

Response of local and commercial tomato cultivars and rootstocks to *Meloidogyne javanica* infestation

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Abstract

Fifty two local Greek tomato (*Solanum lycopersicum*) cultivars and accessions and ten commercial nematode-resistant tomato cultivars and rootstocks were evaluated under controlled environmental conditions for resistance against the root-knot nematode *Meloidogyne javanica*. All tested local tomato cultivars and accessions were susceptible to *M. javanica*. Conversely, the commercial root-knot nematode-resistant tomato cultivars significantly reduced galling and egg mass production of *M. javanica*. Depending on the inoculum level (200 or 400 second stage juveniles (J2) per plant), the tested tomato rootstocks showed a different response to *M. javanica*. When plants were inoculated with 200 *M. javanica* J2 a significantly lower number of galls and egg masses was recorded for all tested rootstocks in comparison to that of the control 6 weeks after inoculation. Plant inoculation with 400 *M. javanica* J2 resulted in reduced root galling on the non-grafted rootstock Multifort 6 weeks after inoculation, whereas a significantly lower gall index was recorded for all non-grafted rootstocks and the grafted rootstocks Multifort and Unifort 12 weeks after inoculation.

Keywords: grafting, integrated pest management, *Meloidogyne javanica*, root-knot nematode, rootstock, *Solanum lycopersicum*.

Introduction

Root-knot nematodes (RKN) (*Meloidogyne* spp.) have been recorded to cause severe yield and economic losses to tomato (*Solanum lycopersicum*) production in Mediterranean countries, where nematode growth is favoured by climatic conditions (Ornat et al., 2001). Until recently, RKN control has primarily relied on the use of chemical nematicides, however, due to environmental concerns and food safety reasons their use has been restricted. Alternatively, integrated nematode management approaches which involve a combination of cultural, chemical and biological methods could more efficiently regulate nematode populations (Sikora et al., 2005). An important tool and key factor to the success of such control strategies is the careful selection and use of cultivars that suppress nematode populations and subsequently yield losses of tomato production (Molinari, 2011). Local tomato cultivars which currently are excluded from modern large-scale agriculture, are lately gaining popularity (Gómez et al., 2001; Rodríguez-Burruezo et al., 2005; Kumar et al., 2007; Adalid et al., 2010). Their high rate of acclimatization to the conditions of the geographical location of their origin often results in a positive response to native pests and diseases. Due to this trait, local tomato cultivars have been used as a source of resistance genes against pests and diseases in breeding programs (Robertson and Labate, 2007). In Greece, a tomato germplasm collection, with more than 200 local cultivars and accessions is kept in reserve at the Greek Gene Bank of the Agricultural Research Centre of Makedonia and Thraki, constituting a genetic resource that has not been evaluated for nematode resistance. Many tomato cultivars with resistance to the three most widespread species of RKN (*M. javanica*, *M. incognita* and

M. arenaria) are commercially available in local markets and used by farmers. These tomato cultivars carry the *Mi*-resistance gene from *Lycopersicon peruvianum* (Fuller et al., 2008), which accounts for a hypersensitive response of the plant. This response results in rapid and localized cell necrosis at the infection site soon after the initiation of nematode feeding and ultimately in the disruption of the nematode life cycle (Roberts, 1992). However, there are several reports of resistance breaking root-knot nematode populations, virulent against the *Mi*-gene worldwide (Eddoudi et al., 1997; Ornat et al., 2001; Tzortzakakis et al., 2005; Devran et al., 2010). Therefore, in order to fully exploit tomato resistant cultivars in an integrated management context and at the same time preserve the durability of the resistance, it is essential to assess in advance the virulence of nematode populations present in a growing region against these cultivars. When resistant tomato cultivars with desirable traits and fruit quality characteristics are not available, susceptible cultivars can be grafted onto nematode-resistant rootstocks. Vegetable grafting is used worldwide as a method to control *Meloidogyne* spp. (Lee, 2003; Oka et al., 2004; Sigüenza et al., 2005; Rivard et al., 2010). Although a range of commercial tomato rootstocks with resistance to *M. javanica*, *M. incognita* and *M. arenaria* is available, recent research indicates increased variability in the response of tomato rootstocks to diverse nematode species and populations. Cortada et al. (2009) tested 31 nematode population-tomato rootstock genotype combinations in pot tests and reported that almost half of the combinations resulted in a highly resistant response, 11 were moderately resistant, and five were susceptible responses. In another

similar greenhouse study two resistant rootstocks exhibited high differences in root galling and final nematode populations after exposure to three *M. incognita* populations (López-Pérez et al., 2006). In this framework, the objective of this study was to screen a collection of local Greek tomato cultivars and accessions for resistance against *M. javanica*. Furthermore, the efficacy of commercially available root-knot nematode-resistant tomato cultivars and rootstocks to control *M. javanica* was determined.

