers (+A or +C). Selective amplification was conducted with 1/10 of the first PCR-amplified products, using 3-bp extension-^{33}P-labelled hexacutter primers (*Hind*III+3, *Pst*I+3) and a non-labelled tetracutter primer (*Mse*I+3). The following cycle profile ensured optimal primer selectivity: one cycle of 1 min at 94°C, 1 min 20 s at 65°C, 1 min at 72°C, followed by 12 cycles with an annealing temperature decreasing by 0.7°C at each cycle and 24 cycles with an annealing temperature of 56°C.

The restriction fragments were electrophoresed in 6 or 8% polyacrylamide gels as described by Vos et al. (1995). Gels were transferred to Whatman 3 MM paper, dried and exposed to X-ray films. Every experiment was repeated at least once.

The polymorphic markers were analysed in the DH progeny to locate them in relation to the *Me*$_3$ gene. Genetic distances between the markers and the *Me*$_3$ gene based on pairwise comparisons were calculated using the Mapmaker software, version 3.0 (Lander et al. 1987). A minimum LOD score of 3 and a maximum recombination rate of 0.3 were chosen. The recombination values were converted into map units (cM) using Kosambi's mapping function (Kosambi 1944).

Mapping

One intraspecific map of the pepper genome has been developed using the DH population obtained from the F_1 hybrid of the cross 'Per'×'YW' (Lefebvre et al. 1995, 1997) (Table 1). Two AFLP primer combinations that were polymorphic between the bulks were mapped on this population.

Results

Screening RAPDs linked to *Me*$_3$

DNA pools from resistant and susceptible DH lines, and the parental lines 'YW' and 'PM687', were screened with RAPDs (presence vs absence). Each primer revealed an average of five scorable amplicons (4,000 amplicons scored ranging in size from 0.2 to 2.9 kb). About half of the 800 random decamer primers tested revealed polymorphism between the parental lines. But four primers (that were not located on our reference pepper map) gave four reproducible amplicons that were present in the resistant pools C and D (seven DH lines per pool), and absent from the susceptible pools A and B (seven DH lines per pool). When the four primers were

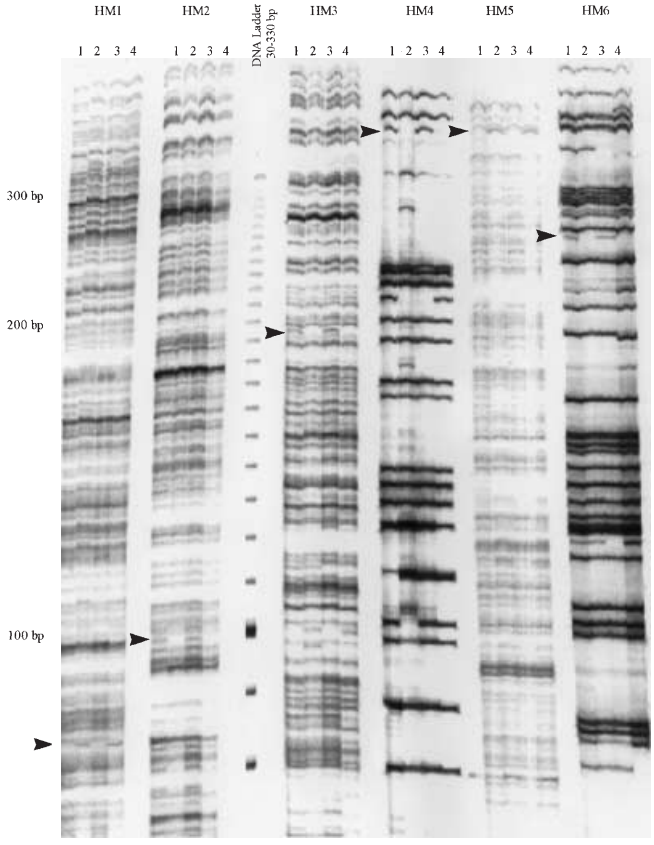

Fig. 1 Linked AFLP markers to the *Me*$_3$ locus. A representative example of six of the 400 *HIND*III+3/*Mse*I+3 primer combinations is shown (*Lane 1*, susceptible parent to RKN 'YW'; *lane 2*, resistant parent 'PM687'; *lane 3*, a pool of 20 susceptible DH lines; and *lane 4*, a pool of 20 resistant DH lines. A candidate AFLP marker is indicated by an *arrow*)

tested on the four pools of ten DH lines, no convincing polymorphism was retrieved. The tests carried out with these four primers on the whole DH progeny indicated that the markers were skewed. These four RAPDs perfectly co-segregated in the DH progeny (data not shown). The distance between Me_3 and these four markers was close to the detection limit (over 40% recombination). The chi-square test of independence showed that they were only slightly linked to the resistance gene.

