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Differentiation of *Meloidogyne incognita* and *M. arenaria* novel resistance phenotypes in *Lycopersicon peruvianum* and derived bridge-lines

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Abstract *Lycopersicon peruvianum* PI 270435 clone 2R2 and PI 126443 clone 1MH were crossed reciprocally with three *L. esculentum-L. peruvianum* bridge-lines. The incongruity barrier between the two plant species was overcome; F_1 progeny were obtained from crosses between four parental combinations without embryo-rescue culture. Hybridity was confirmed by leaf and flower morphology and by the production of nematode-resistant F_1 progeny on homozygous susceptible parents. Clones of the five F_1 bridge-line hybrids were highly resistant to *Mi*-avirulent root-knot nematode (*Meloidogyne incognita*) at both 25°C and 30°C soil temperatures. However, only clones from PI 270435-3MH and PI 126443-1MH, and hybrids from PI 126443-1MH, were resistant to *Mi*-virulent *M. incognita* isolates at high soil temperature. Clones and hybrids from PI 270435-2R2 were not resistant to two *Mi*-virulent *M. incognita* isolates at high soil temperature. A source of heat-stable resistance was identified in bridge-line EPP-2, and was found to be derived from *L. peruvianum* LA 1708. Accessions of the *L. peruvianum* 'Maranon races', LA 1708 and LA 2172, and bridge-line EPP-2, segregated for heat-stable resistance to *Mi*-avirulent *M. incognita*, but were susceptible to *Mi*-virulent *M. incognita* isolates. Clone LA 1708-I conferred heat-stable resistance to *M. arenaria* isolate W, which is virulent to heat-stable resistance genes in *L. peruvianum* PI 270435-2R2, PI 270435-3MH, and PI 126443-1MH. Clone LA 1708-I has a distinct heat-stable factor for resistance to *Mi*-avirulent *M. arenaria* isolate W, for which the gene symbol *Mi-4* is proposed. A *Mi*-virulent *M. arenaria* isolate Le Grau du Roi was virulent on all *Lycopersicon* spp. accessions tested, including those with novel resistance genes.

Key words Heat-sensitivity · Virulence · Tomato · Root-knot nematodes · Bridge-line

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Introduction

Host plant resistance to *Meloidogyne* spp. is used widely in processing and in fresh market tomato (*Lycopersicon esculentum* Mill.) varieties. Resistance to root-knot nematodes, *Meloidogyne* spp., in tomato cultivars is derived from a single resistant F_1 plant (Smith 1944), and is conferred by the dominant gene *Mi* (Gilbert and McGuire 1956), located on chromosome 6 (Messeguer et al. 1991). Intensive use of this resistance could result in selection for *Mi*-breaking virulent populations of the nematode (Roberts 1992). There are reports of both selected and non-selected *Mi*-virulent populations of root-knot nematodes (Netscher 1978; Berthou et al. 1989; Roberts and Thomason 1986, 1989; Jarquin-Barberena et al. 1991; Castagnone-Sereno 1994). In addition, the resistance conferred by *Mi* breaks down in soil above 28°C (Holtzmann 1965; Dropkin 1969).

Ammati et al. (1985) found sources of resistance, expressed at soil temperatures above 28°C, within some accessions of *L. peruvianum* L. Additionally, a *L. peruvianum* PI 270435×*L. p.* var. *glandulosum* PI 126443 hybrid showed resistance against *M. incognita* isolates selected for virulence on tomato cultivars with the gene *Mi* (Roberts et al. 1990).

The wild tomato *L. peruvianum* is a highly polymorphic species that is quite remote from, and not cross-compatible with, the cultivated tomato *L. esculentum* (Taylor 1986). Unilateral incompatibility between the two tomato species occurs when self-compatible *L. esculentum* is used as the pollen parent in crosses with self-incompatible *L. peruvianum* (Hogenboom 1979; Lefrancois et al.1993). In interspecific crosses where *L. peruvianum* is the male parent, some plants have been produced successfully by embryo culture, depending on the genotype of the accession (Thomas and Pratt 1981; Barbano and Topoleski 1984; Gradziel and Robinson 1991). Backcrossing to *L. esculentum* of hybrid lines carrying the heat-stable nematode resistance created by Cap et al. (1991) was impeded because the embryo-rescue hybrids were sterile (Veremis 1995).

