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# Relationships between *Meloidogyne incognita* resistance genes in *Lycopersicon peruvianum* differentiated by heat sensitivity and nematode virulence

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**Abstract** Resistance to *Meloidogyne incognita* (Kofoid and White) Chitwood in clones of *Lycopersicon peruvianum* (L.) Mill. PI 126443-1MH, 270435-2R2 and 270435-3MH, their $F_1$, a field-produced $F_2$, and their test-cross ($TC_1$) populations, was evaluated based on egg masses and eggs produced on root systems. Reactions to *M. incognita* isolates differing in virulence to gene *Mi* were determined at 25°C (*Mi* expressed) and 32°C (*Mi* not expressed). PI 126443-1MH, 270435-2R2, 270435-3MH, and their $F_1$ progenies were resistant to *Mi*-virulent and *Mi*-avirulent isolates. At 32°C with a *Mi*-avirulent isolate and at 25°C with a *Mi*-virulent isolate, four $TC_1$ generations segregated into resistant: susceptible (R:S) ratios close to 3:1. These results indicated resistance to *Mi*-(a)virulent *M. incognita* isolates is conferred by different non-allelic dominant genes in PI 126443-1MH, 270435-2R2 and 270435-3MH. The $F_2$ progeny of PI 126443-1MH × EPP-1, challenged with *Mi*-avirulent *M. incognita* at 32°C and with *Mi*-virulent *M. incognita* at both 25°C and 32°C, segregated with a ratio of 3:1 (R:S), indicating expression of a single dominant resistance gene in PI 126443-1MH in each case. In dual screenings on clones of the same individual plants from the $TC_1$ and $F_2$ segregating populations, some individual plants were susceptible at 32°C to a *Mi*-avirulent isolate but resistant to the *Mi*-virulent isolate, and *vice versa*, suggesting that different but linked genes confer heat-stable resistance to *Mi*-avirulent *M. incognita* and resistance to *Mi*-virulent *M. incognita*. We propose the symbol *Mi-5* for the gene in PI 126443 clone 1MH and the symbol *Mi-6* for the gene in PI 270435 clone 3MH which both confer resistance to *Mi*-avirulent *M. incognita* isolates at high temperature. We propose the symbol *Mi-7* for the gene in PI 270435 clone 3MH and the symbol *Mi-8* for the gene in PI 270435 clone 2R2 that both confer resistance to the *Mi*-virulent *M. incognita* isolate 557R at moderate (25°C) temperature. The novel resistance genes are linked and reside in a genomic region in each parental clone that is independent from the *Mi* locus.

**Key words** Heat-sensitivity · Virulence · Tomato · Root-knot nematodes · Test-crossing

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## Introduction

Host plant resistance to nematodes is a powerful tool for crop protection, and will play an increasingly important role in managing plant parasitic nematodes, more particularly since the use of many effective nematicides has now been restricted. Root-knot nematodes (*Meloidogyne* spp.) cause severe damage to the tomato (*Lycopersicon esculentum* Mill.) root system (Lamberti 1979), especially in tropical, sub-tropical and warm temperate areas of the world (Sasser 1977). Resistance to root-knot nematodes in commercial tomato cultivars is conferred by the single dominant gene *Mi* (Roberts and Thomason 1989), located on chromosome 6 (Messeguer et al. 1991). Reports of both selected and non-selected *Mi*-virulent nematode populations raise concerns over the durability of *Mi* (Castagnone-Sereno 1994). In addition, the resistance conferred by *Mi* is temperature sensitive, and breaks down above 28°C (Dropkin 1969).

Ammati et al. (1985) identified and cloned plants with new sources of heat-stable resistance in some accessions of the wild tomato *Lycopersicon peruvianum* L. Additional indications of new resistance differing from gene *Mi* were found in hybrids between *L. peruvianum* PI 270435-3MH and *L. peruvianum* var. *glandulosum* 126443-1MH, that were resistant to *M. incognita* isolates selected for virulence on the gene *Mi* (Roberts et al. 1990). Heat-stable resistance to a *Mi*-avirulent *M. incognita* isolate in *L. peruvianum* PI 270435-2R2, PI 270435-3MH, and PI 126443-1MH, was shown to be homozygous dominant, monogenic, and non-allelic to the gene *Mi* in each case (Cap 1991). The

symbol *Mi*-2 was given for the gene conferring this heat-stable resistance to *Mi*-avirulent *M. incognita* in clone PI 270435-2R2 (Cap et al. 1993). Yaghoobi et al. (1995) used a segregating backcross population of PI 126443-1MH to show that resistance to a *Mi*-virulent isolate (557R) of *M. incognita* is controlled by a single dominant gene, located on chromosome 12, and proposed the symbol *Mi*-3 for this resistance gene.

Further understanding of the genetic relationships of the novel nematode resistance traits in *L. peruvianum* will help to determine their potential for crop protection in agricultural areas where *Mi* is not effective due either to high temperature or because of *Mi*-virulent nematode isolates. The objectives of the present study were (1) to determine the nature, number, and interrelationship of genes conferring heat-stable resistance and resistance to *Mi*-virulent *M. incognita* isolates in *L. peruvianum* PI 270435-2R2, PI 270435-3MH, and PI 126443-1MH, by test-crossing; (2) to further test for independent assortment of the nematode resistance genes in *L. peruvianum* PI 126443-1MH, by using $F_2$ progeny developed from a field-selfed $F_1$ hybrid between PI 126443-1MH and a susceptible bridge line EPP-1; and (3) to determine the genetic relationships between the heat-(un)stable resistance to *Mi*-avirulent *M. incognita* and the resistance to a *Mi*-virulent isolate, by dual screening of clones of the same individual plants from $TC_1$ and $F_2$ progenies.

