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Inheritance of heat-stable resistance to *Meloidogyne incognita* in *Lycopersicon peruvianum* and its relationship to the *Mi* gene

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Summary. The inheritance of heat-stable resistance to the root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood, was studied in crosses between different accessions and clones of *Lycopersicon peruvianum* L. F_1, F_2 and BC_1 generations were evaluated for their index of resistance based on numbers of eggs and infective second-stage juveniles (J_2) per gram of root, and the segregation ratios were determined in experiments carried out at constant soil temperatures of 25 °C and 30 °C. *L. peruvianum* P.I. 270435 clones 3 MH and 2 R2 and P.I. 126443 clone 1 MH, all heat-stable resistant, were crossed with *L. peruvianum* P.I. 126440 clone 9 MH, which is susceptible at both 25 °C and 30 °C. All F_1 progeny were resistant at 25 °C and 30 °C; F_2 and BC_1 generations at 25 °C gave resistant: susceptible (R:S) ratios of 15:1 and 3:1, respectively, which suggests that resistance is conditioned by two independently assorting genes. However, at 30 °C, R:S ratios of 3:1 and 1:1 were observed for the F_2 and BC_1 generations, respectively. These results indicate that heat-stable resistance is conferred by a single dominant gene expressed at 30 °C, while the second resistance gene is heat unstable and not expressed at 30 °C. P.I. 270435 clones 2 R2 and 3 MH and P.I. 126443 clone 1 MH were crossed with P.I. 128657 clone 3 R4 (source of gene *Mi*), which is resistant at 25 °C but susceptible at 30 °C. All of the F_1 progeny were resistant at 25 °C and 30 °C. TC_1 progeny of 270435-2 R2 × 128657-3 R4, 270435-3 MH × 128657-3 R4 and 126443-1 MH × 128657-3 R4 crossed with susceptible 126440-9 MH were all resistant at 25 °C and segregated in a 1:1 ratio at 30 °C. These results also suggest that the heat-stable resistance is monogenic and that it is non-allelic to gene *Mi*. The non-segregation of TC_1 progenies at 25 °C, suggests that the heat-unstable resistance factor in *L. peruvianum* P.I. 270435 clones 2 R2 and 3 MH and in P.I. 126443 clone 1 MH is allelic to or the same as gene *Mi*. We propose the symbol *Mi-2* for the gene in P.I. 270435 that confers heat-stable resistance to *M. incognita*.

Key words. Heat sensitivity – Tomato – Root-knot nematodes – Dominance – Oligogenic resistance – Gene *Mi-2*

Introduction

Root-knot nematodes (RKN), *Meloidogyne* spp., are important pests on many crops and are distributed worldwide (Sasser 1977). They cause severe damage to tomato crops (Lamberti 1979), especially in tropical, sub-tropical and warm temperate areas of the world where soil temperature is high, seasons are long and several nematode generations can be completed, resulting in high population densities. Frequently there is one or more of the four economically most important species of RKN (*M. incognita, M. arenaria, M. javanica* and *M. hapla*) involved in crop damage (Mai 1985).

Resistance to RKN was found 50 years ago in an accession (P.I. 128657) of *Lycopersicon peruvianum*, a wild relative of edible tomato (*L. esculentum*) that grows in the western coastal region of South America (Cap 1991; Cap et al. 1991; Rick 1987; Medina Filho and Stevens 1980). This resistance was transferred to and expressed in F_1 plants derived from crosses made by

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Smith (1944) between *L. peruvianum* P.I. 128657 × *L. esculentum* 'Michigan State Forcing'.

Further work with this material in the tomato breeding programs at the University of California, Davis, and the Hawaii Experiment Station culminated in the release of the first two resistant cultivars of tomato: 'VFN' and 'Anahu'. All of the RKN-resistant tomato cultivars released for commercial use are derived from these two original sources (Medina Filho and Tanksley 1983). Gilbert and McGuire (1956) determined that this resistance to *Meloidogyne* is conferred by a single dominant gene that they designated *Mi* since the original resistance screening was made with *M. incognita*. Further genetics studies confirmed this finding (Frazier and Dennett 1949; Barham and Winstead 1957; Thomason and Smith 1957).

