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The reproductive potential of root-knot nematodes *Meloidogyne incognita* is affected by selection for virulence against major resistance genes from tomato and pepper

Caroline Djian-Caporalino^{a,*} • Sergio Molinari^b • Alain Palloix^c • Aurelio Ciancio^b • Ariane Fazari^a • Nathalie Marteu^a • Nicolas Ris^d • Philippe Castagnone-Sereno^a

^aINRA PACA, UMR1301 INRA/UNSA/CNRS, Interactions Biotiques et Santé Végétale, BP167, F-06903 Sophia Antipolis, France

^bCNR, Istituto per la Protezione delle Piante, I-70126 Bari, Italy

^cINRA PACA, UR1052, Génétique et Amélioration des Fruits et Légumes, F-84143 Montfavet, France

^dINRA PACA, UE1254, Lutte Biologique, BP167, F-06903 Sophia Antipolis, France

*Author for correspondence. E-mail: Caroline.Caporalino@sophia.inra.fr

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Abstract

Root-knot nematodes (RKNs, *Meloidogyne* spp.) are major plant pathogens of vegetables in most production areas, including the Solanaceous crops tomato and pepper. Due to the banning of chemical nematicides, current control strategies are mainly based on the deployment of resistance genes (R-genes), i.e., *Mi*(s) in tomato, *Me*(s) in pepper. These genes are effective against a wide range of RKN species, including *M. arenaria*, *M. incognita* and *M. javanica*, the most common species in temperate and tropical areas. However, the recent emergence of virulent populations able to overcome the resistance conferred by some of these R-genes may constitute a severe limitation to their use in the field. Research has been conducted to evaluate the durability of the *Mi*(s) and *Me*(s) R-genes, by comparing the reproduction of several laboratory-selected and wild virulent *M. incognita* isolates on both susceptible and resistant tomatoes and peppers. We first showed that the *Me1* R-gene in pepper behaves as a robust R-gene controlling avirulent and virulent *Me3* or *Mi-1* isolates. We also confirm that virulence is highly specific to a determined R-gene on which selection has occurred, thus allowing the alternance of R-genes in the rotation as an effective mean to improve soil health. Another significant experimental result is the observation that a reproductive fitness cost is associated to nematode virulence. The adaptative significance of trade-offs between selected characters and fitness-related traits suggests that, although the resistance can be broken, it might prove durable in some conditions if the virulent nematodes are counterselected in susceptible plants, which has important consequences for the management of plant resistance in the field.

Key words:

Meloidogyne spp. *Solanaceae* . *Me* and *Mi* resistance genes . Reproduction potential . Virulence specificity . Fitness cost of virulence .

Introduction

Root-knot nematodes (RKNs), *Meloidogyne* spp., are extremely polyphagous plant parasites, responsible for estimated losses of more than 80 billions Euros/year (Blok et al. 2008). These yield losses are a problem throughout the EU and are exacerbated by the sophisticated and intensive character of EU agriculture. In addition, new regulations have withdrawn the use of most chemical nematicides since 2006, and nematode problems have been increasing in horticultural crops consequently. In this context, cultivation of grafted vegetables is expanding in Europe, and has been adopted as a nonchemical alternative to methyl bromide in several countries (Methyl Bromide Technical Options Committee 2006). Because of this situation, breeding programs for the selection of resistant cultivars (cvs) and/or rootstocks have recently become a major challenge for breeders. However, the diversity of *Meloidogyne* species and isolates infecting vegetables in most major production areas worldwide is a threat to the use of single major resistance genes (R-genes) (Castagnone-Sereno 2002). Since resistance sources against these nematodes are rare, management of the R-genes currently available is of crucial importance to preserve their durability.