## Materials and methods

### Plant material

*L. peruvianum* PI 270435 clone 2R2 and clone 3MH, PI 126443 clone 1MH, all resistant to *Mi*-avirulent isolates at 25°C and 30°C soil temperature (Ammati et al. 1985, 1986; Cap 1991), PI 126440 clone 9MH, susceptible to *Mi*-avirulent isolates (Ammati et al. 1986; Cap et al. 1993), and susceptible bridge line EPP-1-1 (Veremis 1995) were all obtained from the wild *Lycopersicon* spp. clone collection maintained by the Department of Nematology, University of California (U.C.), Riverside. Parental material was propagated agamically, as described previously (Cap et al. 1993).

Pollen from all parents was collected and stored as described by McGuire (1952). $F_1$ progenies were produced by crossing resistant genotypes *L. peruvianum* PI 270435 clone 3MH×PI 270435 clone 2R2, PI 270435 clone 3MH×PI 126443 clone 1MH, and PI 126443 clone 1MH×PI 270435 clone 2R2 (see Table 1). Crosses with emasculated flowers were made under greenhouse conditions between 11 am to 1 pm. Pollinated ovaries were enclosed with paper bags or capsules to prevent any cross-pollination from other exotic tomato pollen.

Production of test-cross ($TC_1$) seed progeny was carried out in spring, as described above for obtaining $F_1$ seeds. The $F_1$ plants were reciprocally crossed with PI 126440-9MH, which is susceptible to both *Mi*-avirulent (Ammati et al. 1986; Cap et al. 1993) and *Mi*-virulent *M. incognita* isolates. Some crosses set fruit, but not all fruit produced mature seeds. In general, seed production was variable with from very few seeds to about 100 seeds per berry.

In a field at the U.C. Coachella Desert Research Station, Indio, California, cuttings of the PI 126443-1MH×EPP-1, $F_1$ bridge hybrids that do not self under greenhouse conditions, formed copious amounts of fruits with viable $F_2$ seeds (Veremis 1995).

### Nematode cultures

The *Mi*-avirulent *M. incognita* race-3 isolate Project 77 was started from a field population on greenhouse-grown tomato, cv Tropic, plants. The *Mi*-virulent *M. incognita* isolate 557R was provided by Dr. V. Williamson (U.C. Davis) as eggs that were then cultured on greenhouse-grown tomato cv VFN-8 plants. Identities of the nematode isolates were confirmed morphologically by microscopic examination of the perineal patterns of adult females (Eisenback 1985), and by isozyme (esterase and malate dehydrogenase) patterns (Cap et al. 1991). For isolate Project 77, identification was also made by the North Carolina differential host test (Hartman and Sasser 1985).

### Resistance screening

Thirty day-old seedlings or rooted cuttings were used for tests of host reaction to nematodes. Single plants were grown in cone-tainers (Stuewe and Sons Inc., 2290 S. E. Kiger Island Drive, Corvalis, Oregon 97333-9461) filled with steam-sterilized loamy sand, fertilized with Osmocote. Experiments that tested heat-stable resistance were carried out in constant-environment growth chambers. Plants were maintained at 32°C for 7 days before inoculation and 30 days after inoculation, and then placed in a greenhouse environment at 25°C (±3°C). Seedlings of the test-crosses were also transferred to growth pouches (Omwega et al. 1988) and maintained in a growth chamber at 30°C constant temperature. Experiments that tested heat-unstable resistance requiring moderate, rather than high, temperature were carried out in a greenhouse at 25°C (±3°C) or in a growth chamber where temperature was maintained constantly at 25°C.

Inoculum was prepared by the sodium hypochlorite method of Hussey and Barker (1973). In cone-tainer experiments, a water suspension of approximately 7000 infective second-stage juveniles ($J_2$) of *Mi*-avirulent *M. incognita* isolate Project 77, or 3000 $J_2$ of the *Mi*-virulent *M. incognita* isolate 557R, was pipetted into the soil around the plant roots. Plants were arranged in each cone-tainer rack in a completely randomized design. Plants in the growth pouches were inoculated with a suspension of approximately 4000 $J_2$ and the plants were arranged in a completely randomized design within the growth chamber.

Nematode egg masses and egg production on roots were evaluated after the accumulation of approximately 1000 degree days (Trudgill 1994), following the methodology of Omwega et al. (1988) and Cap et al. (1993). At the same time, the plant material was propagated agamically from stem cuttings as described for the parental material above.

Plants were considered susceptible if the number of egg masses was equal to, or greater than, 25 and/or the number of eggs per gram of root was equal to, or greater than, 600. Most of the variability could be explained by differences in the size, morphology and growth habits of *L. peruvianum* segregating populations at the time of inoculation. Susceptible tomato cv Tropic, and VFN-8, which possesses the gene *Mi* and expresses resistance below 28°C, but susceptibility above 28°C (Dropkin 1969), were included to check inoculum viability, infectivity and the expression of heat-sensitive genes.

## Results

### Test-cross experiment at 32°C with the *Mi*-avirulent isolate

Preliminary data from $TC_1$ growth-pouch experiments showed that all plants of the resistant parent clones were resistant, whereas all the clones of parent clone PI 126440-9MH were susceptible, as expected. The $F_1$ progenies were all resistant and the $TC_1$ populations indicated a resistant:susceptible (R:S) segregation ratio of 3:1 (data not shown).

**Table 1** Reaction of *L. peruvianum* parental clones, $F_1$, and test-cross ($TC_1$) progenies for resistance to the *Mi*-avirulent *M. incognita* isolate Project 77 at 32°C according to the nematode egg masses and eggs produced on the roots

| Generation | *L. peruvianum* parent clone or cross | Number of plants | | Expected ratios | | $\chi^2$ | *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 | 20 | | | | |
| $F_1$ progeny | | | | | | | |
| $F_{1\,1}$ | $P_2 \times P_1$ | 15 | 0 | | | | |
| $F_{1\,2}$ | $P_2 \times P_3$ | 15 | 0 | | | | |
| $F_{1\,3}$ | $P_3 \times P_1$ | 15 | 0 | | | | |
| Test-crosses | | | | | | | |
| $TC_{1\,1}$ | $P_4 \times F_{1\,1}$ | 31 | 8 | 3 | 1 | 0.41 | 0.70–0.50 |
| $TC_{1\,2}$ | $F_{1\,1} \times P_4$ | 25 | 3 | 3 | 1 | 2.96 | 0.10–0.05 |
| $TC_{1\,1} + TC_{1\,2}$ | Pooled reciprocal data | 56 | 11 | 3 | 1 | 2.62 | 0.20–0.10 |
| $TC_{1\,3}$ | $P_4 \times F_{1\,2}$ | 68 | 28 | 3 | 1 | 0.88 | 0.50–0.30 |
| $TC_{1\,4}$ | $P_4 \times F_{1\,3}$ | 20 | 21 | 3 | 1 | 15.0 | <0.001 |