Inheritance studies carried out on *L. esculentum* lines by Sidhu and Webster (1973, 1975, 1981) suggested the presence of two major genes, $LMiR_1$ in cv 'Nematex', and $LMiR_2$ in cv 'Small Fry', and one recessive gene $LMir_3$ in cv 'Cold Set-1'. Gene $LMiR_1$ is the same as or allelic to gene *Mi*. An incompletely dominant gene was suggested to be present in cv 'Rossol' that seems to be allelic to $LMiR_2$ (Fatunla and Salu 1977; Sidhu and Webster 1981). These studies were not conclusive, require confirmation, and it is generally considered that resistance to RKN is conditioned by a single dominant gene. Gene *Mi* does not allow *M. incognita*, *M. arenaria* and *M. javanica* to reproduce, but it is not effective against *M. hapla*. Also there is a breakdown of the resistance when the soil temperature is above 28 °C (Dropkin 1969; Holtzmann 1965). There are reports of the presence of natural populations and selected virulent isolates of *Meloidogyne* spp. normally controlled by gene *Mi* that are able to reproduce on tomatoes bearing *Mi* (Roberts and Thomason 1986, 1989; Jarquin-Barberena et al. 1991; Berthou et al. 1989). These facts and the ban on the use of several important nematicides, including most of the fumigants, due to environmental and health risks (Thomason 1987), prompted Ammati et al. in 1985 to search for new sources of resistance among wild tomato germ plasm.

Ammati et al. (1985) found that some accessions of *L. peruvianum* (P.I. 270435 and P.I. 126443) not only were resistant to *M. incognita*, *M. arenaria* and *M. javanica* but were also resistant to *M. hapla*. Additionally, this resistance was expressed in experiments in which the soil temperature was 30 °C and 32 °C, respectively (Ammati et al. 1986). Roberts et al. (1990) observed that F_1 seedlings from a cross between two of these wild tomato lines, *L. peruvianum* (*L.p.*) P.I. 270435 × *L.p.* var *glandulosum* 126443, were resistant to virulent selected populations of *M. incognita* able to reproduce on tomato plants bearing the *Mi* gene. Further work on these new sources of resistance resulted in the transfer of resistance to F_1s of crosses with *L. esculentum* (Cap et al. 1991).

An understanding about the genetic basis of this heat-stable resistance (HSR) to RKN in *L. peruvianum* will facilitate the incorporation of gene(s) controlling resistance into processing and fresh market tomato cultivars. A knowledge of the genetic basis of this HSR also could be useful in the molecular exploitation of this trait(s). The objectives of the study presented here were: (1) to determine the inheritance of HSR, and (2) to elucidate the allelic relationship of the new resistance factor(s) with gene *Mi*. Resistant and susceptible parents, F_1, F_2, BC_1 and test-cross (TC_1) progenies were screened at normal and high soil temperatures, and their segregation ratios were analyzed.

Materials and methods

Plant material

The genotypes used in this study were *Lycopersicon peruvianum* L. P.I. 270435 clones 2 R2 and 3 MH, both resistant at 25 °C and 30 °C (Ammati et al. 1986); P.I. 128657 clone 3 R4, source of gene *Mi*, resistant at a normal (< 28 °C) soil temperature but susceptible at a soil temperature above 28°–30 °C (Holtzmann 1965; Dropkin 1969; Ammati et al. 1986); P.I. 126443 clone 1 MH, resistant at soil temperatures of 25 °C and 30 °C (Ammati et al. 1985; Ammati et al. 1986; Cap et al. 1991); and P.I. 126440 clone 9 MH, susceptible at both soil temperatures. The genotypes were obtained from the wild *Lycopersicon* spp. clone collection maintained by the Department of Nematology, University of California, Riverside. The number following the P.I. designation refers to a particular resistant or susceptible individual plant within that accession that was screened previously against RKN by Ammati et al. (1985, 1986). The letters MH stand for *M. hapla*, R2 for *M. incognita* race 2, and R4 for *M. incognita* race 4.

Parental material was propagated agamically. Cuttings 3 nodes in length from young stems with a diagonal cut at 45° were taken from the plants and dusted with rooting powder containing 2000 mg/l 1-naphthaleneacetamide, 1000 mg/l indole-3-butyric acid and 4040 mg/l Thiram. The dusted cuttings were planted in speedling trays containing vermiculite and maintained in a mist chamber for at least 2 weeks, by which time the cuttings had started to produce new roots.

