European Journal of Plant Pathology **102:** 585–590, 1996.

Selection for *Meloidogyne incognita* virulence against resistance genes from tomato and pepper and specificity of the virulence/resistance determinants

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Accepted 25 March 1996

Key words: Capsicum annuum, doubled haploid pepper lines, *Lycopersicon esculentum*, *Meloidogyne incognita*, near-isogenic nematode lines, root-knot nematode resistance genes, virulence

Abstract

Experiments were designed to analyze the relationships between the root-knot nematode *Meloidogyne incognita* and resistant tomato and pepper genotypes. From a natural avirulent isolate, near-isogenic nematode lineages were selected with virulence either against the tomato *Mi* resistance gene or the pepper *Me3* resistance gene. Despite the drastic selection pressure used, nematodes appeared unable to overcome the pepper *Me1* gene, therefore suggesting some differences in the resistance conferred by *Me1* and *Me3* in this species. Nematodes virulent on *Mi*-resistant tomatoes were not able to reproduce on *Me1*-resistant nor on *Me3*-resistant peppers, and nematodes virulent on *Me3*-resistant peppers were not able to reproduce on *Mi*-resistant tomatoes nor on *Me1*-resistant peppers. These results clearly demonstrate the specificity of *M. incognita* virulence against resistance genes from both tomato and pepper, and indirectly suggest that gene-for-gene relationships could occur between these two solanaceous crops and the nematode.

Introduction

Host plant resistance is the most efficient and environmentally safe method of controlling root-knot nematodes, *Meloidogyne* spp., and provides an alternative strategy to chemical nematicides when associated with crop rotation. This is especially true in solanaceous crops, for which many resistance genes have been identified in cultivated and wild species and subsequently used in breeding programs to develop nematode-resistant cultivars (Fassuliotis, 1987). The expression of host resistance in the Solanaceae consists of an hypersensitive reaction leading to localized root-cell necrosis and restricted nematode development at the infection site (Kaplan and Keen, 1980).

In tomato, resistance to *M. arenaria*, *M. incognita* and *M. javanica* is controlled by a single dominant gene designated *Mi* (Gilbert and McGuire, 1956). In pepper, at least five genes were shown to be involved in plant incompatibility to root-knot nematodes. Some of these genes (*Me2*, *Me4* and *Me5*) control a specific resistance against one of the main *Meloidogyne* species or against only some strains of one species. The two other genes (*Me1* and *Me3*) respectively were identified in two independant breeding lines and confer the same large resistance spectrum as *Mi* (Hendy et al., 1985b).

Even though plant resistance is increasingly used in agriculture, damage due to root-knot nematodes is still of economic importance. Among other reasons, the development of virulent isolates (i.e. able to reproduce on cultivars carrying resistance genes) may contribute to this situation. Because tomato is associated with many agronomical systems worldwide, and because *Meloidogyne* is a major pest of this crop, extensive information is available on the occurrence of natural populations virulent against the *Mi* resistance gene. In addition to the observation of resistance-breaking biotypes in the field, several experiments have been designed to select and study *Meloidogyne Mi*-virulent genotypes under controlled conditions (reviewed in Castagnone-Sereno, 1994).

In preliminary experiments, we demonstrated that two artificially selected lines of *M. incognita* virulent on resistant tomato were unable to develop on resis-

tant peppers (Castagnone-Sereno et al., 1992). The objectives of the present study were 1) to initiate the selection of *M. incognita* lineages virulent against the pepper resistance genes *Me1* and *Me3*; 2) to use *Mi* and *Me* (s)-virulent nematodes to compare the specificity of host-parasite relationships between tomato and pepper.

Materials and methods

Plant material

Two near-isogenic tomato cultivars were used in the experiments. Saint Pierre is susceptible to *M. arenaria*, *M. incognita* and *M. javanica*, while Piersol, carrying the *Mi* gene, is resistant to the same *Meloidogyne* species.