In Solanaceous species (e.g., tomato, pepper), several R-genes have been identified, that control the three main RKN species, i.e., *M. arenaria*, *M. incognita* and *M. javanica* (Williamson and Kumar 2006), and are currently used in breeding programs. However, the diversity and availability of such R-genes is limited. In tomato, all the modern fresh-market and processing resistant cvs carry the single dominant gene *Mi-1*, introgressed from a single interspecific hybrid (Williamson and Roberts 2009). For more than 60 years, this gene has been the only source of resistance to RKNs in all the available tomato cvs worldwide, and may be considered as a very stable R-gene in terms of durability. However, although this gene has been an excellent example of the use of host resistance to effectively reduce the need for pesticide application (Sorribas et al. 2005), the lack of diversity in the resistance sources exploited explains in part the emergence and spread of virulent nematode populations able to overcome it (Castagnone-Sereno 2002). Indeed, natural virulent isolates have been reported in many areas of the world where tomato is a major crop, and this trend has been increasing recently (e.g., Tzortzakakis et al. 2005, 2008; Verdejo-Lucas et al. 2009; Devran and Sogut 2010), which could significantly reduce the duration of the commercial exploitation of the current R-gene. Moreover, the artificial selection of virulent lineages from progenies of avirulent *Meloidogyne* isolates was demonstrated by repetitive inoculations onto *Mi-1*-resistant tomato genotypes under laboratory and greenhouses conditions (Jarquin-Barberena et al. 1991; Castagnone-Sereno et al. 1994b; Meher et al. 2009).

In pepper, resistance to RKNs is controlled by several linked dominant genes, i.e., the *Me* genes. Three of them (*Me1*, *Me3*, *Me7*), in inbred lines originating from three genetically distant accessions, are effective against a wide range of RKN species (Hendy et al. 1985; Djian-Caporalino et al. 1999; 2001; 2007). However, in laboratory experiments, the selection of virulent variants toward the *Me3* gene was achieved by using drastic selection pressure of avirulent *M. incognita* populations, while the *Me1* gene could not be overcome (Castagnone-Sereno et al. 1996; 2001). Although obtained in experimental assays, these results suggested that these R-genes may differ in their durability if repeatedly used under field conditions.

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Few data are available in literature about the reproductive potential of RKNs virulent against R-genes from Solanaceous vegetables, and reports appear somehow conflicting. Experimental evaluation of the fecundity (i.e., production of egg-masses by mature females) of *Meloidogyne* field populations virulent against the tomato *Mi* R-gene did not show any significant differences when the nematodes were inoculated onto susceptible vs. resistant plants (Castagnone-Sereno et al. 1994a; Tzortzakakis et al. 1998). Conversely, another study indicated that *Mi*-virulent nematodes produced fewer eggs per gram of root on resistant tomatoes compared to susceptible ones (Huang et al. 2004). More recently, a lower reproduction of *M. incognita* was found associated to virulence against *Mi-1* when inoculated onto susceptible tomatoes, using a combination of female fertility, fecundity and egg hatching as a global measure of reproduction (Castagnone-Sereno et al. 2007). In addition, as far as we know, no such information is currently available concerning RKN populations exhibiting virulence against the pepper *Me* R-genes.

The first objective of the present study was to compare the reproduction of several laboratory-selected and wild virulent *M. incognita* isolates on both susceptible and resistant tomatoes and peppers. For that purpose, we evaluated the ability of the juveniles to mature into adult females and produce eggs. A further objective was to determine whether reproductive fitness costs may be associated with the ability of the nematodes to overcome the tomato and pepper R-genes, by comparing the reproductive parameters of *M. incognita* isogenic lines that differ only for their virulence on a set of resistant and susceptible host genotypes. Such costs could have important consequences for the management of plant resistance in the field.

Materials and methods

Plant material

Tomato (*Solanum lycopersicon*) cvs Saint Pierre and Marmande, susceptible to *M. arenaria*, *M. incognita* and *M. javanica*, and cvs Piersol and VFN8, homozygous for the *Mi-1* gene and resistant to the same three *Meloidogyne* species, were used in the experiments.

Pepper (*Capsicum annuum*) genotypes used in this work were inbred lines with differential resistances to the three RKN species. Doux Long des Landes (DLL) is a susceptible cv; Yolo Wonder (YW) exhibits a partial (i.e., a low level of quantitative) resistance to some RKN populations; the two resistant haplo-diploid lines, HD149 and HD330, produced through *in vitro* androgenesis (Dumas de Vaulx et al. 1981) are homozygous for the *Me3* and *Me1* genes, respectively (Hendy et al. 1985); the inbred pepper line Criollo de Morelos 334 (CM334) was selfed in insect-proof cages to eliminate outcrossing and was assumed to be homozygous for the major dominant resistance gene previously named *Me7* (Djian-Caporalino et al. 2007).

