Theor Appl Genet (1996) 93: 894–901

J.C. Veremis · P.A. Roberts

Identification of resistance to *Meloidogyne javanica* in the *Lycopersicon peruvianum* complex

Received: 23 January 1996 / Accepted: 29 March 1996

Abstract Clones of *Lycopersicon peruvianum* PI 270435-2R2, PI 270435-3MH and PI 126443-1MH expressed novel resistance to three *Mi*-avirulent *M. javanica* isolates in greenhouse experiments. Clones from PI 126443-1MH were resistant to the three *M. javanica* isolates at 25°C. The three isolates were able to reproduce on one embryo-rescue hybrid of PI 126443-1MH, but not on three *L. peruvianum-L. esculentum* bridge-line hybrids of PI 126443-1MH when screened at 25°C (*Mi*-expressed temperature). Clones of PI 270435-2R2 and all its hybrids with susceptible genotypes were resistant to the three *M. javanica* isolates at 25°C. The bridge-line hybrid EPP-2×PI 270435-2R2 was susceptible to *M. javanica* isolate 811 at 32°C, whereas PI 270435-2R2 and all other hybrids of PI 270435-2R2 crossed with susceptible genotypes were resistant at 32°C. At 32°C, one F_2 progeny of PI 126443-1MH×EPP-1, and three test-cross progenies of PI 126440-9MH×[PI 270435-3MH×PI 126443-1MH], and reciprocal test-cross progenies of [PI 270435-3MH×PI 270435-2R2]×PI 126440-9MH, each segregated into resistant: susceptible (R:S) ratios close to 3:1. The results from the F_2 progeny indicated that heat-stable resistance to *Mi*-avirulent *M. javanica* in PI 126443-1MH is conferred by a single dominant gene. The results from the test-crosses indicated that this gene in PI 126443-1MH is different from the resistance gene in PI 270435-3MH. The resistance gene in PI 270435-3MH was also shown to differ from the resistance factor in PI 270435-2R2. The expression of differential susceptibility and resistance to *M. javanica* and *M. incognita* in individual plants of the bridge-line hybrid, embryo-rescue hybrid, F_2, and test-crosses indicated that at least some genes governing resistance to *M. javanica* differ from the genes conferring resistance to *M. incognita*. A new source of heat-stable resistance to *M. javanica* was identified in *Lycopersicon chilense*.

Key words Heat-sensitivity · Virulence · Dominant resistance · Tomato · Root-knot nematodes · *M. javanica*

Communicated by G. Wenzel

J. C. Veremis · P. A. Roberts (✉)
Department of Nematology, University of California, Riverside, CA 92521, USA

Introduction

Host-plant resistance to plant-parasitic nematodes is a powerful tool for crop protection, and it is destined to play a more important role than ever before in managing nematode problems in sustainable agriculture. The most effective nematicides have been restricted in agriculture because of high risk to human health and the environment. Root-knot nematodes (*Meloidogyne* spp.) are the most important nematode pests of crops worldwide, with extensive host ranges including most crop plants (Sasser 1977). All tomato (*Lycopersicon esculentum* Mill.) cultivars with the gene *Mi* have been developed from one resistant interspecific hybrid plant from *L. peruvianum* L. (Smith 1944). The resistance conferred by *Mi* is effective against *M. incognita, M. arenaria* (Neal) Chitwood, and *M. javanica* (Treub) Chitwood (Dropkin 1969). Resistance is different from most breeding traits, because the effectiveness is defined by the genetic composition of the pathogen, which can become virulent over time. Intensive use of *Mi*-based resistance raises valid concerns over its durability due to the potential for selecting *Mi*-breaking virulent populations of the nematode (Castagnone-Sereno 1994), or shifting the species composition in the field. Effects of long-term cropping of resistant cultivars may include shifts in nematode races and the occurrence of multiple species of nematodes within the same field (Young 1992).

There are reports of natural or selected virulence to gene *Mi* in isolates of *M. javanica* (Netscher 1978). Natural and selected resistance-breaking or virulent biotypes of *M. javanica* on tomatoes with the gene *Mi* occurred in tomato production areas of Greece (Tzortzakakis and Gowen 1996). Resistance break down is a classic example of evolution by natural selection of the cropping system. Roberts et al. (1990) point out that variation within root-knot for parasitic ability on tomato cultivars bearing the gene *Mi* does not conform to currently recognized species or host-race categorization. The frequent occurrence of two or more species or to host races of root-knot nematodes in the field should be reflected in the focus of screening for resistance in breeding programs. In addition, cultivars with the gene *Mi* express resistance below 28°C but not above 28°C (Holtzmann 1965; Dropkin 1969). The *Mi* gene also breaks down to isolates of *M. javanica* in the field at high temperatures in Cyprus (Philis and Vakis 1977). Variability in the reproduction of different populations of *M. javanica* has been demonstrated on different tomato cultivars possessing the *Mi* gene (Roberts and Thomason 1986, 1989). Novel resistance to *M. incognita* has been identified in clones of several *L. peruvianum* genotypes (Ammati et al. 1985, 1986). Understanding the spectrum of the novel resistance identified in wild donor germ plasm is important before attempts are made to incorporate the most useful resistance traits into an agricultural crop.

The objectives of the present work were: (1) to characterize genetically the inheritance of, and the relationships between novel resistance traits with isolates of *M. javanica* at low and high temperature and; (2) to identify additional novel sources of host plant resistance to *M. javanica* in *Lycopersicon* spp.