[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

Results from the cone-tainer experiment based on assays of eggs per gram of root were similar to the growth-pouch experiments; parent lines $P_1$ (20 mean eggs/g. root), $P_2$ (36 mean eggs/g. root) and $P_3$ (23 mean eggs/g. root), were resistant, whereas all the plants of parent line $P_4$ (6400 mean eggs/g. root) were susceptible (Table 1). To confirm the temperature sensitivity of resistance conferred by the gene *Mi*, cv VFN-8 was included and at high temperature gave a susceptible reaction (1500 mean eggs/g. root). The $F_{1\,1}$, $F_{1\,2}$, and $F_{1.3}$ progenies were all resistant indicating complete dominance of resistance and its homozygous condition in the parental clones.

The $TC_{1\,1}$ and $TC_{1.2}$ reciprocal test-cross populations both segregated for resistance (Table 1); their pooled results gave 56 resistant: 11 susceptible plants, indicating a segregation ratio of 3:1 (R:S) with a $\chi^2$ value of 2.62 ($0.20<P>0.10$). $TC_{1.3}$ contained 68 resistant: 28 susceptible plants also indicating a 3:1 (R:S) ratio ($\chi^2=0.88$; $0.50<P>0.30$). The $TC_{1.4}$ population segregated too, with 20 resistant: 21 susceptible plants. The observed results did not fit a 3:1 (R:S) ratio ($\chi^2=15$; $P<0.001$), although this could occur by chance in this small sample if drawn from a bigger population of *L. peruvianum* with distorted segregation (Table 1).

## Experiment with *Mi*-virulent *M. incognita* at 25°C

All the parental clones of *L. peruvianum* PI 270435-2R2 ($P_1$) (80 mean eggs/g. root), PI 270435-3MH ($P_2$) (85 mean eggs/g. root) and PI 126443-1MH ($P_3$) (4 mean eggs/g. root) were resistant. All plants of *L. peruvianum* PI 126440-9MH ($P_4$) (989 mean eggs/g. root) were susceptible (Table 2). All $F_1$ plants from crosses of the resistant parents, 270435-3MH × 270435-2R2, 270435-3MH × 126443-1MH, and 126443-1MH × 270435-2R2 ($F_{1\,1}$, $F_{1\,2}$ and $F_{1.3}$, respectively), were resistant, indicating that resistance to *Mi*-virulent nematodes in these *L. peruvianum* parental genotypes is carried in the homozygous dominant condition (Table 2).

Test-cross populations $TC_{1\,1}$ and $TC_{1\,2}$ each segregated in a 3:1 (R:S) ratio (Table 2). Their pooled results gave 51 resistant: 16 susceptible plants, further indicating a segregation ratio of 3:1 (R:S) ($\chi^2=0.06$; $0.90<P>0.80$). $TC_{1\,3}$ contained 65 resistant: 31 susceptible plants also, indicating a segregation ratio of 3:1 (R:S) ($\chi^2=2.68$; $0.20<P>0.10$). The test-cross $TC_{1\,4}$ population, with 22 resistant: 19 susceptible plants, did not conform to a 3:1 (R:S) ratio, ($\chi^2=9.9$; $0.010<P>0.001$), although this could occur by chance in this small sample if drawn from a bigger population of *L. peruvianum* with distorted segregation (Table 2).

## $F_2$ experiment with *Mi*-avirulent isolate at 25°C and at 32°C

To test for independent assortment of resistance to *Mi*-avirulent isolate Project 77 in *L. peruvianum* PI 126443 clone 1MH, its bridge-hybrid $F_2$ progeny was screened at 25°C. All plants of the parent line PI 126443-1MH ($P_1$) (0 mean egg masses per root system) were resistant, whereas all plants of parent bridge-line EPP-1 ($P_2$) (112 mean egg masses per root system) were susceptible, as expected. All cuttings of the bridge-line hybrid $F_1$ (0 mean egg masses per root system) were resistant. To confirm gene *Mi* expression, cv VFN-8 was included and, as expected, gave a resistant reaction (0 mean egg masses per root system), compared to the susceptible control cv Tropic (118 mean egg masses per root system) at 25°C. The $F_2$ progeny contained 47 resistant and 3 susceptible plants, indicating a segregation ratio of 15:1 (R:S) ($\chi^2=0.00$; $P>0.95$). This

**Table 2** Reaction of *L. peruvianum* parental clones, $F_1$, and test-cross ($TC_1$) progenies for resistance to *Mi*-virulent *M. incognita* isolate 557R at 25°C according to the nematode egg masses and eggs produced on the roots

| Generation | *L. peruvianum* parent clone or cross | Number of plants | | Expected ratios | | $\chi^2$ | *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 | 20 | | | | |
| $F_1$ progeny | | | | | | | |
| $F_{1\,1}$ | $P_2 \times P_1$ | 15 | 0 | | | | |
| $F_{1\,2}$ | $P_2 \times P_3$ | 15 | 0 | | | | |
| $F_{1\,3}$ | $P_3 \times P_1$ | 15 | 0 | | | | |
| Test-crosses | | | | | | | |
| $TC_{1\,1}$ | $P_4 \times F_{1\,1}$ | 27 | 12 | 3 | 1 | 0.68 | 0.50–0.30 |
| $TC_{1\,2}$ | $F_{1.1} \times P_4$ | 24 | 4 | 3 | 1 | 1.70 | 0.20–0.10 |
| $TC_{1\,1} + TC_{1\,2}$ | Pooled reciprocal data | 51 | 16 | 3 | 1 | 0.06 | 0.90–0.80 |
| $TC_{1\,3}$ | $P_4 \times F_{1\,2}$ | 65 | 31 | 3 | 1 | 2.68 | 0.20–0.10 |
| $TC_{1\,4}$ | $P_4 \times F_{1\,3}$ | 22 | 19 | 3 | 1 | 9.9 | 0.01–0.001 |

