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Reproduction of *Mi*-Virulent *Meloidogyne incognita* Isolates on *Lycopersicon* spp.[1]

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Abstract: Selection of detectable numbers of *Mi*-virulent root-knot nematodes has necessitated a greater understanding of nematode responses to new sources of resistance. During the course of this research, we compared the reproduction of four geographically distinct *Mi*-virulent root-knot nematode isolates on three resistant accessions of *Lycopersicon peruvianum*. Each accession carried a different resistant gene, *Mi-3, Mi*-7, or *Mi-8*. All nematode isolates were verified as *Meloidogyne incognita* using diagnostic markers in the mitochondrial genome of the nematode. Reproduction of *Mi*-virulent isolates W1, 133 and HM, measured as eggs per g of root, was greatest on the *Mi-7* carrying accession and least on the *Mi-8* carrying accession. In general, *Mi-3* behaved similar to the *Mi-8* carrying accession. Reproduction of the four nematode isolates was also compared on both *Mi* and non-*Mi*-carrying *L. esculentum* cultivars and a susceptible *L. peruvianum* accession. Resistance mediated by *Mi* in *L. esculentum* still impacted the *Mi*-virulent nematodes with fewer eggs per g of root on the resistant cultivar ($P \leq 0.05$). Preliminary histological studies suggests that *Mi-8* resistance is mediated by a hypersensitive response, similar to *Mi*.

Key words: Lycopersicon esculentum, L. peruvianum, Meloidogyne spp., *Mi*-virulent root-knot nematodes, reproduction, resistance mechanism, tomato.

Root-knot nematodes, *Meloidogyne* spp., are important agricultural pests worldwide that cause severe damage of many cultivated plant species including tomato, *Lycopersicon esculentum* Mill. Currently, all commercially available root-knot nematode resistance in tomato cultivars is conferred by the single dominant gene *Mi* (Medina-Filho and Tanksley, 1983). *Mi* was originally identified in wild tomato, *L. peruvianum,* and introgressed into cultivated tomato using embryo rescue of the F1 from the cross between *L. esculentum* cv. Michigan State Forcing and *L. peruvianum* USDA Accession (Acc.) PI 128657 (Smith, 1944). The *Mi* gene confers resistance to three major *Meloidogyne* spp., *M. incognita, M. javanica,* and *M. arenaria*. However, not all *Meloidogyne* spp. are avirulent on *Mi* including *M. hapla* (Ammati et al., 1985). The resistance mediated by *Mi* is negatively affected by plant hormone applications and by soil temperatures above 28 °C (Ammati et al., 1986; Dropkin, 1969a).

Over the years, virulent root-knot nematode isolates that are able to reproduce on *Mi*-carrying tomato cultivars have been reported (Berthou et al., 1989; Bost and Triantaphyllou, 1982; Castagnone-Sereno et al., 1993; Riggs and Winstead, 1959; Roberts et al., 1990; Roberts and Thomason, 1989; Sikora et al., 1973; Triantaphyllou and Sasser, 1960; Viglierchio 1978; Prot, 1984). In recent years, the emergence of two *Mi*-virulent root-knot nematode populations, from different geographical locations in California, were also reported (Kaloshian et al., 1996). Some of these *Mi*-virulent isolates have been selected on *Mi*-carrying tomato, either by continuous propagation of avirulent isolates (Castagnone-Sereno et al., 1993) or isolated from tomato roots from fields following repetitive planting of tomato cultivars carrying the *Mi* gene (Kaloshian et al., 1996). However, other isolates have been identified with inherent ability to parasitize tomato with *Mi* (Berthou et al., 1989; Bost and Triantaphyllou, 1982; Riggs and Winstead, 1959; Roberts and Thomason, 1989; Sikora et al., 1973; Triantaphyllou and Sasser, 1960; Viglierchio 1978; Prot, 1984).