Pollen from all of the parents was collected and stored as described by McGuire (1952). F_1 progeny were produced by crossing susceptible and resistant genotypes using pollen collected the previous day. Crosses were made under greenhouse conditions between 11 am and 1 pm by pollinating female flowers that had been emasculated previously. Pollinated ovaries were enclosed with paper bags to avoid any cross-pollination from other exotic tomato plants.

For the production of F_2 seed progeny, the method of McGuire and Rick (1954) was followed. Pollen from individual F_1 plants was used to check for compatible interactions with other F_1 plants, and in this way the S (self-incompatibility gene system) allelic composition of each plant could be deduced.

The production of BC_1 and test-cross (TC_1) seed progeny was carried out as described above for obtaining F_1 seeds. Test-cross seed progeny were produced in order to study the allelic relationships of the *Mi* gene with the new resistance factor(s). Crosses were made as described above between *L. peruvianum* P.I. 128657 (source of *Mi*) × P.I. 270435 clone 2 R2, clone 3 MH and P.I. 126443 clone 1 MH (possessing HSR, Cap et al. 1991). These F_1s were test crossed with the susceptible P.I. 126440 clone 9 MH.

Although most of the crosses set fruits, not all fruits produced mature seeds. In general seed production was variable from very few seeds up to 100 seeds per berry.

Nematode culture

The *M. incognita* race 1 culture was started from a single egg mass on greenhouse-grown tomato cv 'Tropic' plants. The identity of the nematode isolate was confirmed morphologically by microscopic examination of the perineal patterns of adult females (Eisenback 1985), by isozyme (esterase and malate dehydrogenase) phenotypes (Cap and Roberts 1992) and by the North Carolina differential host test (Hartman and Sasser 1985).

Screening tests

One-month-old seedlings or rooted cuttings were used for tests of host reaction to nematodes. Single plants were grown in 1-1 polyethylene plastic cups (Louisiana Plastic, Saint Louis, Mo.), filled with steam-sterilized loamy sand and fertilized with Osmocote. The experiments were carried out in water-bath temperature tanks, and the soil temperature in the cups was maintained constantly at either 25 °C or 30 °C for each experiment. Seedlings of the test-cross (128657-3 R4 × 126443-1 MH) × 126440-9 MH and (128657-3 R4 × 270435-3 MH) × 126440-9 MH were transferred to growth pouches (Omwega et al. 1988, modified by Cap 1991) and maintained in a growth chamber at 25 °C and 30 °C. Inoculum was prepared by the method of Hussey and Barker (1973). A water suspension of 5,000 eggs and infective second-stage juveniles (J_2) was pipetted into three holes of a half-cup depth in the soil around the plant roots. Plants were arranged in each temperature tank in a completely randomized design. Plants in the growth pouches were inoculated with a suspension of 1,000 J_2, and the plants within the growth chamber were arranged in a completely randomized design. Nematode egg production on the roots was evaluated after the accumulation of approximately 500 degree days (12,000 heat units above the nematode developmental threshold temperature of 10 °C). This allowed completion of one nematode generation (Tyler 1933). Root systems were washed free of soil under tap water, damp-dried with paper towelling and weighed. Eggs were extracted by first macerating the roots in 1% NaOCl solution in a commercial blender and then pouring and rinsing the macerate through a screen series with openings of 850μ, 106μ, and 38μ, respectively. The collected egg suspension was brought to a known volume, and the numbers of eggs were counted under a dissecting microscope (Hussey and Barker 1973). In the growth pouch experiment, the root system was first removed from the pouch, and then the same nematode-extraction procedure was followed as for the plants growing in the plastic cups.

The number of eggs per gram of root was calculated by dividing the total number of eggs per root system by the total root weight; this was used to develop an index of resistance (IR). A plant was considered to be resistant when the number of eggs per gram of root was less than 10% of the eggs per gram of root found on the susceptible control *L. peruvianum* P.I. 126440 clone 9 MH. Due to the poorer growth of root systems of the $TC_{1.2}$ and $TC_{1.3}$ plants tested in growth pouches at 30 °C (Table 4), typical susceptible responses, that included obvious root-galling, had less egg production than in other tests. Therefore, resistance was assigned for plants with eggs per gram of root less than 10% of the highest susceptible score for $TC_{1.2}$ (1,545 eggs per gram of root) or $TC_{1.3}$ (3,215 eggs per gram root) plants, respectively. Tomato cv 'Tropic', susceptible to RKN, and 'VFN' (bearing gene *Mi*), resistant at 25 °C but susceptible at 30 °C, were included in all tests to check inoculum viability and infectivity. Chi-square tests were conducted on F_2, BC_1 and TC_1 data to determine goodness of fit to the hypothetical segregation ratios for resistant and susceptible classes.