[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

**Table 3** Reaction of parents, $F_1$, and $F_2$ segregating progeny of *L. peruvianum* PI 126443-1MH × EPP-1 tested for resistance to *Mi*-avirulent *M. incognita* isolate Project 77 at 25°C and 32°C according to the nematode egg masses and eggs produced on the roots

| Generation and temperature | Parent or progeny | Number of plants | | Expected ratios | | $\chi^2$ | *P* |
|---|---|---|---|---|---|---|---|
| | | R[a] | S[b] | R | S | | |
| $P_1$ at 25°C and 32°C | *L. peruvianum* PI 126443 clone 1MH | 20 | 0 | | | | |
| $P_2$ at 25°C and 32°C | Bridge line[c] EPP-1 | 0 | 20 | | | | |
| $F_1$ at 25°C and 32°C | $P_1 \times P_2$ | 20 | 0 | | | | |
| $F_2$ at 25°C | $F_1$ | 47 | 3 | 15<br>63 | 1<br>1 | 0.00<br>6.39 | >0.950<br>0.50–0.01 |
| $F_2$ at 32°C | $F_1$ | 43 | 17 | 3 | 1 | 0.35 | 0.70–0.50 |

[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*

value was within the theoretical limit predicted for two independent gene loci (Table 3). These data were not as consistent with the segregation model for three independent loci which has a segregation ratio of 63:1 (R:S) ($\chi^2$=6.39; 0.50<*P*>0.01).

At 32°C all clones of the parental line PI 126443-1MH ($P_1$) (0 mean egg masses per root system and 31 mean eggs/g. root) were resistant to the *Mi*-avirulent isolate Project 77, whereas all plants of parent bridge line EPP-1 ($P_2$) (246 mean egg masses per root system and 8945 mean eggs/g. root) were susceptible, as expected. All cuttings of the bridge-line hybrid $F_1$ (PI 126443-1MH × EPP-1) (1 mean egg mass per root system) were also resistant at 32°C (Table 3). To confirm the breakdown of heat-unstable resistance conferred by the gene *Mi*, the cv VFN-8 was included and, as expected, gave a susceptible reaction at 32°C (247 mean egg masses per root system and 3010 mean eggs/g. root). The control cv Tropic gave a susceptible reaction of 470 mean egg masses per root system and 9913 mean eggs/g. root. The $F_1$ clones were resistant to the *Mi*-avirulent isolate (268 mean eggs/g. root) providing further confirmation of complete dominance of heat-stable resistance to *Mi*-avirulent nematodes in clone PI 126443-1MH (Table 3). The $F_2$ progeny at 32°C with the *Mi*-avirulent isolate segregated into 43 resistant: 17 susceptible plants, conforming to a ratio of 3:1 (R:S) ($\chi^2$=0.35; 0.90<*P*>0.50) (Table 3).

**Table 4** Reaction of parents, $F_1$, and $F_2$ segregating progeny of *L. peruvianum* PI 126443-1MH × EPP-1 tested for resistance to *Mi*-virulent *M. incognita* isolate 557R at 25°C and 32°C according to the nematode egg masses and eggs produced on the roots

| Generation and temperature | Parent or progeny | Number of plants | | Expected ratios | | $\chi^2$ | $P$ |
|---|---|---|---|---|---|---|---|
| | | R[a] | S[b] | R | S | | |
| $P_1$ at 25°C and 32°C | *L. peruvianum* PI 126443 clone 1MH | 20 | 0 | | | | |
| $P_2$ at 25°C and 32°C | Bridge line[c] EPP-1 | 0 | 20 | | | | |
| $F_1$ at 25°C and 32°C | $P_1 \times P_2$ | 20 | 0 | | | | |
| $F_2$ at 25°C | $F_1$ | 43 | 7 | 3 | 1 | 3.22 | 0.10–0.05 |
| $F_2$ at 32°C | $F_1$ | 33 | 6 | 3 | 1 | 1.92 | 0.20–0.10 |

[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*

### $F_2$ experiment with *Mi*-virulent isolate at 25°C and at 32°C

All the parental clones of *L. peruvianum* PI 126443 clone 1MH (0 mean eggs/g. root) were resistant to the *Mi*-virulent isolate 557R at 25°C. All plants of bridge line EPP-1 (1350 mean eggs/g. root) were susceptible (Table 4). Circumvention of *Mi* resistance in cv VFN-8 was confirmed by a susceptible reaction of 1400 mean eggs/g. root. The susceptible control cv Tropic gave a reaction of 1500 mean eggs/g. root. The $F_1$ cuttings were all resistant (85 mean eggs/g. root), providing confirmation of the complete dominance of the gene blocking reproduction of the *Mi*-virulent isolate (Table 4). The $F_2$ progeny contained 43 resistant and 7 susceptible plants, indicating a segregation ratio of 3:1 (R:S) ($\chi^2$=3.22; 0.10<*P*>0.05) (Table 4).

All the parental clones of *L. peruvianum* PI 126443-1MH (0 mean eggs/g. root) were resistant to the *Mi*-virulent isolate 557R at 32°C. All plants of bridge line EPP-1 (1235 mean eggs/g. root) were susceptible (Table 4). Circumvention of gene *Mi* resistance in cv VFN-8 was confirmed by a susceptible reaction (1142 mean eggs/g. root). The susceptible control cv Tropic gave a reaction of 1600 mean eggs/g. root. The $F_1$ cuttings were all resistant (95 mean eggs/g. root), providing confirmation of the complete dominance of the gene that blocks the *Mi*-virulent isolate at 32°C (Table 4). The $F_2$ progeny contained 33 resistant and 6 susceptible plants, also conforming to a segregation ratio of 3:1 (R:S) ($\chi^2$=1.92; 0.20<*P*>0.10) (Table 4).