Nematode isolates

Twelve *M. incognita* isolates were used. Their geographical origin and (a)virulence against the tomato *Mi-1* or pepper *Me3* and *Me7* R-genes are reported in Table 1. Prior to multiplication, each isolate was specifically identified according to its isoesterase electrophoretic pattern (Dalmaso and Bergé, 1978) and/or by SCAR-PCR (Zijlstra et al. 2000). All nematode isolates were maintained in the greenhouse. Hatched second-stage

juveniles (J2s) were obtained in a mist chamber from previously inoculated roots. Nematodes were collected in water every 48 hours and used immediately.

The avirulent nematode isolates used in this study were collected originally from heavily infested fields or greenhouses, in areas where resistant tomatoes and peppers have not been cultivated.

From the Morelos *M. incognita* avirulent isolate, virulent lines were laboratory-selected and reared by successive re-inoculation on the *Mi-1*-tomato cv Piersol, or the *Me3*-pepper HD149 line, or the *Me7*-pepper CM334 line for more than 25 generations according to the procedure of Jarquin-Barberena et al. (1991). The virulent isolates (A, B, C) from Hungary have been naturally selected on resistant peppers carrying *Me3* in greenhouses. The *Mi-1* virulent SM1 and SM2V isolates have been selected under controlled greenhouse conditions from the avirulent wild-type isolates avir Mifield1 and avir MifieldV, respectively, by repeated inoculations on resistant tomato for about 40 generations.

Experimental procedures and infestation parameters

Tomato and pepper seedlings of cvs susceptible and resistant to RKNs were grown individually in 100 ml pots containing steam-sterilized sandy soil covered by a 1 cm layer of loam. Six to twenty replicates were performed for each cultivar and each nematode isolate tested. Experiments were conducted in climatic chambers maintained at 24°C ($\pm 2^\circ\text{C}$) with a 12-h light cycle and a relative humidity of 60–70%. For each nematode isolate, six to seven-week-old plants (4-6 true leaves) were inoculated with a water suspension of 300 to 500 J2s.

Six to seven weeks after inoculation (i.e., a duration that allowed completion of the nematode life cycle), plants were harvested, carefully washed individually with tap-water, and stained for 10 minutes in an aqueous solution of 0.45% eosin yellow, to specifically stain egg masses (EMs) in red. The roots were then rinsed and examined under a magnifying glass and the number of EMs counted for each plant.

For each plant, up to 10 EMs were removed at random from the root system and the eggs were separated from the gelatinous matrix in 0.9% NaOCl, mounted in water between a glass slide and a cover slip and counted under a stereomicroscope. Three repeats per plant were achieved.

Three parameters of infestation were calculated:

- the Infestation Frequency (IF) was calculated according to the following ratio: $\text{IF} = \text{number of EMs} / \text{number of J2s inoculated}$. IF theoretically ranged from 0 (no reproduction at all) to 1 (each juvenile inoculated could develop into a gravid female and generate one EM);
- the Female Fertility (FF) was evaluated as the average number of eggs per EM (i.e., the number of eggs produced by each female);
- the Reproduction Potential (RP) of the nematode population defined as the number of eggs / number of J2s inoculated, following formula: $\text{RP} = \text{IF} \times \text{FF}$.

The virulence costs (C_h) of the laboratory-selected virulent lines on the susceptible host were estimated as follows: $C_h (\%) = 1 - \text{RP}(\text{virulent line}) / \text{RP}(\text{avirulent line})$ (Castagnone-Sereno et al. 2007).

Statistical analysis

In order to evidence a possible cost of virulence, the Reproduction Potentials (RP) on the susceptible tomato of a restricted set of genotypes (avir F, avir Mifield1, avir MifieldV, vir SM1, vir SM2V, vir *Me3*, vir *Me7*) were compared. A Kruskal-Wallis test was firstly carried out. Wilcoxon-Mann-Whitney unilateral tests were then used for post hoc comparisons in order to check if, for a similar genetic background, avirulent strains have higher RP than virulent ones. This led us to perform four pairwise comparisons (avir F *versus* vir *Me3* or vir *Me7*; avir Mifield1 *versus* vir SM1; avirMifieldV *versus* vir SM2V). Bonferroni correction was consequently applied (significance level at $\varepsilon=0.05/4=0.0125$ instead of $\alpha=0.05$). Analyses were performed using the free software R (<http://www.r-project.org/>).