Materials and methods

Plant material

Plant genotypes used in this study were *L. peruvianum* PI 270435 clone 2R2, PI 270435 clone 3MH and PI 126443 clone 1MH (Ammati et al. 1986); embryo-rescue hybrids developed by Cap (1991); the *L. peruvianum-L. esculentum* bridge-line hybrids, F_2 progeny of PI 126443-1MH×EPP-1, test-crosses of PI 126440-9MH×[PI 270435-3MH×PI 126443-1MH] and reciprocal test-crosses of [PI 270435-3MH×PI 270435-2R2]× PI 126440-9MH (Veremis 1995). Plant material was propagated agamically as described previously (Cap et al. 1993).

Seeds of the exotic tomato accessions with the designation LA were obtained from the Tomato Genetics Stock Center (TGSC) at U.C. Davis, those with a PI number from the Northeast Regional Plant Introduction Station (NERPIS) at Geneva, New York, while seeds of the bridge-lines EEP-1, EPP-1 and EPP-2 were provided by Dr. V. Poysa (1990). The exotic tomato seeds were pretreated as described by Rick and Borgnino (1989), and then germinated in speedling trays containing vermiculite, together with untreated seeds of *L. esculentum* cultivars. Seeded speedling trays were maintained in a mist chamber for at least 1 week to enhance germination.

Nematode cultures

Cultures of three *M. javanica* isolates, 811, Bettencourt, and Cox-Perez, were started from field populations on greenhouse-grown tomato cv Tropic plants. The identities of the nematode isolates were confirmed morphologically by microscopic examination of the perineal patterns of adult females (Eisenback 1985), by isozyme (esterase and malate dehydrogenase) phenotypes (Cap et al. 1991), and by the North Carolina differential host test (Hartman and Sasser 1985).

Screening tests

One-month-old seedlings or rooted cuttings were used for tests of host reaction to nematodes. Single plants were grown in cone-tainers (SC-10 Super Cell, Stuewe and Sons Inc.) filled with steam-sterilized loamy sand and fertilized with Osmocote. Experiments that tested heat-sensitivity of resistance were carried out in constant environment growth chambers where temperature was maintained constantly at 32°C for 7 days before and 30 days after inoculation, and then placed for an additional 25–30 days in a greenhouse environment 25±3°C). The experiments requiring moderate rather than high temperature were carried out in a growth chamber held constantly at 25°C or in a greenhouse at 25±3°C.

Inoculum was prepared by the sodium hypochlorite method of Hussey and Barker (1973). A water suspension of approximately 6000 infective second-stage juveniles (J_2) per plant, in tests with *M. javanica* isolate 811 and isolate Bettencourt, or 3000 J_2, in tests with *M. javanica* isolate Cox-Perez, was pipetted into the soil around the plant roots. Plants were arranged in cone-tainer racks in a completely randomized design. Nematode egg production on roots was evaluated after the accumulation of approximately 1000 degree days (base temperature of 10°C). This allowed completion of at least one nematode generation before evaluation of the experiment (Trudgill 1994). Root systems were washed free of soil under tap water, damp-dried with paper towel, weighed and stained overnight with 75 mg/l of erioglaucine (Aldrich Co.) solution to highlight egg masses for counting (Omwega et al. 1988). At the same time the material was propagated agamically as described for the parental material above. Eggs were extracted by macerating the roots in 1% NaOCl solution in a commercial blender, and pouring and rinsing the macerate through a screen series with openings of 850 mμ, 106 mμ, and 38 mμ, respectively. The collected egg suspension was adjusted to a known volume and the numbers of eggs were counted under a dissecting microscope.

The numbers of eggs per gram of root were calculated by dividing the total number of eggs per root system produced by the total fresh root weight. A plant was considered resistant when the number of egg masses per root system and the number of eggs per gram of root were less than 10% of the egg masses per root system and the eggs per gram of root, respectively, on the susceptible control. Susceptible tomato cv Tropic and cv VFN-8, which possesses the gene *Mi* (expressed below 28°C but not above 28°C; Dropkin 1969), were included to check inoculum viability, infectivity and the expression of heat-sensitive resistance genes.

Results

Resistance of *Lycopersicon* spp. to *M. javanica* isolate 811 at 32°C

A range of *Lycopersicon* species accessions was tested for resistance to *M. javanica* isolate 811 at 32°C. The number of plants within each entry accession, cultivar, and hybrid tested, and the mean number of egg masses per root system produced, are given in Table 1. The *L. esculentum* cvs Peto 94 and VFN-8 gave a susceptible reaction with 455 and 284 mean egg masses per root system, respectively, and a high eggs/g. root number and root-galling response. The high level of reproduction on VFN-8 at 32°C confirmed that high-temperature induction of susceptibility to the *M. javanica* isolate (breakdown of the *Mi* gene) had occurred at 32°C in this experiment.