Results

Response of local tomato cultivars and accessions to *M. javanica* infestation

There was no significant effect of the cultivar on the plant growth parameters measured after infestation with *M. javanica* (data not shown). For none of the tested Greek tomato cultivars and accessions, a reduction in root galling or production of egg masses was found (data not shown).

Response of nematode-resistant tomato cultivars to *M. javanica* infestation

There were significant differences in the number of galls ($F = 37.963$; $p < 0.001$) and egg masses ($F = 38.780$; $p < 0.001$) between the nematode-resistant tomato cultivars tested and the susceptible cultivar Ace when challenged with 200 *M. javanica* J2. For all resistant cultivars tested, root galling was in all cases significantly lower compared to control but no differences between cultivars were found. (Figure 1). Similarly, when challenged with 400 *M. javanica* J2 cultivars Formula, Elpida, Rally, Mirsini and Optima showed a significantly lower gall index compared to the susceptible control at 6 ($F = 22.314$; $p < 0.001$) and 12 weeks ($F = 17.696$; $p < 0.001$) post-inoculation. (Figure 2A and 2B, respectively).

Response of nematode-resistant tomato rootstocks to *M. javanica* infestation

When plants were challenged with 200 *M. javanica* J2, all rootstocks tested showed a significantly lower number of galls ($F = 3.778$; $p = 0.002$) and egg masses ($F = 4.526$; $p = 0.001$) in comparison to the control, 6 weeks after inoculation (Table 3). Plant inoculation with 400 *M. javanica* J2 resulted in no differences in root galling and number of egg masses between the tested rootstocks and the control, 6 weeks after inoculation, except for non-grafted Multifort, which showed a significantly lower number of galls ($p = 0.041$) and egg masses ($p = 0.003$) compared to control (Table 4). For grafted and non-grafted rootstock Resistar, significantly lower numbers of egg masses ($p < 0.04$) were found 6 weeks after inoculation (Table 4). Twelve weeks after inoculation all non-grafted rootstocks ($F = 5.698$; $p = 0.002$) and grafted Multifort ($p = 0.042$) and Unifort ($p = 0.035$) showed a significantly lower gall index (Table 4).

Discussion

Local cultivars can be a valuable source of genetic material for modern breeding programs and screening local cultivars against important pests and diseases can be helpful for the development of new cultivars with improved traits (Robertson and Labate, 2007). In this study, a collection of local Greek tomato cultivars and accessions was tested against a native population of *M. javanica*. The tomato

cultivars and accessions tested originated from various regions or Greece, as well as from Greek islands which conventionally represent genetically isolated populations, in order to assure sufficient genetic diversity. However, for all tested local cultivars and accessions severity of root galling and nematode damage was similar to the susceptible cultivar Ace, indicating that all cultivars were highly susceptible to *M. javanica*, thus having no potential as gene source for nematode resistance breeding. In contrast, all commercial nematode-resistant cultivars tested showed a protective response against *M. javanica*, as in both experiments and at all evaluation dates root galling was significantly reduced in comparison to control. These results demonstrate their potential for use in integrated management of *M. javanica* in tomato production systems in combination with other cultural and biological methods, especially when nematicides cannot be used, e.g. in organic farming systems. Previous studies have shown that the incorporation of resistant cultivars in crop rotations has reduced nematode damage and increased the yield value. In a recent trial in plastic houses in Spain the integration of a resistant tomato cultivar in the rotation scheme for three consecutive cropping seasons, resulted in significant suppression of the nematode densities and subsequent reduction of yield losses for the succeeding susceptible cultivar (Talavera et al., 2009). Similarly, Sorribas et al. (2005) reported that the suppression of *M. javanica* populations after three consecutive crops of a resistant cultivar was analogous to the methyl bromide treatment resulting in high yield and economic profits. However, although all nematode-resistant tomato cultivars tested in this study reduced nematode damage, they were not immune to the *M. javanica* population but instead supported some level of nematode reproduction. The ability of root-knot nematode populations to reproduce on resistant tomatoes has also been demonstrated in previous studies and has been attributed to the interaction between the plant genotype and the nematode species (Jacquet et al., 2005). Ornat et al. (2001) tested the reproductive potential of Spanish populations of *M. javanica*, *M. incognita* and *M. arenaria* on resistant tomato cultivars and found that *M. javanica* populations had a higher reproduction rate on resistant tomato cultivars than those of *M. incognita* and *M. arenaria*. Roberts and Thomason (1986) have also demonstrated the increased virulence of *M. javanica* populations. The reproduction of *M. javanica* populations on resistant tomato cultivars, together with the fact that *M. javanica* is one of the most common root-knot nematode species in many tomato-growing areas (Ornat et al., 2001) raise concerns about the durability of the resistance mediated by the *Mi* gene in tomato cultivars and rootstocks. Repeated cultivation of resistant tomato cultivars or rootstocks may lead to the selection of virulent nematode populations (Verdejo-Lucas et al., 2009). Therefore, the careful integration of resistant cultivars in the cropping rotation schemes as well as the testing of the resistant cultivars against local nematode populations prior to their addition in root-knot nematode management programs is essential. Information on the response of nematode-resistant tomato rootstocks to root-knot nematode species and populations is still limited to few studies that show increased variability in terms of nematode infectivity and reproduction among the rootstocks. For instance, the resistant rootstock Big Power displayed high resistance in soils naturally infested with *M. javanica* (Cortada et al., 2008) and *M. incognita* (Rivard et al., 2010). In the same study, Maxifort and Beaufort showed partial resistance to the Southern root-knot nematode compared to non- and self-grafted controls (Rivard et al., 2010).