Screening AFLPs linked to Me_3

The four DNA pools (E, F, G, H) from ten resistant or ten susceptible DH lines, together with the parental lines ('YW' and 'PM687'), were first screened with 400 AFLP *Hind*III/*Mse*I primer combinations. Approximately 60,000 amplicons ranging in size from 40 to 400 bp were scored (an average of 150 products per primer combination). Ninety products (0.15%) obtained with 65 primer combinations (16.3%) revealed a polymorphism between the resistant and susceptible pools and between the parental lines. The maximum number of polymorphic amplicons per primer combination was three. We analysed two additional pools composed each of 20 DH lines (I, J) to identify markers more closely linked to Me_3. Only half of the markers (42 products, i.e. 0.07%, obtained with 28 primer combinations, i.e. 7%) were confirmed as being polymorphic between these new bulks.

The presence or absence of the amplification products in 103 DH progenies was registered to evaluate genetic distances between the markers and the locus Me_3. Given the number of the progenies, we used a strict threshold of 30% of recombination as recommended by Lefebvre et al. (1995). Six polymorphic amplified fragments were scored as AFLP markers with the required accuracy; they were tightly linked on a single linkage group. The pattern of amplification products obtained with those six AFLP primer combinations is shown in figure 1. Four *Hind*III/*Mse*I AFLPs were linked in repulsion with the Me_3 resistance allele. Two markers [HM5 (R)-300 bp and HM1 (R)-58 bp] were linked in coupling with the Me_3 resistance allele. To increase the marker density in the vicinity of Me_3, 112 AFLP *Pst*I/*Mse*I primer combinations were used to detect polymorphism between the susceptible and resistant pools I, J (composed of 20 DH lines). Each primer combination generated on average 125 fragments (14,000 *Pst*I/*Mse*I amplification products were scored). Fifty eight products (0.43%) obtained with 35 primer combinations (31.2%) were polymorphic. Genetic distances between the markers and Me_3 were evaluated in the DH progeny. Six new markers were placed on the target linkage group. Four were linked in repulsion with the Me_3 resistance allele. Two [PM5 (R)-250 bp and PM6 (R)-255 bp] were linked in coupling with the Me_3 resistance allele.

The data were analysed with Mapmaker using the Kosambi function. The best log-likelihood corresponds to the order given in Fig. 2. The Me_3 gene resided in an approximately 56.1-cM interval covered by 12 markers. Among those 12 markers, seven mapped less than 10.4 cM from the target gene and 4 were located 0.5±0.7, 1.0±0.9, 1.5±1.2 and 3.0±1.7 centimorgans (cM) on both sides of the gene.

The AFLP analysis was also performed in the 163 F_2 individuals from the same cross with the nearest coupling-phase marker [HM1 (R)-58 bp, 0.5±0.7 cM from Me_3 in the DH progeny] to confirm linkage with Me_3 and to obtain an accurate estimate of the recombination fraction. The signal of the amplification product appeared often less intense in the F_2 individuals than in the DH lines. The marker was confirmed to be tightly linked to the Me_3 locus: we obtained five recombinant individuals, indicating that the marker was located 3.0±1.4 cM from Me_3 in the F_2 progeny (data not shown).

Location of Me_3 on the intraspecific DH_{200} pepper map

The repulsion-phase marker HM4 (S)-355 bp and the coupling-phase marker HM1 (R)-58 bp were polymorphic between 'YW' and 'Per'. So they could be mapped on our pepper intraspecific map (Lefebvre et al. 1995, 1997) generated from the cross 'Per' and 'YW'.

Both AFLP markers were assigned to a 24.2-cM linkage group of the DH_{200} intraspecific map, separated by a distance of 12.3 cM (Fig. 2). The coupling-phase marker HM1 (R)-58 bp was 2.7±1.7 cM from the RFLP marker CT135.

Co-localization of Me_3 and Me_4

Me_4 (from 'PM687'), controlling only *M. arenaria* Ain Taoujdate, seemed to be linked to Me_3 (Hendy 1984). We have considered the data of Hendy (1984) developed on 54 lines (included in the 103 DH lines we analysed), together with our AFLP mapping data. On the 54 DH lines, the AFLP markers were located on one side of the

Fig. 2 AFLP mapping of Me_3 on the 'PM687'×'YW' pepper map (103 DH lines) and on our intraspecific pepper map 'Per'×'YW' (94 DH lines). Positions of RFLP marker CT135 linked to the Me_3 locus on the tomato map (Tanksley et al. 1992), of the PCR-specific marker (PCR-NR14) linked to the *Mi-3/Mi-5* RKN resistance genes (Yaghoobi et al. 1995), and of the Gpa_2 resistance gene (Rouppe von der Voort et al. 1997) on the distal end of the short arm of tomato chromosome 12 or potato chromosome XII. (*R*) and (*S*) indicate the allelic origin of the mapped band (R for alleles coming from 'PM687' and *S* for alleles coming from 'YW'): H+ACA/M+AGT encoding HM1(R)-58 bp, H+ACT/M+ATG encoding HM2 (S)-70 bp, H+AGA/M+ACC encoding HM3 (S)-200 bp, H+AGC/M+ACG encoding HM4 (S)-355 bp, H+ATA/M+AAG encoding HM5(R)-300 bp, H+ATC/M+ACG encoding HM6 (S)-217 bp, P+ATA/M+CGG encoding PM1 (S)-120 bp, P+ATA/M+CGG encoding PM2 (S)-121 bp, P+ATA/M+CGG encoding PM3 (S)-122 bp, P+ATA/M+CGG encoding PM4 (S)-123 bp, P+ATT/M+AAC encoding PM5 (R)-250 bp, P+ATT/M+ACG encoding PM6 (R)-255 bp. * According to Yaghoobi et al. (1995). ** According to Rouppe von der Voort et al. (1997)