The pepper cultivar Doux long des Landes was chosen as susceptible control in this study. Two pepper resistant inbred lines, PM217 and PM687, originating from the Institut National de la Recherche Agronomique (INRA) collection in Montfavet, were used. PM217, originating from population PI 201234, carries two independant genes, one (*Me1*) controlling resistance to *M. arenaria*, *M. incognita* and *M. javanica* and the second (*Me2*) active against *M. javanica* and *M. hispanica* (Hendy et al., 1985b). PM687, derived from population PI 322719, also carries two genes, one (*Me3*) active against the three main species, except one *M. arenaria* isolate, the second (*Me4*) controlling just this particular isolate (Hendy et al., 1985b). These two lines were crossed with the susceptible pepper cultivar Yolo Wonder, and doubled haploid (DH) progenies were obtained from the F1 plants through *in vitro* androgenesis (Dumas de Vaulx et al., 1981). Two DH lines, HDA149 and HDA330, each one carrying a single gene with large *Meloidogyne* resistance spectrum (*Me3* and *Me1* respectively), were chosen for this study.

The characteristics of the tomato and pepper genotypes used in this study, and the resistance genes involved, are summarized in Table 1.

Nematode lines

A pair of near-isogenic *M. incognita* lineages, respectively avirulent and virulent against the tomato *Mi* resistance gene, were chosen from the INRA collection in Antibes, France. These two lineages had previously been selected for avirulence/virulence from a single female egg mass deriving from a natural isolate from Adiopodoumé, Ivory Coast, according to an already described procedure (Jarquin-Barberena et al., 1991). The tomato cultivars used for this selection process were Saint Pierre and Piersol.

From the avirulent nematode line, selection for virulence against the pepper resistance genes *Me1* and *Me3* was initiated on the autodiploid lines HDA330 and HDA149 respectively, according to the same selection procedure, except that the inoculum levels ranged from a single egg mass to 5.10^3 second-stage juveniles per plant.

Virulence tests

All experiments were conducted in a climate controlled room at a mean temperature of 23 °C. Reproduction of nematode lines was evaluated on both susceptible and resistant tomato and pepper genotypes using previously described miniaturized tube test culture and inoculation conditions (Castagnone-Sereno el al., 1993). As observed in a previous study (Castagnone-Sereno et al., 1992), inoculum levels needed to be different between tomato and pepper to obtain reproducible results: each tomato plant received 25 second-stage juveniles and each pepper plant 500 second-stage juveniles of each nematode genotype. Plants were arranged in a randomized complete block design with 10 replicates for each nematode × plant combination tested, and the experiment was repeated twice.

Eight weeks after inoculation, the washed root systems were placed in cold eosin yellow (0.1 g/l of water) and stirred for 30 min to stain egg masses. Numbers of egg masses per root system were counted and a reproductive index (Ri) was calculated according to the following ratio: Ri = no. egg masses/no. juveniles inoculated. Ri theoretically ranged from 0 (no reproduction at all) to 1 (each juvenile inoculated gave one egg mass).

Statistical analysis

An analysis of variance (ANOVA) was performed on the data, and Scheffe's F-test was used to determine differences among the mean values. Because no significant differences existed between the repeated treatments in the experiments, results were pooled together for the statistical analysis. All the statistical work was done using Statview™ software.

Table 1. Nematode-susceptible and resistant tomato and pepper genotypes used in this study

Plant species	Line or cultivar	RKN resistance[a]	Gene(s) involved
L. esculentum	Saint Pierre	S (Susceptible)	–
"	Piersol	R (Resistant)	*Mi*
C. annuum	Doux long des Landes	S	–
"	PM217	R	*Me1*, *Me2*
"	PM687	R	*Me3*, *Me4*
"	HDA149	R	*Me3*
"	HDA330	R	*Me1*

[a] RKN: root-knot nematode.

Results

Selection for virulence against pepper Me*(s) genes*

Selection for virulence against *Me1* and *Me3* was initiated on the DH resistant lines HDA330 and HDA149 respectively, by inoculating individual pepper plants with increasing numbers of second-stage juveniles of the avirulent *M. incognita* lineage. Results of this first generation of selection are reported in Table 2.

