Plant Pathology (2005) **54**, 93–99 Doi: 10.1111/j.1365-3059.2005.01143.x

Variation in resistance to the root-knot nematode *Meloidogyne incognita* in tomato genotypes bearing the *Mi* gene

M. Jacquet[a], M. Bongiovanni[b], M. Martinez[a], P. Verschave[a], E. Wajnberg[c] and P. Castagnone-Sereno[b]*†

[a]*Vilmorin, Centre de Recherche de la Costière, 30210 Lédenon;* [b]*INRA, UMR 1064;* [c]*INRA, UMR 1112, BP167, 06903 Sophia-Antipolis cedex, France*

Root-knot nematodes (*Meloidogyne* spp.) are among the main pathogens of tomato (*Lycopersicon esculentum*) worldwide. Plant resistance is currently the method of choice for controlling these pests and all the commercially available resistant cultivars carry the dominant *Mi* gene, which confers resistance to the three main species *Meloidogyne arenaria*, *M. incognita* and *M. javanica*. However the emergence of virulent biotypes able to overcome the tomato resistance gene may constitute a severe limitation to such a control strategy. To date, little was known of the possible influence of the homozygous *vs* heterozygous allelic state of the *Mi* locus, or the tomato genetic background, on the expression of the resistance. In order to test both these factors, the resistance was evaluated of a large panel of *L. esculentum* genotypes (selected from the Vilmorin germplasm stock collection) to seven *M. incognita* lines avirulent or virulent against the *Mi* gene. Plant resistance was estimated by counting the egg masses on the root systems after inoculation with second-stage juveniles (J_2). Reproduction of the nematodes was similar or, more often, significantly higher on heterozygous tomato genotypes than on homozygous ones, suggesting a possible dosage effect of the *Mi* gene. Data also indicated that the tomato genetic background had a major effect on the variations observed in nematode reproduction, especially when tomato genotypes were heterozygous for the *Mi* gene. These results have important consequences in terms of breeding strategies and durability of the resistance conferred by the *Mi* gene.

Keywords: gene dosage, *Meloidogyne* spp., *Mi* resistance gene, tomato genotypes, virulence

Introduction

Root-knot nematodes of the genus *Meloidogyne* are among the main pathogens of tomato (*Lycopersicon esculentum*) crops worldwide. Infested plants show an aberrant development of the root system characterized by the formation of typical galls, which alter the uptake of water and nutrients and interfere with the translocation of minerals and photosynthates (Williamson & Hussey, 1996). As a result, aboveground deficiency symptoms appear, which may lead to severe yield decreases, depending on the severity of the infestation.

Because of the adverse effects associated with the use of chemical nematicides, plant resistance is currently considered as the method of choice for controlling root-knot nematodes. Resistance to *Meloidogyne* spp. was observed originally in some accessions of the wild tomato species *Lycopersicon peruvianum* (Bailey, 1941), and subsequently shown to be due to a single dominant gene named *Mi* (Gilbert & McGuire, 1956). Further studies demonstrated that this gene controls the three major species *Meloidogyne arenaria*, *M. incognita* and *M. javanica* (Barham & Winstead, 1957). The *Mi* gene was transferred from *L. peruvianum* PI128657 into *L. esculentum* using embryo rescue (Smith, 1944). From the initial interspecific cross, one single F_1 plant was used for further breeding by repeated backcrossing, and all the modern fresh-market and processing resistant tomato cultivars are derived from this single F_1 plant (Williamson, 1998).

Although highly efficient in most cases, the intensive use of the *Mi* gene, along with the pathogenic variability of root-knot nematodes, raises concern about the durability of the resistance (Roberts, 1995; Castagnone-Sereno, 2002). First, although the *Mi* gene should block nematode development at an early stage, occurrence of and variation in *Meloidogyne* spp., reproduction on *Mi*-resistant tomato genotypes has been documented (Roberts & Thomason,

*To whom correspondence should be addressed.