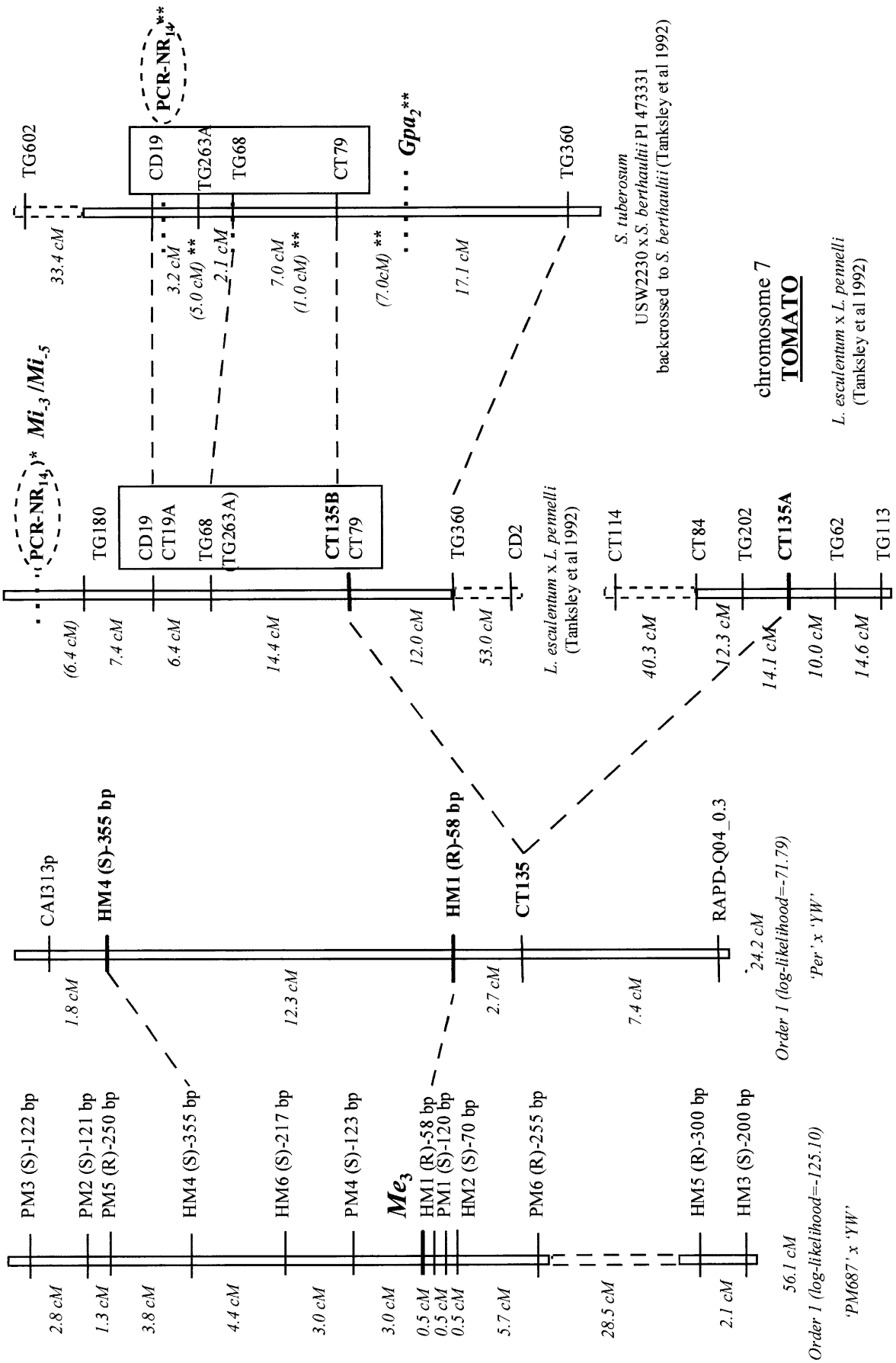

Fig. 2 Legend see page 595

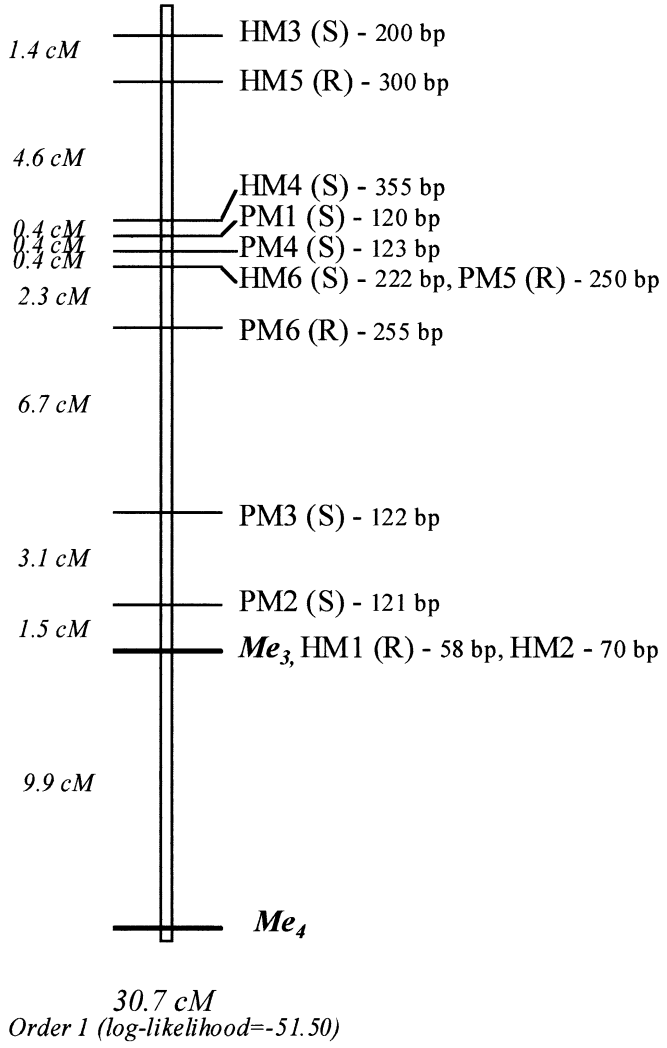

Fig. 3 AFLP mapping of *Me*$_4$ on the 'YW'×'PM687' pepper map (54 DH lines analysed)

Me$_3$ gene. *Me*$_4$ maps 10±4 cM from *Me*$_3$, HM1 (R)-58 bp and HM2 (S)-70 bp . These two AFLP cosegregate with *Me*$_3$ on the 54 DH lines (Fig. 3).

Discussion

We have shown that the combined strategy using BSA and AFLP markers is an efficient method for identifying markers tightly linked to the *Me*$_3$ resistance gene to RKN and for the mapping of this gene on the pepper genome.

In this study, two kinds of PCR-based markers, RAPD and AFLP, were compared. While Moury et al. (2000) succeeded to target the *Tsw* pepper gene with RAPDs, DNA fingerprinting by RAPD analysis did not allow detecting markers linked to the *Me*$_3$ gene. The percentage of polymorphic amplicons between the bulks was rather low for RAPDs, and the four RAPD markers found with bulks of seven lines were not confirmed. However, they co-segregated in the DH progeny. Clusters of RAPD markers have already been found during the construction of pepper maps (Lefebvre et al. 1997).

AFLP-marker saturation of the target interval was accomplished using the frequent-cutter *Mse*I in combination with two different rare-cutting restriction enzymes (*Hind*III and *Pst*I). Comparison of the both hexacutters revealed that *Pst*I (a GC-rich recognition sequence) detected a higher level of polymorphic sites (31.2%) than *Hind*III (an AT-rich recognition sequence) (7%). This confirms the results of Powell et al. (1997), Young (1998) and Haanstra et al. (1999) which indicated that GC methylation-sensitive primers are more efficient in detecting polymorphisms in barley, soybean and tomato, respectively, and are more evenly distributed than GC methylation non-sensitive primers. *Pst*I