The tomato *Mi-1* gene confers resistance to both root-knot nematodes and potato aphids

Pieter Vos*†, Guus Simons†, Taco Jesse, Jelle Wijbrandi, Leo Heinen, René Hogers, Adri Frijters, John Groenendijk, Paul Diergaarde, Martin Reijans, Joke Fierens-Onstenk, Michiel de Both, Johan Peleman, Tsvetana Liharska[1‡], Jan Hontelez[1], and Marc Zabeau[§]

*Keygene N.V., Agro Business Park, P.O. Box 216, 6700 AE Wageningen, The Netherlands. [1]Department of Molecular Biology, Wageningen Agricultural University, Dreyenlaan 3, 6709 DB Wageningen, The Netherlands. †These authors contributed equally to this work. ‡Present address: Department of Genetics, Free University of Amsterdam, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands. §Present address: Helix, Onafhankelijkheidslaan 38, 9000 Gent, Belgium. *Corresponding author (e-mail: keygene@euronet.nl).*

Received 23 June 1998; accepted 12 October 1998

***Mi-1*, a *Lycopersicon peruvianum* gene conferring resistance to the agricultural pests, root-knot nematodes, and introgressed into tomato, has been cloned using a selective restriction fragment amplification based strategy. Complementation analysis of a susceptible tomato line with a 100 kb cosmid array yielded a single cosmid clone capable of conferring resistance both to the root-knot nematode *Meloidogyne incognita* and to an unrelated pathogen, the potato aphid *Macrosiphum euphorbiae*. This resistance was stable. The *Mi-1* gene encodes a protein sharing structural features with the nucleotide-binding site leucine-rich repeat–containing type of plant resistance genes.**

Keywords: agricultural engineering, plant biology

Plants are hosts to a great variety of phytopathogenic viruses, bacteria, fungi, and nematodes. The plant parasitic nematodes of the family *Heteroderidae* cause severe economic damage to crops and can be divided into two groups: The cyst nematodes that include the genera *Heterodera* and *Globodera*, and the root-knot nematodes of the genus *Meloidogyne*. The latter genus has a worldwide distribution and is capable of attacking more than 2000 plant species[1]. Of 70 known *Meloidogyne*, only *M. incognita*, *M. javanica*, *M. arenaria*, and *M. hapla* are of major economic importance. Root-knot nematode larvae invade the roots of host plants, and once established in contact with the vascular system, induce the formation of giant cells from which they feed. The most characteristic symptom of infected plants is the appearance of many dozens of root galls, in which the nematodes are embedded. The root systems of infected host plants are thus severely affected, and the plants become susceptible to wilting, growth reduction, and infection by other pathogens. Naturally occurring host resistance against *Meloidogyne* spp. has been found in several wild plant species, including *Lycopersicon* spp.[2]. Older varieties of tomato (*L. esculentum*) are fully susceptible, but certain accessions of the wild species *L. peruvianum* show a high level of resistance[3]. This resistance is effective against *M. incognita*, *M. arenaria,* and *M. javanica* and the gene encoding it was designated *Mi-1*. All modern cultivars of tomato contain the *Mi-1* gene introgressed from *L. peruvianum*. Moreover, these nematode-resistant tomato lines display resistance to infestations of the potato aphid *Macrosiphum euphorbiae* (a gene designated *Meu-1* [ref. 4]). This potato aphid, although primarily a parasite of *Rosa* sp., is highly polyphagous on *Solanaceae*, especially potato (*Solanum tuberosum*)[5]. A heavy infestation of aphids leads to growth reduction, leaf malformation, and premature death of the host plant through depletion of phloem exudate. Moreover, *M. euphorbiae* is known to transmit over 40 different viruses, some of which, such as potato leaf roll virus, are carried in a persistent manner[5].