Results

All the *M. incognita* isolates tested were able to infest both the susceptible tomatoes and peppers, with IF ranging from 0.10 to 0.87 (Table 2). The avirulent isolates avir MifieldV and avir F produced fewer egg masses on the partially resistant pepper cv YW compared to the susceptible pepper DLL and tomato St Pierre or Marmande plants. As expected, i) avirulent isolates (avir MifieldV and avir F) were unable to reproduce on the resistant tomatoes or peppers (IF values ranging from 0 to 0.002); ii) selected isolates virulent on *Mi-1*-tomato exhibited a high infestation frequency on this resistant tomato ($0.35 \leq IF \leq 0.82$); and iii) both selected and natural isolates virulent on *Me3* or *Me7*-pepper exhibited a high infestation frequency on the *Me3*-pepper ($0.23 \leq IF \leq 0.81$). Nevertheless, none of the virulent isolates (overcoming either tomato or pepper R genes) was able to reproduce on *Me1*-pepper, and both selected and natural isolates virulent on resistant peppers were unable to reproduce on *Mi-1*-tomato. One or two egg-masses were obtained on a few *Me1*-peppers, but these egg masses contained very few eggs (i.e., < 65 eggs; data not shown) compared to egg masses obtained on *Me3*-peppers (i.e., > 800 eggs; data not shown) and they did not survive to a successive inoculation, so it was not possible to obtain a virulent isolate.

Considering the RP of the nematodes, we first observed that the RP of avirulent isolates was higher on susceptible tomato than on susceptible pepper (496.78 ± 46.30 and 371.21 ± 71.77 for the avir F on tomato and pepper, respectively; 238.78 ± 35.13 and 157.73 ± 17.93 for the avir MifieldV on tomato and pepper, respectively) (data not shown). When comparing the RP of a restricted set of the avirulent and virulent nematode populations on susceptible plants, we firstly evidenced significant differences between nematode populations ($\chi^2_{6df}=40.8$, $p < 10^{-3}$) (Figure 1). More interestingly, the hypothesis of a virulence cost was strongly supported in three of the four pairwise comparisons: avir F *versus* vir *Me3* ($W=200$; $p < 10^{-3}$); avir F *versus* vir *Me7* ($W=279$; $p < 10^{-3}$); avir MifieldV *versus* vir SM2V ($W=34$; $p=0.004$). In these three cases, the estimated fitness cost ranged from 54 to 71%. In the last comparison (avir Mifield1 *versus* vir SM1), the same tendency was observed but the difference was not significant ($W=23$; $p=0.24$); it is noteworthy that the sample size was nevertheless low.

Finally, when comparing the RP of virulent *M. incognita* isolates selected in the laboratory and in the field on both susceptible and resistant tomatoes and peppers (Figure 2),

we observed high levels of variability between virulent lines on a same host, the number of eggs produced ranging from 106 to 496 times the number of J2s inoculated on susceptible cvs and from 0 to 435 times the number of J2s inoculated on resistant cvs. The *Me3* virulent isolates from Hungary (pop A, B and C) reproduced much more on all susceptible and *Me3*-resistant pepper genotypes (RP ranging from 200 to 496) than the *Me3* virulent isolate issued from a single year of selection in greenhouse (vir CREAT) (RP ranging 117 from to 209), and than the laboratory selected isolates (vir *Me3* and vir *Me7*) (RP ranging from 62 to 322).

