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Evidence for a Dosage Effect of the *Mi* Gene on Partially Virulent Isolates of *Meloidogyne javanica*

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Abstract: The reproduction of single egg-mass isolates of *Meloidogyne javanica* from Crete that differed in virulence were compared on tomato (*Lycopersicon esculentum*) genotypes homozygous or heterozygous for the *Mi* gene. The reproduction of three isolates with partial virulence was much greater on tomato genotypes heterozygous for the *Mi* gene (cultivars Scala, Bermuda, and 7353) than on two homozygous genotypes (F8 inbred lines derived from Scala). The reproduction of a highly virulent isolate on the homozygous and heterozygous genotypes was similar to that on a susceptible cultivar. These results pose questions regarding the nature of partial virulence and indicate a quantitative effect of the *Mi* gene in relation to such virulence.

Key words: heterozygous resistance, homozygous resistance, *Lycopersicon esculentum, Meloidogyne javanica, Mi* gene, nematode, resistance, root-knot nematodes, tomato, virulence.

Resistance to root-knot nematodes in tomato (*Lycopersicon esculentum* Mill.) is generally thought to be conferred by a single dominant gene (allele) designated *Mi* (Roberts, 1992). Whether only one or a small group of very tightly linked alleles is involved is uncertain but should soon be resolved (V. Williamson, pers. comm.). The *Mi* allele is effective in the heterozygous state against many populations of *Meloidogyne incognita* (Kofoid & White) Chitwood, *M. javanica* (Treub) Chitwood, and *M. arenaria* (Neal) Chitwood (Roberts, 1992). However, the resistance conferred by the *Mi* gene is not always effective; it is greatly decreased at soil temperatures above 28 °C (Dropkin, 1969), and naturally virulent populations of all three *Meloidogyne* species have been identified (Castegnone-Sereno et al., 1993; Castegnone-Sereno et al., 1994; Roberts et al., 1990). With a minority of populations and lines, it has been possible to progressively increase virulence from a low level by repeated selection on *Mi* resistant tomato (Jarquin-Barberena et al., 1991; P. A. Roberts, pers. comm.). As these three *Meloidogyne* species reproduce by mitotic parthenogenesis (Triantaphyllou, 1985), it is difficult to account for such increases in virulence, or for partial virulence (Tzortzakakis and Gowen, 1996), in populations derived from single egg-masses.

Our objective was to compare the resistance of tomato homozygous and heterozygous for the *Mi* gene against single egg-mass isolates of *M. javanica* that differed in virulence. Of particular interest were single egg-mass isolates with partial virulence previously described by Tzortzakakis and Gowen (1996).

Materials and Methods

Nematode isolates: Four single egg-mass isolates of *M. javanica* that originated from different fields close to the south coast of Heraklion province, Crete, were used. Isolate 1 was previously characterized as highly virulent because it reproduced at a similar high rate on both resistant and susceptible cultivars of tomato (Tzortzakakis and Gowen, 1996). Isolates 2 and 3a did not reproduce on the resistant tomato hybrids and were classified as avirulent. Isolate 3b came from the same field as 3a, but was characterized as having low virulence (Tzortzakakis and Gowen, 1996) because it had a low rate of reproduction on the resistant cultivars of tomato. Prior to the experiments described below these isolates were separately maintained on susceptible tomato cv. Rutgers.

Plant material and experiments: Two inbred

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F8 lines (F8A, F8B) of tomato homozygous for the *Mi* allele were developed from the heterozygous cv. Scala (P. L. J. Egelmeers, pers. comm.). The resistance of these inbred lines to the populations of *M. javanica* that differed in virulence was compared with that of cv. Scala in three experiments using cv. Dombito as the susceptible control.

Experiment 1 was in a growth room at 22 to 26 °C with 16 hours light and used only Isolates 1 and 3a. Experiments 2 and 3 were conducted concurrently and compared the same tomato genotypes as Experiment 1, plus cultivars Bermuda and 7353 (both F1 hybrids heterozygous for *Mi*). All four *M. javanica* isolates were used. Experiment 2 was in the growth room with the same conditions as Experiment 1, whereas Experiment 3 was in a glasshouse where temperatures ranged from 10 to 35 °C.

Experimental methods: Tomato seeds were germinated in trays of commercial, sterilized compost soil in a glasshouse and transplanted at the two-leaf stage into plastic pots filled with steam-sterilized sandy loam soil. The plants were watered as necessary, and liquid fertilizer (5-8-10 NPK) diluted 200 times in water was applied twice per month. One week after transplanting, the tomato plants in the growth room experiments were inoculated with 300 and those in the glasshouse with 600 second-stage juveniles (J2)/pot. The J2 were obtained from egg masses incubated on extraction filters at 25 to 26 °C (Southey, 1986); only those collected between 24 and 96 hours of incubation were used.