### Multiple screening of cloned plants from an $F_2$ segregating population

The relationship between the heat-unstable and the heat-stable *Mi*-avirulent *M. incognita* resistance factors in *L. peruvianum* PI 126443-1MH was examined by dual resistance screenings on clones of the same individual plants from an $F_2$ segregating population at 25°C and 32°C (Table 5). Three individual plants were identified as susceptible at both temperatures (Table 5). Among the resistant plants, the genotype of the resistant individuals at 25°C can be *Mi_* or *mimi* because resistance can be expressed by the heat-stable *Mi-5_* (Table 5).

Expression of resistance to *Mi*-virulent *M. incognita* isolate 557R in *L. peruvianum* PI 126443-1MH was also examined by dual resistance screenings at 25°C and 32°C on clones of the same individual plants from the $F_2$ segregating population (Table 5). Three individuals were resistant at 25°C and susceptible at 32°C; the susceptibility at 32°C could be due to high-temperature sensitivity or the individuals concerned could have been escapes. Some individual $F_2$ genotypes were susceptible at 32°C to the *Mi*-avirulent *M. incognita* isolate but resistant to the *Mi*-virulent *M. incognita* isolate, and *vice versa* (Table 5). Therefore, the heat-stable resistance to the *Mi*-avirulent *M. incognita* isolate (gene *Mi-5*) and the resistance to the *Mi*-virulent *M. incognita* isolate (gene *Mi-3*) are not conferred by the same factor in PI 126443-1MH. However, the majority of the $F_2$ individuals being dual-resistant suggested that these two resistant genes are linked, but still able to recombine (Table 5).

### Multiple screening of cloned plants from $TC_1$ segregating populations

Dual resistance screenings were made on clones of the same individual plants from segregating populations of the $TC_{1.1}$, $TC_{1.2}$, $TC_{1.3}$ and $TC_{1.4}$ generations (Tables 6 and 7). In the $TC_{1.1}$ and $TC_{1.2}$ populations a total of three individual plants were susceptible to *Mi*-avirulent *M. incognita* at

**Table 5** A summary of resistance phenotypes and assigned genotypes based on multiple screening of individual plants from *L. peruvianum* $F_2$ (PI 126443-1MH × EPP-1) segregating progeny by use of vegetative propagation

| No. of individual plants[a] | Nematode and temperature | | | | | |
|---|---|---|---|---|---|---|
| *L. peruvianum* PI 126443-1MH × EPP-1 | Isolate Project 77 at 25°C | | Isolate 557R at 25°C and 32°C | | Isolate Project 77 at 32°C | |
| | Genotype | Index[b] | Genotype | Index | Genotype | Index |
| 2 | *mimi* | S | *mi-3mi-3* | S | *mi-5mi-5* | S |
| 1 | *mimi* | S | *Mi-3_* | R | *mi-5mi5* | S |
| 5 | *Mi_* | R | *Mi-3_* | R | *mi-5mi-5* | S |
| 1 | *Mi_* | R | *mi-3mi3* | S | *Mi-5_* | R |
| 7 | *Mi_* | R | *Mi-3_* | R | *mi-5mi5* | S |
| 26 | *Mi_* or *mimi* | R | *Mi-3_* | R | *Mi-5_* | R |

[a] Only $F_2$ plants screened for all three phenotypes are included
[b] Resistant (R), Susceptible (S): see text for symbol assignment

**Table 6** A summary of resistance phenotypes and assigned genotypes based on multiple screening of individual plants from *L. peruvianum* $TC_{1\,1}$ and $TC_{1\,2}$ segregating progenies by use of vegetative propagation

| Number of individual plants | | Nematode and temperature | | | |
|---|---|---|---|---|---|
| | | Isolate Project 77 (*Mi*-avirulent) at 32°C | | Isolate 557R (*Mi*-virulent) at 25°C | |
| $TC_{1\,1}$[a] | $TC_{1\,2}$[b] | Genotype | Index[c] | Genotype | Index |
| 6 | 2 | *Mi-2_mi-6mi-6* or *Mi-2_Mi-6_* or *mi-2mi-2Mi6_* | R | *mi-7mi-7* *mi-8mi-8* | S |
| 2 | 1 | *mi-2mi-2* *mi-6mi6* | S | *Mi-7_mi-8mi-8* or *Mi-7_Mi-8* or *mi-7mi-7Mi-8_* | R |
| 6 | 2 | *mi-2mi-2* *mi-6mi-6* | S | *mi-7mi-7* *mi-8mi-8* | S |
| 25 | 23 | *Mi-2_mi-6mi-6* or *Mi-2_Mi-6_* or *mi-2mi-2Mi-6_* | R | *Mi-7_mi-8mi-8* or *Mi-7_Mi-8_* or *mi-7mi-7Mi-8_* | R |

[a] $TC_{1\,1}$ is PI 126440-9MH × [PI 270435-3MH × PI 270435-2R2]
[b] $TC_{1\,2}$ is [PI 270435-3MH × PI 270435-2R2] × PI 126440-9MH
[c] Resistant (R), Susceptible (S): see text for symbol assignment

32°C, but resistant to the *Mi*-virulent isolate at 25°C, and a total of eight individual plants were susceptible to the *Mi*-virulent isolate at 25°C, but resistant to the *Mi*-avirulent isolate at 32°C (Table 6). This demonstrated that heat-stable resistance to *Mi*-avirulent *M. incognita* (genes *Mi-2* in 270435-2R2 and *Mi-6* in 270435-3MH) and resistance to *Mi*-virulent *M. incognita* (genes *Mi-7* in 270435-3MH and *Mi-8* in 270435-2R2) are not conferred by the same factor in PI 270435 clones 2R2 and 3MH. However, in the segregating populations the majority of the individuals (25 of 39 $TC_{1.1}$ and 23 of 28 $TC_{1.2}$) expressed dual resistant phenotypes, suggesting that these two resistance gene clusters are linked, but still able to recombine (Table 6).