cleavage is inhibited by methylation of the recognition sequence (McClelland et al. 1994) and transcribed plant DNA is generally hypo-methylated (Antequera and Bird 1988). Therefore, *Pst*I probably cleaves predominantly in gene-rich chromosome segments. Young (1998) observed effectively that the use of a methylation-sensitive marker in the soybean genome increased the chance of placing markers in regions containing actively expressed genes. This would explain the higher rate of polymorphism linked to the *Me*$_3$ resistance gene observed with *Pst*I-based primer combinations (six markers for 400 *Hind*III/*Mse*I primer combinations and six markers for only 112 *Pst*I/*Mse*I primer combinations).Twelve dominant AFLP markers were found to be linked to *Me*$_3$ in a 56.1-cM interval. The nearest are 0.5, 1.0, 1.5 and 3.0 cM on both sides of the gene. Four markers were linked in coupling to the *Me*$_3$ resistance locus. *Me*$_4$ that has a very limited activity (only against *M. arenaria* Ain Taoujdate) was nevertheless linked to *Me*$_3$, active against the main species of *Meloidogyne*. Histological studies (Hendy et al. 1985a) indicated that DH lines containing only *Me*$_4$ develop a delayed resistance reaction (some juveniles penetrated roots and initiated a few imperfect giant cells) whereas the hypersensitive reaction due to *Me*$_3$ starts immediately, sometimes during the first contact with the root epidermis, as is observed with the *Mi-1* gene in resistant tomato plants. Future knowledge of the mode of action of these different genes in pepper might reveal a number of steps in the signal cascade leading to nematode resistance in plants.

The most tightly *Me*$_3$-linked coupling-phase marker was mapped on the intraspecific pepper map of Lefebvre et al. (1997) constructed from the DH lines 'Per'×'YW', 2.7 cM from the RFLP marker CT135.