Table 1. List of local Greek tomato cultivars and accessions used in this study.

Cultivar / Accession number	Origin	Cultivar / Accession number	Origin	Cultivar / Accession number	Origin
1. VG-004/83	Serres	19. GRC 1936/04	Samothraki	37. VE-032/83	Drama
2. GRC 1925/04	Evros	20. Plataniani	Crete	38. 14/8/1996	Ksanthi
3. VE-027/83	Thasos	21. GRC 1807/04	Evros	39. GRC 242/99	Evros
4. Pantaroza	Kefalonia	22. GRC 069/04	Crete	40. Karabola	Kozani
5. Mylati	Kefalonia	23. GRC 1594/04	Magnisia	41. VG-001/83	Drama
6. Lainates	Lasithi	24. GRC 2114/04	Drama	42. VG-002/83	Serres
7. GRC 2062/04	Kos	25. GRC-1388/04	Arta	43. GRC 1113C/04	Fokida
8. Bourneli	Ikaria	26. GR-076/94	Karpathos	44. VE-029/83	Drama
9. Mylo	Chalkidiki	27. GR-014/99	Samos	45. GR 068/99	Ikaria
10. GRC 1480/04	Ioannina	28. VE-022/83	Rodopi	46. VE-021/83	Kavala
11. Tomataki	Santorini	29. VG-003/83	Serres	47. GR 301/99	Pella
12. GR 279/99	Drama	30. VE-026/83	Samothraki	48. VE-035/83	Kavala
13. GRC 2118/04	Ksanthi	31. GR-093/93	Samos	49. GRC 1032/04	Naupaktos
14. GRC 2079/04	Limnos	32. GR-240/99	Evros	50. VE 030/83	Thasos
15. GRC 1429/04	Ioannina	33. VE-034/83	Kavala	51. GR-025/99	Samos
16. GRC 1112/04	Fokida	34. Fylloto	Samos	52. GR-069/94	Karpathos
17. GRC 1927/04	Evros	35. VE-023/83	Evros		
18. GRC 1592/04	Magnisia	36. VE-031/83	Thasos		