Additional sources of resistance to *M. incognita, M. javanica, M. arenaria,* and *M. hapla* were identified in *L. peruvianum* Acc. PI 126443 clone 1MH, PI 270435 clones 3MH and 2R2 (Ammati et al., 1986). Unlike *Mi,* the resistance in these accessions was heat-stable at 32 °C. However, these plants were less resistant to an aggressive isolate of *M. javanica*. Roberts et al. (1990) reported that an F1 hybrid from a cross between *L. peruvianum* Acc. PI 126443 clone 1MH and *L. peruvianum* Acc. PI 270435 clone 3MH was resistant to one *Mi*-virulent *M. incognita* isolate but susceptible to another *Mi*-virulent *M. incognita* isolate. Both nematode isolates were selected on *Mi*-carrying tomatoes. The F1 was also susceptible to naturally occurring *Mi*-virulent isolates of *M. incognita* and *M. arenaria* (Roberts et al., 1990). In addition, *L. peruvianum* Acc. PI 126443 clone 1MH was resistant to *Mi*-virulent *M. incognita* isolate 557R (Yaghoobi et al., 1995). Similarly, *L. peruvianum* Acc. PI 270435 clones 3MH and 2R2 were resistant to isolate 557R (Veremis and Roberts, 1996). Resistance to *Mi*-virulent root-knot nematodes in these three accessions of *L. peruvianum* is mediated by three distinct genes (Veremis and Roberts, 1996). Resistance in Acc. PI 126443 clone 1MH is mediated by *Mi-3,* which is localized to chromosome 12 (Yaghoobi et al., 1995), whereas resistance in Acc. PI 270435 clones 3MH and

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2R2 are mediated by *Mi-7* and *Mi-8,* respectively (Veremis and Roberts, 1996). The chromosomal locations of these genes are not known.

Plant resistance to a pathogen is manifested in a variety of ways and is often correlated with a hypersensitive response (HR). Hypersensitive response typically results in localized cell death in the host plant at the site of infection, which is thought to be responsible for limiting pathogen growth (Staskawicz et al., 1995). Even though root-knot nematode resistant interaction has been reported in the absence of HR (Bendezu and Starr, 2003; Potenza et al., 1996), the most common host resistance involves HR in the cells where the infective stage juvenile attempts to feed (Dropkin, 1969b; Frazier and Dennett, 1949; Kaplan and Davis, 1987; Kaplan and Keen, 1980).

To evaluate the breadth of the resistance in resistant accessions of *L. peruvianum* to *Mi*-virulent *Meloidogyne* spp., and compare the virulence of these nematode isolates on both *Mi*- and non-*Mi*-carrying *L. esculentum* cultivars, we assessed the reproduction of four *Mi*-virulent root-knot nematode isolates on these hosts. In addition, the mechanism of the resistance to *Mi*-virulent *M. incognita* in *L. peruvianum* PI 270435 clone 2R2 mediated by *Mi-8* was analyzed.

Materials and Methods

Plant material and growth conditions: The genotypes used in the study were *L. peruvianum* Acc. PI 126443 clone 1MH, and Acc. PI 270435 clones 2R2 and 3MH, all resistant to *Mi*-virulent root-knot nematodes at soil temperatures of 25 °C (Ammati et al., 1985; Ammati et al., 1986; Cap et al., 1993); Acc. PI 126440 clone 9MH, susceptible to *Mi*-virulent root-knot nematodes at 25 °C (Ammati et al., 1986); *L. esculentum* cv. VFN (*Mi/Mi*) and cv. UC82B (*mi/mi*), both susceptible to *Mi-1*-virulent root-knot nematodes at 25 °C. The susceptible *L. peruvianum* Acc. PI 126440 clone 9MH was included in these experiments because it is used as the susceptible parent in our genetic crosses to map the resistance genes in PI 270435 clones 2R2 and 3MH. Seeds of *L. esculentum* cvs. were planted in Sunshine mix (SunGro Horticulture, Bellevue, WA) in a flat and allowed to grow for 4 weeks before seedlings were transplanted to sand in 1-liter plastic cups with one plant/pot. Transplanted seedlings were allowed to grow for an additional 2 weeks before nematode inoculation. *Lycopersicon peruvianum* material from different genotypes were propagated agamically (Cap et al., 1993). Cuttings of three nodes in length from young stems were used. One cutting/cup for PI 270435 clones 2R2 and 3MH and 2 cuttings/cup for PI 126443 clone 1MH and PI 126440 clone 9MH were propagated. Two cuttings/cup for these accessions were necessary because of the poor root growth of these plants. Cuttings were allowed to grow 6 to 8 weeks to establish adequate root systems before nematode inoculation. Tomato seedlings and cuttings were fertilized with Osmocote (17-6-10) (Sierra Chemical Company, Milpitas, CA) and biweekly with Tomato MiracleGro (Stern's MiracleGro Products, Port Washington, NY), and maintained in a greenhouse at 22 to 25 °C.