†E-mail: pca@antibes.inra.fr

Accepted 23 October 2004

Table 1 Main characteristics of tomato genotypes used in this study

Type	Code	Parent code	*Mi* allelic condition	Branching pattern (*sp* gene)	Fruit shape	Modernity
Fixed lines	976168		*Mi*/*Mi*	Indeterminate (+/+)	Round	Ancient
	984027		*Mi*/*Mi*	Indeterminate (+/+)	Round	Modern
	984032		*Mi*/*Mi*	Indeterminate (+/+)	Round	?
	984034		*Mi*/*Mi*	Determinate (*sp*/*sp*)	Round	Ancient
	984046		*Mi*/*Mi*	Determinate (*sp*/*sp*)	Elongated	Ancient
	984051		*Mi*/*Mi*	Determinate (*sp*/*sp*)	Elongated	Modern
F_1 hybrids	931263	984034	*Mi*/+	Determinate (*sp*/*sp*)	Round	Ancient
	931265	984027	*Mi*/+	Indeterminate (+/+)	Round	Intermediate
	980468	976168	*Mi*/+	Indeterminate (+/+)	Round	Ancient
	980472	984051	*Mi*/+	Determinate (*sp*/+)	Elongated	Modern
	980473	984027	*Mi*/+	Determinate (*sp*/+)	Round	Intermediate
	980474	984051	*Mi*/+	Determinate (*sp*/*sp*)	Elongated	Modern
	980476	984032	*Mi*/+	Determinate (*sp*/+)	Round	?
	980478	?	*Mi*/+	Indeterminate (+/+)	Round	Ancient
Controls	Saint Pierre		+/+	?	Round	Ancient
	Piersol		*Mi*/*Mi*	?	Round	Ancient

1986, 1989). Second, nematode biotypes virulent against the *Mi* gene have recently been described from most of the tomato-growing areas in the world (Kaloshian *et al*., 1996; Eddaoudi *et al*., 1997; Ornat *et al*., 2001).

The objectives of the present study were to test whether the homozygous *vs* heterozygous allelic condition of the *Mi* locus could explain some variation in nematode reproduction on resistant genotypes, and to evaluate the influence of the genetic background of tomatoes on the expression of the resistance. In order to provide a more comprehensive view of the plant–nematode interaction, and because the consequences in terms of management of the resistance would be of prime importance, avirulent and *Mi*-virulent *M. incognita* isolates were used in the experiments.

Table 2 *Meloidogyne incognita* isolates used in this study

Code	Origin	Virulence against *Mi*
Mia1	Calissane, France	None
Mia2	Kursk, Russia	None
Mivs1	Calissane, France	Selected[a]
Mivs2	Kursk, Russia	Selected[a]
Mivn1	N'Gorom, Senegal	Natural
Mivn2	the Netherlands	Natural
Mivn3	Valbonne, France	Natural

[a]Virulent isolate, laboratory-selected from avirulent one of same origin.

Materials and methods

Plant material

Fourteen tomato (*L. esculentum*) genotypes originating from the Vilmorin stock collection were used in the experiments. Their main characteristics are given in Table 1. Six were fixed lines homozygous for the *Mi* resistance gene; the remainder were F_1 hybrids heterozygous for the *Mi* gene. They were chosen according to differences in their genetic background as evaluated by three main criteria: the branching pattern (determinate *vs* indeterminate) resulting from expression of the *sp* gene (Pnueli *et al*., 1998); the fruit shape (round *vs* elongated); and the modernity (ancient, modern or intermediate) of the genotype. Two near-isogenic tomato cultivars, Saint Pierre (susceptible) and Piersol (resistant, homozygous for the *Mi* gene), were used as controls (Laterrot, 1975).

Nematode isolates

The nematode isolates used in this study were collected originally from heavily infested tomato fields or greenhouses, in areas where resistant tomatoes have not been cultivated. In order to eliminate any potential within-population heterogeneity, an isolate was raised from each field population, starting from the progeny of a single female, as follows: single females were carefully dissected from the root tissues with their egg mass, which was then used to reinoculate a tomato plant. Because of the mitotic parthenogenetic mode of reproduction of *M. incognita* (Triantaphyllou, 1985), all the second-stage juveniles (J_2) that hatched from each egg mass were considered as a clonal line. Seven *M. incognita* isolates were used. Their geographical origin and (a)virulence against the tomato *Mi* resistance gene are reported in Table 2. The *M. incognita* virulent isolates from Calissane (France) and Kursk (Russia) were laboratory-selected and reared on the *Mi*-resistant tomato cultivar Piersol for more than 25 generations according to the procedure of Jarquin-Barberena *et al*. (1991). The three other virulent isolates that did not result from such artificial selection were considered as natural virulent isolates. Prior to multiplication, each isolate was specifically identified according to its isoesterase electrophoretic pattern (Dalmasso & Bergé, 1978).