The bridge-line hybrid PI 126443-1MH×EPP-1 was resistant to isolate 811 at 32°C as indicated by low mean-egg-mass production (6 per root system). Isolate 811 overcame the resistance in the bridge hybrid EPP-2×PI 270435-2R2, indicated by a high level of reproduction (199 mean egg masses per root system), but it did not overcome the resistance conferred by the embryo-rescue hybrid of

Table 1 A broad screen of *Lycopersicon* spp. germ plasm for resistance to *M. javanica* isolate 811 at 32°C in a cone-tainer experiment based on means of egg masses per root system produced

Tomato genotype	(*n*)	Means of egg masses
L. esculentum		
VFN-8	6	284 fghijk[a]
Peto 94	6	455 nop
Interspecific hybrids		
UC82×PI 270235-2R2	4	1 a
PI 126443-1MH×EPP-1	5	6 a
EPP-2×PI 270435-2R2	5	199 cdefg
Bridge lines		
EPP-2 clone II	4	1 a
EPP-1 clone I	3	266 defghij
L. peruvianum		
PI 128656-11R3	4	1 a
PI 126441	4	2 a
PI 270435-3MH	4	6 a
PI 127829	3	14 a
LA 111	5	19 a
PI 126443	4	40 ab
PI 126926	4	117 abcd
PI 126443-2R2	4	141 abcde
PI 129146	3	144 abcde
PI 129152	8	152 bcde
PI 247087	4	173 bcdef
PI 128653	4	216 defgh
PI 126440-9MH	4	217 defgh
PI 128646	3	227 defgh
PI 199380	3	232 defgh
PI 251312	3	270 efghijk
LA 110	5	292 fghijk
PI 251307	4	323 ghijklm
PI 128648	4	346 hijklmn
L. chmielewskii		
LA 1316	5	161 bcde
LA 1306	5	188 cdefg
LA 1028	3	454 mnop
L. chilense		
LA 2884	3	0 a
LA 1969	3	3 a
LA 2750	3	6 a
LA 2930	3	13 a
LA 2759	3	51 ab
LA 1938	3	53 ab
LA 2733	3	108 abcd
LA 1963	3	166 bcdef
LA 1932	3	181 bcdefg
LA 1965	3	240 defghi
LA 1960	3	426 klmno
L. pimpinellifolium		
PI 379058	5	85 abc
PI 126436	3	248 dfghi
PI 375937	3	276 efghijk
LA 1269	4	291 fghijk
PI 230327	3	296 fghijkl
PI 390691	5	344 hijklm
PI 251320	3	349 hijklmn
PI 126947	3	391 ijklmno
LA 412	4	441 lmno
LA 1280	4	508 op
PI 126932	4	528 op
PI 143524	4	596 p
L. pennellii		
LA 1732	3	251 defghij

Table 1 (Continued)

Tomato genotype	(*n*)	Means of egg masses
L. cheesmanii		
PI 231257	7	332 hijklmn
L. hirsutum		
LA 1624	8	192 cdefg
LA 2090	5	246 defghi
PI 415127	5	304 fghijkl
PI 134417	4	309 fghijklm
PI 390514	4	341 hijklmn
PI 134418	4	349 hijklmn
PI 126449	4	398 jklmno
PI 127826	7	408 klmno

[a] Egg mass values followed by the same letter are not significantly different for $P=0.05$ according to a LSD test

PI 270435-2R2 with UC82 (1 mean egg mass per root system) (Table 1). Isolate 811 at 32°C did not overcome the resistance conferred by the clones PI 128656–11R3 (1 mean egg mass per root system) and PI 270435-3MH (6 mean egg masses per root system) (Table 1). The bridge-line EPP-2 clone II was also resistant with a mean of 1 egg mass per root system. *Lycopersicon chilense* LA 2884 supported no nematode reproduction (0 mean egg masses per root system), which was significantly lower than most other treatments (Table 1). *L. chilense* LA 1960 supported the highest rate of reproduction with a mean of 78412 eggs/g root, and a high, but not the highest, rate of reproduction according to the mean number of egg masses per root system (426) (Table 1). The *Lycopersicon* accessions LA 111, LA 2930, LA 2759, LA 1938 and PI 126443 segregated, with individual entries as susceptible or resistant in each case. However, the morphology and quality of the roots in the wild tomato accessions varied within the species and accessions, and in some cases the overall reaction to infection, including root-galling, and the number of egg/g of root produced was helpful in supplementing counts of egg masses when classifying plants as resistant or susceptible.

Resistance of *L. esculentum*×*L. peruvianum* hybrids to *M. javanica* isolates at 25°C

The *L. peruvianum* parental clones, their bridge-line hybrids and embryo-rescue hybrids were screened for resistance to *M. javanica* isolates 811, Bettencourt, and Cox-Perez at 25°C. The *L. esculentum* cv Tropic produced a susceptible reaction, as expected, and cv VFN-8 was resistant to all three *Mi*-avirulent *M. javanica* isolates at 25°C, also as expected for *Mi* gene expression at this temperature (Table 2). The *L. peruvianum* donor parent clones were resistant to all isolates of *M. javanica*. However, the interspecific embryo-rescue hybrid ms-1×PI 126443-1MH was partially susceptible to all three *M. javanica* isolates, although it was only significantly different with the isolate Bettencourt (Table 2). This hybrid was different

Table 2 Reaction of *L. esculentum*, embryo-rescue hybrids, bridge-line hybrids, and clones of *L. peruvianum* to *Mi*-avirulent *M. javanica* isolates 811, Bettencourt, and Cox-Perez at 25°C