Eight weeks after inoculation, virulence and resistance were assessed in the following manner. Tomato plant roots were carefully washed free of soil, stained in a solution of phloxine B (Hartman and Sasser, 1985), and the number of visible egg masses counted under a stereo microscope.

All experiments contained at least four replicates arranged in randomized blocks. The data were subjected to analysis of variance, and the least significant differences ($P < 0.05$) between the treatment means were calculated. Data were transformed to square roots to standardize the variances (Mead and Curnow, 1990).

Results

Experiment 1: The results from Experiment 1 (Table 1) confirmed the high virulence of *M. javanica* Isolate 1 and the low virulence of Isolate 3a (despite the previous classification as avirulent) on resistant tomato cv. Scala heterozygous for the *Mi* allele. Isolate 1 was also completely virulent on the two inbred tomato genotypes (F8A and F8B) homozygous for *Mi,* but Isolate 3a was completely avirulent on these genotypes. The analysis of variance confirmed that there were highly significant interactions between the isolates of *M. javanica* and tomato genotypes ($P < 0.001$). The numbers of egg masses produced by Isolate 1 were not significantly different for tomato genotypes heterozygous and homozygous for the *Mi* allele. In contrast, Isolate 3a produced significantly fewer ($P < 0.01$) egg masses on the tomato genotypes homozygous for the *Mi* allele than on the heterozygous genotypes.

Experiments 2 and 3: The results with Isolates 1 and 3a for Experiments 2 and 3 (Tables 2,3) confirmed those obtained in Experiment 1, except that, in the glasshouse, Isolate 3a produced an occasional egg mass on the *Mi* homozygous genotypes (Table 3). There were significant *M. javanica* isolate by tomato genotype interactions in both experiments (Table 4). Isolate 1 produced many egg masses (>20) on all of the tomato genotypes. However, there were

Table 1. Square root-transformed mean numbers of egg masses produced on four genotypes of tomato by two single egg-mass isolates of *Meloidogyne javanica* in a growth room (Experiment 1).

Tomato genotype	*Mi* allelic condition	Nematode isolates	
		1 (virulent)	3a (avirulent)
Dombito	Susceptible	9.48 (89.9)[a]	7.34 (53.9)
F1 Scala	Heterozygous	9.21 (84.8)	4.32 (18.7)
F8 A	Homozygous	8.63 (74.5)	0.33 (0.1)
F8 B	Homozygous	8.36 (69.9)	0.00 (0.0)
LSD 5%		0.98	

[a] ( ): detransformed data.

TABLE 2. Square root-transformed mean numbers of egg masses produced by four single egg-mass isolates of *Meloidogyne javanica* on six genotypes of tomato in a growth room (Experiment 2).

Tomato genotype	*Mi* allelic condition	Nematode Isolate 1 (virulent)	2 (avirulent)	3a (avirulent)	3b (semi-virulent)
Dombito	Susceptible	6.93 (48)[a]	6.12 (37)	5.50 (30)	8.01 (64)
F1 Scala	Heterozygous	6.96 (48)	2.18 (5)	1.96 (4)	2.13 (5)
F8 A	Homozyrous	5.66 (32)	0.43 (<1)	0.00 (0)	0.00 (0)
F8 B	Homozygous	6.63 (44)	1.04 (1)	0.00 (0)	0.50 (<1)
F1 73 53	Heterozygous	7.56 (57)	3.55 (13)	0.25 (<1)	3.77 (14)
F1 Bermuda	Heterozygous	7.04 (50)	2.43 (6)	0.50 (<1)	0.60 (<1)
LSD 5%			1.09		

[a] ( ): detransformed results.

fewer egg masses ($P < 0.05$) on homozygous F8A in Experiment 2 and the heterozygous cv. Bermuda in Experiment 3 than on the susceptible cv. Dombito. In contrast, the numbers of egg masses produced by Isolate 3a were decreased significantly ($P < 0.01$) by increasing *Mi* copy number, except that in Experiment 2 heterozygous F1 73 53 and F1 Bermuda were significantly ($P < 0.01$, 0.05) more resistant than Scala.

Although Isolate 2 was previously characterized as completely avirulent, in both Experiments 2 and 3 it showed some virulence on the heterozygous resistant tomatoes; Isolate 3b also showed some virulence. Even so, they both consistently produced the fewest egg masses on the *Mi* homozygous lines.

Using the results from Experiments 2 and 3, a more detailed analysis was made of the interactions between the isolates of *M. javanica* and host genotypes by partitioning the interaction sum of squares into: i) comparison of heterozygous cv. Scala and the two *Mi* homozygous genotypes derived from it; and ii) interaction with the remaining host genotypes (Table 4). Results for virulent Isolate 1 were omitted because the *Mi* allele had a marked lack of effect on it compared with the other three isolates of *M. javanica*. This analysis showed that there was a significant effect of different *Mi* copy number ($P < 0.001$), but no interaction between isolates of *M. javanica* and cv. Scala and the two F8 lines. The results in both experiments confirmed that all three isolates produced more egg masses on the heterozygous cv. Scala than on the homozygous F8 lines. The only significant interactions were between the isolates of *M. javanica* and Dombito, F1 7353, and Bermuda tomatoes.