Results

Experiment at a soil temperature of 25 °C

All plants of *L. peruvianum* P.I. 270435 clones 2R2 (a mean of 180 eggs/g root) and 3 MH (a mean of 250 eggs/g root) and P.I. 126443 clone 1 MH (a mean of 91 eggs/g root) (parental classes) were classified as resistant. All plants of *L. peruvianum* P.I. 126440 clone 9 MH (a mean of 15,300 eggs/g root) (parental class) were classified as susceptible (Table 1). All F_1 progeny between the susceptible 126440-9 MH and the HSR parents, 270435-3 MH and 126443-1 MH ($F_{1.1}$ and $F_{1.2}$, respectively), were resistant, indicating that resistance in both *L. peruvianum* genotypes is dominant (Table 1).

Table 1. Segregation of parents, F_1, F_2 and BC_1 progenies for resistance to *M. incognita* race 1 at a soil temperature of 25 °C

Generation	Parent or cross	Number of plants			Expected ratios		χ^2	*P*
		Total	R	S	R	S		
P_1	*L. peruvianum* P.I. 270435 clone 3 MH	10	All					
P_2	*L. peruvianum* P.I. 126443 clone 1 MH	10	All					
P_3	*L. peruvianum* P.I. 126440 clone 9 MH	10		All				
$F_{1.1}$	$P_1 \times P_3$	38	All					
$F_{1.2}$	$P_2 \times P_3$	45	All					
$F_{2.1}$	$F_{1.1} \times F_{1.1}$	28	27	1	15	1	0.037	0.80–0.90
$F_{2.2}$	$F_{1.2} \times F_{1.2}$	20	19	1	15	1	0.053	0.80–0.90
$BC_{1.1}$	$F_{1.1} \times P_3$	41	32	9	3	1	0.073	0.80–0.70
$BC_{1.2}$	$F_{1.2} \times P_3$	27	25	2	3	1	3.56	0.10–0.05

R, Resistant; S, susceptible

F_2 progeny derived from $F_{1.1}$ contained 27 resistant:1 susceptible plants, indicating a segregation ratio of 15:1 (R:S) with a chi-square value of 0.037 ($0.80 < P < 0.90$) (Table 1). F_2 progeny of $F_{1.2}$ contained 19 resistant:1 susceptible plants, indicating a segregation ratio again of 15:1 (R:S) with a chi-square value of 0.053 ($0.80 < P < 0.90$) (Table 1). The backcross progeny of $F_{1.1}$ crossed with the recurrent susceptible parent P_3 contained 32 resistant: 9 susceptible plants, indicating a segregation ratio of 3:1 (R:S) with a chi-square value of 0.073 ($0.80 < P < 0.70$) (Table 1).

The $BC_{1.2}$ progeny ($F_{1.2} \times P_3$) contained 25 resistant:2 susceptible plants, indicating a segregation ratio again of 3:1 (R:S) with a chi-square value of 3.56 ($0.10 < P < 0.05$) (Table 1).

Experiment at a soil temperature of 30 °C

Plants of heat-stable resistant parents P_1 (a mean of 125 eggs/g root) and P_2 (a mean of 85 eggs/g root) were all resistant, whereas those of P_3 (a mean of 16,600 eggs/g root) were all susceptible (Table 2).

$F_{1.1}$ and $F_{1.2}$ progeny were all resistant (Table 2), providing further confirmation of the complete dominance of HSR in both *L. peruvianum* genotypes.

The $F_{2.1}$ progeny contained 25 resistant:7 susceptible plants, indicating a segregation ratio of 3:1 (R:S) with a chi-square value of 0.16 ($0.70 < P < 0.50$) (Table 2).