Tomato genotype	Means of *M. javanica* egg masses/root system		
	811	Bettencourt	Cox-Perez
L. esculentum			
Tropic	290 b[a]	125 c	165 b
VFN-8	0 2 a	0.7 a	0 a
Embryo-rescue hybrids			
UC-82×PI 270435-2R2	0 a	0 a	0 a
ms-1×PI 126443-1MH	30 a	32 b	33 a
Bridge-line hybrids			
PI 126443-1MH×EPP-1	0.5 a	0.7 a	0 2 a
EPP-1×PI 270435-2R2	0 a	0 a	0 a
EEP-1×PI 126443-1MH	0 a	0 a	0 a
EPP-2×PI 270435-2R2	0 a	0 a	0 a
L. peruvianum			
PI 128657-3R4	0 a	0 a	0 a
LA 1708-I	0 a	0 a	0 a
PI 126443-1MH	0 a	0 a	0 a
PI 270435-2R2	0 a	0 a	0 a
PI 270435-3MH	0.5 a	0 a	0 a

[a] Egg mass values within a column followed by the same letter are not significantly different for alpha=0.05 according to a LSD test. Values are the means of four replicates

from the others with mean numbers of 30, 32 and 33 egg masses per root system produced with *M. javanica* isolates 811, Bettencourt, and Cox-Perez. respectively. at 25°C. The *L. peruvianum* parental clones and their other bridge-line hybrids and embryo-rescue hybrids were all resistant to the *M. javanica* isolates with reproduction levels ranging from 0 to 0.7 mean egg masses per root system which were significantly lower than on the susceptible control. The parental clone PI 126443-1MH and the other hybrids were highly resistant to the three *M. javanica* isolates at 25°C (Table 2). The clones of *L. peruvianum* LA 1708-I, PI 128657-3R4 and PI 270435-2R2 were also resistant (Table 2).

F_2 experiment with *M. javanica* isolate 811 at 32°C (*Mi* not expressed)

All clones of the parental line PI 126443-1MH (P_1) (0 mean egg masses per root system) were resistant to the *M. javanica* isolate 811, whereas all the plants of parent bridge-line EPP-1 (P_2) (221 mean egg masses per root system) were susceptible, as expected. All cuttings of the bridge-line hybrid F_1 (PI 126443-1MH×EPP-1) (mean of 1 egg mass per root system) were also resistant at 32°C, indicating complete dominance of heat-stable resistance to *Mi*-avirulent nematodes in clone PI 126443-1MH (Table 3). To confirm the breakdown of the heat-sensitive resistance conferred by gene *Mi*, cv VFN-8 was included and, as expected, gave a susceptible reaction at high temperature (196 mean egg masses per root system). The control cultivar Tropic gave a susceptible reaction of 236 mean egg masses per root system. The F_2 progeny screened at 32°C with *M. javanica* isolate 811 segregated into 25 resistant: 9 susceptible plants, indicating a ratio of 3:1 (R:S) with a chi-square value of 0.29 ($0.90<P>0.80$) (Table 3).

Table 3 Reaction of parents, F_1, and F_2 segregating progeny of *L. peruvianum* PI 126443-1MH×EPP-1 tested for resistance to *M. javanica* isolate 811 at 32°C in a cone-tainer experiment according to nematode egg masses and eggs produced on roots

Generation	Parent or progeny	Number of plants		Expected ratios		χ^2	P
		R[a]	S[b]	R	S		
P_1	*L. peruvianum* PI 126443 clone 1MH	20	0				
P_2	Bridge-line[c] EPP-1	0	20				
F_1	$P_1 \times P_2$	20	0				
F_2	F_1	25	9	3	1	0 29	0.90–0.80

[a] Resistant (R), fewer than 25 egg masses per root system and/or less than 600 eggs per gram of root
[b] Susceptible (S), 25 or more egg masses per root system and/or 600 or more eggs per gram of root
[c] Bridge-line developed by Dr V Poysa (1990) for interspecific gene transfer between *L. peruvianum* and *L. esculentum*

Test-cross experiment with *M. javanica* isolate 811 at 32°C (*Mi* is not expressed)

Parent lines PI 270435-2R2 (P_1) (0 mean egg masses per root system), PI 270435-3MH (P_2) (0 mean egg masses per root system) and PI 126443-1MH (P_3) (0 mean egg masses per root system) were all resistant to the *M. javanica* isolate 811, whereas all the plants of parent line PI 126440-9MH (P_4) (217 mean egg masses per root system) were susceptible (Table 4), as expected. To confirm the breakdown of heat-sensitive resistance conferred by the *Mi* gene, cv VFN-8 was included and, as expected, gave a susceptible reaction at high temperature (283 mean egg masses per root system).