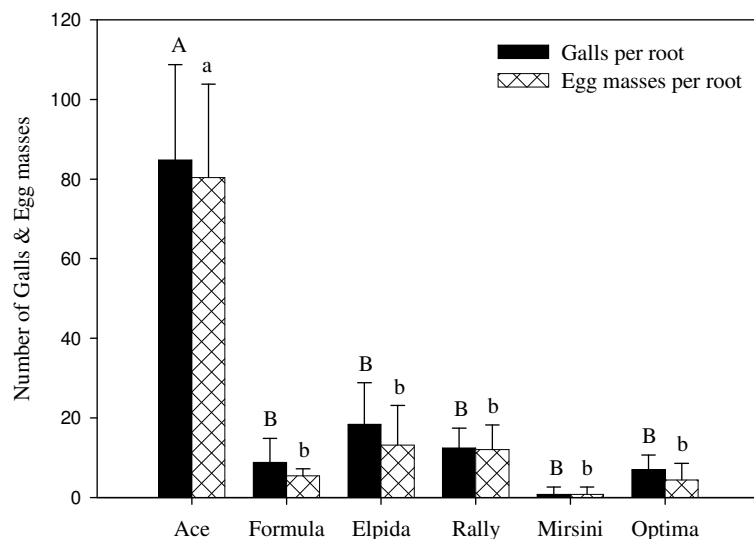


Fig 1. Number of galls and egg masses per root of *M. javanica* on five nematode-resistant tomato cultivars 6 weeks after inoculation with 200 *M. javanica* J2 in a pot experiment. Tomato cultivar Ace was used as susceptible control. Values are means of five replicate plants (n = 5). Columns with different uppercase (Galls per root) or lowercase (Egg masses per root) letters are significantly different according to Tukey's HSD test ($p < 0.05$).

Intermediate resistance response to *M. javanica* was provided also by the resistant tomato rootstock SC 6301, which allowed moderate levels of nematode reproduction in an artificially infested plastic house in Spain (Verdejo-Lucas and Sorribas, 2008). This study provides information on the response of new nematode-resistant rootstocks to *M. javanica* infestation. Although the results presented here are preliminary and limited to pot experiments, they are in agreement with other reports on the differential response of the root-knot nematode-resistant tomato rootstocks to nematode infestation. Multifort, grafted or not, significantly

reduced root galling 6 and 12 weeks after inoculation with 200 and 400 *M. javanica* J2, respectively, however, in all cases, it supported some level of nematode reproduction. Conversely, inoculation of grafted Resistar, Maxifort and Eldorado with 400 *M. javanica* J2 resulted in a susceptible response 12 weeks after inoculation. The resistance mechanism of the Mi-gene can be lost at soil temperatures above 28°C (Williamson, 1998), however in this study breakdown of the resistance due to high soil temperatures was discarded because soil temperatures remained below 28°C throughout the study. In a similar study, in which 10

Table 2. List of commercial tomato cultivars and rootstocks used in this study.

Cultivar	Seed Company	Resistances ^a	Use
Formula	Spirou House of Agriculture, Greece	ToMV/Va/Vd/Fol:0,1/Ma/Mi/Mj	Fruit production
Elpida	Spirou House of Agriculture, Greece	ToMV/TSWV/Cf:1-5/Va/Vd/Fol:0,1/For/Ol/Ma/Mi/Mj	“
Rally	Spirou House of Agriculture, Greece	ToMV/TSWV/Cf:1-5/Va/Vd/Fol:0,1/Ma/Mi/Mj	“
Mirsini	Spirou House of Agriculture, Greece	TYLCV/TSWV/Va/Vd/Fol/Ss/Aal/Ma/Mi/Mj	“
Optima	Geniki Fitotechniki, Greece	ToMV/Va/Vd/Fol:1,2/Ma/Mi/Mj	“
Resistar	Hazera Genetics	ToMV/Fol:1,2/For/Pl/Vd/Ma/Mi/Mj	Rootstock
Eldorado	Spirou House of Agriculture, Greece	ToMV/Cf:1-5/Fol:0,1/For/Va/Vd/Ma/Mi/Mj	“
Maxifort	De Ruiters Seeds, Netherlands	ToMV/Fol:0,1/For/Pl/Va/Vd/Ma/Mi/Mj	“
Multifort	De Ruiters Seeds, Netherlands	ToMV/Fol:0,2/For/Pl/Va/Vd/Ma/Mi/Mj	“
Unifort	De Ruiters Seeds, Netherlands	ToMV/Ff:1-5/Fol:0,1/For/Va/Vd/Ma/Mi/Mj	“

^a Information from the seed companies' descriptions. ToMV: *Tomato mosaic virus*; TYLCV: *Tomato yellow leaf curl virus*; TSWV: *Tomato Spotted Wilt Virus*; Ff: 1–5: *Fulvia fulva* races 1, 2, 3, 4 and 5; Cf: 1-5: *Cladosporium fulvum* races 1, 2, 3, 4 and 5; Fol 0–2: *Fusarium oxysporum* f. sp. *lycopersici* races 0, 1 and 2; For: *Fusarium oxysporum* f. sp. *radicis-lycopersici*; Ol: *Oidium neolycopersici*; Ss: *Stemphylium solani*; Aal: *Alternaria alternata* f.sp. *lycopersici*; Pl: *Pyrenochaeta lycopersici*; Va: *Verticillium albo-atrum*; Vd: *Verticillium dahliae*; Mi: *Meloidogyne incognita*, Ma: *M. arenaria*, Mj: *M. javanica*.