$BC_{1.1}$ progeny contained 23 resistant:22 susceptible plants, indicating a segregation ratio of 1:1 (R:S) with a chi-square value of 0.02 ($0.90 < P < 0.80$) (Table 2).

Table 2. Segregation of parents, F_1, F_2 and BC_1 progenies for resistance to *M. incognita* race 1 at a soil temperature of 30 °C

Generation	Parent or cross	Number of plants			Expected ratios		χ^2	P
		Total	R	S	R	S		
P_1	*L. peruvianum* P.I. 270435 clone 3 MH	5	All					
P_2	*L. peruvianum* P.I. 126443 clone 1 MH	10	All					
P_3	*L. peruvianum* P.I. 126440 clone 9 MH	10		All				
$F_{1.1}$	$P_1 \times P_3$	29	All					
$F_{1.2}$	$P_2 \times P_3$	28	All					
$F_{2.1}$	$F_{1.1} \times F_{1.1}$	32	25	7	3	1	0.16	0.70–0.50
$BC_{1.1}$	$F_{1.1} \times P_3$	45	23	22	1	1	0.02	0.90–0.80
$BC_{1.2}$	$F_{1.2} \times P_3$	31	18	13	1	1	0.80	0.50–0.30

R, Resistant; S, susceptible

Table 3. Segregation of parents, F_1, and test-cross progenies for resistance to *M. incognita* race 1 at a soil temperature of 25 °C

Generation	Parent or cross	Number of plants			Expected ratios	
		Total	R	S	R	S
P_1	*L. peruvianum* P.I. 270435 clone 2 R2	10	All			
P_2	*L. peruvianum* P.I. 270435 clone 3 MH	10	All			
P_3	*L. peruvianum* P.I. 128657 clone 3 R4 *Mi* source	13	All			
P_4	*L. peruvianum* P.I. 126443 clone 1 MH	10	All			
P_5	*L. peruvianum* P.I. 126440 clone 9 MH	10		All		
$F_{1.1}$	$P_1 \times P_5$	72	All			
$F_{1.2}$	$P_3 \times P_1$	14	All			
$F_{1.3}$	$P_2 \times P_3$	30	All			
$F_{1.4}$	$P_4 \times P_3$	14	All			
$TC_{1.1}$	$F_{1.2} \times P_5$	99	99	0	All	
$TC_{1.2}$	$F_{1.3} \times P_5$	45	45	0	All	
$TC_{1.3}$	$F_{1.4} \times P_5$	52	52	0	All	

R, Resistant; S, susceptible

$BC_{1.2}$ progeny contained 18 resistant:13 susceptible plants, also indicating a segregation ratio of 1:1 (R:S) with a chi-square value of 0.80 ($0.50 < P < 0.30$) (Table 2).

Test-cross experiment at a soil temperature of 25 °C

All plants of the parental lines *L. peruvianum* P.I. 270435 clone 2R2 (P_1) (a mean of 192 eggs/g root), P.I. 270435 clone 3MH (P_2) (a mean of 275 eggs/g root) and P.I. 126443 clone 1 MH (P_4) (a mean of 120 eggs/g root), all possessing HSR, and P.I. 128657 clone 3R4 (P_3) (a mean of 350 eggs/g root), source of gene *Mi*, were resistant (Table 3). All plants of the test-crosser, *L. peruvianum* P.I. 126440 clone 9 MH (P_5) (a mean of 14,800 eggs/g root), were susceptible (Table 3).

The $F_{1.1}$ (72 plants), $F_{1.2}$ (14 plants), $F_{1.3}$ (30 plants) and $F_{1.4}$ (14 plants) progeny were all resistant (Table 3). The test-cross $TC_{1.1}$, $TC_{1.2}$ and $TC_{1.3}$ progeny (99, 45, and 52 plants, respectively) were all resistant (Table 3).

Test-cross experiment at a soil temperature of 30 °C

All plants of the HSR parental lines P_1 (a mean of 210 eggs/g root, P_2 (a mean of 300 eggs/g root) and P_4 (a mean of 130 eggs/g root) were resistant, whereas all the plants of parental line P_3 (a mean of 10,500 eggs/g root) were susceptible (Table 4), as was expected due to the breakdown of heat-unstable resistance conferred by gene *Mi*, as was the susceptible P_5 (a mean of 13,500 eggs/g root).