Nematode isolates and inoculum preparation: Four *Mi*-virulent *Meloidogyne* isolates, W1, 133, 246R, and HM, were used. These nematodes were collected and maintained in the laboratory of V. M. Williamson at the University of California, Davis. All *Mi*-virulent nematode isolates were maintained in the greenhouse on *L. esculentum* cv. VFN carrying the *Mi* gene. Cultures of *Mi*-avirulent *M. incognita* and *M. javanica* were maintained on UC82B (*mi/mi*) tomato.

To obtain nematode inoculum, eggs were extracted from infected roots by processing in 0.52% NaOCl in a Waring blender, for 2 minutes at high speed (Hussey and Barker, 1973). Eggs and root debris were collected on a 25-µm-pore sieve. Second-stage juveniles (J2) were obtained by hatching the eggs in modified Baermann funnels. Wire mesh baskets were lined with two layers of paper towels, set in a glass petri dish, filled with the egg mixture, and incubated at 25 °C. Nematodes were collected in water every 48 hours and used immediately or stored at room temperature for an additional 48 hours with aeration.

Nematode identification using molecular markers: Meloidogyne spp. identification was performed using diagnostic markers developed by Powers and Harris (1993) from the mitochondrial genome of the nematode. Previously described protocols (Powers and Harris, 1993) were performed with slight modifications. Single J2 of each isolate were transferred with an insect pick (minuetin) to 10 µl sterile water in a petri dish and the nematode was crushed using the tip of the minuetin. The nematode lysate was immediately transferred to a 15-µl PCR amplification mixture containing 0.8 µM each of primer pair C2F3 and 1108 (Powers and Harris, 1993), 2 mM $MgCl_2$, 200 µM dNTP, 1× PCR buffer, and 1.0 unit *Taq* polymerase (Promega, Madison, WI) in a 0.2-ml microcentrifuge tube placed on ice. Samples were placed into the thermocycler PTC-200 (MJ Research, Watertown, MA) already set at 94 °C. The amplification program included a denaturation step of 3 minutes at 94 °C followed by 45 cycles of the following steps: 1 minute at 94 °C, 45 seconds at 48 °C, and 2 minutes at 70 °C. The last cycle was followed by 5 minutes at 72 °C to complete the extension of any partially synthesized second strands. The PCR products were then digested with 2 units of *Hinf* I (Promega) at 37 °C for 2 hours, and 5 to 10 µl was resolved and visualized on a 1.5% agarose gel in TBE buffer (100 mM Tris, 100 mM Boric acid, 2 mM EDTA) by ethidium bromide staining (0.5 µg/ml) and examined by UV transillumination. At least 10 individual J2 from each of the four virulent nematode isolates were used as templates in PCR. Second-

stage juveniles from known isolates of *M. incognita* and *M. javanica* also were included.