Complex hybrids between *L. esculentum* and *L. peruvianum* and their potential use in promoting interspecific gene transfers depends on the genotype of the accession (Taylor and Al-Kummer 1982). Rick (1983) identified two *L. peruvianum* 'Maranon race' accessions LA 1708 and LA 2172, which if used as pollen parents set 0.2 seed/fruit in crosses with *L. esculentum*. Poysa (1990) used LA 1708 and embryo-callus culture to develop bridging genotypes of *L. esculentum* × *L. peruvianum* to facilitate inter-specific gene transfer.

The objectives of the present work were (1) to obtain hybrids between the "bridging genotypes" developed by Poysa (1990) and the *L. peruvianum* genotypes that possess gene(s) controlling the expression of heat-stable nematode resistance and resistance to *Mi*-virulent nematode isolates; and (2) to identify and characterize the phenotype of additional novel sources of host-plant resistance to *M. arenaria* and *M. incognita* isolates in *Lycopersicon* spp.

Materials and methods

Plant material

Plant genotypes used in this study were *L. peruvianum* L. PI 270435 clone 2R2, PI 270435 clone 3MH and *L. peruvianum* var. *glandulosum* PI 126443 clone 1MH (Rick 1976; Warnock 1988), resistant to *Mi*-avirulent nematode isolates at 25°C–30°C (Ammati et al. 1986). The *L. peruvianum* parent material was propagated agamically, as described previously (Cap et al. 1993).

Seeds of the following genotypes were kindly provided for study: *L. esculentum-L. peruvianum* bridge-lines EEP-1, EPP-1 and EPP-2 from Dr. V. Poysa, Research Station, Harrow, Ontario, Canada; *L. peruvianum* LA 2157, LA 1708 and LA 2172 from Dr. C. M. Rick, Dept. of Vegetable Crops, University of California, Davis, California; nematode-susceptible *L. esculentum* cultivars UC82, UC204B, Hunt-100, Tropic, Peto 94 and Peto 95 from Petoseed Company Inc., Saticoy, California; and Apex-1000, New Yorker and Tracy from Ferry Morse Seed Co., Modesto, California. Seeds were planted in trays containing vermiculite, and maintained in a mist chamber for at least 1 week to enhance germination. Plants were grown in UC Mix II soil in 6-l nursery pots (Western Pulp Products Co., Corvallis, Oregon) in the greenhouse with a day/night temperature regime of 27°C±5°C, and fertilized with Osmocote (Sierra Chemical Co., Corvallis, Oregon).

Hybrid crosses

Pollen from all parents was collected and stored as described by McGuire (1952). F_1 hybrids were produced by crossing the *L. esculentum-L. peruvianum* bridge-lines with *L. peruvianum* resistance gene donors during the spring (Table 1). Three *L. esculentum-L. peruvianum* bridge-lines, EEP-1, EPP-1 and EPP-2, were used as parents in 100 reciprocal crosses for each of the six possible combinations with the two resistance donors: *L. peruvianum* PI 270435 clone 2R2 and PI 126443 clone 1MH.

One day before anthesis (when the petals changed color from green yellow to light yellow), flowers of *L. esculentum-L. peruvianum* bridge-lines and *L. peruvianum* plants were emasculated, and hand-pollinated. Crosses were made under greenhouse conditions between 11 am and 1 pm. Pollinated ovaries were enclosed with paper bags or gelatin capsules to prevent cross-pollination from other exotic tomato pollen. Crosses between *L. esculentum* plants (pistilate parents) and the resistant bridge hybrid F_1s were carried out in the spring as described above (see Table 1).

Nematode cultures

Cultures of *M. incognita* isolate Project 77, and *M. arenaria* isolate W were started from field populations on greenhouse-grown tomato cv Tropic plants. The *M. incognita Mi*-virulent isolate Cote d'Ivoire had been laboratory-selected for virulence to the gene *Mi* and was cultured on greenhouse-grown tomato cv Piersol plants (Roberts et al. 1990). The culture of *M. arenaria* isolate Le Grau du Roi was started from a subculture on greenhouse-grown tomato cv VFN-8 plants. The identities of the nematode isolates were confirmed as described previously (Cap et al. 1993). The *M. incognita Mi*-virulent isolate 557R was kindly provided by Dr. V. M. Williamson (U.C. Davis, California) as eggs, which were then cultured on greenhouse-grown tomato cv VFN-8 plants.