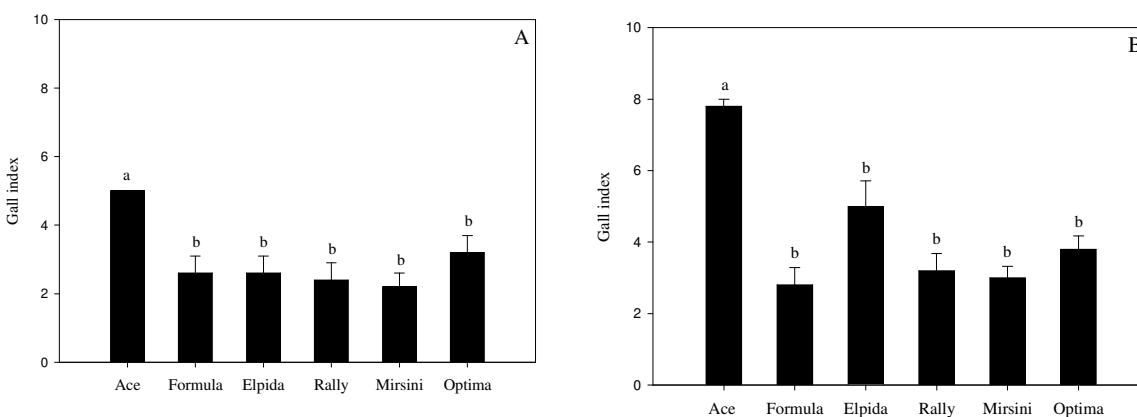


Fig 2. Gall index of *M. javanica* on five nematode-resistant tomato cultivars 6 (A) and 12 (B) weeks after inoculation with 400 *M. javanica* J2 in a pot experiment. Tomato cultivar Ace was used as susceptible control. Values are means of four replicate plants (n = 4). Gall index scale: 0-10, where 0 = no galls and 10 = dead plant. Columns with different uppercase (Galls per root) or lowercase (Egg masses per root) letters are significantly different according to Tukey's HSD test ($p < 0.05$).

tomato rootstocks carrying the *Mi* resistance gene were challenged with a single isolate of *M. javanica*, the authors reported increased variation in the infectivity and reproduction of *M. javanica*, some rootstocks being highly susceptible, and attributed this effect to the genetic background of the rootstocks (Cortada et al., 2008). The aforementioned results alongside with the ones of our study highlight the importance of the rootstock-nematode interaction and its implications in root-knot nematode management. The effect of growing resistant tomato rootstocks aiming at nematode suppression and tomato yield increase in nematode infested fields could vary depending on the tomato rootstocks and the *Meloidogyne* populations present in an area, thus limiting their resistance value as an efficient nematode management tool. Therefore, it is crucial to perform trials in order to assess the interaction of the resistant rootstocks against local root-knot nematode

populations ahead of their exploitation in root-knot nematode management strategies.

Materials and methods

Nematode and plant material

A field population of *M. javanica* (Treb) Chitwood was isolated from a field (Volos, Central Greece) planted for the first time with tomato and identified by cutting perineal patterns of adult females. *M. javanica* was multiplied continuously on the susceptible tomato cultivar Ace before initiation of the experiments. Eggs of *Meloidogyne javanica* were extracted from infected tomato roots using the sodium hypochlorite method (Hussey and Barker, 1973). To induce hatching, air was supplied with an aquarium pump in the egg suspension for 7 days at room temperature in the dark. Freshly hatched second stage juveniles (J2) were separated

Table 3. Effect of *Meloidogyne javanica* infestation on dry shoot weight, root fresh weight and number of galls and egg masses of five commercial nematode-resistant tomato rootstocks grafted or not with the susceptible tomato cultivar Ace 6 weeks after inoculation with 200 *M. javanica* J2 per pot.