The $F_{1.1}$ progeny contained only resistant plants (32 individuals), providing further confirmation of the complete dominance of HSR (Table 4). The $F_{1.2}$ progeny were all resistant (14 plants), as were all the plants of the $F_{1.3}$ (15 plants) and $F_{1.4}$ (11 plants).

The test-cross $TC_{1.1}$ population contained 27 resistant:36 susceptible plants, indicating a segregation ratio of 1:1 (R:S) with a chi-square value of 1.28 ($0.30 < P < 0.20$) (Table 4). Although poor root growth of the $TC_{1.2}$ and $TC_{1.3}$ plants limited total egg production, a clear assignment of individuals to resistant or susceptible classes was possible as described, aided by the higher numbers of egg masses and root-galls in susceptible reactions. The $TC_{1.2}$ contained 16 resistant:19 susceptible plants, indicating also a segregation ratio of 1:1 (R:S) with a chi-square value of 0.44 ($0.75 < P < 0.50$). The test-cross $TC_{1.3}$ population contained 61 resistant: 39 susceptible plants, indicating a segregation ratio again of 1:1 (R:S) with a chi-square value of 4.84 ($0.05 < P < 0.25$) (Table 4).

Discussion

The results obtained with the F_1 populations at normal (25 °C) and high (30 °C) soil temperatures indicate that the nature of HSR to *M. incognita* race 1 is completely dominant (Tables 1 and 2). This is in agreement with the results obtained by Cap et al. (1991), in which F_1 hybrids between *L. esculentum* and HSR *L. peruvianum* lines P.I. 270435 and P.I. 126443 produced through tissue culture were all resistant.

Results obtained in the experiment conducted at a normal (25 °C) soil temperature show segregation ratios

Table 4. Segregation of parents, F_1, and test-cross progenies for resistance to *M. incognita* race 1 at a soil temperature of 30 °C

Generation	Parent or cross	Number of plants			Expected ratios		χ^2	P
		Total	R	S	R	S		
P_1	*L. peruvianum* P.I. 270435 clone 2R2	8	All					
P_2	*L. peruvianum* P.I. 270435 clone 3MH	10	All					
P_3	*L. peruvianum* P.I. 128657 clone 3R4 *Mi* source	13		All				
P_4	*L. peruvianum* P.I. 126443 clone 1MH	14	All					
P_5	*L. peruvianum* P.I. 126440 clone 9MH	10		All				
$F_{1.1}$	$P_1 \times P_5$	32	All					
$F_{1.2}$	$P_3 \times P_1$	14	All					
$F_{1.3}$	$P_2 \times P_3$	15	All					
$F_{1.4}$	$P_4 \times P_3$	11	All					
$TC_{1.1}$	$F_{1.2} \times P_5$	63	27	36	1	1	1.28	0.30–0.20
$TC_{1.2}$	$P_5 \times F_{1.3}$	35	16	19	1	1	0.44	0.75–0.50
$TC_{1.3}$	$P_5 \times F_{1.4}$	100	61	39	1	1	4.84	0.05–0.025

R, Resistant; S, susceptible

for resistance of 15:1 (R:S) and 3:1 (R:S) in the F_2 and BC_1 populations, respectively, indicating that two independently segregating dominant genes are expressed at this temperature (Table 1). However, when the soil temperature was high (30 °C), F_2 and BC_1 populations segregated in a 3:1 (R:S) and 1:1 (R:S) fashion, respectively (Table 2). These ratios are expected for a single dominant gene with a major effect.

These results indicate that each of the genotypes possess two independent dominant resistance genes of major effect; one which is heat stable and another which is heat unstable. Both genes are expressed at a normal (25 °C) soil temperature, but only the heat-stable gene is expressed at a high (30 °C) soil temperature. We propose the symbol *Mi-2* for this heat-stable gene in *L. peruvianum* P.I. 270435.