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 (Stuewe and Sons Inc., 2290 S. E. Kiger Island Drive, Corvalis, Oregon 97333-9461) filled with steam-sterilized loamy sand and fertilized with Osmocote. Experiments that tested the heat-stability of resistance were carried out in environment growth chambers where temperature was maintained constantly at 30° or 32°C for 7 days before, and 30 days after inoculation and then placed for an additional 25–30 days in a greenhouse at 27°±5°C. In growth pouch experiments, single seedlings or rooted cuttings of the tomato plants were transferred to growth pouches (Omwega et al. 1988) and maintained in a growth chamber maintained constantly at 30°C. The experiments requiring moderate temperatures were carried out in a greenhouse at 25°±3°C or in a growth chamber set constantly at 25°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) for tests with isolates *M. incognita* Project 77 and *M. arenaria* W, or 3000 J_2 for tests with isolates *M. incognita* 557R, Cote d'Ivoire and *M. arenaria* Le Grau du Roi, were pipetted into the soil around the plant roots. Plants were arranged in cone-tainer racks in a completely randomized design. Plants in the growth pouches were inoculated with a suspension of 2000 J_2 per plant and the plants within the growth chamber were arranged in a completely randomized design. Nematode egg production on roots was evaluated after the accumulation of approximately 1000 degree days with a base temperature of 10°C (Trudgill 1994) following the method of Cap et al. (1993). At the same time all the test plants were propagated agamically as described above for the parental material.

A plant had at least five replications to determine the ability of the nematode to reproduce, and 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 VFN-8 [possessing the *Mi* gene, and resistant below 28°C but susceptible above 28°C (Dropkin 1969)] were included to check inoculum viability, infectivity and the expression of heat-sensitive resistance genes.

Results

Hybrids via bridge-lines

The numbers of seeds set in each cross were highly variable and some crosses did not produce viable F_1 seeds. Most of the mature seeds did not germinate; only five produced plantlets that grew vigorously. Two plants (EEP-1 × 1MH-I and EEP-1 × 1MH-II) were obtained from the crosses between bridge-line EEP-1 × *L. peruvianum* parent PI 126443 clone 1MH. Only one plant was obtained

Table 1 Cross compatibility of three bridge hybrids used as staminate parents with seven *L. esculentum* cultivars used as pistillate parents

L. esculentum cultivars	Bridge-line hybrids								
	1MH × EPP-1			EPP-1 × 2R2			EEP-1 × 1MH		
	FS[a]	S[b]	P[c]	FS[a]	S[b]	P[c]	FS[a]	S[b]	P[c]
New Yorker	0.2	23	0	0	0	0	–	–	–
Tracy	0.1	35	0	0.1	74	0	0.2	81	0
Hunt-100	0	0	0	0	0	0	0	0	0
UC-204-B	0.05	37	0	0.02	12	0	0	0	0
PETO-94	0.1	93	0	0.08	4	0	0	0	0
PETO-95	0.1	55	0	0.09	98	0	0.2	316	0
APEX-1000	0.2	36	0	0.2	96	0	0.1	229	0

[a] FS. Percentage of pollinated flowers which set seed
[b] S. Total number of seed set
[c] P. Total number of nematode-resistant plants

Table 2 Reaction of *L. esculentum*, *L. peruvianum* bridge-line parental accessions, embryo-rescue hybrids, bridge-line hybrids, and bridge-lines to *Mi*-avirulent *M. incognita* isolate Project 77 at 25°C and 30°C in growth-pouch and cone-tainer experiments

Tomato	Response to *Mi*-avirulent *M. incognita* isolate Project 77	
	25°C	30°C
L. esculentum		
Tropic	S[a]	S
VFN-8	R	S
Bridge lines		
EPP-1	S	S
EEP-1	S	S
EPP-2	R/S[b]	R/S
EPP-2-I	R	R
L. peruvianum		
PI 128646-6	–	S
LA 2172	R/S	R/S
LA 2172-I	R	R
LA 1708	R/S	R/S
LA 1708-I	R	R
Embryo rescue hybrids		
UC-82 × 2R2	R	R
ms-1 × 1MH	R	R
Bridge-line hybrids		
1MH × EPP-1	R	R
EPP-1 × 2R2	R	R
EEP-1 × 1MH	R	R
EPP-2 × 2R2	R	R