Discussion

Selection for RKN resistance in crops, including tomato and pepper, is a major challenge for plant breeders. Therefore, because the introgression of R-genes into cvs of agronomic interest by successive back-crosses generally takes several years, a long-term goal of breeders is to select the genotypes (robust R-genes, their combination, and associated genetic backgrounds) that is expected to exhibit the best durability under field conditions (late emergence and low fitness of nematode virulent populations). In pepper, the *Me1* R-gene appears to be a good candidate for such a purpose. Previous studies had shown that none of the 22 RKN avirulent isolates tested, belonging to the three major species (*M. incognita*, *M. arenaria* and *M. javanica*), were able to overcome this R-gene under laboratory artificial conditions (Castagnone-Sereno et al., 1996; 2001). In the present study, we further showed that neither natural nor selected virulent RKN isolates (overcoming either *Mi-1* from tomato or *Me3* or *Me7* from pepper) were able to reproduce on *Me1*-peppers. Such a result is in good agreement with previous data indicating that these R-genes are not equally overcome by RKNs, and that *Me1* behaves as a robust R-gene. Conversely to *Mi-1*, *Me3* and *Me7*, *Me1* induces a late hypersensitive reaction in the vascular cylinder of infested roots, thus inhibiting the development of egg-laying females (Bleve-Zacheo et al. 1998 ; Pegard et al. 2005). Our observations here support previous data suggesting that late expression of the hypersensitive reaction by *Me1* prevents (or at least strongly reduces) the frequency of emergence of virulent nematode genotypes. Additionally, this study demonstrates that both natural and selected RKN isolates virulent on resistant peppers are unable to reproduce on *Mi-1* tomato, and confirmed that selected isolates virulent on *Mi-1* tomato are unable to reproduce on *Me3* and *Me1* resistant peppers. The virulent isolate overcoming *Me7* was able to reproduce on *Me3*-pepper, which may indicate that *Me3* and *Me7* maybe one single allele at the same gene. Indeed, two independent allelism tests performed with 385 test-cross lines [F1(CM334xHD149) x DLL] strongly suggested that *Me3* and *Me7* are allelic, since no susceptible plant was obtained on these 385 lines inoculated with 500 avirulent nematodes (Djian-Caporalino & Castagnone-Sereno, unpublished). Moreover, the fact that RKN selected for virulence over *Me3* also brokedown *Me7* (and reciprocally) indicates these are a single allele. So, RKN isolates virulent on one resistant crop are definitively not virulent on a different resistant crop. These findings indicate that virulence is highly specific to the R-gene on which selection has occurred. As a result of such strict specificity of virulence, once virulent isolates are selected on a determined R-gene, it is very likely that alternance in the

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rotation with a different gene will improve soil health, except if multivirulence can be progressively selected, which remains to be demonstrated.

The reproduction potential of virulent laboratory-selected and wild *M. incognita* isolates exhibited high levels of variability on a same host, either susceptible or resistant. As nematode reproduction on resistant genotypes is generally explained by the interaction between the plant genotype and nematode isolate, but not by either factor alone (Ornat et al. 2001; Jacquet et al. 2005; Lopez-Perez et al. 2006; Cortada et al. 2008), we can hypothesize that the variability observed here in the infection and reproduction of virulent lines on a same host can be attributed to the nematode genetic background, because they did not originate from the same nematode population and they were not selected on the same resistant plants in laboratory and in fields, even if the R-gene in these plants was the same. The natural virulent isolates selected on resistant peppers seemed to reproduce better on susceptible and resistant *Me3*-peppers than laboratory-selected isolates. This observation may be correlated to the way these isolates acquired their virulence, and suggests that the genetic changes induced under managed pressure in artificial conditions are different from those occurring in natural *Me3* resistance-breaking biotypes, as previously reported for laboratory-selected or natural isolates virulent against the tomato *Mi-1* R-gene (Castagnone-Sereno et al. 1994a; Roberts 1995). It may also result from the fact that laboratory-selected virulent isolates are very recent and have not yet been submitted to alternative hosts, conversely to natural virulent isolates that may be older and whose genetic background may have co-evolved with the virulence, thus restoring the nematode fitness.