Pepper, tomato and potato are related species of the *Solanaceae* family and nematodes are common parasites. Therefore, it is interesting to investigate the map-position orthologies between nematode resistance genes that have a similar phenotype. Colinearity between RFLP markers already indicated a high synteny between the 12 chromosomes of the *Solanaceae* species (Tanksley et al. 1988, 1992; Gebhardt et al. 1991). Moreover, without exception, homologues of cloned resistance genes

mapped to syntenic positions in other solanaceaous genomes, indicating that there is conservation of both the position and the sequence of these genes (Tanksley et al. 1992; Prince et al. 1993; Hammond-Kosack and Jones 1997; Grube 1999; Pflieger et al. 1999). Pepper homologues to the tobacco resistance gene *N* to mosaic virus (Whitham et al. 1994; Dinesh-Kumar et al. 1995), to the potato resistance gene *Ry* to potato virus Y (Brigneti et al. 1997) and to the tomato resistance genes *Sw-5* controlling tospovirus (Stevens et al. 1995; Brommonschenkel and Tanksley 1997), *Pto* and *Prf* controlling *Pseudomonas syringae* pv tomato (Martin et al. 1993; Salmeron et al. 1996), *I2c* controlling *Fusarium oxysporum* (Bournival et al. 1989), were mapped in the same pepper genomic regions as those observed in tobacco, potato and tomato (Caranta et al. 1999b; Grube 1999, Pflieger et al. 1999).

In tomato, the cDNA probe CT135 (linked to Me_3 in pepper) mapped to two distinct genomic regions: on the long arm of tomato chromosome 7 (CT135 A) and on the telomeric region of the short arm of the tomato chromosome 12 (CT135B) (Fig. 2). CT135B is tightly linked to the RFLP marker CT79 and 28.7 cM from the RFLP marker TG180 (Tanksley et al. 1992). Yaghoobi et al. (1995) mapped the PCR-specific marker (NR_{14}) of the nematode resistance genes *Mi-3* and *Mi-5* from *Lycopersicon peruvianum* var. *glandulosum* PI 126443–1MH in this same genomic region of chromosome 12, 6.4 cM from TG180 (Fig. 2). *Mi-3* confers resistance to some *Mi-1* naturally virulent *M. incognita* isolates (Roberts and Thomason 1986; Roberts et al. 1990; Veremis 1995; Veremis et al. 1999) as the pepper gene Me_3 does to artificially selected lines of *Mi-1* virulent *M. incognita* (Castagnone-Sereno et al. 1996). The *Mi-5* gene confers heat-stable resistance in *L. peruvianum* PI 126443–1MH (Veremis 1995; Veremis et al. 1999) when *Mi-1* is ineffective (Ammati et al. 1986), as does Me_3 (Djian-Caporalino et al. 1999). Thus tomato *Mi-3* and *Mi-5* genes have a similar phenotypic response to the pepper Me_3 gene. If we consider nematode resistance genes from potato, the major dominant gene Gpa_2, effective against the pathotype Pa_2 of the potato cyst nematode *Globodera pallida*, was mapped on the distal end of potato chromosome XII (Rouppe van der Voort et al. 1997) that is orthologous to the tomato chromosome 12 (Gebhardt et al. 1991). Gpa_2 was mapped 13 cM distal from the PCR-specific marker NR_{14} (linked to *Mi-3/Mi-5)* and 5 cM from the RFLP marker CT79 to RFLP-CT79 (Fig. 2).

To further examine the relationship between the dominant resistances to the different nematode genera in pepper (Me_3), in tomato (*Mi-3, Mi-5*) and in potato (Gpa_2), we attempted to map other markers located on the tomato chromosome 12 on pepper maps. NR_{14}–specific primers did not generate any fragments with pepper DNA (unpublished data). The RFLP tomato markers CT19, CT79, TG68, TG263 and TG360 were monomorphic between 'Per'- and 'YW'-DNA digested with five enzymes. The RFLP tomato probe CD19 was multicopy and the polymorphic marker was unlinked. The RFLP tomato marker TG180 mapped in a pepper group gathering other RFLP tomato markers located on the tomato chromosomes 5 (TG379), 11 (TG47, CT120) and 12 (CT120). Thus, TG180 is not linked to CT135 on the pepper map. Without two collinear markers, we cannot align the CT135 pepper region to any part of the tomato chromosomes. Several authors (Tanksley et al. 1988; Lefebvre et al. 1995, 1997; Livingstone et al. 1999) demonstrated that a lot of rearrangements differentiate the pepper and the tomato genome. Indeed, phenotypically similar resistance genes, like the pepper *L* gene, the tomato *Tm-2* and the tobacco *N* gene for resistance to tobacco mosaic virus, or the pepper *Tsw* gene and the tomato *Sw-5* gene for resistance to tomato spotted wilt virus, mapped in distinct genomic regions (Caranta et al. 1999b). A part of the tomato chromosome 12 could be duplicated in the pepper genome. Presently, our results did not allow affirming or disapproving that Me_3 is in a syntenic region of *Mi-3/Mi-5* or Gpa_2. Other RFLP markers of tomato chromosomes 12 and 7, and of potato chromosome XII (Fig. 2), will therefore be localised on our pepper intraspecific map to determine the real relationship between nematode resistance genes in the *Solanaceae*.