The susceptible control Doux long des Landes produced an average of more than 220 egg masses per plant when inoculated with second-stage juveniles from a single egg mass. In contrast, no egg masses were produced on either resistant line using this inoculum level (78 and 60 plants individually tested for HDA149 and HDA330, respectively). With higher inoculum levels, very occasional egg masses were produced on HDA149 after inoculation with 1,000 and 5,000 second-stage juveniles (Table 2). One of these progenies was arbitrarily selected, and 14 successive nematode generations were repeatedly propagated on HDA149 between December, 1992 and December, 1994. The whole progeny obtained was used as the inoculum for the next generation. The 15th *M. incognita* generation selected this way was used and referred to in the following experiments as the *Me3*-virulent line.

Conversely, no reproduction at all was observed on HDA330, whatever inoculum level was used. Thus, selection for *M. incognita* virulence against *Me1* proved to be impossible under our conditions.

Specificity of nematode virulence against Me1

As expected, the *M. incognita* avirulent line reproduced well on the susceptible cultivar Doux long des Landes (Ri = 0.498). In contrast, few or no egg masses were produced on the four resistant pepper genotypes tested (PM217, PM687, HDA149, HDA330). No significant differences (P = 0.05) were observed between their respective Ri values (Table 3).

Table 2. Reproduction of the avirulent *Meloidogyne incognita* line on the two resistant pepper genotypes HDA149 and HDA330

Pepper genotype	Inoculum	Plants tested	Egg-masses obtained
Doux long des Landes	1 egg-mass[a]	20	4414 (220.7/plant)
HDA149	1 egg-mass	78	0
	1000 J2[b]	28	1
	5000 J2	120	6[c]
HDA330	1 egg-mass	60	0
	1000 J2	24	0
	5000 J2	96	0

[a] about 300–500 infective second-stage juveniles.
[b] J2: second-stage juvenile.
[c] six plants with one egg-mass each.

On the susceptible pepper, the previously selected *Me3*-virulent *M. incognita* line produced significantly more (P = 0.05) egg masses and had a higher reproductive rate (Ri = 0.880) than the near-isogenic, avirulent nematode line. The two pepper resistant lines carrying at least the *Me1* gene, but not the *Me3* gene (PM217 and HDA330), showed no significant nematode attack on their root system after inoculation with the *Me3*-virulent line (Ri values of 0.003 and 0.004 respectively). In contrast, the two resistant genotypes carrying the *Me3* gene (PM687 and HDA149) exhibited large numbers of egg masses on their roots, with Ri values similar (P = 0.05) to that of the avirulent line on the susceptible control pepper (Table 3).

Table 3. Reproduction of near-isogenic, avirulent and virulent *Meloidogyne incognita* lines on susceptible and resistant pepper genotypes

Nematode lines		Pepper genotypes				
		Doux long des Landes	PM217 (*Me1*,*Me2*)	HDA330 (*Me1*)	PM687 (*Me3*,*Me4*)	HDA149 (*Me3*)
avirulent *M. incognita*	egg masses per plant[a]	248.95	0.05	0.26	0.05	0
	Ri[ab]	0.498xy	10^{-4}w	0.001w	10^{-4}w	0w
M. incognita selected on HDA149	egg masses per plant	433.57	1.32	1.75	213.20	292.80
	Ri	0.880z	0.003w	0.004w	0.426x	0.586y

[a] Values are the means of 20 replicate plants

[b] Values followed by the same letter are not significantly different at P = 0.05 according to Scheffe's F-test.

Relationships between Mi and Me(s) genes

To evaluate the relationship between nematode resistance genes in tomato and pepper, tomatoes carrying *Mi* and peppers carrying *Me1* or *Me3* were inoculated with both the *Mi*-virulent and the *Me3*-virulent *M. incognita* near-isogenic genotypes. The natural avirulent isolate from which these two virulent lines were selected, and the susceptible cultivars Saint Pierre and Doux long des Landes were used as controls.

