

Tomato Rootstocks for Management of *Meloidogyne*

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Abstract

Studies on factors affecting root-knot nematode reproduction in tomato rootstocks with the *Mi*-resistance gene indicated that the resistant response is highly variable and the rootstocks show nematode-isolate specific resistant responses. Repeated cultivation of resistant tomatoes selected *Mi*-virulent populations from an avirulent one over three cropping cycles. Bioassays to determine the stability of the acquired *Mi*-virulence indicated that the *Mi*-virulence remained stable for at least two nematode generations in susceptible tomato irrespective of whether the virulent populations were generated on a *Mi*-resistant rootstock or cultivar. Management of *Meloidogyne* with resistant tomatoes should consider the differential efficacy of the rootstocks in suppressing nematode reproduction.

INTRODUCTION

The *Mi*-gene in tomato confers resistance but not immunity to *M. arenaria*, *M. incognita* and *M. javanica*. Resistant cultivars are an effective mean for nematode control (Sorribas et al., 2005), but are not extensively used probably because fruit characteristics do not always meet market demands or acceptance by consumers (e.g., hard peel). Therefore, grafting susceptible cultivars onto *Mi*-resistant rootstocks is an attractive way of producing commercially desirable tomato cultivars in pathogen infested soils as the rootstocks usually incorporate resistance to several pathogens including *Meloidogyne*.

Previous investigations indicated that the efficacy of the *Mi*-gene in tomato rootstocks is highly variable and the resistant response ranged from highly resistant to fully susceptible (López-Pérez et al., 2006; Cortada et al., 2008, 2009). Repeated cultivation of resistant tomatoes in a *M. javanica* infested field selected *Mi*-virulent populations able to overcome the resistance (Verdejo-Lucas et al., 2009). This paper presents the results of bioassays conducted to determine i) the *Mi*-avirulence status of the original population of *M. javanica* infesting the experimental field site, and ii) the stability of the acquired *Mi*-virulence after repeated cultivation of resistant tomatoes in the same land.

MATERIALS AND METHODS

To determine the *Mi*-a/virulence status of the field population infesting the experimental site, infested soil was collected and mixed with sterilized sand (1/3; v/v). The average infestation level (P_i) of the soil mixture was 487 second-stage juveniles (J2) per pot (1 L capacity). Tomato seedlings of the rootstocks 'PG76' and 'Brigeor' the *Mi*-resistant cultivar 'Monika', and susceptible cultivar 'Durinta' were transplanted into the pots and each plant treatment was replicated seven times. Plants were maintained in a glasshouse for 10 weeks. The number of eggs/g root was determined by macerating the entire root system in a 0.5% NaOCl solution in a food blender at 1000 rpm for 10 min. The multiplication rate (P_f/P_i) of the nematode was calculated as the number of eggs per plant (P_f) divided by J2 inoculum (P_i).

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The stability of the *Mi*-virulence generated after repeated cultivation of the resistant tomatoes in the field was tested following the scheme described in Figure 1. As indicated, three subpopulations of the nematode were generated; one per each resistant tomato. Soil samples were collected from the field site the following spring after termination of three consecutive cropping cycles of rootstocks 'PG76' and 'Brigeor', *Mi*-resistant cultivar 'Monika', and susceptible 'Durinta' (control for reference), and J2 were extracted using Baermann trays. Ten replicated plants of susceptible 'Durinta' were inoculated with 600 J2 of the respective subpopulations. After 10 weeks of growth in a glasshouse, eggs produced on the susceptible plants were extracted and used to inoculate a new set of resistant 'Monika' and susceptible 'Durinta'. The inoculum level was 3000 eggs per plant and ten replicated plants were prepared per each subpopulation-cultivar combination. Plants were maintained in a glasshouse and harvested after completion of one nematode generation. The number of egg masses and egg/g of root were determined. Eggs were extracted as previously described. The nematode multiplication rate was calculated as number of eggs per plant (Pf) divided by egg inoculum (Pi).

Data on numbers of eggs/g root and Pf/Pi were subjected to analysis of variance and means were separated by LSD test ($P < 0.05$) in the test to determine the *Mi*-avirulent status of the field population. The stability of virulence was analyzed by comparing reproduction of each subpopulation in the resistant and susceptible cultivar using Student's t-test ($P < 0.05$).

RESULTS

The *Mi*-avirulent status of the field population of *M. javanica* infesting the experimental site was confirmed since the nematode produced lower ($P < 0.05$) eggs/g root in the *Mi*-resistant tomatoes than in the susceptible one (Table 1). The nematode did not increase the initial levels in rootstocks PG76 and 'Brigeor' ($Pf/Pi < 1$).

The acquired *Mi*-virulence of the subpopulations generated after repeated cultivation of *Mi*-resistant tomatoes remained stable for at least two nematode generations in susceptible tomato (maximum time tested) irrespective of whether it was generated on a *Mi*-resistant rootstock or cultivar; there was no difference in egg masses, egg production or Pf/Pi in the *Mi*-resistant compared with the susceptible cultivar (Table 2). Only the subpopulation from susceptible tomato, unexposed to selection pressure of the *Mi*-gene, remained *Mi*-avirulent.

DISCUSSION

The acquired *Mi*-virulence after repeated cultivation of resistant tomatoes in the same land remained stable for the time tested (two nematode generations). Further studies are needed to ascertain the stability of the virulence through a longer time since the virulence might be reversed after cultivation of nematode susceptible crops. The selection of virulence exerted by the *Mi*-gene may not occur in every situation because it depends on the genetic pool of the nematode population (Castagnone-Sereno et al., 1994), the rate of *Mi*-virulent individuals present in a field population (Roberts, 2002) and the frequency of cropping (Verdejo-Lucas et al., 2009). In addition, the genetic background of the plant genotype also affects the efficacy of the resistant tomatoes as some genotypes are less effective than others in suppressing the nematode (Cortada et al., 2008), and others had shown a nematode-isolate specific resistant response (Cortada et al., 2009).