Cultivar		Dry Shoot weight (g)	Fresh Root Weight (g)	Galls per root	Egg masses per root
Ace (non-inoculated)		3.0 ± 0.2	3.4 ± 0.3	-	-
Ace (inoculated)		3.2 ± 0.1	3.8 ± 0.4	25.5 ± 3.9	14.7 ± 2.9
Grafted	Resistar/Ac	3.3 ± 0.2	3.8 ± 0.3	7.0 ± 5.2 *	3.0 ± 2.4 *
	Multifort/A	2.8 ± 0.1	3.6 ± 0.2	4.0 ± 3.1*	1.3 ± 0.7 *
	Maxifort/Ac	n.t.	n.t.	n.t.	n.t.
	Unifort/Ace	2.9 ± 0.2	3.8 ± 0.4	1.0 ± 1.0 *	1.0 ± 1.0 *
	Eldorado/A	3.1 ± 0.2	3.5 ± 0.3	2.5 ± 1.0 *	2.0 ± 0. *
Non-Grafted	Resistar	3.7 ± 0.2	5.2 ± 0.5	8.0 ± 8.0 *	2.0 ± 2.0 *
	Multifort	4.3 ± 0.2 *	4.0 ± 0.4	1.5 ± 0.5 *	0.5 ± 0.5 *
	Maxifort	3.9 ± 0.3	5.0 ± 0.4	3.5 ± 1.5 *	3.0 ± 1.3 *
	Unifort	3.8 ± 0.2	5.7 ± 0.4 *	9.5 ± 9.5 *	4.5 ± 4.5 *
	Eldorado	3.5 ± 0.3	3.9 ± 0.5	1.5 ± 1.5 *	0.5 ± 0.5 *

Values are means (± SEM) of five (n = 5) replicate plants. Values in the same column with asterisks (*) are significantly different from the inoculated control cv. Ace, according to Dunnett's two-sided *t*-test ($p < 0.05$); n.t.: not tested.

from the unhatched eggs with a modified Baermann dish technique applied (Dababat and Sikora, 2008). Tomato seeds were sown in multi-pot trays filled with commercial seedling substrate. Two weeks post-germination, seedling roots were gently washed free of substrate and transplanted in pots ($\varnothing = 10$ cm) with a sand:soil:seedling substrate mix (2:1:1, v/v) content, one plant per pot. The field soil used was sandy loam (8% clay, 24% silt, 68% sand) with organic matter content of 0.4% and pH of 8. Plants were allowed two weeks to re-establish prior to experiment initiation.

Response of local tomato cultivars and accessions to *M. javanica* infestation

Fifty two local Greek tomato cultivars and accessions (Table 1) provided by the Greek Gene Bank of the Agricultural Research Centre of Makedonia and Thraki were challenged with 200 J2 of *M. javanica*. Nematodes were inoculated with 3 ml distilled water into three holes around the stem base, approximately 2 cm deep. The tomato cultivar Ace served as susceptible control and 5 plants per cultivar were used. Pots were arranged in a completely randomized design on a bench in a growth chamber at $25 \pm 1^\circ\text{C}$ and a 16 h photoperiod. Plants were watered daily and fertilized weekly with 50 ml of a 0.1% fertilizer solution (20-20-20, N:P:K). Six weeks after inoculation, tomato plants were uprooted, roots washed free of soil and plant growth parameters recorded. The number of galls and egg masses per root system were counted after staining with a 0.015% Phloxine B (Sigma-Aldrich Chemie GmbH, Munich, Germany) solution (Hussey and Janssen, 2002).

Response of nematode-resistant tomato cultivars to *M. javanica* infestation

Five commercial tomato cultivars (Table 2), resistant to *M. incognita*, *M. javanica* and *M. arenaria* according to

specifications offered by the seed companies, were challenged with 200 J2 of *M. javanica*, which were inoculated as described above. The tomato cultivar Ace served as susceptible control and 5 plants per cultivar were used. Pots were arranged in a completely randomized design on a bench in a growth chamber at $25 \pm 1^\circ\text{C}$ and a 16 h photoperiod. Plants were watered daily and fertilized weekly as described above. Six weeks after inoculation the experiment was terminated and plant growth parameters as well as the number of galls and egg masses per root were counted after staining as described above. The experiment was repeated and ten plants of each cultivar were inoculated with 400 *M. javanica* J2 per plant. Five tomato plants from each cultivar were harvested 6 weeks after inoculation, while the remaining plants were evaluated 12 weeks after inoculation. Plant growth parameters as well as gall index (0-10) according to Bridge and Page (1980) were estimated.

Response of nematode-resistant tomato rootstocks to *M. javanica* infestation

Five commercial tomato rootstocks (Table 2) carrying the Mi resistance gene, were challenged with *M. javanica*. The susceptible cultivar Ace was used both as control and scion cultivar. There were two treatments for each rootstock: Ace grafted onto the rootstock and non-grafted rootstock. Four-week-old seedlings of similar size were used for tube grafting (Rivard et al., 2010). Plants were kept at $25 \pm 1^\circ\text{C}$ in a plastic chamber with high humidity away from direct sunlight. Throughout the following week, plants were gradually exposed to lower humidity levels in order to get acclimatized to the growth chamber conditions. Ten days later 200 *M. javanica* J2 were inoculated around the stem base of the seedlings and 5 plants per rootstock were used. Plants were maintained at $25 \pm 1^\circ\text{C}$ in a growth chamber with a 16-hour photoperiod on a bench in a completely randomised design. Plants were watered daily and fertilized weekly as described above. Six weeks after inoculation the

Table 4. Effect of *Meloidogyne javanica* infestation on dry shoot weight, root fresh weight and number of galls and egg masses of five commercial nematode-resistant tomato rootstocks grafted or not with the susceptible tomato cultivar Ace 6 and 12 weeks after inoculation with 400 *M. javanica* J2 per pot.