A number of natural plant resistance genes (*R* genes) have been cloned and divided into five classes based on their structural features[6–8]. One class represented by the tomato *Pto* gene, which confers resistance to *Pseudomonas syringae*, encodes a serine/threonine protein kinase capable of autophosphorylation. A second class consisting of the *Arabidopsis RPS2* and *RPM1* genes (encoding resistance to *P. syringae*) and the tomato *I2* gene (encoding resistance to *Fusarium oxysporum* f.sp. *lycopersici*, race 2) encode proteins with a possible leucine zipper region and a potential nucleotide-binding site domain (NBS). The C-terminal end of these proteins comprises a leucine-rich repeat motif (LRR). The *Prf* gene of tomato, which is required for *Pto* activity, also belongs to this class. Another class of *R* genes encodes proteins with NBS and LRR domains, and N-terminal regions with homology to the cytoplasmic domains of *Drosophila* Toll protein and the mammalian interleukin-1 receptor protein (TIR domain). Members of this group are the tobacco *N* gene (resistance against tobacco mosaic virus), the flax *L6* gene (resistance against the rust fungus *Melamspora lini*) and the *Arabidopsis RPP5* gene (active against the downy mildew *Peronospora parasitica*). A fourth group of *R* genes encodes extracellular proteins without an apparent NBS, but with an LRR domain. The C-termini of these proteins consist of a transmembrane region and a small cytoplasmic tail. This group includes the tomato *Cf-2, Cf-4, Cf-5*, and *Cf-9* genes conferring resistance to different races of the leaf mold *Cladosporium fulvum* and the sugar beet gene *HS1*$^{pro-1}$ for resistance to the beet cyst nematode *Heterodera schachttii*. The fifth group is formed by the rice late blight resistance gene *Xa21* encoding a protein with an extracellular LRR domain as well as an intracellular serine/threonine kinase domain.

We describe the positional cloning of the *Mi-1* gene of tomato and show that this gene has a dual specificity: Resistance to the root-knot nematode *M. incognita* and to the potato aphid *M. euphorbiae.* Based on its structural features, *Mi-1* belongs to the NBS-LRR class of *R* genes.

Results

A single cosmid clone confers resistance to *Meloidogyne incognita* and *Macrosiphum euphorbiae*. The *Mi-1* locus was mapped by selective restriction fragment amplification (AFLP) analysis[9,10] using the enzyme combinations PstI/MseI (PM) and EcoRI/MseI (EM). Five codominant AFLP markers (PM110, PM21, PM19, EM47, and PM22) (Fig. 1A) were used in further analysis of recombinants[11], thereby narrowing down the *Mi-1* region to a section flanked by EM47 and PM22. AFLP markers PM19, PM21, and PM22 were used to screen a 2.2 genome equivalent tomato yeast artificial chromosome (YAC) library. A YAC contig of approximately 1.7 Mb covering the complete Motelle region was assembled (Fig. 1B). Subsequently, part of YAC 1/1172 and YAC 2/1256 were subcloned in a T-DNA cosmid vector and organized into a single array spanning the Motelle region with two small gaps (Fig. 1C). Following genetic fine mapping, cosmids covering the region between markers EM47 and PM22 (approximately 100 kb, Fig. 1C) were selected from YAC 2/1256 and used to transform the susceptible tomato line 52201.

For each cosmid, 10 to 15 transformants were generated, of which 54 selfed progenies were tested for nematode resistance. Seedlings of the resistant control genotype Motelle developed either no galls or occasionally a single gall on their root systems, whereas plants of the susceptible control genotype 52201 developed at least 20 galls and usually more than 50. Only the roots of plants transformed with cosmid 11, with an insert size of 19 kb, showed a total absence of galls (Table 1; Fig. 2). The roots of these resistant plants had an otherwise normal morphology. All nine R_1 populations of cosmid 11 segregated for resistance. In most cases, the ratio of resistance to susceptibility best fitted 3:1, as expected when only one copy of the gene had been incorporated.

The transgenic R_1 lines were also tested for resistance to the potato aphid *M. euphorbiae* (Table 1). Resistant control plants of Motelle harbored no aphids, or occasionally a single female but no juveniles. On all tested susceptible control plants of 52201, the aphids had reproduced, and more than 20 adults and numerous juveniles were present on the upper leaves and stem (Fig. 3). Again, only plants transformed with cosmid 11 showed resistance. All eight R_1 populations tested segregated for aphid resistance. The segregation ratios of four of these lines best fitted a 3:1 segregation of a single gene ($p>0.7$), whereas the other four lines best fitted a 3:1 segregation ($p>0.3$).

A number of resistant R_1 plants in which resistance to nematodes was observed were selfed or backcrossed (BC) to the parental tomato 52201. In these R_2 and R_1BC populations, the resistance to both nematodes and aphids was stably inherited (Table 1). In the nematode assay, either all individuals of a given R_2 population were resistant, indicating that the parental R_1 plants were homozygous for the introduced gene, or the resistance segregated (in most cases 3:1, $p\geq0.50$). Eight of 11 R_1BC lines also showed resistance in the nematode assay (Table 1). In three BC lines, many plants were scored as