Table 3 Average reproduction of seven root-knot nematode (*Meloidogyne incognita)* isolates on 16 tomato genotypes[a]

Type		Mia1	Mia2	Mivs1	Mivs2	Mivn1	Mivn2	Mivn3
Fixed lines	976168	1·4 ± 0·96	0	23·4 ± 0·51	19·9 ± 1·45	15·2 ± 1·57	19·5 ± 0·92	21·4 ± 1·96
	984027	0·1 ± 0·08	0	22·7 ± 1·05	18·1 ± 1·95	22·8 ± 0·90	21·1 ± 0·88	23·4 ± 0·53
	984032	0·06 ± 0·06	0·1 ± 0·12	24·4 ± 0·39	18·2 ± 0·74	14·0 ± 1·06	15·3 ± 1·06	24·2 ± 0·66
	984034	0	0·2 ± 0·11	22·0 ± 0·89	22·7 ± 0·88	12·7 ± 1·92	21·6 ± 0·87	20·9 ± 1·64
	984046	0·3 ± 0·25	0·1 ± 0·06	24·1 ± 0·36	20·7 ± 2·05	20·0 ± 2·13	21·7 ± 0·63	19·1 ± 1·33
	984051	0	0	24·2 ± 0·35	19·4 ± 1·09	19·2 ± 0·85	16·8 ± 0·67	23·1 ± 0·49
F_1 hybrids	931263	0·8 ± 0·19	0·5 ± 0·15	23·0 ± 0·77	23·0 ± 1·21	16·1 ± 2·62	23·3 ± 1·40	24·5 ± 0·35
	931265	0	0·7 ± 0·21	22·0 ± 0·59	23·0 ± 1·43	19·6 ± 1·56	21·5 ± 0·76	24·5 ± 0·53
	980468	0·1 ± 0·13	0·3 ± 0·14	23·4 ± 0·53	21·4 ± 1·12	18·5 ± 1·51	18·9 ± 1·24	22·1 ± 1·21
	980472	0	0	23·3 ± 0·52	22·9 ± 0·56	18·5 ± 0·88	18·5 ± 0·93	23·4 ± 0·52
	980473	0	0·1 ± 0·06	20·7 ± 1·13	17·5 ± 1·71	21·9 ± 0·85	15·1 ± 0·83	23·7 ± 0·65
	980474	0	0·2 ± 0·10	24·0 ± 0·56	20·6 ± 1·26	21·9 ± 0·98	21·6 ± 1·02	23·5 ± 0·50
	980476	0·2 ± 0·10	0·8 ± 0·21	23·5 ± 0·67	20·6 ± 1·21	20·0 ± 0·92	20·9 ± 0·63	22·4 ± 0·62
	980478	1·5 ± 0·50	2·6 ± 0·64	24·8 ± 0·18	22·4 ± 0·81	22·1 ± 0·60	18·6 ± 1·23	23·2 ± 0·76
Controls	Saint Pierre	21·2 ± 0·87	17·1 ± 0·97	22·7 ± 0·85	20·6 ± 1·20	22·0 ± 1·51	21·9 ± 1·26	23·1 ± 1·35
	Piersol	0	0·1 ± 0·07	21·3 ± 1·22	21·2 ± 1·36	11·7 ± 1·50	22·1 ± 1·34	23·9 ± 0·89

[a]For each plant × nematode combination, reproduction was evaluated as the average number of egg masses per plant produced 8 weeks after inoculation with 25 juveniles, ± standard error.

Experimental procedures

Experiments were conducted in a climate room at a mean temperature of 20°C. Tomato seeds were germinated in steam-sterilized, sandy soil in seed trays, and 2-week-old seedlings were transplanted singly into 50 mL plastic tubes containing the same substrate and allowed to establish for 2–3 weeks before inoculation. Nematode reproduction was evaluated on all tomato genotypes using previously described miniaturized test-tube culture and inoculation conditions (Castagnone-Sereno *et al*., 1993). Plants of each tomato genotype received 25 J_2 of each nematode isolate. Plants were arranged in a block design with 20 replicates for each nematode × plant combination tested.