The most-tightly linked AFLP markers we have identified so far are separated from Me_3 by 0.5 cM on one side and 3 cM on the other side, which corresponds, on average, to a physical distance of 0.6–1.1 Mb and 3.6–6.6 cM, respectively. These calculations suggest that the physical linkage of at least one of both identified markers is not yet sufficient to identify a single BAC or YAC clone covering the target locus. In any case, the seven AFLP markers linked to Me_3, and located less than 10 cM from the resistance gene, will be converted to PCR-specific markers and will be used for marker-assisted selection to introgress the Me_3 gene into a desired genetic background through backcross breeding programmes.

Acknowledgements This work was supported by an 'Action Incitative Programmée' grant from INRA as part of the 'Disease Resistance Genes' research programme. The DH progenies were obtained from Daniel Chambonnet and Thérèse Phaly, Unité de Génétique et Amélioration des Fruits et Légumes of Institut National de la Recherche Agronomique at Montfavet (France), and from Guiseppe Nervo, Istituto Sperimentale per l'Orticultura Sezione Operativa, Monatanaso-Lombardo (Italy). The corresponding authors thanks Michèle Pierre, CNRS Gif/Yvette (France) for introducing us to the AFLP technique and providing valuable information during the start of this project.

References

Ammati M, Thomason IJ, McKinney HE (1986) Retention of resistance to *Meloidogyne incognita* in *Lycopersicon* genotypes at high soil temperature. J Nematol 18:491–495

Antequera F, Bird AP (1988) Unmethylated CpG islands associated with genes in higher plant DNA. EMBO J 7:2295–2299

Arumuganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9:208–218

Bernatzky R, Tanksley SD (1986) Genetics of actin-related sequences in tomato. Theor Appl Genet 72:314–321

Bournival BL, Vallejos CE, Scott JW (1989) An isozyme marker for resistance to race 3 of *Fusarium oxysporum* f.sp. *lycopersici* in tomato. Theor Appl Genet 78:489–494

Bradshaw JE, Hackett CA, Meyer RC, Milboune D, McNicol JW, Philips MS, Waugh R (1998) Identification of AFLP and SSR markers associated with quantitative resistance to *Globodera pallida* (Stone) in tetraploid potato (*Solanum tuberosum* subsp. *tuberosum*) with a view to marker-assisted selection. Theor Appl Genet 97:202–210

Brigneti G, GarciaMas J, Baulcombe DC (1997) Molecular mapping of the potato virus resistance gene *Ry* (sto) in potato. Theor Appl Genet 94:198–203

Brommonschenkel SH, Tanksley SD (1997) Map-based cloning of the tomato genomic region that spans the *Sw-5* tospovirus resistance gene in tomato. Mol Gen Genet 256:121–126

Burke D, Carle G, Olson G (1987) Cloning of large segments of exogenous DNA into yeast by means of artificial chromosome vectors. Science 236:806–812

Caranta C, Thabuis A, Palloix A (1999a) Development of a CAPS marker for the *Pvr4* locus: a tool for pyramiding potyvirus resistance genes in pepper. Genome 42:1111–1116

Caranta C, Pflieger S, Lefebvre V, Palloix A, Blattes A (1999b) Comparative mapping of virus resistance genes in *Solanaceous* species. In: VIIth Int Conf Status of Plant and Animal Genome Research, January 17–21, 1999, San Diego, USA, P349 (Poster)

Castagnone-Sereno P, Bongiovanni M, Palloix A, Dalmasso A (1996) Selection for *Meloidogyne incognita* virulence against resistance genes from tomato and pepper and specificity of the virulence/resistance determinants. Eur J Plant Pathol 102:585–590

Di Vito MF, Saccardo F, La Gioia N, Carella A, Cecchini G, Papacchioli V (1985) Lines of *Capsicum annuum* resistant to root-knot nematodes obtained by interspecific hybridization *(C. annuum×C. frutescens*) (abstract). Genet Agraria 39:322–324

Dinesh-Kumar SP, Whitham S, Choi D, Hehl R, Corr C, Baker B (1995) Transposon tagging of tobacco mosaic virus resistance gene *N*: its possible role in the TMV-*N*-mediated signal transduction pathway. Proc Natl Acad Sci USA, 92:4175–4180

Djian-Caporalino C., Pijarowski L, Januel A, Lefebvre V, Phally T, Palloix A, Dalmasso A, Abad P (1999) A spectrum of resistance to root knot nematodes (*Meloidogyne* spp.) in sweet pepper (*Capsicum annuum* L.) and inheritance of heat-stable resistance in the PM687 line derived from PI 322719. Theor Appl Genet 99:496–502

Eun-Young Y, JinKyung K, Byung-Dong K (1999) Hot pepper bacterial artificial chromosome (BAC) library. In: VIIth Int Conf Status of Plant and Animal Genome Research, January 17–21, 1999, San Diego, USA, P104 (Poster)