The test-cross $TC_{1.1}$ population contained 14 resistant: 3 susceptible plants, indicating a segregation ratio of 3:1 (R:S) with a chi-square value of 0.49 ($0.50<P>0.30$) (Table 4). The $TC_{1.2}$ population contained 12 resistant: 6 susceptible plants, also indicating a segregation ratio of 3:1 (R:S) with a chi-square value of 0.66 ($0.50<P>0.30$). When pooled the $TC_{1.1}$ and $TC_{1.2}$ reciprocal crosses contained 26 resistant: 9 susceptible plants, further supporting a segregation ratio of 3:1 (R:S) with a chi-square value of 0.00 ($P>0.95$). $TC_{1.3}$ contained 16 resistant: 4 susceptible plants also giving a 3:1 (R:S) ratio (χ^2=0.26; $0.70<P>0.50$). The highly non-significant chi-square value was a good fit between the observed results and the calculated expectancy of a 3:1 (R:S) ratio, although it could occur by chance in this small sample if drawn from a larger

Table 4 Reaction of *L. peruvianum* parental clones, F_1 and test-cross (TC_1) progenies for resistance to the *Mi*-avirulent *M. javanica* isolate 811 at 32°C soil temperature according to nematode egg masses

Generation	*L. peruvianum* parent clone or cross	Number of plants		Expected ratios		χ^2	*P*
		R[a]	S[b]	R	S		
Parents							
P_1	PI 270435-2R2	10	0				
P_2	PI 270435-3MH	10	0				
P_3	PI 126443-1MH	10	0				
P_4	PI 126440-9MH	0	10				
F_1 progeny							
$F_{1\,1}$	$P_2 \times P_1$	5	0				
$F_{1\,2}$	$P_2 \times P_3$	5	0				
Testcrosses							
$TC_{1\,1}$	$P_4 \times (P_2 \times P_1)$	14	3	3	1	0.49	0.50–0.30
$TC_{1\,2}$	$(P_2 \times P_1) \times P_4$	12	6	3	1	0.66	0.50–0.30
$TC_{1\,1}$+$TC_{1\,2}$	Pooled reciprocal data	26	9	3	1	0.00	>0.95
$TC_{1\,3}$	$P_4 \times (P_2 \times P_3)$	16	4	3	1	0.26	0.70–0.50

[a] Resistant (R), fewer than 25 egg masses per root system
[b] Susceptible (S), 25 or more egg masses per root system

population of *L. peruvianum* with distorted segregation (Table 4).

Discussion

Significant differences in the level of reproduction of *M. javanica* isolates were observed among accessions of several wild *Lycopersicon* species relative to the susceptible controls. The reproduction levels on control plants indicated that the inoculation procedures and the experimental conditions were adequate. These results indicate that the majority of selected accessions, representing *L. chmielewskii*, *L. penellii* Corr., *L. hirsutum* Humb. and Bonpl., *L. pimpinellifolium* (Jusl.) Mill. and *L. cheesmanii* Riley, do not include promising sources of resistance to the root-knot isolates that were used. However, one *L. chilense* Dun. selection, accession LA 2884, has a very promising heat-stable resistance trait to *M. javanica* isolate 811, expressed at 32°C, which needs to be characterized further (Table 1). *L. chilense* is the southern-most distributed species of the genus *Lycopersicon*; it may have co-evolved with root-knot nematode populations at high temperatures and, as a result, apparently possesses heat-stable nematode resistance for its survival. *L. chilense* hybridizes with *L. peruvianum* and these species form the 'peruvianum-complex' (Rick and Lamm 1955). One may speculate that the resistance to root-knot nematodes in *L. chilense* and *L. peruvianum* may have originated in a common ancestor of the 'peruvianum-complex'. It was interesting to find low parasitism rates of the warm climate root-knot species *M. javanica* on a southern *L. chilense* accession, but we do not know the relationship of the *L. chilense* heat-stable resistance to the novel heat-stable resistance in *L. peruvianum*. From a breeding perspective, *L. chilense* has the advantage that it hybridizes fairly readily with the cultivated tomato *L. esculentum*, compared with *L. peruvianum* (Rick and Lamm 1955).

With the use of embryo-rescue culture (Cap et al. 1991) and the bridge-lines developed by Poysa (1990), additional novel resistance traits to *M. javanica* from *L. peruvianum* PI 270435 clone 2R2 and PI 126443 clone 1MH that differ from the heat-sensitive *Mi* gene have been incorporated into a partial *L. esculentum* background. Data from resistance screening suggest that the expression of the new resistance to *M. javanica* may be partially affected by the *L. esculentum* background, because the level of nematode reproduction on one embryo-rescue hybrid (ms-1× PI 126443 clone 1MH) was higher (i.e., partially susceptible) than on the resistant parent and on the other bridge-line hybrids derived from the same resistant parent (Table 2). This same embryo-rescue hybrid expresses resistance to *M. incognita* at 32°C (Cap et al. 1991; Veremis and Roberts 1996a) and is known to possess genes *Mi* (heat-sensitive), *Mi-3* and *Mi-5* (heat-stable) for resistance to *M. incognita* (Veremis and Roberts 1996b). Cap et al. (1993) suggested that the heat-unstable resistance factor in PI 126443-1MH is allelic to, or the same as, the gene *Mi*. Clones of hybrid ms-1×PI 126443-1MH were partially susceptible at 25°C when *Mi* should be expressing resistance, and at 32°C when *Mi-5* should be expressing resistance to *M. javanica*. There is some evidence that the *Mi* region may actually contain more than one resistance allele (Sidhu and Webster 1981) which differentiate (a)virulent phenotypes (Netscher 1978). The susceptibility of the hybrid to isolates 811, Cox-Perez and Bettencourt, indicated the presence of an unknown factor for resistance that is different from *Mi* in clone PI 126443-1MH. The *M. javanica* isolate 811 was able to reproduce on one F_1 hybrid produced from PI 270435-2R2 when screened at 32°C, but not on the parent and the other hybrids produced from it. The factor conferring heat-unstable resistance to *M. javanica* in EPP-2×PI 270435-2R2 (fully resistant to isolate 811 at 25°C but susceptible at 32°C) has a phenotype that is similar to the gene *Mi*, but not to the gene *Mi-2* that is also present in this hybrid and confers heat-stable resis-