At 8 weeks after inoculation, the root systems were carefully washed under water and stored at –20°C until analysis. For analysis, roots were placed in cold eosin yellow (0·1 g L^{-1} water) and stirred for 30 min to stain egg masses in order to facilitate counting. Numbers of egg masses per root system ranged from 0 (no reproduction at all) to 25 (each juvenile developed into a female that produced one egg mass).

Statistical analysis

Due to poor growing conditions in the 50 mL plastic tubes, some tomato plants died before the end of the experiments, which resulted in missing values in the analyses. However, the rate of this mortality was verified to be independent of the tomato genotypes tested (logistic regression with binomial error: $\chi^2 = 13{\cdot}15$; df = 13; $P = 0{\cdot}4363$). Because the egg mass numbers were counts, values were square-root transformed before analysis to standardize the variances. Data were then analysed by means of a two-way ANOVA using the tomato genotypes, the nematode isolates and their interaction as the tested effects, and the number of egg masses as the dependent variable. Results from the two cultivars used as controls (Saint Pierre and Piersol) were not included in the analyses. Preplanned comparisons between means were done on transformed data with Student's *t* least significant difference test at $P = 0{\cdot}05$ or 0·01). All computations were done using the PROC GLM procedures of the SAS/STAT package (SAS Institute Inc., 1990).

Results

The two tomato cultivars used as controls confirmed the (a)virulence of the seven *M. incognita* isolates against the *Mi* resistance gene. As expected, the two avirulent isolates Mia1 and Mia2 reproduced well on the susceptible cultivar Saint Pierre (Table 3). Conversely, both were controlled by the resistant cultivar Piersol, although a very few J_2s developed into fecund females for the Mia2 isolate. On the other hand, all the virulent nematodes reproduced well on both susceptible and resistant control tomato cultivars, except for the isolate Mivn1, which produced fewer egg masses on Piersol compared with the other virulent isolates. Reproduction of the *M. incognita* isolates on either the fixed lines or the F_1 hybrids was as expected, ranging on average from 0 to 2·6 ± 0·64 egg masses per plant for the avirulent isolates, and from 12·7 ± 1·92 to 24·8 ± 0·18 egg masses per plant for the virulent isolates, respectively (Table 3). The two-way ANOVA performed on the data showed that nematode reproduction was strongly influenced by either the tomato genotype ($F = 7{\cdot}94$; df = 13; $P < 0{\cdot}0001$) or the nematode isolate ($F = 3882{\cdot}80$; df = 6; $P < 0{\cdot}0001$), and also that a very significant interaction occurred between these two factors ($F = 4{\cdot}41$; df = 78; $P < 0{\cdot}0001$). This indicates that neither the tomato genotype component alone, nor the nematode

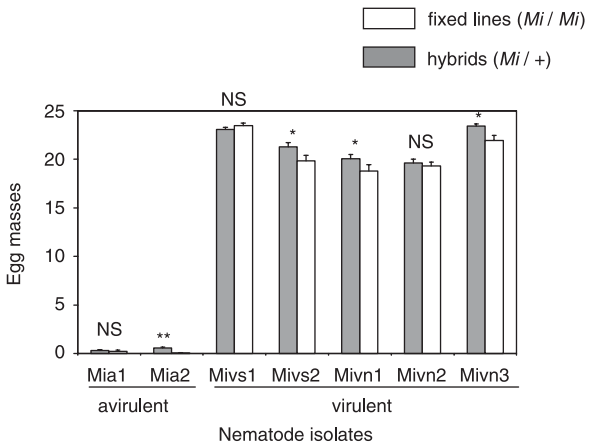

Figure 1 Average reproduction of *Meloidogyne incognita* isolates on tomato fixed lines or hybrids, homozygous or heterozygous, respectively, for the *Mi* resistance gene. Bars, standard errors; NS, not significant; *, significant at $P = 0{\cdot}05$; **, significant at $P = 0{\cdot}01$. Isolate codes given in Table 2.

isolate component alone, is sufficient to explain the differences observed in *M. incognita* reproduction.