The avirulent *M. incognita* isolate reproduced well on both susceptible cultivars, producing significantly more (P = 0.05) egg masses on tomato than on pepper (Ri values of 0.882 and 0.470 respectively). As observed previously, very few or no egg masses at all were counted on the roots of the resistant tomato and pepper genotypes (Table 4).

The *Mi*-virulent nematode lineage exhibited an equally (P = 0.05) high reproduction rate on both resistant and susceptible tomato cultivars (Ri values of 0.724 and 0.756 respectively). These values were slightly but significantly (P = 0.05) less than the Ri value of the near-isogenic avirulent isolate on the susceptible tomato cultivar. The two resistant DH pepper lines were not attacked by the *Mi*-selected nematode. A few egg masses and immature *M. incognita* females were present on the roots of the susceptible pepper cultivar, but the overall reproduction rate observed was nevertheless not significantly (P = 0.05) different from zero (Table 4).

The *Me3*-virulent *M. incognita* lineage reproduced equally well (P = 0.05) on the susceptible tomato and pepper cultivars (Ri values of 0.927 and 0.835 respectively). The egg mass production observed on the resistant autodiploid line HDA149 was not so high (Ri = 0.482), but close to the Ri of the avirulent nematode isolate inoculated onto the susceptible pepper cultivar. The *Mi*-resistant tomato cultivar and the *Me1*-resistant autodiploid pepper line exhibited no significant (P = 0.05) egg mass production on their roots (Table 4).

Discussion

The results presented here demonstrate the specificity of *M. incognita* virulence against resistance genes from tomato (*Mi*) and pepper (*Me1* and *Me3*). In fact, this specificity is observed at two levels: i) between resistance genes originating from different botanical species (nematodes virulent on *Mi*-resistant tomatoes were not able to reproduce on *Me1* or *Me3*-resistant pepper, and nematodes virulent on *Me3*-resistant peppers were not able to reproduce on *Mi*-resistant tomatoes); ii) between resistance genes belonging to the same botanical species (i.e. nematodes virulent on *Me3*-resistant peppers were not able to reproduce on *Me1*-resistant peppers). Concordant observations had already been made for tomato, in which resistance was found against selected *Mi*-virulent *M. incognita* isolates (Roberts et al., 1990), and later identified as partly due to the gene *Mi-2* (Cap et al., 1993). Very recently, a new resistance locus was identified in *Lycopersicon peruvianum*, and this gene, designated *Mi-3*, was shown to confer resistance against a *M. incognita* strain that can infect plants carrying *Mi* (Yaghoobi et al., 1995). In every case, virulence in the parasite is strictly directed against one single plant resistance gene, providing some indirect evidence that a gene-for-gene complementarity may be involved between *M. incognita* and tomato or pepper. Even if such relationships have been suggested,

Table 4. Reproduction of *Meloidogyne incognita* near-isogenic lines on susceptible and resistant toamato and pepper genotypes

Nematode lines		Tomato genotypes		Pepper genotypes		
		Saint Pierre	Piersol (*Mi*)	Doux long des Landes	HDA149 (*Me3*)	HDA330 (*Me1*)
avirulent *M. incognita*	egg masses per plant[a]	22.05	1.95	235.14	0.09	0
	Ri[ab]	0.882z	0.078v	0.470w	2.10^{-4}v	0v
M. incognita selected on Piersol	egg masses per plant	18.90	18.09	6.39[c]	0.04	0
	Ri	0.756xy	0.724x	0.013v	8.10^{-5}v	0v
M. incognita selected on HDA149	egg masses per plant	23.18	0.19	417.65	241.13	1.35
	Ri	0.927z	0.008v	0.835xyz	0.482w	0.003v

[a] Values are the means of 20 replicate plants
[b] Values followed by the same letter are not significantly different at P = 0.05 according to Scheffe's F-test.
[c] Immature females were also observed on the roots.