In order to examine the relationship between the heat-unstable resistance gene in these *L. peruvianum* genotypes to the heat-unstable gene *Mi*, we hypothesized that the heat-unstable gene in P.I. 270435 and P.I. 126443 is allelic to or the same as gene *Mi*. If this is the case, then segregation ratios for two independent genes would be expected at 25 °C. However, if the unstable resistance locus was independent of the *Mi* gene, then segregation ratios for three independent genes – *Mi* + a new heat-unstable gene + *Mi-2* – would be expected. In the first case, assuming that the heat-unstable gene is allelic to or the same as the *Mi* gene, the test-cross ($TC_{1.1}$, $TC_{1.2}$, and $TC_{1.3}$) progeny will not segregate in a 3:1 (R:S) ratio, as is expected for an independent assortment of two genes, because the F_1 used in the cross with the susceptible tester line will be homozygous dominant for the heat-unstable gene. The TC_1 will contain four genotypes, and all of them will have at least one dominant allele for resistance present. Thus all TC_1-derived plants should be resistant.

According to the results discussed previously, the heat-stable resistant genotypes contain two dominant genes. If the heat-unstable gene present in these genotypes was independent of gene *Mi*, the segregation ratio expected in the TC_1-derived progeny will contain eight genotypes, and in seven out of the eight at least one dominant allele for resistance will be present, whereas there will be only one possible gamete combination in which a triple recessive condition of susceptibility will be present, giving a segregation ratio of 7:1 (R:S). Test-cross populations at a normal soil temperature did not segregate, and all of the individuals were resistant (Table 3). Thus, the hypothesis that the heat-unstable gene is allelic to or the same as gene *Mi* is supported. Results from experiments run at a soil temperature of 30 °C showed that test-cross populations segregated in a 1:1 ratio (Table 4). This confirms the expression of one dominant gene at a high soil temperature (*Mi-2*) as the basis of the heat-stable resistance and further supports the independence of *Mi-2* from *Mi*.

Additional circumstantial evidence for our proposed genetic relationship (same as or allelic to) of the heat-unstable resistance gene (HUR) in *L.p.* P.I. 270435 clone 2R2 to gene *Mi* is given by the presence in this genotype of the variant allele $^{1/1}$ for the isozyme acid phosphatase 1 (*Aps1*) (Cap et al. 1991). The *Aps1* locus is tightly linked with the *Mi* locus and the $Aps1^{1/1}$ allele is associated with the presence of the *Mi* dominant allele (Rick and Fobes 1974). This is found both in the original *Mi* donor genotype *L. peruvianum* 128657 and in most *L. esculentum* cultivars bearing the *Mi* dominant allele, except for the rare occurrence where breeding has resulted in recombination between *Mi* and *Aps1*. The presence of $Aps1^{1/1}$ in *L.p.* P.I. 270435-2R2 (see Cap et al. 1991) supports the hypothesis that the HUR gene in this genotype could be the same as *Mi*.

Table 5 is a summary of our findings about the genetics of HSR in these exotic tomato genotypes. The relationship of the HSR gene in P.I. 126443 to gene *Mi-2* in P.I. 270435 is unknown. It seems likely that the HSR gene in both genotypes is the same, but the possibility does exist that they could be different. Differences certainly would be desirable and important for the improvement of tomato genetic resources with respect to RKN resistance. It will be important to see whether *Mi-2* is expressed against the other economically important root-knot nematode species *M. javanica*, *M. arenaria* and *M. hapla*, and also whether *Mi-2* is expressed against selected virulent populations of RKN such as those selected on *Mi* gene-bearing plants. Some of the virulent isolates selected were unable to reproduce on F_1 hybrid progeny of the cross *L.p.* P.I. 270435-3 MH and *L.p.* P.I. 126443-1 MH (Roberts et al. 1990) when screened at a normal soil temperature only. This HSR conferred by the *Mi-2* gene has been transferred to F_1s with *L. esculentum* (Cap et al. 1991), and this very definitely constitutes an important preliminary step toward the improvement of genetic diversity in adapted tomato cultivars.

Table 5. A summary of genetic relationships among RKN (*M. incognita*) resistance genes in exotic tomato genotypes

Genotype and clone	Number of genes[a]		Relationship of HUR to *Mi*
	Heat-unstable	Heat-stable	
L. peruvianum P.I. 128657 (*Mi* source)	One (*Mi*)	None	Same
P.I. 270435-2R2	One	One (*Mi-2*)	Same locus
P.I. 270435-3 MH	One	One (*Mi-2*)	Same locus
L.p. var *glandulosum* P.I. 126443 1 MH	One	One (*Mi-2* ?)	Same locus

[a] All genes expressed in a dominant fashion

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