Nematode virulence assays: For the virulence test, plants were inoculated with 3,000 J2 using a modified pipet (Martinez de Ilarduya et al., 2001). Each combination of host genotype and nematode isolate was replicated five times. After inoculation, the plants were distributed in a completely randomized design on a greenhouse bench and maintained at 22 °C to 25 °C for 6 weeks. The accumulated degree-days during this period ensured the completion of one root-knot nematode generation (Tyler, 1933). The plants were then uprooted. Roots were washed, fresh weights of the roots were recorded, and eggs were extracted from individual root systems using the bleach- and blending-method described above. Egg suspensions were stored at 5 °C and the volume in each sample was brought to 200 ml before counting. After appropriate dilution, eggs in two 1-ml aliquots from each sample were counted, using a dissecting microscope, and the number of eggs per sample calculated. The experiment was repeated, and a representative data set was used for further analysis. Eggs per g of root data were $\log_{10}$ (x+1) transformed to stabilize the variances. Variable means were separated by the least significant difference *t*-test at the 0.05 probability level (SAS Institute Inc., Cary, NC).

Histology: For histological analysis, 4-week-old rooted cuttings of *L. peruvianum,* Acc. PI 270435 clone 2R2 and PI 126440 clone 9MH, were inoculated with root-knot nematode *Mi*-virulent isolate W1 at 25 °C, and then destructively sampled 1, 2, 3, 14, and 30 days after inoculation. As a positive control for hypersensitive response, VFN tomato seedlings were infected with VW4, an *Mi*-avirulent root-knot nematode. To visualize the nematodes inside the roots, tissue was first cleared in 0.5% NaOCl for 1 and 5 minutes for *L. peruvianum* and VFN roots, respectively. Roots were rinsed in water and nematodes were then stained by briefly boiling in 1:30 dilution of acid fuchsin staining solution (3.5g acid fuchsin in 250 ml of acetic acid and 750 ml of H_2O) in a microwave (Hussey, 1990). Plant tissue was destained in a solution of equal volume of acetic acid:glycerol:water. Stained roots were examined with a stereo microscope to identify infection sites. Root segments containing infection sites were mounted in 50% glycerol and examined using differential interference contrast optics on a Leica DMR compound microscope. Images were captured using a SpotRT digital camera (Diagnostics Instruments, Sterling Heights, MI).

Results

Nematode identification using molecular markers: The origin of the nematodes used in this study and the virulence against tomatoes containing the *Mi* gene are listed in Table 1. Since the *Mi* gene confers resistance to *M. incognita, M. javanica,* and *M. arenaria,* but not to *M. hapla,* we identified the species of the *Mi*-virulent root-knot nematodes. Using primer pair C2F3 and 1108, a 1.7-kb DNA fragment was amplified in all reactions indicating that the *Mi*-virulent root-knot nematodes were either *M. incognita* or *M. javanica* but not *M. arenaria* or *M. hapla* (data not shown). Restriction digestion with *Hinf* I of the PCR products of the controls resulted in two fragments of 1.32 kb and 0.38 kb for *M. incognita,* whereas *M. javanica* remained uncut (Fig. 1). Restriction digests of all *Mi*-virulent isolates resulted in two fragments of 1.32 kb and 0.38 kb, indicating that they were all *M. incognita* (Fig. 1).

Table 1. *Meloidogyne* spp. isolates and their virulence against *Mi* gene in tomato.

Isolate	Origin	Virulence on *Mi*
W1	Woodland, California	field selected
133	Kettleman City, California	field selected
HM	Harris Ranch, California	field selected
246R	T. Triantaphyllou, North Carolina State University	selected, unknown origin
VW4	V. M. Williamson, University of California, Davis	avirulent

Reproduction of Mi-virulent root-knot nematode isolates on L. esculentum cultivars and accessions of L. peruvianum: For both tomato genotypes and nematode isolates, analyses of variance found that fresh root weight, number of egg masses per g root, and number of eggs per g of root were significant at the 0.05 probability level. All *Mi*-virulent root-knot nematode isolates reproduced poorly on the three resistant *L. peruvianum* accessions (Table 2; Fig. 2). The number of egg masses per root system was equivalent for *L. peruvianum* Acc. PI 240435 clone 2R2, 3MH and PI 126443 clone 1MH (Table 2). The susceptible *L. peruvianum* Acc. PI 126440 clone 9MH had the smallest root system of all the tomato genotypes ($P = 0.05$) and thus provided the fewest root tips for nematode penetration. Although susceptible to