FAO (1996) 1995 FAO production yearbook. Food and Agriculture Organisation of the United Nations, Rome 49:129–138

Fery RL, Dukes PD (1984) Southern root-knot nematode of pepper: studies on the value of resistance (abstract). Hort Science19:211

Gebhardt C, Ritter E, Barone A, Debener T, Walkemeier B, Schatschabel U, Kaufmann H, Thompson RD, Bonierbale MW, Ganal MW, Tanksley SD, Salamini F (1991) RFLP maps of potato and their alignment with the homologous tomato genome. Theor Appl Genet 83:49–57

Giovannoni JJ, Wing RA, Ganal MW, Tanksley SD (1991) Isolation of molecular markers from specific chromosomal intervals using DNA pools from existing mapping populations. Nucleic Acids Res 19:6553–6558

Grube RC (1999) Genetics of virus resistance in *Capsicum* and comparative analysis of disease resistance in the *Solanaceae*. PhD thesis, Cornell University, USA

Haanstra JPW, Wye C, Verbakel H, Meijer-Dekens F, van den Berg P, Odinot P, van Heusden AW, Tanksley S, Lindout P, Peleman J (1999) An integrated high-density RFLP-AFLP map of tomato based on two *Lycopersicon esculentum×L. pennellii* F_2 populations. Theor Appl Genet 99:254–271

Hammond-Kosack KE, Jones JDG (1997) Plant disease resistance genes. Annu Rev Plant Physiol Plant Mol Biol 48:575–607

Hendy H (1984) Contribution à l'étude des relations hôte-parasite chez les nématodes phytophages du genre *Meloidogyne*. Génétique et mécanismes de la résistance chez *Capsicum* spp. Thèse de Doctorat, USTL Montpellier, France

Hendy H, Dalmasso A, Cardin C (1985a) Differences in resistant *Capsicum annuum* attacked by different *Meloidogyne* species. Nematologica 31:72–78

Hendy H, Pochard E, Dalmasso A (1985b) Transmission héréditaire de la résistance aux *Meloidogyne* portée par deux lignées de *Capsicum annuum*: études de descendances d'homozygotes issues d'androgénèse. Agronomie 5:93–100

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

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

Lefebvre V, Palloix A, Caranta C, Pochard E (1995) Construction of an intraspecific integrated linkage map of pepper using molecular markers and doubled-haploid progenies. Genome 38, 1:112–121

Lefebvre V, Caranta C, Plieger S, Moury B, Daubèze AM, Blattes A, Ferriere C, Phaly T, Nemouchi G, Ruffinatto A, Palloix A (1997) Updated intraspecific maps of pepper. *Capsicum* and Eggplant Newslett 16:35–41

Lefebvre V, Goffinet B, Chauvet JC, Caromel B, Signoret,P, Brand R, Palloix A (2001) Evaluation of genetic distances between pepper inbred lines for cultivar protection purposes: comparison of AFLP, RAPD and phenotypic data. Theor Appl Genet (in press)

Lindsey DL, Clayshulte MS (1982) Influence of initial population densities of *Meloidogyne incognita* on three chili cultivars. J Nematol 14:353–358

Livingstone KD, Lackney VK, Blauth JR, van Wijk R, Jahn MK (1999) Genome mapping in *Capsicum* and the evolution of genome structure in the Solanaceae. Genetics 152:1183–202

Lu ZX, Sosinski B, Reighard GL, Baird WV, Abbott AG (1998) Construction of a genetic linkage map and identification of AFLP markers for resistance to root-knot nematodes in peach rootstocks. Genome 41:199–207

Martin GB, Brommonschenkel SH, Chunwongse J, Frary A, Ganal MW, Spivey R, Wu T, Earle ED, Tanksley SD (1993) Map-based cloning of a protein kinase gene conferring disease resistance in tomato. Science 262:1432–1436

McClelland M, Nelson M, Raschke E (1994) Effect of site-specific modification on restriction endonucleases and DNA modification methyltransferases. Nucleic Acids Res 22:3640–3659

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease- resistance genes by bulked segregant analysis: a rapid method to detect markers in specific regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Moury B, Pflieger S, Blattes A, Lefebvre V, Palloix A (2000) A CAPS marker to assist selection of tomato spotted wilt virus (TSWV) resistance in pepper. Genome 43:1–6

Pflieger S, Lefebvre V, Caranta C, Blattes A, Goffinet B, Palloix A (1999) Disease resistance gene analogs as candidates for QTLs involved in pepper-pathogen interactions. Genome 42:1100–
1110

Pickersgill B (1977) Chromosomes and evolution in Capsicum. In: "*Capsicum* 77", CR 3ème congrès Eucarpia sur la génétique et la sélection du piment, 5–8 Juillet 1977, Montfavet Avignon, France, Pochard E (eds), pp 27–38

Pierre M, Noel L, Lahaye T, Ballvora A, Veuskens J, Ganal M, Bonas U (2000) High-resolution genetic mapping of the pepper resistance locus *Bs3* governing recognition of the *Xanthomonas campestris* pv *vesicatoria* AvrBs3 protein. Theor Appl Genet 101:255–263