Although cropping *Mi*-resistant tomato cultivars or rootstocks reduced root galling (Miguel, 2002; Cortada et al., 2008) some rootstocks do not prevent population increases (López-Pérez, 2006; Cortada et al., 2008, 2009), and thus, they will not be an effective mean for nematode control on a long term basis. For instance, the rootstock 'Beaufort' and 'Maxifort' consistently supported high population densities of several populations of *M. incognita* and *M. javanica* (López-Pérez et al., 2006; Cortada et al., 2009). Grafting susceptible tomato cultivars onto resistant rootstocks increases yield compared to non-grafted plants (Miguel, 2002; López-Pérez et al., 2006; Cortada et al., 2008) probably due to their vigorous root systems that provide tolerance to the nematode. Nevertheless, the

stability of the yield through successive cropping cycles needs to be investigated.

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Tables

Table 1. Number of eggs per gram of root and multiplication rate of a population of *Meloidogyne javanica* in rootstocks 'PG76' and 'Brigeor' and in the *Mi*-resistant cultivar 'Monika' and susceptible 'Durinta' in a glasshouse to test for the *Mi*-virulence status of the field population infesting the soil of the experimental site used for field trials.

Tomato	Egg/g root	Multiplication rate ¹
Rootstock		
PG76 (R) ²	48±39 b	0.8±0.6 b
Brigeor (R)	79±55 b	0.9±1.0 b
Cultivar		
Monika (R)	340±240 b	3±2 b
Durinta (S)	26000±3200 a	496±64 a

Values are mean ± standard deviation of seven replicated plants. Values in each column followed by different letters are different according to the LSD test ($P<0.05$).

¹ Eggs per plant/juvenile inoculum.

² (R) *Mi*-resistant plant; (S) susceptible plant.

Table 2. Number of egg masses per plant, egg per gram of root and multiplication rate of four subpopulations of *Meloidogyne javanica* on resistant tomato cultivar 'Monika' and susceptible 'Durinta' generated after exposure to tomatoes with the *Mi*-gene for three consecutive cropping cycles in a nematode-infested field site.

Origin of the populations	Inoculated plant	Egg masses per plant	Eggs /g root	Pf/Pi ¹
PG76 (R) ³	Resistant	107±25	3048±549	26±4
	Susceptible	54±17	5500±820	36±7
Brigeor (R)	Resistant	830±62	17220±1590	149±13
	Susceptible	778±82	18100±1629	144±11
Monika (R)	Resistant	131±16	6947±800	51±8
	Susceptible	175±29	6273±1353	43±10
Durinta (S)	Resistant	22±7*	660±229*	5±1*
	Susceptible	134±26	6330±1185	47±9

Values are mean ± standard error of 10 replicated plants. Values for each nematode population followed by a * are different between the resistant and susceptible cultivar according to the Student's t-test ($P<0.05$).

¹ Eggs per plant/egg inoculum.

² (R) *Mi*-resistant plant; (S) susceptible plant.

Figures

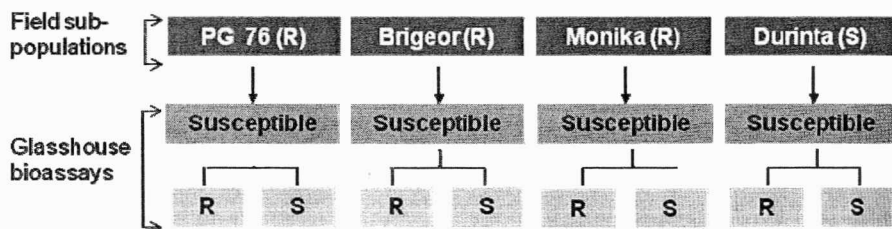


Fig. 1. Schematic diagram to indicate the origin of the subpopulations of *Meloidogyne javanica* generated after exposure to tomatoes with the *Mi*-gene for three consecutive cropping cycles in a nematode-infested field site, and bioassays conducted in a glasshouse to test the stability of the acquired *Mi*-virulence. (R) *Mi*-resistant plant (S) susceptible plant.

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Photographs on the front cover by courtesy of A. Crescenzi:

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1. Stem Rot (Southern Blight) by *Sclerotium rolfsii* on tomato plant grown under greenhouse conditions: white mycelium growth in which small spherical sclerotia, around 1-2 mm in diameter, are produced develops on the stem at the soil line.
2. Wonderful tomato field in the agro of Palazzo San Gervasio (Potenza, Italy).
3. *Clavibacter michiganensis* subs. *michiganensis* on tomato: "Bird's eyes" on fruits (spots with raised brown centers surrounded by an opaque white halo) (Fanigliulo et al., 2011; Acta Hort. 914:43-46).
4. Symptoms of abortion and necrosis of flowers, and fruit deformation associated with irregular ripening in Tomato 'San Marzano', artificially infected by *Columnea latent viroid*, CLVd (Crescenzi et al., 2011; Acta Hort. 914: 149-152).
5. Leaves of tomato 'Santa' with symptoms of interveinal necrosis and leaf deformation, induced by the necrotic strain of *Potato Virus Y* PVY-LF02, in natural infection (Crescenzi et al., 2005. Acta Hort. 695:331-338).
6. Fern-leaf and shoestring-like symptoms in tomato 'San Marzano', induced by a wild-type strain of *Cucumber mosaic virus* in natural infection (Crescenzi et al. 1993. Plant Disease 77: 28-33).

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