[a] Resistant (R). Susceptible (S); see text for symbol assignment
[b] Accessions LA 2172 and LA 1708 and the bridge-line EPP-2 segregated for resistance with individual entries classified as R or S Based on values of at least five replicates

from each of the following crosses: PI 126443 clone 1MH × EPP-1 (1MH × EPP-1), EPP-1 × PI 270435 clone 2R2 (EPP-1 × 2R2), and EPP-2 × PI 270435 clone 2R2 (EPP-2 × 2R2). Their morphological characters of leaf shape, short sepals, hirsuteness, indeterminate growth habit, and length of style were intermediate between the two parents. The bridge-line hybrids were self-incompatible under greenhouse conditions, resembling the *L. peruvianum* parents.

Bridge hybrid cross-compatibility with *L. esculentum*

The numbers of seeds set in crosses were highly variable and some crosses did not produce any seeds. In general, the amount of seed production was variable, ranging from very few seeds up to 100 seeds per berry, depending on the genotype of the parents (Table 1). Most mature seeds did not germinate; all plants from these crosses were nematode-susceptible, and might have been selfed *L. esculentum*.

Bridge-line resistance to *Mi*-avirulent *M. incognita*

The bridge-lines of Poysa (1990) were tested for resistance to *Mi*-avirulent *M. incognita* isolate Project 77 at both 25°C and 30°C (Table 2). Heat-stable resistance to *M. incognita* was found in bridge-line EPP-2-I and its putative resistance donor parent LA 1708, with 0 eggs per g of root at both 25°C and 30°C. Clones of PI 128646-6 and the bridge-lines EPP-1 and EEP-1 were all susceptible at both temperatures. The 'Maranon race' accessions LA 2172 and LA 1708 and bridge-line EPP-2 segregated for heat-stable resistance to the *Mi*-avirulent *M. incognita* isolate Project 77.

Bridge-line × *L. peruvianum* hybrid resistance to *Mi*-avirulent *M. incognita*

Bridge hybrids (bridge-line × *L. peruvianum* resistance donor) and the resistant controls (embryo rescue hybrids) were resistant to *M. incognita* isolate Project 77 at both 25°C and 30°C (Table 2). A few individual nematodes within the population were able to reproduce on resistant plants, especially at 30°C, with an average of 0.7–1.7 egg masses per root system; however, their egg masses contained fewer eggs than found in a typical compatible reaction on the susceptible parents. Resistance in the bridge

Table 3 Reproduction of *Mi*-virulent *M. incognita* isolates Cote d' Ivoire and 557R at 24°–32°C and *Mi*-avirulent *M arenaria* isolate W at 25°C and 32°C in *Lycopersicon esculentum, L. peruvianum* clones. bridge-lines and their hybrids in cone-tainer experiments

Tomato	*Mi*-virulent *M. incognita*		*Mi*-avirulent *M. arenaria*	
	Cote d'Ivoire	557R	Isolate W	
	24°C–32°C	24°C–32°C	25°C	32°C
	Means of eggs/gram	Means of eggs/gram	Means of egg masses	Means of eggs/gram
L. esculentum				
Tropic	1590 d[a]	2477 de	60 b	3513 abcd
VFN-8(*Mi*)	1400 d	2191 d	13 a	15104 g
L. peruvianum				
PI 126443-1MH	205 a	34 a	–	–
PI 270435-3MH	232 a	85 a	5 a	7359 cde
LA 1708-I	2692 e	1115 bc	7 a	15 a
PI 270435-2R2	–	1050 bc	4 a	2959 abc
LA 2157-I	–	8796 f	–	–
Bridge line				
EPP-1 clone I	6268 f	–	129 c	–
EPP-2 clone I	–	4016 e	4 a	82 a
Hybrids				
EEP-1 × 1MH	82 a	109 ab	0 a	4859 bcde
1MH × EPP-1	193 a	57 a	1 a	1513 ab
EPP-1 × 2R2	744 c	1959 cd	11 a	7970 def
EPP-2 × 2R2	528 b	832 abc	0 a	9089 ef
ms-1 × 1MH	84 a	35 a	8 a	12253 fg
EPP-2 × 2157	–	2386 de	2 a	17 a

[a] Values within a column followed by the same letter are not significantly different for alpha=0.05 according to a LSD test. Values are means of four replicates for isolate Cote d' Ivoire and five replicates for isolates 557R and W

hybrids provided additional confirmation that they were true hybrids, with the heat-stable resistance genes incorporated from the *L. peruvianum* resistance donor genotypes.