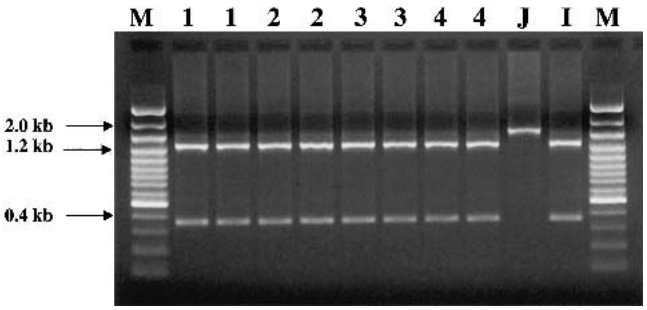

Fig. 1. PCR amplification of mitochondrial DNA from single juveniles of *Mi*-virulent isolates of root-knot nematodes. Single juveniles from four *Mi*-virulent isolates were used in PCR with primers C2F3 and 1108, which flank the intergenic spacer region between the cytochrome oxidase subunit II gene and 16S rRNA gene. After amplification, DNA was digested with *Hinf* I and electrophoresed on a 1.5% agarose gel in TBE buffer. Lanes marked M = GeneRuler 100 bp DNA ladder plus, 1 = W1, 2 = 133, 3 = 246R, 4 = HM, I = *M. incognita* control, J = *M. javanica* control.

TABLE 2. Average number of egg masses of *Mi*-virulent *M. incognita* isolates and average root weight of tomato accessions and cultivars.

Plant	Fresh root weight (g)	Number of egg masses/root			
		246R	W1	133	HM
PI 270435 clone 2R2	27.8 b	1.2 a	1.6 a	0.4 a	1.0 a
PI 126443 clone 1MH	21.1 a	1.6 a	1.6 a	1.2 a	1.5 a
PI 270435 clone 3MH	21.5 a	2.2 a	21.4 a	1.4 a	2.3 a
PI 126440 clone 9MH	19.2 a	99.3 b	85.6 b	108.0 b	74.0 b
VFN	62.2 c	214.0 c	331.0 c	313.8 c	89.0 c
UC82B	70.3 d	485.6 d	633.8 d	535.0 d	228.2 d

Means within a column followed by the same letter do not differ at $P \leq 0.05$.

the *Mi*-virulent isolates, the smaller root system resulted in fewer egg masses than the susceptible *L. esculentum* cultivars (Table 2). The number of egg masses on VFN was lower than UC82 for all nematode isolates.

Eggs per g of root is a more precise measure of nematode reproduction than egg masses per root system. More of the means for eggs per g of root were statistically distinct for both the nematode isolates (Fig. 2) and the tomato genotypes (Fig. 3) than the egg mass per root system data (Table 2). For every nematode isolate there were fewer eggs per g of root on the resistant *L. peruvianum* Acc. PI 240435 clone 2R2, 3MH and the PI 126443 clone 1MH (Fig. 2). In general, the PI 270435 clone 3MH had the highest eggs per g of root among the resistant *L. peruvianum* accessions. For the *L. esculentum* cultivars, nematode isolates W1 and 246R produced more eggs per g of root on UC82B than VFN (Fig. 2). Although nematode isolates 133 and HM did not result in higher number of eggs per g of root on UC82B than VFN (Fig. 2), significant differences were observed with the untransformed data (data not shown).

All four nematode isolates differed in the number of eggs per g of root on the susceptible *L. peruvianum* Acc.