Powell W, Thomas WTB, Baird E, Lawrence P, Booth A, Harrower B, McNicol JW, Waugh R (1997) Analysis of quantitative traits in barley by the use of amplified fragment length polymorphisms. Heredity 79:48–59

Prince JP, Pochard E, Tanksley SD (1993) Construction of a molecular linkage map of pepper and a comparison of synteny with tomato. Genome 36:404–417

Roberts PA, Thomason IJ (1986) Variability in reproduction of isolates of *M. incognita* and *M. javanica* on resistant tomato genotypes. Plant Dis 70:547–551

Roberts PA, Dalmasso A, Cap GB, Castagnone-Sereno P (1990) Resistance in *Lycopersicum peruvianum* to isolates of *Mi* gene-compatible *Meloidogyne* populations. J Nematol 22:585–589

Rouppe van der Voort J, Wolters P, Folkertsma R, Hutten R, van Zanvoort P, Vinke H, Kanyuka K, Bendahmane A, Jacobsen E, Janssen R, Bakker J (1997) Mapping of the cyst nematode resistance locus *Gpa2* in potato using a strategy based on comigrating AFLP markers. Theor Appl Genet 95:874–880

Salmeron JM, Oldroyd GE, Rommens CM, Scofield SR, Kim HS, Lavelle DT, Dahlbeck D, Staskawicz BJ (1996) Tomato *Prf* is a member of the leucine-rich repeat class of plant disease resistance genes and lies embedded within the *Pto* kinase gene cluster. Cell 86:123–33

Shizuya h, Birren B, Kim UJ, Mancino V, Slepak T, Tachiiri Y, Simon M (1992) Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in *Escherichia coli* using an F-factor-based vector. Proc Natl Acad Sci USA 89:8794–8797

Stevens MR, Lamb EM, Rhoads DD (1995) Mapping the Sw-5 locus for tomato spotted wilt virus resistance in tomatoes using RAPD and RFLP analyses. Theor Appl Genet 90:451–456

Tai T, Staskawicz BJ (1999) Construction of a yeast artificial chromosome library of pepper (*Capsicum annuum* L.) and identification of clones from the *Bs2* resistance locus. Theor Appl Genet 100:112–117

Tanksley SD (1984) Linkage relationships and chromosomal locations of enzyme-coding genes in pepper, *Capsicum annuum*. Chromosoma 89:352–360

Tanksley SD, Bernatzky R, Lapitan NL, Prince JP (1988) Conservation of gene repertoire but not gene order in pepper and tomato. Proc Natl Acad Sci USA 85:6419–6423

Tanksley SD, Ganal MW, Prince JP, de Vicente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Paterson AH, Pineda O, Röder MS, Wing RA, Wu W, Young ND (1992) High-density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141–1160

Tanksley SD, Ganal MW, Martin GB (1995) Chromosome landing: a paradigm for map-based gene cloning in plants with large genomes. Trends Genet 11:63–68

Thies JA, Mueller JD, Fery RL (1997) Effectiveness of resistance to southern root-knot nematode in 'Carolina Cayenne' pepper (*Capsicum annuum* L.) in greenhouse, microplot, and field tests. J Am Soc Hort Sci 122:200–204

Thomas CM, Vos P, Zabeau M, Jones DA, Norcott KA, Chadwick BP, Jones JDG (1995) Identification of amplified restriction fragment polymorphism (AFLP) markers tightly linked to the tomato *Cf-9* gene for resistance to *Cladosporium fulvum*. Plant J 8:785–794

Van Eck HJ, Rouppe van der Voort Draaistra J, van Zandvoort P, van Enckevort E, Segers B, Peleman J, Jacobsen E, Helder J, Bakker J (1995) The inheritance and chromosomal localization of AFLP markers in a non-inbred potato offspring. Mol Breed 1:397–410

Veremis JC (1995) Genetic characterization of novel resistance to root-knot nematode (*Meloidogyne* spp.) in wild tomato (*Lycopersicum peruvianum*). PhD thesis, University of California, Riverside, California

Veremis JC, van Heusden AW, Roberts PA (1999) Mapping a novel heat-stable resistance to *Meloidogyne* in *Lycopersicon esculentum*. Theor Appl Genet 98:274–280

Vos P, Hogers R, Bleeker M, Reijans M, VanDelee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new concept for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Whitham S, Dinesh-Kumar SP, Choi D, Hehl R, Corr C, Baker B (1994) The product of the tobacco mosaic virus resistance gene *N*: similarity to toll and the interleukin-1 receptor. Cell 78:1101–1115

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535

Yaghoobi J, Kaloshian I, Wen Y, Williamson VM (1995) Mapping a new nematode resistance locus in *Lycopersicon peruvianum*. Theor Appl Genet 91:457–464

Young WP (1998) DNA methylation and AFLP marker distribution in the soybean genome. VIth Int Conf Status of Plant and Animal Genome Research, January 18–22, 1998, San Diego, USA, P227 (Poster)

Zabeau M, Vos P (1993) Selective restriction fragment amplification: a general method for DNA fingerprinting. European Patent Application, 92402629.7 (Publication number: 0 534 858 A1