Bridge-line × *L. peruvianum* hybrid resistance to *Mi*-virulent *M. incognita*

The *L. peruvianum* parental clones and their bridge hybrids were screened for resistance to the *Mi*-virulent *M. incognita* isolates Cote d'Ivoire and 557R at greenhouse summer temperature (24°C–32°C) (Table 3). The high numbers of eggs per gram of root produced on VFN-8 confirmed its susceptibility to the *Mi*-virulent *M. incognita* isolates. The numbers of egg masses per root system and eggs per gram of root produced by the *Mi*-virulent *M. incognita* isolates Cote d'Ivoire and 557R on the resistant PI 126443-1MH and PI 270435-3MH were significantly less than on the susceptible VFN-8. The clone LA 1708-I was susceptible to the *Mi*-virulent isolate Cote d'Ivoire and 557R (Table 3). Intermediate numbers of egg masses and eggs produced on roots of clone PI 270435-2R2 and its hybrids indicated the partial breakdown of the resistance to isolate 557R at high temperature (Table 3). In contrast, PI 126443-1MH and its hybrids were resistant to the isolate 557R at temperatures as high as 38°C (data not shown).

Resistance of *L. esculentum* × *L. peruvianum* hybrids to *Mi*-avirulent *M. arenaria* at 25°C and 32°C

The *L. peruvianum* donor parents and their bridge-line and embryo-rescue hybrids were resistant to *M. arenaria* isolate W at 25°C (Table 3). Clones of LA 1708 and EPP-2 were also resistant to *M. arenaria* isolate W at this moderate temperature. The high numbers of egg masses per root system produced on cv Tropic and on the bridge-line EPP-1 indicated a fully susceptible reaction at 25°C. as expected in the absence of gene *Mi* or other resistance genes. The high level of reproduction of *M. arenaria* isolate W (15104 eggs/g. root) on VFN-8 at 32°C, compared to that at 25°C, confirmed the high-temperature induction of gene *Mi* susceptibility (Table 3). The *L. peruvianum* donor parents PI 270435-2R2 and PI 126443-1MH, and their bridge-line hybrids and embryo-rescue hybrids were susceptible to *M. arenaria* isolate W at 32°C, supporting high numbers of eggs per gram of root (Table 3). Clones of LA 1708 and EPP-2 (which have heat-stable resistance to *M. incognita*) were highly resistant to *M. arenaria* isolate W at high temperature. Therefore, *M. arenaria* isolate W overcame the heat-stable resistance conferred by *Mi-2* (in PI 270435-2R2) (Cap et al. 1993) and by *Mi-3* (in PI 126443-1MH) (Yaghoobi. et al. 1995), but not the resistance carried by LA 1708 (Table 3). The bridge-line hybrid EPP-2×2R2 was susceptible to *M. arenaria* isolate W at 32°C. This con-

Table 4 Reproduction of *Mi*-virulent *M. arenaria* isolate Le Grau du Roi in different *Lycopersicon* accessions and hybrids at 32°C soil temperature in cone-tainer experiments