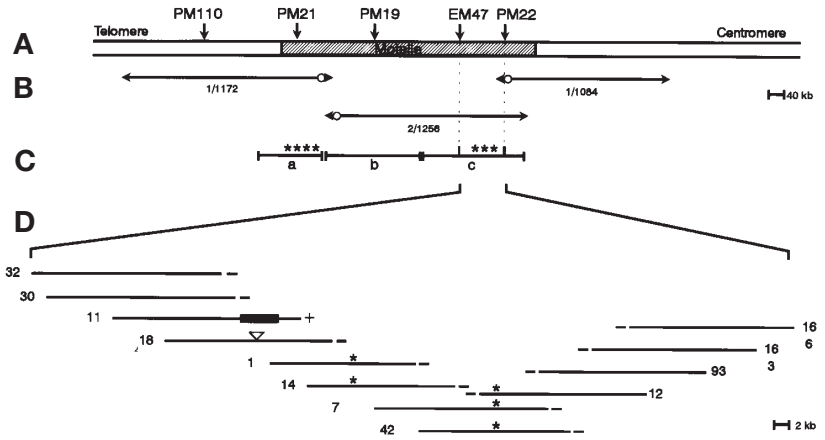

Figure 1. Positional cloning of *Mi-1*. (A) Position of AFLP markers with respect to the Motelle introgression region. (B) YAC contig spanning the Motelle region. The circle-arrowhead combination represents the left arm of pYAC4. (C) Cosmid contigs a, b, and c. The asterisks in contig a and c indicate four and three *Mi-1* homologs, respectively. (D) Position of the overlapping set of cosmids between markers EM47 and PM22 (+ and – refer to the ability or inability of a cosmid to confer resistance in transgenic plants). The location of *Mi-1* is indicated as a solid box and the two additional *Mi*-homologs are indicated with asterisks. The open triangle indicates the large deletions that occurred after transformation of cosmid 18 to plants.

Table 1. Nematode and aphid assays.

Root–knot nematode assay

Genotype(s)	Generation	Number of lines tested	All resistant[a] ($p<0.05$)	Segregating[a] ($p>0.1$)	All susceptible[a] ($p<0.05$)
52201 (control)	-	1			1
Motelle (control)	-	1	1		
pCLD04541 (transgene)	R_1	11			11
Mi-32 (transgene)	R_1	6			6
Mi-30 (transgene)	R_1	9			9
Mi-11 (transgene)	R_1	9		9	
	R_2	28	10	18	
	R_1BC	11	1	7	3[b]
Mi-18 (transgene)	R_1	9			9
Mi-01 (transgene)	R_1	10			10

Potato aphid assay

Genotype(s)	Generation	Number of lines tested	All resistant[a] ($p<0.05$)	Segregating[a] ($p>0.1$)	All susceptible[a] ($p<0.05$)
52201 (control)	-	1			1
Motelle (control)	-	1	1		
pCLD04541 (transgene)	R_1	5			5
Mi-32 (transgene)	R_1	4			4
Mi-30 (transgene)	R_1	8			8
Mi-11 (transgene)	R_1	8		8	
	R_2	27		25	2[c]
	R_1BC	8		8	
Mi-18 (transgene)	R_1	9			9
Mi-01 (transgene)	R_1	6			6

[a] All seedlings were tested for segregation (3:1 and 1:1) using the chi-square test. All resistant: *p*-value less than 0.05, significantly deviating from a 3:1 and 1:1 segregation; segregating: *p*-value larger than 0.1, not significantly deviating from a 3:1 and 1:1 segregation; all susceptible: *p*-value less than 0.05, significantly deviating from a 3:1 and 1:1 segregation.
[b] Half of the plants of two lines had an intermediate phenotype; i.e., 4–10 galls.
[c] Half of the plants of both lines had an intermediate phenotype; i.e., a few living adult aphids.
R_1 is the selfed progeny of a primary transformant; R_2 is the selfed progeny of a resistant R_1 plant; R_1BC is the progeny of a resistant R_1 plant backcrossed to the susceptible line 52201.

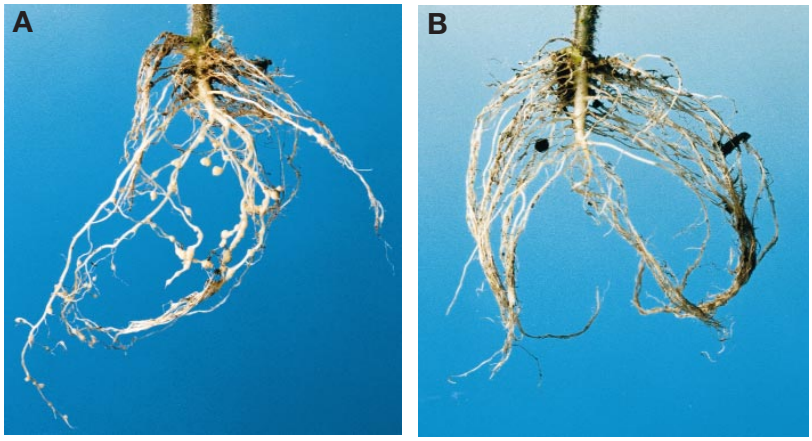

Figure 2. Root-knot nematode (*Meloidogyne incognita*) resistance. (A) The root system of a susceptible control plant (52201). (B) Roots of a resistant R_1 plant.