		6 weeks after inoculation				12 weeks after inoculation		
		Dry Shoot weight (g)	Fresh Root Weight (g)	Galls per root	Egg masses per root	Dry Shoot weight (g)	Fresh Root Weight (g)	Gall index
Ace (non-inoculated)		4.3 ± 0.8	4.6 ± 0.2	-	-	5.4 ± 0.7	5.5 ± 0.3	-
Ace (inoculated)		3.8 ± 0.4	4.9 ± 0.6	90.5 ± 16.1	69.0 ± 13.8	3.7 ± 0.4	9.1 ± 0.9	6.5 ± 0.3
Grafted	Resistar/Ace	3.5 ± 0.4	3.3 ± 0.5	79.0 ± 22.5	20.7 ± 9.9 *	3.5 ± 0.8	10.8 ± 0.1	6.0 ± 0.0
	Multifort/Ace	3.1 ± 0.5	3.5 ± 0.2	127.2 ± 10.3	56.2 ± 11.8	3.4 ± 0.3	10.8 ± 1.3	5.0 ± 0.3 *
	Maxifort/Ace	4.2 ± 0.8	4.6 ± 0.4	108.5 ± 12.6	55.0 ± 8.2	3.3 ± 0.2	7.9 ± 0.3	5.5 ± 0.4
	Unifort/Ace	3.7 ± 0.2	3.9 ± 0.2	63.0 ± 19.0	43.7 ± 13.7	3.5 ± 0.2	9.4 ± 1.7	4.7 ± 0.3 *
	Eldorado/Ace	3.4 ± 0.8	3.9 ± 0.2	98.3 ± 5.7	48.7 ± 7.3	3.6 ± 0.4	8.2 ± 0.8	5.8 ± 0.6
Non-Grafted	Resistar	2.6 ± 0.1	3.2 ± 0.5	58.0 ± 6.1	11.0 ± 1.3 *	3.3 ± 0.5	4.9 ± 0.5 *	3.8 ± 0.5 *
	Multifort	2.9 ± 0.1	3.1 ± 0.4 *	34.5 ± 8.1 *	10.0 ± 3.9 *	3.2 ± 0.4	4.8 ± 0.2 *	3.5 ± 0.5 *
	Maxifort	2.7 ± 0.2	2.5 ± 0.2 *	69.7 ± 15.9	28.7 ± 7.1	3.3 ± 0.3	5.4 ± 0.6	4.2 ± 0.4 *
	Unifort	2.4 ± 0.2	3.9 ± 0.2	62.5 ± 13.4	30.0 ± 6.8	4.5 ± 0.1	9.2 ± 0.2	4.5 ± 0.3 *
	Eldorado	3.2 ± 0.8	3.4 ± 0.4	119.7 ± 30.2	65.0 ± 26.7	4.3 ± 0.4	8.0 ± 0.8	4.8 ± 0.6 *

Values are means (± SEM) of four

experiment was terminated and plant growth parameters as well as the number of galls and egg masses per root were counted after staining as described above. The experiment was repeated and eight plants of each rootstock were inoculated with 400 *M. javanica* J2 per plant. Four tomato plants of each rootstock were harvested 6 weeks after inoculation, while the remaining plants were evaluated 12 weeks after inoculation. Plant growth parameters and gall index were estimated as described above.

Statistical analysis

For all three experiments, data on plant growth parameters and root galling were subjected to one-way analysis of variance (ANOVA) using SPSS 15.0 (SPSS Inc., Chicago). When ANOVA showed significant effects, mean separation was done using Tukey's HSD test ($p < 0.05$) or Dunnett's two sided *t*-test ($p < 0.05$).