In the $TC_{1\,3}$ population, a total of 14 individual plants were susceptible to *Mi*-avirulent *M. incognita* at 32°C, but resistant to the *Mi*-virulent isolate at 25°C, and a total of 16 individual plants were susceptible to the *Mi*-virulent isolate at 25°C, but resistant to the *Mi*-avirulent isolate at 32°C (Table 7). This demonstrated that the heat-stable resistance to *Mi*-avirulent *M. incognita* (genes *Mi-5* in 126443-1MH and *Mi-6* in 270435-3MH) and the resistance to *Mi*-virulent *M. incognita* (genes *Mi-3* in 126443-1MH and *Mi-7* in 270435-3MH) are not conferred by the same factors in PI 126443-1MH and PI 270435-3MH. However, in the $TC_{1\,3}$ population the majority of the individuals (51 of 95) being dual-resistant suggested that these two resistance genes are linked but able to recombine within each clone (Table 7).

In the $TC_{1.4}$ population a total of seven individual plants were susceptible to the *Mi*-avirulent *M. incognita* isolate at 32°C, but resistant to the *Mi*-virulent isolate at 25°C, and a total of five individual plants were susceptible to the *Mi*-virulent isolate at 25°C, but resistant to the *Mi*-aviru-

**Table 7** A summary of resistance phenotypes and assigned genotypes based on multiple screening of individual plants from *L. peruvianum* $TC_{1\,3}$ and $TC_{1\,4}$ segregating progenies by use of vegetative propagation

| Number of individual plants | Nematode and temperature | | | |
|---|---|---|---|---|
| | Isolate Project 77 (*Mi*-avirulent) at 32°C | | Isolate 557R (*Mi*-virulent) at 25°C | |
| | Genotype | Index[a] | Genotype | Index |
| Test-cross$_{1\,3}$ PI 126440-9MH × [PI 270435-3MH × PI 126443-1MH] | | | | |
| 17 | *Mi-5_mi-6mi-6* or *Mi-5_Mi-6_* or *mi-5mi-5Mi6_* | R | *mi-7mi-7* *mi-3mi-3* | S |
| 14 | *mi-5mi-5* *mi-6mi6* | S | *Mi-7_mi-3mi-3* or *Mi-7_Mi-3_* or *mi-7mi-7Mi-3_* | R |
| 14 | *mi-5mi-5* *mi-6mi-6* | S | *mi-7mi-7* *mi-3mi-3* | S |
| 51 | *Mi-5_mi-6mi-6* or *Mi-5_Mi-6_* or *mi-5mi-5Mi-6_* | R | *Mi-7_mi-3mi-3* or *Mi-7_Mi-3_* or *mi-7mi-7Mi-3_* | R |
| Test-cross$_{1\,4}$ PI 126440-9MH × [PI 126443-1MH × PI 270435-2R2] | | | | |
| 5 | *Mi-5_mi-2mi-2* or *Mi-5_Mi-2_* or *mi-5mi-5Mi2_* | R | *mi-3mi-3* *mi-8mi-8* | S |
| 7 | *mi-5mi-5* *mi-2mi-2* | S | *Mi-3_mi-8mi-8* or *Mi-3_Mi-8_* or *mi-3mi-3Mi-8_* | R |
| 14 | *mi-5mi-5* *mi-2mi-2* | S | *mi-3mi-3* *mi-8mi-8* | S |
| 15 | *Mi-5_mi-2mi-2* or *Mi-5_Mi-2_* or *mi-5mi-5Mi-2_* | R | *Mi-3_mi-8mi-8* or *Mi-3_Mi-8_* or *mi-3mi-3Mi-8_* | R |

[a] Resistant (R), Susceptible (S); see text for symbol assignment

lent isolate at 32°C (Table 7). This demonstrated that the heat-stable resistance to *Mi*-avirulent *M. incognita* (gene *Mi-2* in 270435-2R2 and gene *Mi-5* in 126443-1MH) and the resistance to *Mi*-virulent *M. incognita* (gene *Mi-8* in 270435-2R2 and gene *Mi-3* in 126443-1MH) are not conferred by the same factor in PI 270435-3MH and PI 126443-1MH. That some individual $TC_1$ clones were susceptible at 32°C but resistant to the *Mi*-virulent isolate, and *vice versa*, suggested that the heat-stable resistance to the *Mi*-avirulent *M. incognita* isolate and the resistance to the *Mi*-virulent *M. incognita* isolate are not conferred by the same gene in the tomato genome, but that the two resistance traits are controlled by independent weakly linked genes.

## Discussion

The results obtained with the $F_1$ populations at 25°C (*Mi* expressed) and 32°C (*Mi* not expressed) indicate that the nature of heat-stable resistance to the *Mi*-avirulent *M. incognita* isolate is homozygous dominant in the resistant parental clones (the $F_1$ must be heterozygous). This is in agreement with the results for heat-stable resistance to a *Mi*-avirulent *M. incognita* isolate obtained by Cap et al. (1991, 1993), in which $F_1$ hybrids of *L. peruvianum* clones PI 270435-2R2, PI 270435-3MH and PI 126443-1MH were all resistant. In addition, the results obtained with the $F_1$ populations at 25°C indicate that the nature of resistance to *Mi*-virulent *M. incognita* is also completely dominant and present in the homozygous condition in the parental clones that carry these resistance genes. This is in agreement with the results obtained by Roberts et al. (1990), in which $F_1$ hybrids of *L. peruvianum* clones PI 126443-1MH and PI 270435-3MH were resistant to two *Mi*-virulent isolates. Thus the *L. peruvianum* parental clones PI 270435-2R2, PI 270435-3MH and PI 126443-1MH used in the test-crossing experiments are homozygous dominant for the resistance, because none of their $F_1$ hybrids were susceptible.