Figure 3. Potato aphid (*Macrosiphum euphorbiae*) resistance. A leaf of a susceptible control plant (52201) (left) and a leaf of a resistant R_1 plant (right).

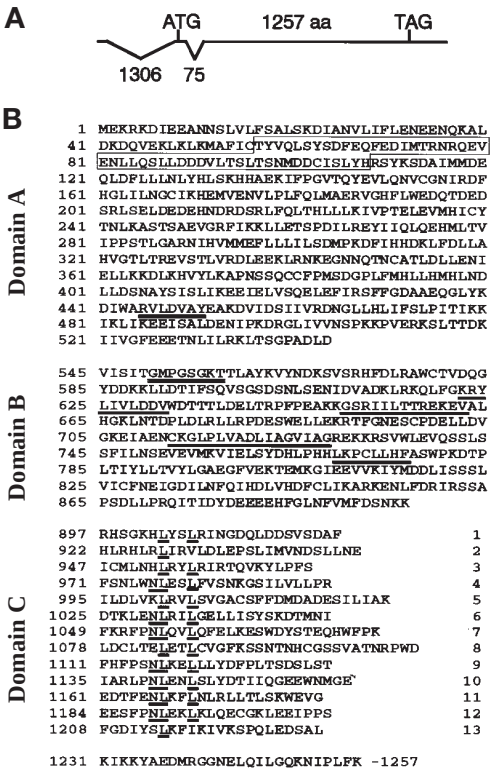

Figure 4. Structure of the *Mi-1* gene. (A) The *Mi-1* transcript. Introns are indicated by lines angled downward. The initiator (ATG) and terminator (TAG) codons are indicated. (B) Deduced Mi-1 protein sequence. The four domains are labeled A–D. Numbers on the left and at the end of the protein sequence refer to the positions of amino acid residues in the Mi-1 protein sequence. In domain A, the PCI domain is boxed and the tyrosine phosphorylation site is underlined. In domain B, the P-loop is double underlined, and the kinase 2a domain and the conserved domains 1, 2, and 3 are underlined. The numbers on the right of domain C refer to the individual LRRs. The asparagine (N) at position 6 and the leucine residues (L) at position 7 and 10 of the consensus LRR are underlined. The *Mi-1* sequence is available under GenBank accession number AF091048.

intermediate rather than resistant, with a few galls, but galls were far fewer than observed in the susceptible controls. In the potato aphid assays, all R_2 and R_1BC populations showed resistance except for two R_2 lines that were scored as intermediate.

The *Mi-1* gene. The complete insert of cosmid 11 (19 kb) was sequenced and only one open reading frame (ORF) could be predicted. Various primer sets scattered throughout the 19 kb insert were tested in a PCR analysis on cDNA from the resistant cultivar Motelle. Only primers corresponding to the predicted ORF amplified fragments from cDNA, verifying that cosmid 11 carries a single transcribed gene.

Furthermore, a large part of cosmid 11 outside the predicted ORF (60% of the insert) overlapped with cosmids 30 and 32, neither of which conferred resistance after transformation. This suggests that no functional resistance genes reside in this overlapping part of cosmid 11. Another cosmid, no. 18, overlapped with cosmid 11 in the region of the predicted ORF. PCR with primer sets derived from the predicted ORF was used to show that all plants transformed with cosmid 18 contained internal deletions in the predicted ORF, explaining its inability to confer resistance. The inability of the partially overlapping cosmids to confer resistance combined with the transcript analysis indicate that only one active gene is present on cosmid 11. Therefore, *Mi-1* and *Meu-1* must be the same gene.

The sequence of 5′ and 3′ rapid amplification of cDNA ends (RACE) products of the transcript of the predicted ORF revealed two introns: one of 1306 nucleotides in the 5′-untranslated region and one of 75 nucleotides in the 5′-end of the coding region. The deduced ORF encodes a predicted polypeptide of 1257 amino acids (Fig. 4).