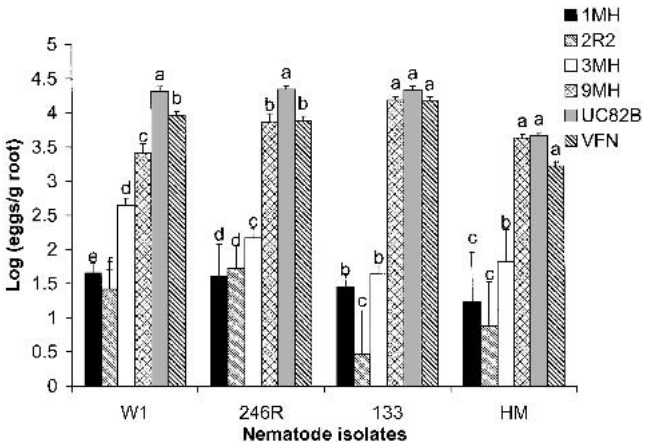

FIG. 2. Reproduction of *Mi*-virulent *Meloidogyne incognita* isolates (133, 246R, HM and W-1) on accessions of *Lycopersicon peruvianum* (1 MH, 2R2, 3MH, and 9MH) and cultivars of *L. esculentum* (UC82B and VFN). Means within the same nematode isolate with the same letter are not different ($P \leq 0.05$).

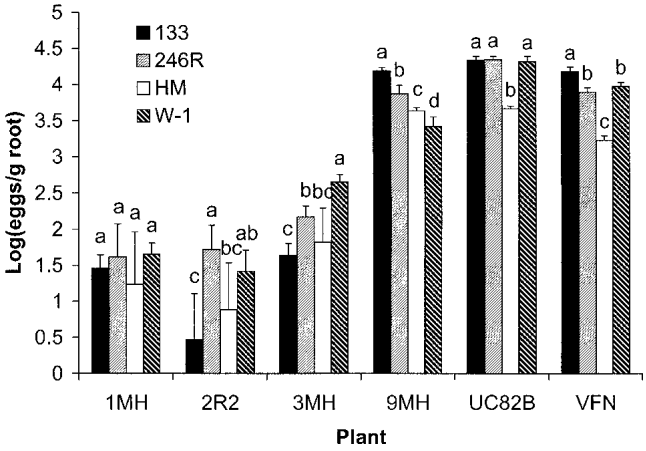

FIG. 3. Reproduction of *Mi*-virulent *Meloidogyne incognita* isolates (133, 246R, HM, and W-1) on accessions of *Lycopersicon peruvianum* (1MH, 2R2, 3MH and 9MH) and cultivars of *L. esculentum* (UC82B and VFN). Means within the same plant accession or cultivar with the same letter are not different ($P \leq 0.05$).

PI 126440 clone 9MH (Fig. 3). All four nematode isolates produced similar numbers of eggs per g of root on PI 126443 clone 1MH. Despite differences in the number of eggs per g of root of the four nematode isolates on PI 240435 clone 2R2 (Fig. 3), this *L. peruvianum* accession had the lowest reproduction of both isolates W1 and 133 among all the genotypes tested (Fig. 2).

Histology: Two days after inoculation, the resistance response in PI 270435 clone 2R2 was manifested as apparent cell death or HR in cells near the head of feeding J2 (Fig. 4A). Cell death began to occur at about the same time after inoculation regardless of whether it was mediated by *Mi-8* or by the *Mi* gene (Fig. 4B). However, in susceptible *L. peruvianum* Acc. PI 126440 clone 9MH, within the same time period, J2 established feeding sites and caused swelling of the roots (Fig. 4C).