TABLE 3. Square root-transformed mean numbers of egg masses on tomatoes inocluated with single egg-mass isolates of *Meloidogyne javanica* in a glasshouse (Experiment 3).

Tomato genotypes	Nematode Isolate 1 (virulent)	2 (avirulent)	3a (avirulent)	3b (semi-virulent)
Dombito	6.30 (40)[a]	6.07 (37)	5.68 (32)	6.45 (42)
F1 Scala	5.74 (33)	3.54 (13)	3.28 (11)	3.67 (13)
F8 A	5.57 (31)	1.08 (1)	1.17 (1)	0.88 (<1)
F8 B	6.30 (40)	1.42 (2)	0.48 (<1)	1.99 (4)
F1 73 53	6.08 (37)	2.17 (5)	2.31 (5)	2.76 (8)
F1 Bermuda	4.81 (23)	2.08 (4)	3.07 (9)	3.87 (15)
LSD 5%		1.00		

[a] ( ): detransformed results.

TABLE 4. Analysis of variance of data from Experiments 2 and 3, excluding the results with virulent Isolate 1.

Source	Mean square	
Experiments	20.46	***
Reps within experiments	0.45	
Meloidogyne isolates	10.15	***
Tomato genotypes	109.81	***
Scala vs. F8 A+B	77.59	***
Deviations	117.85	***
Experiments × *Meloidogyne* isolates	4.78	***
Experiments × tomato genotypes	5.05	***
Experiments × (Scala vs. F8 A+B)	1.43	
Experiment × deviations	5.95	***
Meloidgyne × tomato	1.54	**
Meloidogyne × (Scala vs. F8 A+B)	0.16	
Meloidogyne × deviations	1.88	***
Experiment × *Meloidogyne* × tomato	2.79	***
Experiment × *Meloidogyne* × (Scala vs. F8 A+B)	0.09	
Experiment × *Meloidogyne* × *deviation*	3.48	***
Residual	0.52	

DISCUSSION

Ideally, near-isogenic lines of tomato should be used to test the expression of *Mi* resistance in the homozygous and heterozygous states as this would ensure that any differences in egg-mass production were due to differences in *Mi* copy number and not differences in genetic background. We used the next best approach as our experiments compared a *Mi*-heterozygous genotype (cv. Scala) with two inbred lines derived from it. Thus, the two homozygous genotypes, even though not isogenic, are derived from the same (cv. Scala) genetic background. Even so, the results should be interpreted with caution because the comparison of cv. Scala with the other two genotypes heterozygous for *Mi* indicate interactions involving the isolates of *M. javanica* and environmental factors.

In our experiments, the three isolates of *M. javanica* with low virulence consistently produced more egg masses on heterozygous cv. Scala than on the two *Mi*-homozygous F8 lines. This consistent difference indicates that there is probably a dose effect of the *Mi* gene, as the genetic backgrounds of the two F8 genotypes are likely to be as different from one another as they are from cv. Scala.

These results raise several questions. It is apparent that such an interaction can be detected only with single egg-mass isolates that have intermediate virulence. Virulent Isolate 1 was unaffected by *Mi* copy number, and the reproduction of a completely avirulent isolate would be totally suppressed on all *Mi*-bearing plants. However, it is unclear how partial virulence might be inherited in a nematode that supposedly reproduces by mitotic parthenogenesis, or the mechanisms by which it might be expressed. Selection studies (Jarquin-Barberena et al., 1991; P. A. Roberts, pers. comm.) indicate that partial virulence in *Meloidogyne* spp. can be a quantitatively inherited character that can be increased by selection, and that this selection can lead to other changes in host range (Castagnone-Sereno et al., 1992).

Although the *Mi* allele is regarded as completely "dominant," this perception is of a phenotype usually identified using a completely avirulent population of *Meloidogyne*. If the *Mi* allele is expressed co-dominantly at the genotypic level, then, where virulence is quantitatively inherited, the level of resistance observed may be affected by *Mi* copy number. Testing with partially virulent lines, as we have done, suggests that the level of resistance is influenced by *Mi* copy number, indicating co-dominant inheritance. The *Mi* allele may not be typical of most nematode-resistance genes because, as with resistance in bean (Roberts, 1992), its effectiveness is influenced by temperature. Therefore, the isolation and molecular characterization of the *Mi* allele could provide an interesting comparison with other resistance alleles that have already been cloned and sequenced (Jones, 1996).

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