The *Mi-1* gene family consists of at least 11 members. Southern blot analysis of resistant cultivars indicated that the *Mi-1* gene belongs to a multigene family. The *Mi-1* probe was hybridized to gel blots of DNA of the three YACs and of the three cosmid contigs a, b, and c (Fig. 1C). There were at least 11 major hybridizing bands in the YAC contig, of which at least seven were unique to the Motelle introgressed region carrying *Mi-1* and which could be assigned to cosmids of the cosmid contig (Fig. 5). Southern blot analysis of chromosomal DNA of Motelle (data not shown) did not reveal additional hybridizing bands. The *Mi-1* gene and two additional homologs map within the EM47-PM22 interval, whereas four other homologs are located at the centromeric end of YAC 1/1172, separated by 300 kb from *Mi-1*.

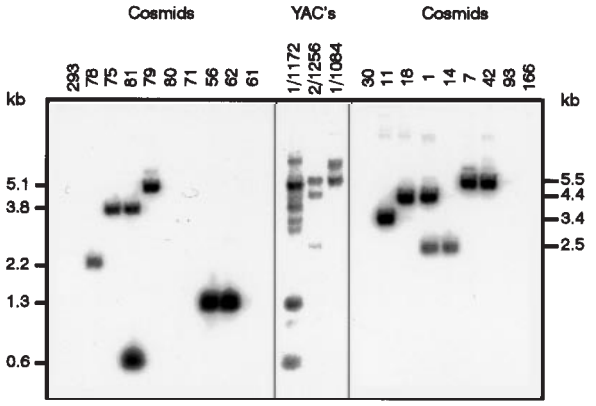

Figure 5. Identification of *Mi-1* homologs. A 116 bp fragment derived from the LRR region was used as a probe in hybridization of EcoRI + PstI digested DNA of YACs 1/1172, 2/1256, and 1/1084 (center) and of cosmids contigs a, b, and c at high stringency. Only two sets of overlapping cosmids hybridized. Cosmids 293 to 61 (on the left) originate from YAC 1/1172 and cosmids 30 to 166 (on the right) are located between the flanking AFLP markers EM47 and PM22.

The Mi-1 protein. The amino acid sequence of *Mi-1* shares structural features with the NBS-LRR type of *R* genes[6–8], and could be dissected into four domains, denoted A–D (Fig. 4). The N-terminal domain A is a leucine-rich region that lacks any known cleavable signal sequence, predicting that the *Mi-1* gene encodes a cytoplasmic protein. This distinct domain shows no homology to cytoplasmic domains of the *Drosophila* developmental gene *Toll* and the mammalian immune response gene encoding the interleukin-1 receptor (TIR). It contains one potential tyrosine phosphorylation site at residue 445–451 (RVLDVAY) and a search against the PROSITE-library[12] revealed a match (residues 57–108) with the PCI domain[13]. This is a recently described α-helical domain of about 200 amino acid residues, which is generally localized at the extreme C-terminus of a protein. This domain is present in five different proteasome subunits, at least two subunits of the plant morphogenic regulator complex COP9, two subunits of the translation initiation factor eIF3, and subunits of other multiprotein complexes[13]. In addition, five possible leucine zipper motifs (L-x(6)-L-x(6)-L-x(6)-L, where L = leucine and x = any amino acid) were predicted (residues 252–273, 259–280, 311–332, 318–339, and 402–423). However, this pattern was found only with 75% similarity. The N-terminal region of *Mi-1* most closely resembles the *L. esculentum Prf* gene product, which is required for resistance of tomato to *P. syringae*[14].

Domain B contains a putative NBS, with a P-loop (GMPGSGK) occurring at residues 550–557, followed by the kinase 2 (KRYLIVLDDV) and 3a (GSRIILTTREKEVAL) regions and the two conserved regions 2 (CKGLPLVADLIAGVIAG) and 3 (LKPCLLHF). Domain C, from position 897, is an LRR region consisting of 13 LRRs[15]. Domain D, the C-terminal region, displays no homology to known protein regions. The LRR of *Mi-1* (axxaxNLxxLxaxxxxxaxxa/Sxxx with L = leucine or isoleucine, a = aliphatic or aromatic amino acids, S = serine, and x = any amino acid) matches the consensus LRR motif of the *Arabidopsis RPM1* gene with a conserved asparagine (N) at position 6 and a poor conservation after residue 12 (ref. 16).