If the genes conferring heat-stable resistance to *Mi*-avirulent isolates present in these genotypes were not independent from each other, i.e., if the same single gene is homozygous in all the resistant parent clones, the $TC_1$-derived progeny should all contain at least one dominant allele for

resistance. The resistant parent clones used in the test-crosses were homozygous for the resistance and the susceptible tester clone (PI 126440-9MH) was homozygous susceptible (Cap et al. 1993). If the heat-stable resistance to the *Mi*-avirulent *M. incognita* isolate 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* not expressed, $TC_1$ populations segregated for resistance in a ratio close to 3:1 (R:S) when challenged with the *Mi*-avirulent *M. incognita* isolate. In this study, by using the susceptible PI 126440-9MH as male parent in $TC_{1.2}$, there was less recombination of the resistance locus compared with when it was used as a female parent in $TC_{1.1}$. Van Ooijen et al. (1994) found a similar effect in *L. peruvianum* where recombination at male meiosis was reduced; therefore, this could be a general phenomenon within *L. peruvianum*. Pooling the data of the $TC_{1.1}$ and $TC_{1.2}$ tests gave a better fit between the observed and the calculated expectancy to correct for the reduced recombination at male meiosis. These ratios are expected for a different single dominant gene for the heat-stable resistance to *Mi*-avirulent *M. incognita* being present within each of the resistant clones. The resistant individuals in the $TC_1$ populations can be visualized as expressing dominant gene(s) *Mi-a_* or *Mi-b_* from the resistant parents, while the susceptible individuals exhibit the double recessive *mi-ami-a mi-bmi-b* genotype.

This hypothesis is supported by the fact that the gene conferring heat-stable resistance to *Mi*-avirulent *M. incognita* is independent and different in each parental resistant clone. We propose the symbol *Mi-5* for the gene in PI 126443 clone 1MH, and the symbol *Mi-6* for the gene in PI 270435 clone 3MH, which each confer resistance to *Mi*-avirulent *M. incognita* isolates at high temperature (i.e., heat-stable resistance). The results from test-crossing confirm the expression of one dominant gene at high temperature as the basis of the resistance to *Mi*-avirulent *M. incognita* in each clone (Cap et al. 1993). Furthermore, susceptible recombinants in the test-cross populations supports the independence between gene *Mi-2* in clone PI 270435-2R2, gene *Mi-5* in clone 126443-1MH, and gene *Mi-6* in clone PI 270435-3MH.

The number of genes conferring resistance to *Mi*-virulent *M. incognita* in clones of PI 270435-2R2, PI 270435-3MH, and PI 126443-1MH could also be determined by test-crossing the $F_1$ hybrids between these clones in different combinations with susceptible PI 126440-9MH. Assuming that the resistance in these *L. peruvianum* genotypes is allelic at the same gene locus, or identical in all resistant clones, then the progeny would not segregate with all individuals being resistant. The test-cross would contain four genotypes, and all of them would have at least one dominant allele *Mi-a_* present for resistance expression. However, the test-cross-derived progenies segregated in a 3:1 (R:S) ratio. This ratio is expected for the presence of a separate single dominant gene with a major effect conferring resistance to *Mi*-virulent *M. incognita* isolate 557R within each of the parental resistant clones.

In order to examine the relationship between the gene for heat-stable resistance to the *Mi*-avirulent isolate and the gene for resistance to the *Mi*-virulent isolate within each the *L. peruvianum*-resistant clones PI 270435-2R2, PI 270435-3MH and PI 126443-1MH, the same cloned $TC_1$ individual plants were used in all resistance screening. Thus if the same gene expresses both resistance phenotypes, there should be no differences in expression by the same individual plants in the segregating test-cross populations ($TC_{1.1}$, $TC_{1.2}$, $TC_{1.3}$, and $TC_{1.4}$). Assuming that the heat-stable *Mi*-avirulent nematode resistance factor is tightly linked to, or the same as, the gene for resistance to the *Mi*-virulent isolate, the test-cross individuals will contain one dominant allele for the expression of both phenotypes present. However, some individual plants in the $TC_{1.1}$, $TC_{1.2}$, $TC_{1.3}$, and $TC_{1.4}$ populations were not resistant for both traits, as expected for an independent assortment of single genes (Tables 6 and 7). That some individual $TC_1$ clones were susceptible at 32°C but resistant to the *Mi*-virulent nematode isolate, and *vice versa*, suggested that different genes confer heat-stable resistance to *Mi*-avirulent *M. incognita* and resistance to *Mi*-virulent *M. incognita*. However, the finding that the large number of individual plants possessed the double-resistance phenotype suggest that the different genes are linked in each resistant *L. peruvianum* clone.

Thus, the resistance to the *Mi*-virulent *M. incognita* isolate is also conferred by single dominant genes and these are different in each resistant parental clone. We propose the symbol *Mi-7* for the gene in PI 270435 clone 3MH and the symbol *Mi-8* for the gene in PI 270435 clone 2R2, which each confer resistance to the *Mi*-virulent *M. incognita* isolate 557R at moderate (25°C) temperature. The test-cross data support the independence between gene *Mi-8* and gene *Mi-7*, and the distinction of each gene from the gene *Mi-3* that was shown to be a single dominant gene on chromosome 12 by Yaghoobi et al. (1995).

The field-produced $F_2$ generation provided an additional independent genetic analysis of resistance in clone PI 126443-1MH. A segregation ratio of 3:1 resistant: susceptible plants was determined with *Mi*-avirulent *M. incognita* at 32°C, and the $F_2$ progeny also segregated in a 3:1 (R:S) ratio with *Mi*-virulent *M. incognita* at both 32°C and 25°C (Table 4). These ratios indicated that the different resistance phenotypes are based on the expression of a single dominant gene in each case. However, extreme distorted segregation ratios caused by the selection of one locus are common in *L. peruvianum* (Sandbrink et al. 1995).