To test the influence of the status of the *Mi* gene (homozygous *vs* heterozygous) on nematode reproduction, tomato genotypes were separated into two categories according to this criterion, and the new data set was submitted again to a two-way ANOVA. Results indicated that both the allelic condition of the *Mi* gene ($F = 24{\cdot}73$; df = 1; $P < 0{\cdot}0001$) and the nematode isolate ($F = 3264{\cdot}03$; df = 6; $P < 0{\cdot}0001$) significantly influenced nematode reproduction, and also that a significant interaction occurred between these two factors ($F = 3{\cdot}17$; df = 6; $P = 0{\cdot}0043$). When significant differences were observed, comparison of the numbers of egg masses indicated that reproduction of the nematode was always significantly higher on heterozygous tomato genotypes than on homozygous ones (Fig. 1). This was true for one avirulent isolate (Mia2), one virulent isolate artificially selected (Mivs2), and two virulent isolates of natural origin (Mivn1 and Mivn3). However, no difference in nematode reproduction was detected for the other isolates.

Finally, to analyse in more detail the influence of the genetic background of the tomato genotypes on expression of the *Mi* resistance gene, data from the homozygous fixed lines on the one hand, and from the heterozygous F_1 hybrids on the other, were analysed separately, and infestations with either avirulent or virulent nematodes were considered as independent experiments. Results of the two-way ANOVAs performed are shown in Table 4. From these analyses it appeared that the genetic background of the tomato always had a significant effect on reproduction of the nematode when the *Mi* gene was in a heterozygous allelic condition. The same significant influence was observed when homozygous resistant plants were infested with avirulent nematodes. On the other hand, the genetic background of homozygous resistant genotypes had no effect on reproduction of the virulent *M. incognita* isolates.

In good agreement with the previous analyses, nematode isolates showed a significant effect in all the situations tested, and their interaction with the genetic background of the tomato genotypes always had a significant influence on the reproduction of the nematodes. In order to assess the individual effects of tomato genotypes, the reproduction levels of avirulent and virulent nematodes were compared separately, for each of the fixed lines and of the hybrids, respectively. Results of these analyses are shown in Figs 2 and 3. Clearly, significant differences (at $P = 0{\cdot}05$) were observed in all four interactions, indicating an effect of the genetic background on nematode reproduction, although the range of variation always appeared higher in the hybrids than in the fixed lines.

Table 4 Two-way ANOVA testing the effects of tomato genetic background, nematode isolate and their interaction on *Meloidogyne incognita* reproduction

	Source	Variance	df	*F*	*P* > F
Fixed lines (*Mi*/*Mi*)	Genetic background (1)	0·5237	5	1·48	0·1951
Virulent isolates	Nematode isolate (2)	5·5432	4	15·66	<0·0001
	Interaction (1) × (2)	1·7270	20	4·88	<0·0001
	Error	0·3539	428		
	Total	0·4576	457		
Fixed lines (*Mi*/*Mi*)	Genetic background (1)	0·3896	5	3·69	0·0034
Avirulent isolates	Nematode isolate (2)	0·6376	1	6·04	0·0150
	Interaction (1) × (2)	0·6144	5	5·82	<0·0001
	Error	0·1056	173		
	Total	0·1227	184		
F_1 hybrids (*Mi*/+)	Genetic background (1)	0·7387	7	3·49	0·0011
Virulent isolates	Nematode isolate (2)	4·7616	4	22·50	<0·0001
	Interaction (1) × (2)	0·6523	28	3·08	<0·0001
	Error	0·2116	569		
	Total	0·2698	608		
F_1 hybrids (*Mi*/+)	Genetic background (1)	3·8258	7	17·01	<0·0001
Avirulent isolates	Nematode isolate (2)	2·8385	1	12·62	0·0005
	Interaction (1) × (2)	0·5697	7	2·53	0·0157
	Error	0·2249	236		
	Total	0·3468	251		

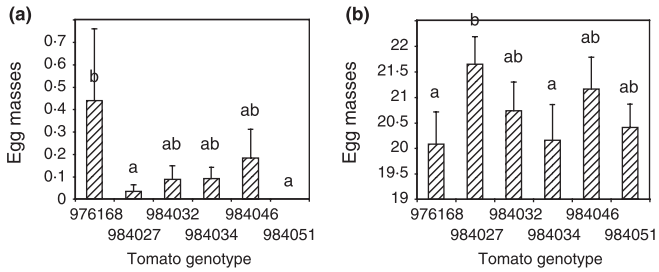

Figure 2 Average reproduction of *Meloidogyne incognita* on tomato fixed lines homozygous for the *Mi* resistance gene: (a) avirulent, (b) virulent nematodes. Bars, standard errors. In each graph, values followed by the same letters are not significantly different at $P = 0{\cdot}05$. Tomato genotypes described in Table 1.