Table 5 A summary of genetic interactions of *M. javanica* isolates 811, Bettencourt, and Cox-Perez with resistance genes in selected *L. peruvianum* clones and hybrids

Tomato accession clones and hybrids	Nematode isolates		
	811		Bettencourt and Cox-Perez
	At 25°C	At 32°C[a]	at 25°C
LA 1708-I	R[b]	R	R
PI 270435-3MH	R	R	R
PI 270435-2R2	R	R	R
Hybrids of 270435-2R2			
UC82×270435-2R2	R	R	R
EPP-1×270435-2R2	R	R	R
EPP-2×270435-2R2	R	S	R
PI 126443-1MH	R	R	R
Hybrids of 126443-1MH			
126443-1MH×EPP-1	R	R	R
EPP-1×126443-1MH	R	R	R
ms-1×126443-1MH	Partial[c]	Partial	Partial

[a] Resistance at 32°C indicates heat-stability
[b] Resistant (R): Susceptible (S)
[c] Partial susceptibility about 30 egg masses per root system

tance to *M. incognita* isolates (Veremis and Roberts 1996a) (Table 1).

Table 5 is a summary of the findings concerning the genetic basis of resistance to *M. javanica* in *L. peruvianum* clones and hybrids. This summary is based on an interpretation of the differential expression of resistance in genotypes at moderate and high temperature, and against different *M. javanica* isolates. The results obtained with the F_1 bridge and embryo hybrids at 25°C (*Mi* expressed) and at 32°C (*Mi* not expressed) indicate that the heat-stable resistance to *M. javanica* isolates in clones PI 270435-2R2 and PI 126443-1MH is in a dominant state in each accession (Table 5). The *Mi*-avirulent *M. javanica* isolate 811 was able to reproduce on one progeny of PI 270435-2R2, the bridge-hybrid EPP-2×PI 270435-2R2, but not on the embryo-rescue hybrid UC82×PI 270435-2R2 when screened at 32°C soil temperature. However, another differential reaction was also observed in the other resistant donor parent PI 126443-1MH. The F_1 hybrid of ms-1×PI 126443-1MH was partially susceptible when screened at normal soil temperature with *M. javanica* isolates 811, Cox-Perez and Bettencourt, but the parent PI 126443-1MH and the other hybrids were resistant, indicating different resistance factors inherited from the resistant parental clones (Table 2).

The field-produced F_2 generation was examined to further evaluate resistance to the *Mi*-avirulent *M. javanica* isolate 811 in clone PI 126443-1MH. A segregation ratio of 3:1 (resistant:susceptible) was determined at 32°C soil temperature; this ratio is expected for a single dominant gene expressed at high temperature within the resistant clone PI 126443-1MH (Table 3). In a related study, when the same F_2 individual cloned plants were challenged with *Mi*-virulent and *Mi*-avirulent *M. incognita* isolates at 32°C, they also segregated in a 3:1 (R:S) ratio indicating the expression of a single dominant gene (Veremis 1995; Veremis and Roberts 1996b).

In order to determine the number of genes conferring resistance to the *Mi*-avirulent *M. javanica* isolate 811 in clones of *L. peruvianum* PI 270435-2R2 and PI 270435-3MH, the clones were test-crossed with the susceptible clone *L. peruvianum* PI 126440-9MH. If the heat-stable resistance to *Mi*-avirulent *M. javanica* isolate 811 was conferred by different genes there should be a gamete combination in the TC_1-derived progeny of the double recessive condition of susceptibility. When soil temperature was high and *Mi* was not expressed, TC_1 populations segregated for resistance in a ratio close to 3:1 (R:S) when challenged with the *Mi*-avirulent *M. javanica* isolate 811 at 32°C (Table 4). This ratio is expected for the presence of a separate factor with a major effect conferring resistance to *Mi*-avirulent *M. javanica* isolate 811 within each of the parental resistant clones. Similar conclusions were obtained from the study of genes conferring resistance to *Mi*-(a)virulent *M. incognita* isolates in the same resistant parental clones (Veremis 1995; Veremis and Roberts 1996b). Disturbed segregation is known in *L. peruvianum* (Sandbrink et al. 1995), but the null hypothesis in this case is no segregation at all. The novel traits for heat-stable resistance to *Mi*-avirulent *M. incognita* are also similar phenotypically in the two parental clones, but are controlled by different genes, *Mi-2* in clone 270435-2R2 and *Mi-6* in clone 270435-3MH (Veremis 1995; Veremis and Roberts 1996b).