Genotype	(*n*)	Means of egg masses/root
L. esculentum		
Tropic	8	184 abcd[a]
VFN-8(*Mi*)	8	236 abcd
Hybrids		
EEP-1 × 1MH	5	527 ef
1MH × EPP-1	5	450 bcdef
EPP-1 × 2R2	5	411 abcdef
EPP-2 × 2R2	5	238 abcd
EPP-2 × 2157	5	464 cdef
UC82 × 2R2	5	207 abcd
ms-1 × 1MH	5	393 abcdef
L. peruvianum		
PI 126443	3	244 abcd
PI 126448	3	180 abcd
PI 126926	3	348 abcdef
PI 126440	3	245 abcd
PI 127830	3	130 abc
PI 128646	3	276 abcde
PI 129146	3	245 abcd
PI 129152	4	448 bcdef
PI 199380	3	102 a
PI 247087	6	379 abcdef
PI 251307	3	174 abcd
PI 270435	4	288 abcde
LA 111	3	293 abcde
LA 464	3	85 a
LA 1708	9	290 abcde
LA 2157	5	211 abcd
LA 2770	3	422 abcdef
LA 2745	4	327 abcdef
LA 2744	4	466 cdef
L. hirsutum		
LA 407	4	357 abcdef
LA 1624	5	564 ef
LA 2090	4	411 abcdef
PI 126445	3	119 ab
PI 127826	5	647 f
PI 134417	4	667 f
PI 134418	4	120 ab
PI 251305	6	308 abcde
PI 390514	6	428 bcdef
PI 415127	4	524 ef
L. pimpinellifolium		
LA 412	5	529 ef
LA 1257	3	170 abcd
LA 1269	6	518 ef
LA 1280	3	259 abcde
PI 126436	3	507 ef
PI 126932	4	315 abcde
PI 126947	3	384 abcdef
PI 143524	6	497 ef
PI 230327	4	376 abcdef
PI 375937	3	461 bcdef
PI 379058	4	477 def
PI 390691	4	398 abcdef
L. chilense		
LA 2773	3	488 def
LA 2930	3	493 def
L. cheesmanii		
PI 231257	3	325 abcde
Bridge lines		
EPP-2	4	218 abcd
EPP-1	4	258 abcde
EEP-1	4	406 abcdef

[a] Values followed by the same letter are not significantly different for alpha=0.05 according to a LSD test

firms that heat-stable resistance to *Mi*-avirulent isolates in the hybrid EPP-2×2R2 was incorporated from the *L. peruvianum* parent PI 270435-2R2, which is also susceptible at 32°C to *M. arenaria* isolate W, and not from the bridge-line clone EPP-2 that is resistant to isolate W at 32°C (Table 3).

Susceptibility of *Lycopersicon* spp. and *L. esculentum* × *L. peruvianum* hybrids to *Mi*-virulent *M. arenaria* at 32°C

A range of *Lycopersicon* species accessions were tested for resistance to *M. arenaria* isolate Le Grau du Roi at 32°C, together with the *L. peruvianum* parent clones PI 270435-2R2, PI 126443-1MH and their hybrids (Table 4). All the clones and hybrids supported high numbers of egg masses per root system in each case. All the tested *Lycopersicon* accessions and cultivar VFN-8 were susceptible to *M. arenaria* isolate Le Grau du Roi at 32°C (Table 4). The *L. peruvianum* donor parents and their bridge-line and embryo-rescue hybrids were highly susceptible (Table 4).

Discussion

The development of long-term sustainable solutions in crop protection depends on the identification, introduction, and management of host resistance traits. The novel nematode resistance genes in *L. peruvianum* accession clones PI 126443-1MH and PI 270435-2R2 were transferred to fertile F_1 progeny in crosses made between each clone and *L. peruvianum* × *L. esculentum* bridge lines. The hybrid nature of the F_1 plants derived from the interspecific crosses was confirmed by the expression of nematode-resistance in the hybrid progeny (Table 2). The bridge-lines did not provide an easy method to routinely transfer genes from *L. peruvianum* into *L. esculentum*: we did not recover any nematode resistant individuals with the first attempted crosses. However, the cross compatibility data (Table 1) indicate that some crosses may have greater potential than others for use in combination with seed-rescue culture. Genetically, a bridge-line hybrid may increase the viability and longevity of the embryo to reach a stage that can be rescued or to even produce a viable seedling.

The finding that one bridge-line (EPP-2) developed by Poysa (1990) possesses a heritable root-knot resistance trait, which is derived from the parental accession LA 1708, should be useful. While LA 1708 *per se* is not an effective bridge genome, it does have a cross-compatibility with *L. esculentum* (Rick 1983) and thus the resistance to nematodes could be incorporated directly into an *L. esculentum* background. In one bridge-line *L. esculentum* combination unilateral incompatibility was overcome; self-compatible bridge-line EPP-1 was used successfully as the pollen parent with self-incompatible *L. peruvianum* var. *glandulosum* PI 126443 clone 1MH. High temperature and lateness of the summer season may be components of the environmental interaction with genetic factors in the *L. peruvianum* complex that are likely to have modified the unilateral incompatibility relationship. Bridging lines may possess genetically based mechanism(s) that operate essential components for sexual hybridization within species, and can be useful in the future for introgression of desirable traits from *L. peruvianum* that do not produce fertile hybrids when crossed directly with *L. esculentum*.