Discussion

We have used map-based cloning to identify the *Mi-1* nematode resistance gene of tomato. *Mi-1* has been known as a durable resistance gene, as the frequency at which *Mi-1* breaking strains of nematodes arise is very low. Only recently have variants of the pathogen been isolated that are able to overcome *Mi-1* resistance[17,18]. The *Mi-1* gene had been introgressed from the wild species *L. peruvianum*. Cultivated tomato lines containing *Mi-1* also displayed resistance against the potato aphid *M. euphorbiae*[4], indicating that these loci are linked. Clustering of resistance genes with different specificities has already been demonstrated for the *Cf-2* and *Cf-5* genes for resistance to two distinct isolates of the fungus *C. fulvum*[19], for the potato genes *Gpa2* (resistance to a distinct group of populations of the potato cyst nematode *Globodera pallida*) and *Rx1* (resistance to potato virus X)[20], and for the *Pto* and *Prf* genes giving resistance to *P. syringae*[14]. In the latter case, both genes are required for resistance and reside within a 50 kb region together with four *Pto* homologs. Thus, tight linkage of distinct *R* genes is a common phenomenon. However, the fact that *Mi-1* and *Meu1* are the same gene was unexpected. The complementation data conclusively demonstrated that the *Mi-1* gene confers resistance to both root-knot nematodes and to aphids, two plant pests of totally unrelated phyla of animals.

Based on its structural features, *Mi-1* belongs to the NBS-LRR class of *R* genes without a TIR domain, consisting of the *Arabidopsis RPS2* and *RPM1* genes and the tomato *I2* and *Prf* genes. The *Mi-1* gene product most closely resembles the *L. esculentum Prf* gene product. However, they differ in that the N-terminal region of *Mi-1* shows homology to the PCI domain, whereas that of *Prf* does not. Hence, we suggest that the NBS-LRR class of *R* genes without a TIR domain consists of an *Mi-1* subgroup containing homology to the PCI domain. In addition, the match with the PCI domain suggests that the *Mi-1* gene product is part of a cytoplasmic multiprotein regulator complex and could serve as a structural scaffold by interacting with other proteins[13].

A nematode resistance gene of sugar beet has been cloned[21]. The protein consists of a putative signal peptide, a transmembrane segment, and seven imperfect LRR-units. The small size of the $Hs1^{pro-1}$ gene product deviates strongly from the LRR class of plant *R* genes, products of which generally consist of more than 1000 amino acids. Alignment studies between the gene products of *Mi-1* and $Hs1^{pro-1}$ revealed a very limited similarity with domains A and B, but none with the LRR region (domain C) and the PCI domain of the *Mi-1* gene product. There must be at least two distinct types of nematode resistance proteins and presumably two different mechanisms for nematode recognition: those like *Mi-1* belonging to the NBS-LRR protein family and located intracellularly and those like $Hs1^{pro-1}$, which are leucine-rich and located in the plasma membrane.

The *RPM1* resistance gene of *Arabidopsis thaliana* is another example of a resistance gene with dual specificity[16]. This gene confers resistance to *P. syringae* strains expressing either *avrRpm1* or *avrB*[22]. So far, nematode or aphid *avr* genes and their gene products have not yet been identified. Root-knot nematodes penetrate the roots. A localized necrosis of host cells and a hypersensitive response near the site of the nematode invasion characterize resistance. Very little is known about the mechanism of the aphid resistance. Identification of *Mi-1* should facilitate the study of nematode resistance and help in determining whether similarities exist between the nematode and aphid resistance mechanisms.

Interspecific transfer of resistance genes has great potential if there is no effective resistance in the gene pool of the target species. Evidence has been obtained that *R* genes function in heterologous systems[23]. The success of these interspecific transfers will also depend on the presence of other elements in the signal transduction pathways in the recipient species. Transferring the *Mi-1* gene to important crops such as potato and other *Solanaceae* and testing whether the dual specificity is retained in the new crop will now be important in determining the commercial potential of cloned *R* genes.

Experimental protocol

Plant material. Two sets of near-isogenic tomato lines (NILs): 83M-R (resistant) and 83M-S (susceptible), and the varieties Motelle (resistant) and Mobox (susceptible) have been used in an AFLP screening in search of linked markers. The first set of NILs was obtained from De Ruiter Seeds (Bergschenhoek, The Netherlands), and the second set from INRA (Montfavet, France). Candidate linked AFLP markers were tested on seven homozygous resistant and 11 susceptible tomato breeding lines. For identification of recombinants, a segregating *L. peruvianum* pseudo-F_2 population was used[11], generated from a cross of *L. peruvianum* accession PI-128657-G (Rr), the original source of the *Mi-1* gene, and the susceptible parent LA2964 (rr). A total of 390 F2 plants and 390 F3 plants of this cross were analyzed with the codominant AFLP markers PM110, PM21, PM19, EM47, and PM22, selected from the NIL screening