The relationship between the heat-stable genes for resistance to the *Mi*-(a)virulent *M. incognita* isolates and factors for resistance to the *Mi*-avirulent *M. javanica* isolate was examined in the *L. peruvianum* resistant clones PI 270435-2R2, PI 270435-3MH and PI 126443-1MH. Clones of the same interspecific hybrids, F_2 and TC_1 individual plants that were classified for resistance to *M. incognita* were also screened with *M. javanica*. If the same gene expresses the heat-stable resistance to *Mi*-avirulent *M. javanica* isolates and resistance to *Mi*-(a)virulent *M. incognita* isolates, there should be no difference in expression within the same cloned individual plants of the hybrids, $TC_{1\text{-}1}$, $TC_{1\text{-}2}$, $TC_{1\text{-}3}$, and F_2 progenies. Assuming that the gene conferring heat-stable resistance to *Mi*-avirulent *M. javanica* isolate 811 is tightly linked to, or the same as, the gene for heat-stable resistance to *M. incognita*, the cloned individuals will contain one dominant allele for the expression of both phenotypes. Of seven interspecific hybrid plants obtained from PI 270435-2R2 and PI 126443-1MH that were resistant to *M. incognita* (Veremis and Roberts 1996a), two were susceptible and five were resistant to *M. javanica* (Table 6). Additional evidence for differences between the genes conferring resistance to *M. incognita* and *M. javanica* were found from dual screenings of F_2 and TC_1 individual plants. From 26 F_2 and 56 TC_1 cloned plants that were resistant to *M. incognita* (Veremis and Roberts 1996b) 5 F_2 and 8 TC_1 plants were susceptible to *M. javanica* (Table 6). Thus, a total of 15 individual

Table 6 A summary of assigned resistance phenotypes at 32°C based on double screening of individual plants from interspecific hybrids of PI 270435-2R2 and PI 126443-1MH. F_2, $TC_{1\,1}$ and $TC_{1\,2}$ segregating progenies by use of vegetative propagation

Tomato genotype	Number of plants[a]	*M. incognita* Project 77	*M. javanica* 811
Interspecific embryo and bridge-line hybrids	5	R[b]	R
of PI 270435-2R2 and PI 126443-1MH	2	R	S
F_2 progeny of	21	R	R
PI 126443-1MH×EPP-1	5	R	S
$TC_{1\,1}$, $TC_{1\,2}$ and $TC_{1\,3}$[c]	48	R	R
	8	R	S

[a] Only plants screened for both phenotypes are included; Veremis (1995) and Veremis and Roberts (1996a, b)
[b] Resistant (R)=nematode cannot develop and reproduce; Susceptible (S)=nematode can develop and reproduce
[c] $TC_{1\,1}$ is PI 126440-9MH×[PI 270435-3MH×PI 270435-2R2]; $TC_{1\,2}$ is [PI 270435-3MH×PI 270435-2R2]×PI 126440-9MH; $TC_{1\,3}$ is PI 126440-9MH×[PI 270435-3MH×PI 126443-1MH]

cloned plants from the hybrid, $TC_{1\,1}$, $TC_{1.2}$, $TC_{1.3}$ and F_2 progenies were classified as resistant to *M. incognita*, but were susceptible to *M. javanica* (Table 6). These results confirm that resistance to the two root-knot species is expressed by different genes. This is in agreement with a double screening of a backcross population of PI 126443-1MH and PI 126440-9MH, of which six individual plants were susceptible to the *M. javanica* isolate at 32°C but resistant to the *Mi*-virulent *M. incognita* isolate (Yaghoobi et al. 1995). Thus, specific differences occur between the factors governing the resistance to *M. javanica* and the resistance to *M. incognita*.

The partial susceptibility to *M. javanica* of ms-1×PI 126443-1MH and its high resistance to *M. incognita* indicate the presence of different factors for resistance to these two nematode species, and more detailed genetic evaluation is needed. The results from the other hybrids and the TC_1 and F_2 progenies indicate that the clone PI 126443-1MH possesses a single, independent, dominant gene of major effect which confers resistance to *M. javanica* at 32°C (Table 5). The temperature interaction with the *Mi* genotype in *Lycopersicon* has been reported previously to occur at high-temperature regimes (Dropkin 1969); however, other heat-unstable resistance genes to *M. javanica* may exist with different environmental influences in their expression. The *Mi* allele if present in PI 126443-1MH may be unstable to *M. javanica* at 25°C. Our studies demonstrate that PI 270435-2R2 also possesses heat-unstable resistance to *M. javanica* (Table 5). The diversity of virulence in root-knot populations might be related with a long-term host-parasite relationship and evolution. The divergence of duplicated resistance genes in tomato may have provided an opportunity to acquire resistance. The interaction of novel resistance to *M. javanica* and the other root-knot species in these genotypes of *L. peruvianum* may indicate a divergence of duplicated genes. A cluster of resistance genes with different specificity to root-knot virulence may be present in *Lycopersicon*, which is similar to the genetic complexity found with fungal pathogen systems (Pryor and Ellis 1993). A greater complexity of the host-parasite interaction was suggested in the study of *M. arenaria* and *M. incognita* by the presence of several differential interactions between *Lycopersicon* spp. and *Meloidogyne* spp. (Veremis 1995; Veremis and Roberts 1996a).

The novel heat-stable resistance to *M. javanica* isolates in the *L. peruvianum* complex is different from the *Mi* gene and could be very useful for the future of tomato production. The availability of diverse sources of resistance to root-knot nematodes in *L. esculentum* genotypes is an imperative goal. In tropical, subtropical and warm-temperate (Mediterranean) areas, for example the Greek islands of Crete and Cyprus where *Mi*-gene resistant tomato cultivars are overcome by *M. javanica* because of high soil temperatures (Philis and Vakis 1977; Tzortzakakis and Gowen 1996), the heat-stable *M. javanica* resistance could have an important management utility. Inherently durable resistance would not require changes in the structure of agricultural activities, but would require substitution of the currently used cultivar. Where cultivars with the *Mi* gene are used extensively, additional resistance to *M. javanica* could be effective in suppressing the occurrence of virulent nematode populations selected on heat sensitive *Mi* gene bearing plants and so eliminate the shifting of populations in the field.