The results obtained with the F_1 bridge hybrids at 25°C (*Mi* expressed) and at 30°C (*Mi* not expressed) indicate that the heat-stable resistance to the *Mi*-avirulent *M. incognita* in clones PI 270435-2R2 and PI 126443-1MH is homozygous dominant in each accession (Table 2). This is in agreement with the results obtained by Cap et al. (1991, 1993), in which F_1 hybrids of *L. peruvianum* lines PI 270435-2R2 and PI 126443-1MH with susceptible *L. esculentum* and *L. peruvianum* were all resistant at both temperatures. The finding that resistance to the *Mi*-virulent *M. incognita* isolates (Table 3) was completely dominant in PI 126443-1MH-derived F_1 hybrids is in agreement with results obtained by Roberts et al. (1990), in which F_1 hybrids from a cross between resistant *L. peruvianum* PI 126443 clone 1MH and PI 270435 clone 3MH were resistant to *M. incognita* isolates that had been laboratory selected for virulence to gene *Mi*.

The expression of resistance to *Mi*-(a)virulent *M. incognita* in hybrids at soil temperatures above 28°C provides additional evidence for the presence of new alleles or gene(s) in *L. peruvianum* that are different from the heat-sensitive *Mi* gene. With the use of the bridge-lines developed by Poysa (1990), at least three resistance traits from PI 126443-1MH have been partially incorporated into an *L. esculentum* background: a heat-unstable resistance gene (*Mi* or an allele of *Mi*); a gene that confers heat-stable resistance to *Mi*-avirulent *M. incognita* isolates; and a gene that confers heat-stable resistance to the *Mi*-virulent isolates. Similarly, at least three resistance traits have been incorporated from PI 270435-2R2 into a partial *L. esculentum* background (Veremis 1995; Veremis and Roberts 1996). The gene in clone PI 126443-1MH which is able to block reproduction of *Mi*-virulent isolates is expressed at high temperature, whereas the gene in PI 270435-2R2 that blocks reproduction of *Mi*-virulent isolates is expressed at moderate (Yaghoobi, et al. 1995), but not at high, temperatures (Table 3). Temperature is known to influence the expression of resistance in some pathosystems. The susceptibility of clone PI 270435-2R2 and hybrids to *Mi*-virulent *M. incognita* isolates at high temperature, the heat-stable resistance to *Mi*-avirulent *M. incognita* isolates in the same clones, and the heat-stable resistance to *Mi*-virulent isolates present in clone PI 126443-1MH and its hybrids, indicate the expression of different resistance genes in the two clones. Our studies demonstrate that the PI 270435-2R2 and PI 126443-1MH genotypes possesses different novel resistance phenotypes (Veremis 1995; Veremis and Roberts 1996).

The finding that clone LA 1708-I was resistant to *Mi*-avirulent *M. incognita* at high temperature (Table 2), but susceptible to *Mi*-virulent *M. incognita* isolates (Table 3), provides evidence that the gene conferring heat-stable resistance to the *Mi*-avirulent *M. incognita* isolate is assorting independently from the factor(s) conferring resistance to the *Mi*-gene virulent *M. incognita* isolates in this genotype. Additional evidence for different genes conferring heat-stable resistance and those conferring resistance to gene *Mi*-virulent isolates was found in experiments with clones of *L. peruvianum* PI 129152 (unpublished results). It seems likely that the heat-stable resistance in both *L. peruvianum* 'Maranon race' accessions LA 1708 and LA 2172 may be derived from a common ancestor and conferred by the same gene(s), but the possibility also exists that they are different. For example, heat-stable resistance in the clones 3MH and 2R2 of *L. peruvianum* accession PI 270435 is conferred by different genes (Veremis 1995; Veremis and Roberts 1996). These genetic differences in resistance are important for the improvement of tomato via the development of a broad-based root-knot resistance. Clones of LA 1708 were resistant at 32°C to *M. arenaria* isolate W, indicating that an independent resistance gene (symbol *Mi-4*) occurs in this genotype.

