

Resistance response of the tomato rootstock SC 6301 to *Meloidogyne javanica* in a plastic house

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Abstract Nematode reproduction on the nematode-susceptible tomato cv. Durinta grafted onto the *Mi*-resistance gene tomato rootstock SC 6301 was compared to the *Mi*-resistance gene tomato cv. Monika in a plastic house infested with *Meloidogyne javanica*. The ungrafted susceptible cv. Durinta was included as a control for reference. Final soil population densities were lower ($P \leq 0.05$) on the resistant than susceptible cultivar but intermediate values were recorded on the rootstock SC 6301. The lowest numbers of eggs per gram root were recorded on the resistant cultivar followed by those on the rootstock; in both cases, they were lower ($P < 0.05$) than on the susceptible control. Cumulative yield (kilogram per square meter) was higher ($P < 0.05$) on the resistant than susceptible cultivar whether or not it had been grafted. The rootstock SC 6301 provided an intermediate resistance response to *M. javanica* and was less effective than the resistant cultivar in

suppressing nematode populations and plant damage under the experimental conditions of this study.

Keywords Control · Root-knot nematodes · *Solanum lycopersicum*

Vegetable crops are intensively grown in many areas of the world and several crops are cultivated at the same site throughout the year. Root-knot nematodes, *Meloidogyne* spp. are major pest of vegetable crops of intensive agriculture because of the damage they cause and their rapid spread in several countries including Spain (Eddoudi et al. 1997; Tzortzakakis and Gowen 1996; Verdejo-Lucas et al. 2002). Conventionally, control of root-knot nematodes has been mainly based on the use of broad-spectrum soil fumigants and nematicides. However, the phasing out of methyl bromide, restrictions in the use of nematicides and increased concern for the environment and human health has encouraged research to find non-chemical alternative methods of nematode control.

Plant resistance is an effective, economic and environmentally compatible method of controlling pests and pathogens. The *Mi*-gene in tomato suppresses development or reproduction of root-knot nematodes and confers high levels of resistance to the most common species, *Meloidogyne incognita*, *M. javanica* and *M. arenaria*. The *Mi*-gene was introgressed from *Solanum peruvianum* to *S. lycopersicum* (Smith 1944) and it is present in all resistant commercial tomato cultivars. Tomatoes with the *Mi*-

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resistance gene can be grown on most nematode-infested soil without significant yield losses (Philis and Vakis 1977; Rich and Olson 1999; Sorribas et al. 2005). In addition, resistant cultivars cost the same price as the susceptible ones and they do not require supplementary management practices. However, resistant tomatoes are not extensively used, probably because markets demand fruits with specific characteristics and qualities that are not matched by the resistant cultivars.

Grafting has been used traditionally in agriculture because it provides resistance or tolerance to soil-borne pathogens, growth promotion, and yield increases when compared to non-grafted plants (Lee 1994; Ioannou 2001; Miguel 2002). Also, it allows cultivation in pathogen-infested soils without using chemical compounds, and cultivation in land unsuitable for the grafted scion. Cultivation of grafted vegetables is currently expanding in Europe, and has been adopted alone or in combination with other control methods as a replacement to methyl bromide fumigation in several countries including those of the Mediterranean region (report MBTOC 2006). In Spain, grafting vegetables is a common practice mainly for cucurbits but also for solanaceous crops (Miguel 2002). The tomato rootstocks are selections of *S. lycopersicum* or interspecific hybrids between *S. lycopersicum* x *S. habrochites*, and they are compatible with cultivars of tomato and eggplant. Many seed companies offer tomato rootstocks (Marin Rodriguez 2005), and these are grafted with the desired cultivar in commercial nurseries by trained personnel. Grafted plants are sold as peat blocks and about 45 million grafted tomato plants were produced in 2005 (de la Torre Martinez 2005). Grafting in Spain has been used primarily to increase vigour of cherry and marmande tomatoes (Miguel 2002), and more recently to offset plant damage caused by soil-borne fungi and nematodes as a result of continuous cultivation of the same crop on the same site.

The objective of this study was to determine the efficacy of the *Mi*-resistance gene tomato rootstock SC 6301 in reducing population densities of *M. javanica* in comparison to a *Mi*-resistance gene tomato cultivar in a plastic house infested with the nematode.