Disease assay. Tomato seeds or germinated seedlings were planted in a greenhouse in soil infected with the root-knot nematode *M. incognita*. Of each R_1 line, 10–24 seedlings were assayed and of each R_2 and R_1BC line 20 to 60 seedlings. After 6 weeks, resistance or susceptibility was determined by examining the roots for appearance of galls. Plants were scored "resistant" when 0–3 galls were visible on the roots and "susceptible" when more than 10 galls were formed. Very rarely, an intermediate number of 4–10 galls was found. For purpose of statistical analysis of segregation ratios, this class was considered susceptible. Four-week-old tomato plants were inoculated with five females of the potato aphid *M. euphorbiae*. Of each tomato line 12–15 plants were assayed. After 14 days, resistance or susceptibility to aphid infestation was determined by examining the stem and leaves for newborn aphids. Colonization of *M. euphorbiae* is such that the aphids and their offspring rarely migrate away from susceptible hosts, whereas resistant plants harbor, at the most, only a few passing adults and no offspring. Plants were scored "resistant" when no living aphids were present and "susceptible" when a colony had established with several newborn aphids present.

Statistical analysis of segregation ratios. The numbers of resistant and susceptible plants within each line were analyzed using a chi-square test for an expected segregation ratio of 3:1 and 1:1. Lines with a probability (p-value) less than 0.05 for both tested ratios were assumed to be nonsegregating.

AFLP analysis. The AFLP technique is based on the amplification of subsets of genomic fragments using PCR[5]. To identify polymorphic markers linked to the *Mi-1* gene, amplification reactions were performed using DNA templates for the enzyme combinations PstI/MseI (PM) and EcoRI/MseI (EM). AFLP fingerprinting of tomato lines was performed with two selective nucleotides at the rare cutter site and three selective nucleotides at the frequent cutter site[5].

Library constructions. The tomato line *L. esculentum* E22 was used to construct a YAC library. Construction of the library and screening with AFLP markers was as described[10]. Cosmid libraries of YAC clones 1/1127 and 2/1256 were constructed in the binary cosmid vector pCLD04551 (ref. 24). Cosmids were arranged into contigs using AFLP fingerprinting[25].

Complementation analysis. Cosmids were introduced in *Agrobacterium tumefaciens* strain AGL1 (ref. 26) through conjugative transfer in a triparental mating with helper strain HB101 (pRK2013)[27]. Cotyledon explants of 8-day-old seedlings of the susceptible tomato line 52201 were transformed by the various *Agrobacterium* strains carrying these cosmids[28]. Plants containing pCLD04551 were included as a control.

DNA sequencing and computer analysis. The sequence of cosmid clone *Mi-11* was determined as described[25]. Sequence assembly and analyses were performed with the 1994 version of the STADEN sequence analysis program[29].

Acknowledgments

We thank Jerina Pot and Maarten van Riel for artwork. We acknowledge the staff of De Ruiter Seeds and Enza Zaden for performing the nematode tests and of Rijk Zwaan for the aphid tests. This work was financed by Keygene N.V. and three Dutch breeding companies: De Ruiter Seeds, Enza Zaden, and Rijk Zwaan. Furthermore, this work was subsidized by the Dutch Ministry of Economic Affairs through a Programmatische Bedrijfsgerichte Technologie Stimulering grant. AFLP is a registered trademark of Keygene N.V.