The $F_2$ progeny produced segregation ratios closer to 15:1 than to 63:1 (R:S), when challenged with *Mi*-avirulent *M. incognita* at moderate temperature (*Mi* expressed), indicating that two, rather than three, independently segregating dominant genes are expressed at this temperature. A three-gene pattern of segregation in the $F_2$ would be expected if gene *Mi*, resistance gene *Mi-5* (for heat-stable resistance to *Mi*-avirulent *M. incognita*) and gene *Mi-3* (for resistance to *Mi*-virulent *M. incognita*) were all expressed and assorting independently. The findings in test-cross and $F_2$ segregating populations suggest that *Mi-3* and *Mi-5* are

**Table 8** A summary of genes conferring resistance to root-knot nematodes (*Meloidogyne* spp.) identified in *L. peruvianum* based on experiments at moderate and high temperatures and with *Mi*-gene – virulent and avirulent nematode isolates

| Cultivar or accession and clone | Nematode isolates | | |
|---|---|---|---|
| | Project 77 (*Mi*-avirulent) | | 557R (*Mi*-virulent) |
| | Heat-unstable | Heat-stable | |
| Tropic | Absent[a] | Absent | Absent |
| VFN-8 | *Mi*[b] | Absent | Absent |
| LA 1708-I | *Mi*? | *Mi-4*[c] | Absent |
| PI 270435-2R2 | *Mi*? | *Mi-2* | *Mi-8* |
| PI 270435-3MH | *Mi*? | *Mi-6* | *Mi-7* |
| PI 126443-1MH | *Mi*? | *Mi-5* | *Mi-3* |

[a] Absent – susceptible reaction, no R gene expressed
[b] All genes expressed in a dominant fashion
[c] *Mi-4* described in Veremis and Roberts (1996)

at least weakly linked, and probably operate as a 'single-locus' resistance cluster under most conditions. This is in agreement with a molecular study of clone PI 126443-1MH that positioned *Mi-3* in the telomeric region of chromosome 12 in tomato (Yaghoobi et al. 1995), i.e., on a different chromosome distinct from the *Mi* gene which is on chromosome 6.

Additional examination was made of these resistance gene relationships in *L. peruvianum* PI 126443-1MH by multiple phenotype screening of the same individual plants using vegetative cuttings. If the same gene expressed heat-stable resistance to the *Mi*-avirulent isolate and resistance to the *Mi*-virulent isolate, there should be no differences in phenotypic expression within the same individual cloned plants in $F_2$ progeny; the $F_2$ should contain four genotypes and three of them will have at least one dominant allele for the expression of both phenotypes present. However, not all individual plants in $F_2$ segregating progeny were resistant to both the *Mi*-avirulent isolate and the *Mi*-virulent isolate, and individuals segregated for the three possible phenotypes in $F_2$-derived progeny. A double-recessive condition of susceptibility to the *Mi*-avirulent isolate was present. However, within the segregation ratio of 3:1 (R:S) for the heat-stable resistance traits there were more individual cloned plants that were susceptible to the *Mi*-avirulent isolate but resistant to the *Mi*-virulent isolate. Individuals possessing only the gene (*Mi*) for heat-sensitive resistance to *Mi*-avirulent isolates without the heat-stable *Mi*-avirulent resistance gene confirmed the independence of these loci as determined from the test-crosses where PI 126443-1MH was used as one of the resistant parents. The $F_2$ analyses further support the expression of two dominant genes as the basis of the heat-stable resistance to *Mi*-avirulent *M. incognita* (gene *Mi-5*) and resistance to *Mi*-virulent *M. incognita* (gene *Mi-3*).

The findings reveal a spectrum of *Meloidogyne* resistance genes in *L. peruvianum*, which are expressed in single dominant gene fashion (Table 8). Their arrangement within the *L. peruvianum* genome appears to be that of different genomic locations, at most of which linked genes are present. The original *Mi* gene is located on chromosome 6 (Messeguer et al. 1991), and there is some evidence that the *Mi* region may actually contain more than one resistance gene locus (Sidhu and Webster 1975, 1981) that differentiate (a)virulent phenotypes (Netscher 1978). In PI 126443-1MH the gene *Mi-3* was positioned in the telomeric region of chromosome 12 (Yaghoobi et al. 1995), and our data show the presence of a linked additional gene (*Mi-5*) for heat-stable resistance in this same region. We have found that weakly linked pairs of genes (*Mi-2* and *Mi-8* in PI 270435-2R2; *Mi-6* and *Mi-7* in PI 270435 clone 3MH) occur in two additional *L. peruvianum* genotypes. Although the two genomic locations of these pairs of resistance genes are not known, our analysis indicates that they are independent of each other and of the *Mi* region on chromosome 6, and also independent from the *Mi-3*/*Mi-5* region on chromosome 12.

Our findings suggest that wild tomato has a genetic system capable of generating variation at the nematode resistance locus, leading to the creation of new resistance specificities, similar to other plant resistance loci that contain multiple numbers of resistance genes that are clustered in certain genomic regions, and to which pathogens develop virulent forms to overcome them. For example, *Cladosporium fulvum* is a biotropic imperfect fungal pathogen of tomato that fits a gene-to-gene relationship (de Wit 1992). Mapping experiments have revealed two complex resistance loci, one on chromosome 6, of which *Cf-2*, *Cf-5* and possibly *Mi* are members (Dickinson et al. 1993), and another on chromosome 1, of which *Cf-4*, *Cf-9* and *Cf-1* are members (Jones et al. 1993). Evolutionary and structurally related gene clusters and multiple alleles like the *Mi* gene pattern summarized in Table 8 are not uncommon in tomato; for example, the *cab* (Kellmann et al. 1993), *Cf* (de Wit 1992) and the *Pto* (Martin et al. 1993) gene clusters. Duplicated combinations of parental resistance characters suggest that in *L. peruvianum* the genetic control of resistance to *M. incognita* appears in as many as four multiple allelic series, similar to the resistance of some other plant pathogens. Biochemical or molecular markers (isozymes, RFLPs, AFLPs, and RAPD) tightly linked to these novel genes are needed to expedite their incorporation into cultivated tomato and a combination of molecular and genetic approaches will probably be necessary to elucidate the relationship of these genes in detail.

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