References

Ammati M, Thomason IJ, Roberts PA (1985) Screening *Lycopersicon* spp. for new genes imparting resistance to root-knot nematodes (*Meloidogyne* spp.). Plant Dis 69:112–115

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

Cap GB (1991) Inheritance of heat-stable resistance to *Meloidogyne incognita* in *Lycopersicon peruvianum*. PhD thesis, University of California, Riverside

Cap GB, Roberts PA, Thomason IJ, Murashige T (1991) Embryo culture of *Lycopersicon esculentum* × *L. peruvianum* hybrid genotypes possessing heat-stable resistance to *Meloidogyne incognita*. J Am Soc Hort Sci 116:1082–1088

Cap GB, Roberts PA, Thomason IJ (1993) Inheritance of heat-stable resistance to *Meloidogyne incognita* in *Lycopersicon peruvianum* and its relationship to the *Mi* gene. Theor Appl Genet 85:777–783

Castagnone-Sereno P (1994) Genetics of *Meloidogyne* virulence against resistance genes from Solanaceous crops. In: Lamberti F, De Giorgi C, Bird D McK (eds) Advances in molecular plant nematology. Plenum Press, New York, pp 261–276

Dropkin VH (1969) The necrotic reaction of tomatoes and other hosts resistant to *Meloidogyne*: reversal by temperature. Phytopathology 59:1632–1637

Eisenback JD (1985) Diagnostic characters useful in the identification of the four most common species of root-knot nematodes (*Meloidogyne* spp.). In: Sasser JN, Carter CC (eds) An advanced treatise on *Meloidogyne*. Biology and control. North Carolina State University Graphics 1:95–112

Hartman KM, Sasser JN (1985) Identification of *Meloidogyne* species on the basis of differential host test and perineal pattern morphology. In: Barker KR, Carter CC, Sasser JN (eds) An advanced treatise on *Meloidogyne*. Methodology. North Carolina State University Graphics 2:69–78

Holtzmann OV (1965) Effect of soil temperature on resistance of tomato to root-knot nematode (*Meloidogyne incognita*). Phytopathology 55:990–992

Hussey RS, Barker KR (1973) A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Dis Rep 57:1025–1028

Netscher C (1978) Morphological and physiological variability of *Meloidogyne* in west Africa and implications for their control. Mededelingen Landbouwhogeschool, Wageningen 78–3:1–46

Omwega CO, Thomason IJ, Roberts PA (1988) A non-destructive technique for screening bean germ plasm for resistance to *Meloidogyne incognita*. Plant Dis 72:970–972

Philis J, Vakis N (1977) Resistance of tomato varieties to the root-knot nematode *Meloidogyne javanica* in Cyprus. Nematol Medit 5:39–44

Poysa V (1990) The development of bridge-lines for interspecific gene transfer between *Lycopersicon esculentum* and *L. peruvianum*. Theor Appl Genet 79:187–192

Pryor T, Ellis J (1993) Genetic complexity of fungal genes. Adv Plant Pathol 11:281–305

Rick CM, Lamm R (1955) Biosystematic studies on the status of *Lycopersicon chilense*. Am J Bot 42:663–675

Rick CM, Borgnino FH (1989) A method for improving seed germination of solanaceous species. Dept Vegetable Crops, University of California, Davis

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

Roberts PA, Thomason IJ (1989) A review of variability in four *Meloidogyne* spp. measured by reproduction on several hosts including *Lycopersicon*. Agric Zool Rev 3:225–252

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

Sandbrink JM, van Ooijen JW, Purimahua CC, Vrielink M, Verkerk R, Zabel P, Lindhout P (1995) Localization of genes for bacterial canker resistance in *Lycopersicon peruvianum* using RFLPs. Theor Appl Genet 90:444–450

Sasser JN (1977) Worldwide dissemination and importance of the root-knot nematodes, *Meloidogyne* spp. J Nematol 9:26–29

Sidhu GS, Webster JM (1981) Genetics of plant nematode interactions. In: Zuckerman BM, Rohde RA (eds) Plant parasitic nematodes. Academic Press, London, 3:61–83

Smith PG (1944) Embryo culture of a tomato species hybrid. Proc Am Soc Hort Sci 44:413–416

Trudgill DL (1994) Host and plant temperature effects on nematode development rates and nematode ecology. Nematologica 41:398–404

Tzortzakakis EA, Gowen SR (1996) Occurrence of a resistance-breaking pathotype of *Meloidogyne javanica* on tomatoes in Crete, Greece. Fund Appl Nematol 19:283–288

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

Veremis JC, Roberts PA (1996a) Differentiation of *Meloidogyne incognita* and *M. arenaria* novel resistance phenotypes in *Lycopersicon peruvianum* and derived bridge-lines. Theor Appl Genet 93:960–967

Veremis JC, Roberts PA (1996b) Relationships between *Meloidogyne incognita* resistance genes in *Lycopersicon peruvianum* differentiated by heat sensitivity and nematode virulence. Theor Appl Genet 93:950–959

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 LD (1992) Problems and strategies associated with long-term use of nematode-resistant cultivars. J Nematol 24:228–233