A summary of the genetic basis of heat-stable resistance and resistance to *Mi*-gene virulent populations of *M. incognita* and *M. arenaria* in *L. peruvianum* is given in Table 5. The distinction of the resistance in 'Maranon race' accession LA 1708, confered by gene *Mi-4*, is shown from the genes conferring heat-stable resistance to *M. incognita* in PI 126443-1MH, PI 270435-2R2 and PI 270435-3MH, all of which are susceptible to *M. arenaria* isolate W at 32°C but resistant to the same isolate at 25°C. Furthermore, PI 126443-1MH and PI 270435 clones 2R2 and 3MH are resistant to *Mi*-virulent *M. incognita* isolate 557R, to which LA 1708 is susceptible. The *M. arenaria* isolate Le Grau du Roi overcame the novel heat-stable resistance conferred by *Mi-2* (in PI 270435-2R2), *Mi-3* (in PI 126443-1MH), and by *Mi-4* (LA 1708-I).

On the basis that resistance in the wild tomato *L. peruvianum* is expressed against some *Mi*-virulent populations of root-knot nematodes but not others, we suggest that a gene-for-gene relationship (see Flor 1971; Keen 1992) may exist between the resistance in *L. peruvianum* and the matching (a)virulence in root-knot nematode (*Meloidogyne* spp.) biotypes (Table 5). The pattern of differential reactions includes expression of resistance in *L. peruvianum* genotypes at moderate and high temperature to gene *Mi*-avirulent and gene *Mi*-virulent isolates (biotypes) of

Table 5 A series of genetic differential interactions with temperature and (a)virulent isolates for resistance to *Meloidogyne incognita* and *M. arenaria* in *Lycopersicon peruvianum*

Tomato pathodemes (genotype)	*Meloidogyne* biotypes							
	M. incognita				*M. arenaria*			
	Project 77 (0)[c]		557R (1, 4)		W (0)		Le Gau du Roi (1, 2, 3, 4, 5, 6, 7, 8)	
	25°C	32°C	25°C	32°C	25°C	32°C	25°C	32°C
Tropic[a]	+[d]	+	+	+	+	+	+	+
VFN-8 (*Mi*)[b]	–	+	+	+	–	+	+	+
270435-2R2 (*Mi, Mi-2, Mi-8*[e])	–	–	–	+	–	+	+	+
270435-3MH (*Mi, Mi-6, Mi-7*)	–	–	–	–	–	+	+	+
126443-1MH (*Mi, Mi-3, Mi-5*)	–	–	–	–	–	+	+	+
LA 1708-I (*Mi, Mi-4*)	–	–	+	+	–	–	+	+

[a] *L. esculentum* susceptible control
[b] *L. esculentum* cv with gene *Mi* derived from *L. peruvianum* PI 128657
[c] According to nomenclature of Roberts (1995) for reactions at 25°C
[d] +=compatible, nematode can develop and reproduce, –=incompatible, nematode cannot develop and reproduce
[e] According to Veremis (1995) and Veremis and Roberts (1996) scheme of resistance gene designation

M. incognita and *M. arenaria*. The sequence of resistance gene designations indicated in Table 5 is based on genetic analyses of *L. peruvianum* reported separately in Veremis (1995) and Veremis and Roberts (1996). Some of the nematode biotypes are virulent to more than one gene; for example, the *M. arenaria* Le Grau du Roi biotype is virulent to all resistance genes. The quadratic check of differential interactions was developed based on the three phenotypes of heat-stable resistance, heat-unstable resistance, and resistance to gene *Mi*-virulent isolates, for a non-genetic demonstration of a gene-for-gene interaction. A gene-for-gene interaction has been demonstrated for an amphimictic nematode species using Mendelian approaches to trace the gene for (a)virulence (Janssen et al. 1991). However, for the mitotic parthenogenic root-knot nematodes gene-for-gene interaction has not been demonstrated (Castagnone-Sereno 1994; Roberts 1995). That populations of the two species *M. incognita* and *M. arenaria* are considered together as a group here is justifiable on the basis that they are part of a very closely related sibling-species complex for which species boundaries may be inappropriate (Roberts and Thomason 1989). Thus, a gene-for-gene interaction is indicated for *L. peruvianum* resistance to *Meloidogyne* spp. with the *M. incognita* and *M. arenaria* biotypes (Table 5). However, a complete transagonal matrix of interactions is required (Robinson 1987) to provide a conclusive non-genetic demonstration of a gene-for-gene relationship.

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