Comparing the RP of the avirulent and laboratory-selected virulent nematodes revealed that both *Me3* (or *Me7*) and *Mi-1*-virulent isolates showed significant lower RPs on susceptible plants compared to the avirulent isolates, mainly due to a decrease in female fertility. This result indicates that some fitness cost reduced the nematode reproduction on the susceptible plants because of unnecessary virulence. This fitness cost may be associated to the virulence trait (pleiotropic effect) but may also result in part from the reduction of genetic variability operated by the selection pressure of the R-genes. Experimental evidence of such virulence fitness costs penalizing the aggressivity of virulent pathogens on susceptible plants has been abundantly reported for bacteria, fungi and viruses (e.g., Vera-Cruz et al. 2000; Jenner et al. 2002; Parlevliet 2002; Desbiez et al. 2003; Lecoq et al. 2004; Ayme et al. 2007; Sacristan and García-Arenal 2008; Janzac et al. 2009; 2010). However, similar data on RKNs remain scarce (Petrillo and Roberts 2005; Castagnone-Sereno et al. 2007). The present study reinforces these previous observations and suggests that such a fitness cost of virulent lines on susceptible crops is a general trend in plant pathogens, including nematodes. In our study, the virulence costs were estimated at 33 to 56 % on susceptible tomatoes and at 54 to 71% on susceptible peppers. Thus, *Me3* (or *Me7*) seemed to exert a higher selection pressure than *Mi-1* on RKN populations, resulting in an increased fitness cost of virulence. Moreover, RP of avirulent isolates was higher on susceptible tomatoes than on susceptible peppers, suggesting that the so-called 'susceptible' peppers had some undetermined and partial resistance factors, able to confer a quantitative resistance to some virulent RKN isolates, that are not present in susceptible tomatoes. In that respect, it should be noted that resistance to some isolates of the closely related RKN species *M. chitwoodi* was previously observed in DLL (Berthou et al. 2003; Djian-Caporalino et al. 2007). Further investigations are currently underway in the

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laboratory to confirm this hypothesis, which may have major practical implications in terms of durability of the resistance (Palloix et al. 2009).

Host resistance is considered as an important component of integrated management of RKNs. Because few R-genes acting against these pests are currently available, it is urgently needed to protect them and promote their durability. Since resistance deployment is influenced by the variation in (a) virulence and host range, two primary attributes of host resistance for nematode resistance breeding and management are relevant: i) the value of resistance in crop self-protection, based on the level of resistance to injury caused by initial infection, and ii) the rotational value of resistance in cropping systems for protecting subsequent crops, based on the ability to decrease the nematode population densities in soil by restricting nematode reproduction (Roberts 1995). In pepper, the *Me1* R-gene confers a high level of resistance without affecting the plant growth, is still active at high temperature, conversely to the tomato *Mi-1* resistance gene, is not overcome by both selected and natural virulent isolates overcoming *Mi-1* in tomato nor *Me3* nor *Me7* in pepper. This R-gene therefore looks suitable for either combination with *Me3* in pepper cvs ('pyramiding') or alternance with other R-genes (*Me3* or *Me7*, *Mi-1*) in crop rotations to strengthen and increase the durability of resistance. Experiments are now in progress to validate the obtained results under 3-year-field agronomic conditions comparing i) the alternance of single R-genes in rotation, ii) the mixture of genotypes bearing single R-genes sown in the same plot, and iii) the pyramiding of two R-genes in one genotype. Results will allow the identification of conditions lowering the emergence of virulent biotypes of RKN in the field, and the time required for the improvement of soil health (reduction of parasites under their damage threshold) using the R-plants as RKN 'traps'. This approach will help to promote the durability of those resistances against RKN, making breeders and farmers (resistance users) sensitive to the 'directions for use' necessary to keep their durability.