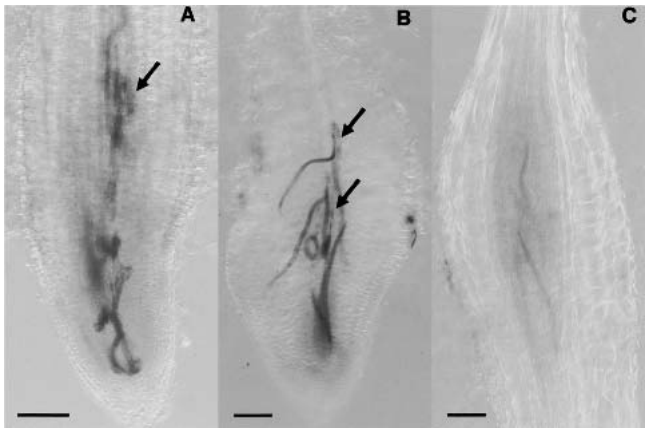

FIG. 4. Resistance to *Mi*-virulent *Meloidogyne incognita* is mediated by hypersensitive response (HR). Whole mounts of roots 2 days after infection with root-knot nematodes stained with acid fuchsin. A, B) Root tips showing HR (arrow) in *Lycopersicon peruvianum* PI 270435 clone 2R2 infected with *Mi*-virulent *M. incognita* (A) and in *L. esculentum* (*Mi/Mi*) infected with *Mi*-avirulent *M. javanica* (B). C) Absence of HR and presence of root swelling in *L. peruvianum* PI 126440 clone 9MH infested with *Mi*-virulent *M. incognita*. Scale bar = 100 µm.

In PI 270435 clone 2R2 roots, HR was also observed at 3 days after inoculation (data not shown). Fourteen and 30 days after inoculation, HR was not seen in roots of VFN or PI 270435 clone 2R2 (data not shown). A few nematodes that were able to develop on the root system of PI 270435 clone 2R2 were third or fourth-stage juveniles at 14 days and mature females at 30 days after inoculation (data not shown).

Discussion

Among the wild relatives of tomato, *L. peruvianum* is a particularly rich source of resistance to root-knot nematodes (Ammati et al., 1986; Veremis and Roberts, 1996; Yaghoobi et al., 1995). Currently, the only commercially available resistance to root-knot nematode is the *Mi* gene. The recent discovery of *Mi* resistance-breaking isolates of root-knot nematodes in California (Kaloshian et al., 1996; Viglierchio, 1978) and other parts of the world (Berthou et al., 1989; Bost and Triantaphyllou, 1982; Castagnone-Sereno et al., 1993; Prot 1984; Sikora et al., 1973; Triantaphyllou and Sasser, 1960) necessitates the incorporation of new sources of resistance into cultivated tomato. Before choosing which genes to pyramid, it is important to identify the breadth of the resistance to make the proper choice for introgressing the most desirable resistance sources.

Our results indicate that all three *L. peruvianum,* Acc. PI 126443 clone 1 MH and PI 270435 clones 2R2 and 3MH, are resistant to the four geographically distinct *Mi*-virulent *M. incognita* isolates. However, some differences in the reproduction of the *Mi*-virulent nematodes on the three accessions were observed. The variation in reproduction on these accessions is not surprising as resistance in these accessions to *Mi*-virulent nematodes is conferred by different single dominant, non-allelic genes (Veremis and Roberts, 1996). Since none of these genes have yet been cloned, their identity and relationship is not known.

Nematode isolate HM reproduced poorly on both tomato varieties, which could be due to its originating as a comparatively weak isolate, as variation in reproduction of root-knot nematodes has been previously reported (Roberts and Thomason, 1986), or that the poor fitness of this particular isolate is the result of the cost of virulence. Unfortunately, no conclusions can be drawn regarding the possible effect of planting history and its selection pressure on the reproductive ability of the HM isolate, as history of the field from which it was isolated is unknown (V. M. Williamson, pers. comm.). However, selected virulence has not been associated with loss of fitness on susceptible tomato cultivars (Castagnone-Sereno et al., 1994; Tzortzakakis and Gowen, 1996). Nematode isolate W-1 was isolated from a field where six crops of tomato had been planted within a 10-year period (Kaloshian et al., 1996). It is possible that selection pressure imposed on the nematode population caused a slow shift in virulence of this population, such that virulence became detectable over a period of time. On the other hand, isolate 133 was isolated from a crop preceded by only two plantings of tomato; therefore, at least a portion of the population must have an inherent capability to infect *Mi*-containing tomato. Planting *Mi*-containing tomato allowed this isolate to become a greater percentage of the overall population in a short period because of its ability to produce viable offspring.