in many other cases, from the genetic study of the host plant only, and the phenotypic observation of the interaction (Thompson and Burdon, 1992), dominance of avirulence (and consequently recessivity of virulence) is generally the rule for the pathogen in most of the well-characterized gene-for-gene situations (Keen, 1990). To this particular point of view, the lack for sexual reproduction in *M. incognita* therefore constitutes a serious challenge to the definite demonstration of this hypothesis, since no classical Mendelian approach can be applied to this parthenogenetic organism. Moreover, these data also suggest that resistance genes in tomato and pepper are triggered by distinct determinants in the nematode, although their spectrum of action is similar. The *Mi*, *Me1* and *Me3* resistance genes seem thus different one from each other in their structure, their mode of action or both. This is in agreement with the previous analysis of F1 and F2 progenies from the cross PM217 × PM687, which suggested that *Me1* and *Me3* are not alleles of the same gene, and are presumably located on the same chromosome (Hendy et al., 1985).

It was quite unexpected to note that the *M. incognita* line selected for virulence against the *Mi*-resistance gene appeared largely unable to reproduce when inoculated on the susceptible pepper cultivar, although its pathogenicity was only slightly affected on the susceptible tomato cultivar. This result is nevertheless in agreement with previous data obtained with two other independant *M. incognita* lines selected for virulence against the *Mi* gene (Castagnone-Sereno et al., 1992). Moreover, it was observed in the latter study that these lines also exhibited a strong reduction in their development on another susceptible pepper cultivar, California Wonder. The mechanism by which virulence against the *Mi* tomato gene is associated in *M. incognita* with the loss of its ability to develop on susceptible pepper seems therefore to be a general rule in the plant-nematode interaction. The fact that the *Mi*-virulence acquisition affects the general pathogenicity towards susceptible pepper but not susceptible tomato indicates that virulence determinants against these two crops may be different. On the contrary, the *Me3*-virulent nematode line reproduced very well on the tomato and pepper susceptible cultivars, even with a significant increase of its Ri index on both of them. All these observations together cannot easily be related to the concept of stabilizing selection as defined by Vanderplank (1968) since in that case the reproductive potential of the virulent lineages should have been altered on the plant genotypes lacking the corresponding resistance gene. Even though calculation of selection coefficients against unnecessary genes for virulence from field data indicated a very low influence of this selective force (Grant and Archer, 1983), the mechanism underlying this inability to reproduce on normally susceptible plants remains unclear. A practical consequence of these experimentations is that the use of the *Me3* gene in large-scale cultivated varieties may favour the development of new *Meloidogyne* strains with increased virulence on both pepper (resistant or susceptible) and tomato. In con-

trast, the resistance controlled by *Me1* seems much more stable, since we were unable to overcome it.

The fact that selection for virulence, in our conditions, was only possible against *Me3* suggests some difference(s) in the resistance mechanisms induced by *Me1* and *Me3*, even though these two genes confer both a large and almost identical resistance spectrum (Hendy et al., 1985b). In fact, preliminary studies showed differential responses when the two genitors PM217 (carrying *Me1*) and PM687 (carrying *Me3*) were infested with *M. incognita* juveniles (Hendy et al., 1985a). A significant reduction of nematode penetration was observed in the root tissues of PM687 compared to those of PM217, in which some juveniles were able to reach the vascular cylinder and to initiate a few imperfect giant cells. In PM687, hypersensitive necrosis occurred very early and prevented any giant cell initiation (Hendy et al., 1985a). Together with our own results, these data suggest that *M. incognita* virulence could be correlated with the ability of the nematode to overcome the early hypersensitive response of the plant. In this hypothesis, *Me3*-resistant peppers (i.e. PM687 and HDA149) should no longer be able to prevent the formation of giant cells as soon as cell necrosis in the external barrier (epidermis and root cortex) has been passed. On the contrary, defence reactions in plants carrying *Me1* (i.e. PM217 and HDA330) could occur later, near or even inside the vascular cylinder, therefore blocking nematode development. Whether such events are indeed responsible for the differences we observed in nematode reproduction on HDA149 and HDA330 is currently unknown, but comparative histological studies are to be carried on this material to elucidate this point.

Acknowledgements

This research was supported in part by a grant from INRA (Institut National de la Recherche Agronomique) AIP 93/4918.

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