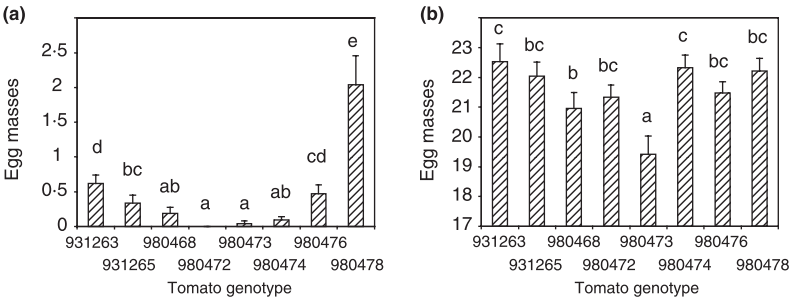

Figure 3 Average reproduction of *Meloidogyne incognita* on tomato hybrids heterozygous for the *Mi* resistance gene: (a) avirulent, (b) virulent nematodes. Bars, standard errors. In each graph, values followed by the same letters are not significantly different at $P = 0{\cdot}05$. Tomato genotypes described in Table 1.

Discussion

Until now, the expression of the tomato *Mi* resistance gene in the homozygous *vs* heterozygous allelic condition had not been extensively studied, and the few available data are not fully in agreement. In earlier experiments, reproduction of one avirulent and one virulent *M. incognita* isolate was the same in tomato genotypes carrying the *Mi* gene in either the homozygous or the heterozygous condition, suggesting lack of a resistance gene-dosage effect (Bost & Triantaphyllou, 1982). More recently, the reproduction of three *M. javanica* isolates with partial virulence (i.e. with a low rate of reproduction on resistant cultivars) was much greater on tomato genotypes heterozygous for the *Mi* gene than on homozygous genotypes, but no difference was observed in the reproduction rate of highly virulent isolates on the same homozygous and heterozygous genotypes (Tzortzakakis *et al*., 1998). These data indicated a dosage effect of the *Mi* gene on partially virulent isolates only. Results reported here, inferred from the analysis of a large number of plant–nematode genotypic relationships, revealed significant interaction between both plant and nematode isolates, and showed that *M. incognita* reproduction was significantly higher on heterozygous than on homozygous tomato genotypes with four of the seven nematode isolates tested (either avirulent or virulent). From these results, it is reasonable to suspect a dosage effect of the *Mi* gene. However, no difference in nematode reproduction was detected for the three other nematode isolates. In two other documented interactions where root-knot nematodes are involved, no evidence for a dosage effect of the resistance gene was provided. The first involves resistance to *M. incognita* conferred by the completely dominant *Mi*-2 gene in the wild tomato *L. peruvianum*, which was equally effective in the resistant parental plants and the F_1 heterozygous progeny (Cap *et al*., 1993). The second is in pepper, a solanaceous crop closely related to tomato, where genotypes homozygous or heterozygous for the *N* gene showed similar resistance to *M. incognita* (Thies & Fery, 2002). However, it should be noted that these two results were obtained after inoculation of the plants with a single avirulent nematode isolate only, which could have hidden some significant genotypic interactions, as observed in the current study. Based on the whole data set available, a general rule that governs the *Mi* resistance response of tomato to *M. incognita* cannot be proposed, and it seems essential to test as many nematode isolates on as many plant genotypes as possible before any conclusion can be reached.

In order to test the possible influence of the genetic background in which the *Mi* gene is present on resistance to *M. incognita*, a collection of tomato genotypes differing in their branching pattern, fruit shape and modernity, alone or in combination, was assembled and the reproductive capability of avirulent and virulent nematodes on them was compared. Tomato lines with different genetic background, either homozygous *Mi*/*Mi* or heterozygous *Mi*/+, showed different numbers of egg masses on their root system when infested with avirulent nematode isolates. On the other hand, reproduction of *Mi*-virulent nematodes was affected on the different F_1 hybrids (*Mi*/+) only. Together, these data nevertheless suggest that tomato genetic background is a major influence in the variations observed.