1. Mai, W.F. 1985. Plant-parasitic nematodes: their threat to agriculture, pp 11–17 in *Advanced treatise on meloidogyne*, Vol. I. Sasser, J.N. and Carter, C.C. (eds.), North Carolina State University Graphics, Raleigh, NC.
2. Bailey, D.M. 1941. The seedling test method for root-knot nematode resistance. *Proc. Am. So. Hortic. Sci.* **38:**573–575.
3. Gilbert, J.C. and McGuire, D.C. 1956. Inheritance of resistance to several root-knot from *Meloidogyne incognita* in commercial type tomatoes. *Proc. Am. So. Hortic. Sci.* **68:**437–442.
4. Kaloshian, I., Harry Lange, W., and Williamson, V. 1995. An aphid-resistance locus is tightly linked to the nematode-resistance gene, *Mi*, in tomato. *Proc. Natl. Acad. Sci. USA* **92:**622–625.
5. Blackman, R.L. and Eastop, V.F. 1984. *Aphids on the world's crops: an identification and information guide.* John Wiley & Sons, New York.
6. Baker, B., Zambryski, P., Staskawicz, B., and Dinesh-Kumar, S.P. 1997. Signaling in plant-microbe interactions. *Science* **276:**726–733.
7. Hammond-Kosack, K.E. and Jones, J.D.G. 1997. Plant disease resistance genes. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **48:**573–607.
8. De Wit, P.J.G.M. 1997. Pathogen avirulence and plant resistance: a key role for recognition. *Trends in Plant Science* **2:**452–458.
9. Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M. et al. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res.* **23:**4407–4414.
10. Simons, G., Van der Lee, T., Diergaarde, P., Van Daelen, R., Groenendijk, J., Frijters, A. et al. 1997. AFLP-based fine mapping of the *Mlo* gene to a 30-kb DNA segment of the barley genome. *Genomics* **44:**61–70.
11. Kaloshian, I., Yaghoobi, J., Liharska, T.B., Hontelez, J., Hanson, D., Hogan, P. et al. 1998. Genetic and physical localization of the root-knot nematode resistance locus *Mi* in tomato. *Mol. Gen. Genet.* **257:**376–385.
12. Bairoch, A., Bucher, P., and Hofmann, K, 1997. The PROSITE database, its status in 1997. *Nucleic Acids Res.* **25:**217–221.
13. Hofmann, K. and Bucher, P. 1998. The PCI domain: a common theme in three multiprotein complexes. *TIBS* **23:**204–205.
14. Salmeron, J.M., Oldroyd, G.E.D., Rommens, C.M.T., Scofield, S.R., Kim, H-S., Lavelle, D.T. et al. 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:**123–133.
15. Jones, D.A. and Jones, J.D.G. 1997. The role of leucine-rich repeat proteins in plant defenses. *Adv. Bot. Res.* **24:**89–167.
16. Grant, M.R., Godiard, L., Straube, E., Ashfield, T., Lewald, J., Sattler, A. et al. 1995. Structure of the *Arabidopsis RPM1* gene enabling dual specificity disease resistance. *Science* **269:**843–846.
17. Yaghoobi, J., Kaloshian, I., Wen, Y., and Williamson, V.M. 1995. Mapping a new nematode resistance locus in *Lycopersicon peruvianum*. *Theor. Appl. Genet.* **91:**457–464.
18. Veremis, J.C. and Roberts, P.A. 1996. Relationships between *Meloidogyne incognita* resistance genes in *Lycopersicon peruvianum* differentiated by heat sensitivity and nematode virulence. *Theor. Appl. Genet.* **93:**950–959.
19. Dickinson, M.J., Jones, D.A., and Jones, J.D.G. 1993. Close linkage between the *Cf-2/Cf-5* and *Mi* resistance loci in tomato. *Mol. Plant. Microbe Interact.* **6:**341–347.
20. Rouppe van der Voort, J., Wolters, P., Folkertsma, R., Hutten, R., Van Zandvoort P., Vinke H. et al. 1997. Mapping of the cyst nematode resistance locus *Gpa2* in potato using a strategy based on comigrating AFLP markers. *Theor. Appl. Genet.* **95:** In press.
21. Cai, D., Kleine, M., Kilfe, S., Harloff, H-J., Sandral, N.N., Marcker, K.A. et al. 1997. Positional cloning of a gene for nematode resistance in sugar beet. *Science* **275:**832–835.
22. Bisgrove, S., Simonich, M., Smith, N., Sattler, N., and Innes, R. 1994. A disease resistance gene in Arabidopsis with specificity for two different pathogen avirulence genes. *Plant Cell* **6:**927–933.
23. Hammond-Kosack, K.E., Tang, S., Harrison, K., and Jones, J.D.G. 1998. The tomato *Cf-9* resistance gene functions in tobacco and potato to confer responsiveness to the fungal avirulence gene product Avr9. *Plant Cell* **10:**1251–1258.
24. Jones J.D.G., Shlumukov, L., Carland, F., English, J., Scofield, S.R., Bishop, G.J. et al. 1992. Effective vectors for transformation, expression of heterologous genes, and assaying transposon excision in transgenic plants. *Transgenic Res.* **1:**285–297.
25. Simons, G., Groenendijk, J., Wijbrandi, J., Reijans, M., Groenen, J., Diergaarde, P. et al. 1998. Dissection of the Fusarium *I2* gene cluster in tomato reveals six homologs and one active gene copy. *Plant Cell* **10:**1055–1068.
26. Lazo, G.R., Stein, P.A., and Ludwig, R.A. 1991. A DNA transformation-competent *Arabidopsis* genomic library in *Agrobacterium*. *Bio/Technology* **9:**963–971.
27. Deblaere, R., Reynaerts, A., Höfte, H., Hernalsteens, J.P., Leemans, J., and Van Montagu, M. 1987. Vectors for cloning in plant cells. *Methods. Enzymol.* **153:**277–292.
28. Koornneef, M., Jongsma, M. Weide, R., Zabel, P., and Hille, J. 1987. Transformation of tomato, pp. 169–178, in *Tomato biotechnology.* Nevins, D.J. and Jones, R.A. (eds.) Alan R. Liss, New York.
29. Dear, S. and Staden, R. 1991. A sequence assembly and editing program for efficient management of large projects. *Nucleic Acids Res.* **14:**3907–3911.