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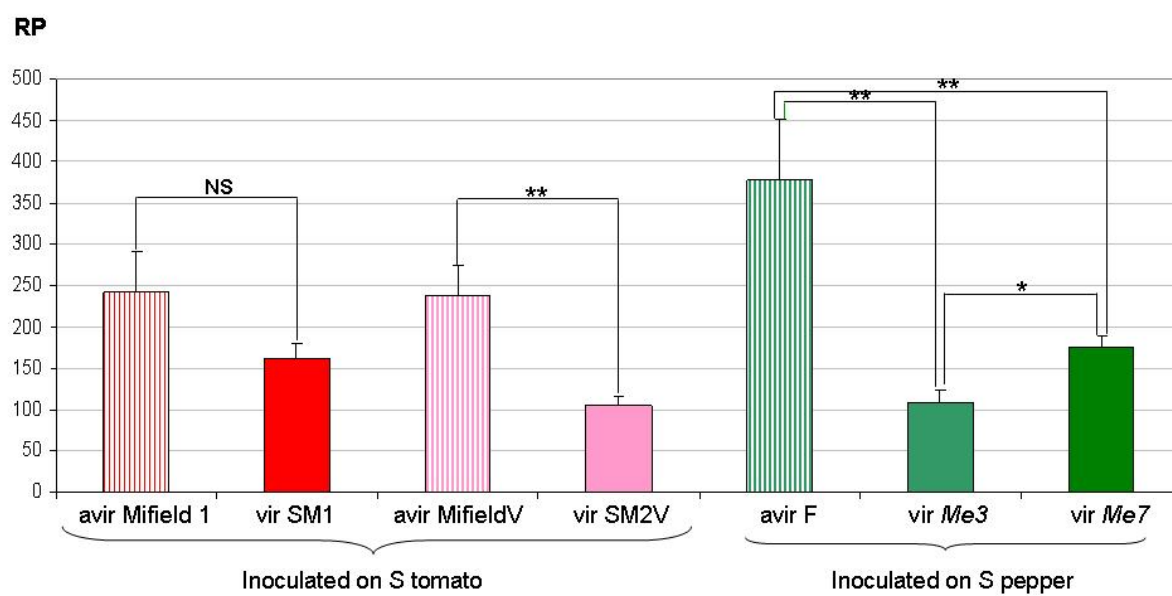
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Figure 1 Comparison of the reproduction potential (mean RP of 6 to 20 replicates \pm standard error) of avirulent and virulent laboratory-selected *M. incognita* isolates on tomato and pepper susceptible genotypes. NS = no significant difference; * = significant difference; ** = highly significant difference.

Figure 1

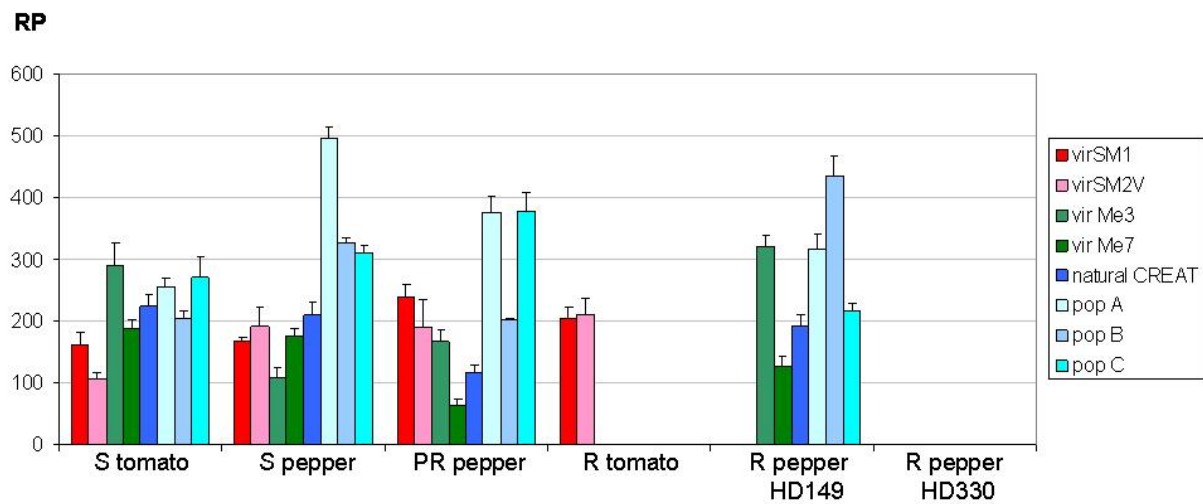


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Figure 2 Comparison of the reproduction potential (mean RP of 6 to 20 replicates \pm standard error) of laboratory-selected and natural *M. incognita* virulent isolates on both susceptible and resistant tomatoes and peppers.