In a recent study, screening of mutated *Mi*/*Mi* tomato populations allowed the isolation of independent mutants with altered root-knot nematode resistance, among which some had reduced resistance, and one, *rme1*, had levels of infestation comparable with those on susceptible tomatoes. Molecular and genetic data indicated a single mutation in the *Rme1* locus, which is not closely linked to *Mi* (Martinez de Ilarduya *et al*., 2001). These data showed that at least one additional locus is required for the

expression of *Mi*. Moreover, experimental results confirmed that *Rme1* does not play a general role in disease resistance, but may be specific for *Mi*-mediated resistance (Martinez de Ilarduya *et al*., 2001). Such information clearly supports the assumption that other factors may be needed in the tomato genome to interact with the *Mi* gene, and thus play a role, either qualitatively or quantitatively, in expression of the resistance.

Little information is available in the literature on the influence of plant genetic background on the expression of resistance to nematodes. However, another example is the case of the interaction involving the wild potato *Solanum vernei* and the cyst nematode *Globodera pallida*. In a test of progenies resulting from the crossing of a resistant parent with different susceptible potato genotypes, variable levels of nematode reproduction were observed (D. Mugniéry, INRA, 35653 Le Rheu, France, personal communication).

Although it is a parthenogenetic organism, it has been shown in the laboratory that *M. incognita* is able to respond to the selection pressure of the *Mi* gene, leading to the selection of virulent lineages from the progeny of avirulent females (Castagnone-Sereno *et al*., 1994). Nevertheless, although it was introgressed into cultivated tomato more than 50 years ago and is still the only nematode-resistance gene used in all commercially available tomato cultivars worldwide (Williamson, 1998), the *Mi* gene remains efficient in most agronomic situations. However, the current emergence of *Mi*-virulent root-knot nematode populations, reported in all tomato-growing areas in the world, raises questions about its durability in the near future (Castagnone-Sereno, 2002). The results reported here could, in part, explain this phenomenon as it has been shown that a dosage effect of the *Mi* gene can occur and, consequently, that some nematodes can reproduce on resistant plants, which is a necessary starting point for the development of virulent populations. As a large proportion of modern tomato cultivars are F_1 hybrids, with *Mi* in the heterozygous condition, this could promote the selection of virulent *M. incognita* biotypes in field conditions. Better knowledge of the parasitic interactions between tomato cultivars and root-knot nematodes should provide new insights into the genetic factors that help sustain or lead to a decline in the durability of plant resistance.

Acknowledgements

The authors thank Didier Mugniéry for sharing unpublished results. This work was financially supported by a grant from the Comité Technique Permanent de la Sélection (6-MCB-C98·05-Conv.61·21·43), which is gratefully acknowledged.

References

Bailey DM, 1941. The seedling test method for root-knot nematode resistance. *Proceedings of the American Society for Horticultural Sciences* **38**, 573–5.

Barham WS, Winstead NN, 1957. Inheritance of resistance to root-knot nematodes in tomatoes. *Proceedings of the American Society for Horticultural Sciences* **69**, 372–7.

Bost SC, Triantaphyllou AC, 1982. Genetic basis of the epidemiologic effects of resistance to *Meloidogyne incognita* in the tomato cultivar Small Fry. *Journal of Nematology* **14**, 540–4.

Cap GB, Roberts PA, Thomason IJ, 1993. Inheritance of heat-stable resistance to *Meloidogyne incognita* in *Lycopersicon peruvianum* and its relationship to the *Mi* gene. *Theoretical and Applied Genetics* **85**, 777–83.

Castagnone-Sereno P, 2002. Genetic variability of nematodes: a threat to the durability of plant resistance genes? *Euphytica* **124**, 193–9.

Castagnone-Sereno P, Bongiovanni M, Dalmasso A, 1993. Stable virulence against the tomato resistance *Mi* gene in the parthenogenetic root-knot nematode *Meloidogyne incognita*. *Phytopathology* **83**, 803–5.