The study was conducted in an unheated plastic house at Cabrils, Barcelona, Spain. The site had been artificially infested with *M. javanica* (code Mj-05) in 1998 by pre-inoculating tomato seedlings before

transplanting. The avirulent status for the *Mi*-resistance gene of this *M. javanica* isolate had been confirmed in greenhouse tests (Ornat et al. 2001). The soil was a sandy loam with 85.8% sand, 8.1% silt and 6.1% clay, pH 8.1, 0.9% organic matter (w/w), and 0.40 dS m⁻¹ electric conductivity. Individual plots were 3.4 m×1.5 m and consisted of two rows with six plants per row spaced 50 cm within the row and 55 cm between rows. Three treatments were investigated. They included: (1) resistant tomato cv. Monika, (2) susceptible tomato cv. Durinta grafted on rootstock SC 6301, and (3) ungrafted susceptible tomato cv. Durinta. Monika has resistance to *Tobacco mosaic virus*, *Verticillium dahliae*, *Fusarium oxysporum* f. sp. *lycopersici* race 1, and *Meloidogyne* spp. the rootstock SC 6301 has resistance to *V. dahliae*, *F. oxysporum* f. sp. *lycopersici* race 1 and 2; *F. oxysporum* f. sp. *radicis lycopersici*, and *Meloidogyne* spp. and Durinta has resistance to *Tobacco mosaic virus*, *V. dahliae*, *F. oxysporum* f. sp. *lycopersici* race 1 and 2. The rootstock SC 6301 was selected for its resistance to *Meloidogyne* among those available in the market. Grafting was performed by a commercial nursery. Each treatment was replicated five times according a stratified randomised block design. Plants were transplanted on March 4th, and harvested on July 12th, 2002.

To estimate initial and final population densities of *M. javanica*, composite soil samples were collected from each plot at the beginning and at the end of the crop, respectively. Individual samples consisted of five soil cores taken at 30 cm depth with a sampling tube (2.5 cm diam). Samples were mixed thoroughly and nematodes were extracted from a 500 cm³ soil subsample using Baermann trays. Nematodes that migrated to the water were collected 1 week later, concentrated on a 25-µm pore sieve, counted and expressed per 250 cm³ of soil. Average initial populations were 225±144 nematodes 250 cm⁻³ soil (mean ± standard deviation). The assessment of the nematode damage was based on the root gall index of tomato plants at the end of the experiment. Eight plants per plot were dug from the soil, examined, and rated on a scale of 0 to 10, where 0 = a complete and healthy root system (no galls observed) and 10 = plants and roots dead (Zeck 1971). Roots from each plot were then bulked, chopped in 1 cm-long segments and two 10-g subsamples used to extract eggs by blender maceration in a 0.5% NaOCl solution for

10 min (Hussey and Barker 1973). The number of eggs is expressed gram^{-1} of fresh root weight. The reproduction index of the nematode was calculated as eggs g^{-1} root on the resistant materials divided by eggs g^{-1} root on the susceptible cultivar $\times 100$.

Tomatoes produced by eight plants in each plot were harvested once per week for 6 weeks and the cumulative yield expressed as kg m^{-2} . Plants received water through a drip irrigation system and were fertilized weekly with a solution consisting of NPK (15–5–30), iron chelate and micronutrients at rates of 31 and $0.9 \text{ kg}^{-1} \text{ ha}$, respectively. Weeds were removed manually during the crop. Soil temperatures were recorded daily at 30-min intervals with temperature probes placed at a depth of 15 cm. The general linear model of the SAS software version 8 (SAS institute Inc., Cary, NC) was used for statistical analyses. The number of J2 in soil and eggs g^{-1} of root were transformed to $[\log (x+1)]$ and then subjected to analysis of variance along with data on gall ratings and yields of tomato. When the overall F test was significant ($P \leq 0.05$), means were separated by the least significant difference (LSD) method.

Soil temperatures were below 28°C from March 4th to July 12th 2002, and they ranged from 10.9 to 27.9°C ($x=18.9^\circ\text{C}$). Final population densities of *M. javanica* were lower ($P \leq 0.05$) on the resistant than susceptible cultivar but intermediate values were recorded on the rootstock SC 6301 (Table 1). The lowest numbers of eggs g^{-1} root were recorded on the

resistant cultivar followed by those on the rootstock; in both cases, they were lower ($P < 0.05$) than on the susceptible control. Plant damage, measured as root gall rating, was 1, 3, and 6 on the resistant, rootstock, and susceptible tomatoes, respectively. All plants of the rootstock and the susceptible cultivar showed galls in their roots but only in one third of the plants of the resistant cultivar. The reproductive index of the nematode was 9 and 35% on the resistant cultivar and rootstock, respectively. When final population densities of *M. javanica* in soil and roots were referred to the susceptible control (100% reproduction), the rootstock SC 6301 reduced population increases by 58 and 65%, respectively, whereas the resistant cultivar did so by 75 and 91%, respectively. Both the resistant cultivar and the rootstock carried the *Mi*-resistance gene but the former was more effective in suppressing population densities and plant damage than the rootstock. Therefore, the degree of resistance to *M. javanica* of the rootstock SC 6301 was intermediate under the conditions prevailing in the plastic house used for this study. Nevertheless, the rootstock showed much less root galling than the susceptible cultivar and egg production was about one third of that on the susceptible control.