Figure 2



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Table 1 *Meloidogyne incognita* isolates used in this study

Code	Geographic origin	Features	(a)virulence
avir F	Morelos, Mexico	Reference isolate from the INRA collection	Avirulent
avir Mifield1	Italy	Field isolate	Avirulent
avir MifieldV	Venezuela	Field isolate	Avirulent
vir <i>Mi-1</i>	France	Laboratory-selected on <i>Mi-1</i> from avir F	Virulent <i>Mi-1</i>
vir SM1	Italy	Greenhouse-selected on <i>Mi-1</i> from avir Mifield1	Virulent <i>Mi-1</i>
vir SM2V	Italy	Greenhouse-selected on <i>Mi-1</i> from avir MifieldV	Virulent <i>Mi-1</i>
vir <i>Me3</i>	France	Laboratory-selected on <i>Me3</i> from avir F	Virulent <i>Me3</i>
vir CREAT	La Baronne, France	Field isolate naturally-selected on <i>Me3</i>	Virulent <i>Me3</i>
vir pop A	Hungary	Greenhouse-selected on <i>Me3</i>	Virulent <i>Me3</i>
vir pop B	Hungary	Greenhouse-selected on <i>Me3</i>	Virulent <i>Me3</i>
vir pop C	Hungary	Greenhouse-selected on <i>Me3</i>	Virulent <i>Me3</i>
vir <i>Me7</i>	France	Laboratory-selected on <i>Me7</i> from avir F	Virulent <i>Me7</i>

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Table 2 Infestation frequency (IF) of greenhouse and laboratory-selected *Meloidogyne incognita* isolates on susceptible vs. resistant tomato and pepper genotypes. Values are the mean (\pm standard error) of 15 replicates.

Host genotypes ^a	Avirulent populations			Laboratory selection on <i>Mi-1</i> tomato ^b			Laboratory selection on <i>Me3</i> pepper ^b	Laboratory selection on <i>Me7</i> pepper ^b	Greenhouse selection on <i>Me3</i> pepper	Greenhouse selection on <i>Me3</i> -peppers		
	Avir F	avir Mifield1	avir MifieldV	vir <i>Mi-1</i>	Vir SMI	vir SM2V	vir <i>Me3</i>	vir <i>Me7</i>	Vir CREAT	vir pop A	vir pop B	vir pop C
S tomato	0.41 \pm 0.03	0.73 \pm 0.08	0.49 \pm 0.06	0.46 \pm 0.12	0.35 \pm 0.03	0.33 \pm 0.04	0.31 \pm 0.03	0.35 \pm 0.03	0.32 \pm 0.02	0.60 \pm 0.03	0.42 \pm 0.03	0.52 \pm 0.06
R tomato <i>Mi-1</i>	0.00 \pm 0.00	nd	0.002 \pm 0.66	0.82 \pm 0.14	0.43 \pm 0.04	0.35 \pm 0.06	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.42 \pm 0.00	0.00 \pm 0.00
S pepper	0.24 \pm 0.02	nd	0.66 \pm 0.05	Nd	0.44 \pm 0.02	0.38 \pm 0.04	0.17 \pm 0.01	0.26 \pm 0.02	0.27 \pm 0.02	0.71 \pm 0.03	0.87 \pm 0.03	0.67 \pm 0.04
PR pepper	0.10 \pm 0.01	nd	0.27 \pm 0.03	0.00 \pm 0.00	0.51 \pm 0.02	0.41 \pm 0.05	0.18 \pm 0.02	0.12 \pm 0.02	0.16 \pm 0.02	0.71 \pm 0.03	0.66 \pm 0.08	0.43 \pm 0.03
R pepper <i>Me3</i>	0.002 \pm 0.001	nd	0.00 \pm 0.00	0.00 \pm 0.00	nd	0.00 \pm 0.00	0.38 \pm 0.02	0.25 \pm 0.03	0.23 \pm 0.02	0.61 \pm 0.08	0.81 \pm 0.06	0.43 \pm 0.02
R pepper <i>Me1</i>	0.00 \pm 0.00	nd	0.00 \pm 0.00	0.0 \pm 0.00	nd	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00

^aS = susceptible, R = resistant, PR = partially resistant

^bThe virulent isolates were selected from the avirulent ones by successive rearing on resistant tomatoes and peppers, respectively

nd = data not available

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