Nematode isolates W1 and 246R reproduced at higher levels on cultivar UC82B (*mi/mi*) than on VFN (*Mi/Mi*). Laboratory selected *Mi*-virulent isolates of *M. incognita* and *M. arenaria* reproduced at similar levels on *L. esculentum* with or without the introgressed *Mi* gene (Riggs and Winstead, 1959). Similarly, no differences were found between the numbers of egg masses produced on resistant vs. susceptible *L. esculentum* by naturally virulent isolates of *M. incognita* and *M. arenaria* (Castagnone-Sereno et al., 1994; Prot, 1984). In contrast, other reports indicated that laboratory selected *Mi*-virulent *M. incognita* isolates reproduced at lower levels on resistant *L. esculentum* compared to susceptible cultivars (Bost and Triantaphyllou, 1982; Castagnone-Sereno et al., 1994). These results suggest that the genetic mechanism for virulence against *Mi* may be different among virulent isolates. In fact, using DNA fingerprinting no correlation was found between molecular diversity and (a)virulence (Semblat et al., 2000; Tzortzakakis and Gowen, 1996). Further, using near-isogenic strains of root-knot nematodes by selecting virulent clones from avirulent progenitors, two different genes have been isolated that are present in the avirulent strain and absent in the virulent, suggesting that there may be more than one *Mi*-avirulence effector (Semblat et al., 2001; Williamson and Gleason, 2003).

All four *Mi*-virulent isolates were identified as *M. incognita.* In agreement with our results, isolates W1 and 133 had been identified previously as *M. incognita* using isozyme patterns (Kaloshian et al., 1996). Interestingly, however, our diagnostic markers for both *M. incognita* and *M. javanica* were consistently lacking one band in comparison with those of Powers and Harris (1993), where the *Hinf* I restriction of the PCR product using the same primers resulted in two bands (1.0 kb and 0.7 kb) for *M. javanica* and three bands (1.0, 0.4, and 0.3 kb) for *M. incognita.* Further investigation has revealed that the additional bands reported by Powers and Harris (1993) may have been the result of unfavorable PCR conditions and annealing of primers to a different position on the DNA template (Powers, pers. comm.). Therefore, our results are considered the norm for identifying *M. incognita* and *M. javanica.*

Our preliminary analysis indicates that resistance mediated by *Mi-8* to *Mi*-virulent *M. incognita* is accompanied by HR. Further detailed histological analysis is required to substantiate this finding. Nonetheless, our

finding indicates that *Mi-8*-mediated resistance may be similar to *Mi*-mediated resistance where cell death is seen near the head of the feeding J2. Recent information showed that cell death could be the cause of the resistance mediated by *Mi* (Hwang et al., 2000). Two NBS-LRR homologs, *Mi-1.1* and *Mi-1.2,* were present in the *Mi* locus (Milligan et al., 1998). However, only one homolog, *Mi-1.2,* conferred resistance to root-knot nematodes. Using chimeric constructs between the functional *Mi-1.2* and the non-functional homolog *Mi-1.1,* Hwang et al. (2000) showed that certain constructs were able to confer nematode resistance while others did not. Using the same constructs in transient expression in *Nicotiana benthamiana* leaves, it was shown that resistance was always associated with cell death (Hwang et al., 2000). In other plant-pathogen systems, cell death has been uncoupled from resistance. One such example in tomato is the *Cf*-mediated resistance against the fungus *Cladosporium fulvum* (Hammond-Kosack and Jones, 1996). Although HR is not always associated with resistance to root-knot nematodes (Bendezu and Starr, 2003; Potenza et al., 1996), our results indicate that in *Lycopersicon* spp., resistance to root-knot nematodes is accompanied with HR.

In summary, there is variation in the ability of the four different *Mi*-virulent *M. incognita* isolates to reproduce on the new sources of resistance in *L. peruvianum.* Therefore, pyramiding genes with different breadth of resistance and different resistance mechanisms is important for developing durable sources of resistance. *Mi-8* seems to have a similar resistance mechanism as *Mi.* In the future, it will be important to determine the mechanism of resistance mediated by *Mi-3* and *Mi-7* to make proper choices for introgression into *L. esculentum.*

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