Although the results presented here are limited to a single tomato crop cycle, they are in agreement with others reports on the differential response of the *Mi*-resistant tomatoes to root-knot nematodes. Thus, high population densities of several populations of *M.*

Table 1 Final nematode population densities in soil, number of eggs g^{-1} of root, reproduction index, gall rating and cumulative yield of tomato on a *Mi*-resistance gene cultivar and rootstock in a plastic house infested with *Meloidogyne javanica*

Treatment	Nematodes 250 cm^{-3} soil	Eggs gram^{-1} root	Reproduction index ^a	Gall rating ^b	Cumulative yield (kg m^{-2}) ^c	No. fruit/ plant	Average fruit weight (g)
Resistant cv. Monika	$4,140 \pm 3,570^b$	$3,548 \pm 1,740^c$	9 ± 4	1.2 ± 1.1^c	9.98 ± 1.8^a	34 ± 6^a	103 ± 21^a
Susceptible cv. Durinta grafted on rootstock SC6301	$7,010 \pm 4,040^{a,b}$	$13,970 \pm 12,430^b$	35 ± 32	3.2 ± 0.7^b	8.54 ± 0.9^b	31 ± 12^a	93 ± 14^b
Ungrafted susceptible cv. Durinta	$16,540 \pm 5,920^a$	$39,355 \pm 8,070^a$		5.9 ± 0.6^a	8.12 ± 1.8^b	30 ± 11^a	91 ± 18^b

Values are mean \pm standard deviation of five replicated plots, two tomato rows per plot, six plants per row. Values within the same column followed by a different letter are significantly different according to the LSD test ($P \leq 0.05$).

^a Eggs per gram root on the resistant materials divided by eggs per gram root on the susceptible cultivar $\times 100$.

^b Based on a scale from 0 (no galls) to 10 (root completely galled, dead plant; Zeck 1971). Forty plants examined per treatment.

^c Eight central plants per individual plot, four plants per row.

incognita were found on the *Mi*-resistance gene rootstock cv. Beaufort in tests conducted in California, and the authors concluded that this rootstock should be considered tolerant rather than resistant to the nematode (López-Perez et al. 2006). On the other hand, the *Mi*-resistance gene rootstock cv. Brigeor was more effective in reducing root-knot nematode disease incidence at lower than higher temperatures in Cyprus (Ioannou 2001). In Spain, root galling was reduced by grafting susceptible tomatoes onto rootstocks SC 6301 and Brigeor but the effect of these rootstocks on nematode reproduction was not reported (Miguel 2002). The resistant cv. Monika showed low nematode infection but retained its relative resistance level relative to the susceptible cv. Durinta in contrast to the rootstock that showed a much lower resistance level. Root-knot nematodes can reach high levels on tomatoes with the *Mi*-gene at the end of cropping in northeastern Spain because multiple nematode generations occur on a single crop cycle in field conditions (Sorribas and Verdejo-Lucas 1994). Breakdown of the resistance at high soil temperatures was discarded because soil temperatures remained below 28°C during the study. We did not check the avirulent status of the population at the end of the experiment because the recent crop history of the site had been susceptible tomato for the three previous years in the plots used for this study (Sorribas et al. 2005). The moderate reproduction of *M. javanica* on the rootstocks SC 6301 confirms the differences in nematode infection and reproduction reported on tomatoes with the *Mi*-resistance gene (López-Perez et al. 2006).

The cumulative yield of tomato was higher ($P \leq 0.05$) on the resistant cultivar than susceptible control (Table 1) and the yield increase was 1,800 kg ha⁻¹. Although varietal differences may account for yield differences, the yield increase can be largely attributed to the nematode because both cultivars yielded similarly in nematode-free soils (Sorribas et al. 2005). The fruit characteristics of both tomato cultivars are similar (Marín Rodríguez 2005). The cumulative yields of the grafted and ungrafted susceptible cultivar did not differ statistically but there was a yield increase of 420 kg ha⁻¹ on the rootstock. This result confirms others reported in the literature on the increased yield value provided by the rootstocks compared to non-grafted plants (Ioannou 2001; Miguel 2002; López-Perez et al. 2006).

Rootstocks with vigorous root systems can provide tolerance to root-knot nematode damage and substantial yields in nematode-infested soils, and they will be probably useful to limit huge increases in population densities of the nematode and to reduce disease incidence and plant damage, particularly in organic farming or integrated production, since these systems do not allow the use of chemical control. Nevertheless, further research is needed to confirm these preliminary results and to assess the value of tomato rootstocks as a control method for root-knot nematodes in infested soils, by evaluating the suppressive effect of different rootstocks under several experimental conditions. As for the *Mi*-resistance gene in tomato cultivars, the rootstocks should be used in an integrated management context to preserve the durability of the resistance and to prevent the selection of virulent populations. The combination of the rootstocks with other control methods will probably be needed to reduce the remnant nematode inoculum left in the soil after harvesting the crop.

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