Castagnone-Sereno P, Wajnberg E, Bongiovanni M, Leroy F, Dalmasso A, 1994. Genetic variation in *Meloidogyne incognita* virulence against the tomato *Mi* resistance gene: evidence from isofemale line selection studies. *Theoretical and Applied Genetics* **88**, 749–53.

Dalmasso A, Bergé JB, 1978. Molecular polymorphism and phylogenetic relationship in some *Meloidogyne* spp.: application to the taxonomy of *Meloidogyne*. *Journal of Nematology* **10**, 323–32.

Eddaoudi M, Ammati M, Rammah H, 1997. Identification of resistance breaking populations of *Meloidogyne* on tomatoes in Morocco and their effect on new sources of resistance. *Fundamental and Applied Nematology* **20**, 285–9.

Gilbert JC, McGuire DC, 1956. Inheritance of resistance to severe root-knot from *Meloidogyne incognita* in commercial type tomatoes. *Proceedings of the American Society for Horticultural Sciences* **68**, 437–42.

Jarquin-Barberena H, Dalmasso A, De Guiran G, Cardin MC, 1991. Acquired virulence in the plant-parasitic nematode *Meloidogyne incognita*. I. Biological analysis of the phenomenon. *Revue de Nématologie* **14**, 299–303.

Kaloshian I, Williamson VM, Miyao G, Lawn D, Westerdahl BB, 1996. 'Resistance-breaking' nematodes identified in California tomatoes. *California Agriculture* **50**, 18–9.

Laterrot H, 1975. Séries de lignées isogéniques de tomate ne différant que par certains gènes de résistance aux maladies. *Phytopathologia Mediterranea* **14**, 129–30.

Martinez de Ilarduya O, Moore AE, Kaloshian I, 2001. The tomato *Rme1* locus is required for *Mi-1*-mediated resistance to root-knot nematodes and the potato aphid. *Plant Journal* **27**, 417–25.

Ornat C, Verdejo-Lucas S, Sorribas FJ, 2001. A population of *Meloidogyne* javanica from Spain virulent to the *Mi* resistance gene in tomato. *Plant Disease* **85**, 271–6.

Pnueli L, Carmel-Goren L, Hareven D, Gutfinger T, Ajvarez J, Ganal M, Zamir D, Lifschitz E, 1998. The *SELF-PRUNING* gene of tomato regulates vegetative to reproductive switching of sympodial meristems and is the ortholog of *Cen* and *TFL1*. *Development* **125**, 1979–89.

Roberts PA, 1995. Conceptual and practical aspects of variability in root-knot nematodes related to host plant resistance. *Annual Review of Phytopathology* **33**, 199–221.

Roberts PA, Thomason IJ, 1986. Variability in reproduction of isolates of *M. incognita* and *M. javanica* on resistant tomato genotypes. *Plant Disease* **70**, 547–51.

Roberts PA, Thomason IJ, 1989. A review of variability in four *Meloidogyne* spp. measured by reproduction on several hosts including *Lycopersicon*. *Agricultural Zoology Reviews* **3**, 225–52.

SAS Institute Inc, 1990. *SAS/STAT User's Guide, Release 6·07*, Vol. I. Cary, NC, USA: SAS Institute.

Smith PG, 1944. Embryo culture of a tomato species hybrid. *Proceedings of the American Society for Horticultural Sciences* **44**, 413–6.

Thies JA, Fery RL, 2002. Heat stability of resistance to southern root-knot nematode in bell pepper genotypes homozygous and heterozygous for the *N* gene. *Journal of the American Society for Horticultural Sciences* **127**, 371–5.

Triantaphyllou AC, 1985. Cytogenetics, cytotaxonomy and phylogeny of root-knot nematodes. In: Sasser JN, Carter CC, eds. *An Advanced Treatise on* Meloidogyne, Vol. I. Raleigh, NC, USA: North Carolina State University Graphics, 113–26.

Tzortzakakis EA, Trudgill DL, Phillips MS, 1998. Evidence for a dosage effect of the *Mi* gene on partially virulent isolates of *Meloidogyne javanica*. *Journal of Nematology* **30**, 76–80.

Williamson VM, 1998. Root-knot nematode resistance genes in tomato and their potential for future use. *Annual Review of Phytopathology* **36**, 277–93.

Williamson VM, Hussey RS, 1996. Nematode pathogenesis and resistance in plants. *Plant Cell* **8**, 1735–45.