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References

- Adalid AA, Roselló S, Nuez F (2010) Evaluation and selection of tomato accessions (*Solanum* section *Lycopersicon*) for content of lycopene, β -carotene and ascorbic acid. *J Food Compos Anal* 23:613-618.
- Bridge J, Page SLJ (1980) Estimation of root-knot nematode infestation levels on roots using a rating chart. *Trop Pest Manage* 26:296-298.
- Cortada L, Sorribas FJ, Ornat C, Kaloshian I, Verdejo-Lucas S (2008) Variability in infection and reproduction of *Meloidogyne javanica* on tomato rootstocks with the *Mi* resistance gene. *Plant Pathol* 57:1125-1135.
- Cortada L, Sorribas FJ, Ornat C, Andrés MF, Verdejo-Lucas S (2009) Response of tomato rootstocks carrying the *Mi*-resistance gene to populations of *Meloidogyne arenaria*, *M. incognita* and *M. javanica*. *Eur J Plant Pathol* 124:337-343.
- Dababat AA, Sikora RA (2008) Induced resistance by the mutualistic endophyte, *Fusarium oxysporum* strain 162, toward *Meloidogyne incognita* on tomato. *Biocontrol Sci Techn* 17:969-975.
- Devran Z, Söğüt MA (2010) Occurrence of virulent root-knot nematode populations on tomatoes bearing the *Mi* gene in protected vegetable-growing areas of Turkey. *Phytoparasitica* 38:245-251.
- 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. *Fund Appl Nematol* 20:285-289.
- Fuller VL, Lilley CJ, Urwin PE (2008) Nematode resistance. *New Phytol* 180:27-44.
- Gómez R, Costa J, Amo M, Alvarruiz A, Picazo M, Pardo JE (2001) Physicochemical and colorimetric evaluation of local varieties of tomato grown in SE Spain. *J Sci Food Agric* 81:1101-1105.
- Hussey RS, Barker KR (1973) A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. *Plant Dis Rep* 57:1025-1028.
- Hussey RS, Janssen GJW (2002) Root-knot nematodes: *Meloidogyne* species. In: Starr JL, Cook R, Bridge J (eds) Plant resistance to plant parasitic nematodes, CABI Publishing, Wallingford, UK.
- Jacquet M, Bongiovanni M, Martinez M, Verschave P, Wajnberg E, Castagnone-Sereno P (2005) Variation in resistance to the root-knot nematode *Meloidogyne incognita* in tomato genotypes bearing the *Mi* gene. *Plant Pathol* 54:93-99.
- Kumar R, Klein D, Krumbeinz A, Köpke U (2007) Product quality of greenhouse tomatoes: Effect of cultivars, organic N-fertilization and harvest time. *Eur J Hortic Sci* 72: 46-51.
- Lee JM (2003) Current status of grafted vegetable cultivation. *Chronica Horticulturae* 43:13-19.
- López-Pérez J-A, Strange ML, Kaloshian I, Ploeg AT (2006) Differential response of *Mi* gene-resistant tomato rootstocks to root-knot nematodes (*Meloidogyne incognita*). *Crop Prot* 25:382-388.
- Molinari S (2011) Natural genetic and induced plant resistance, as a control strategy to plant-parasitic nematodes alternative to pesticides. *Plant Cell Rep* 30: 311-323.
- Oka Y, Offenbach R, Pivonia S (2004) Pepper rootstock graft compatibility and response to *Meloidogyne javanica* and *M. incognita*. *J Nematol* 36:137-141.
- Ornat C, Verdejo-Lucas S, Sorribas FJ (2001) A population of *Meloidogyne javanica* from Spain virulent to the *Mi* resistance gene in tomato. *Plant Dis* 85:271-276.
- Rivard CL, O'Connell S, Peet MM, Louws FJ (2010) Grafting tomato with interspecific rootstock to manage diseases caused by *Sclerotium rolfsii* and southern root-knot nematode. *Plant Dis* 94:1015-1021.
- Roberts PA (1992) Current status of the availability, development, and use of host plant resistance to nematodes. *J Nematol* 24:213-227.
- Roberts PA, Thomason IJ (1986) Variability in reproduction of isolates of *M. incognita* and *M. javanica* on resistant tomato genotypes. *Plant Dis* 70:547-551.
- Robertson LD, Labate JA (2007) Genetic resources of tomato (*Lycopersicon esculentum* Mill.) and wild relatives. In: Razdan MK, Matoo AK (eds) Genetic improvement of solanaceous crops, Vol. 2: Tomato, Science Publishers, New Hampshire, USA.
- Rodríguez-Burruezo A, Prohens J, Roselló S, Nuez F (2005) "Heirloom" varieties as sources of variation for the improvement of fruit quality in greenhouse-grown tomatoes. *J Hortic Sci Biotech* 80:453-460.
- Sigüenza C, Schochow M, Turini T, Ploeg A (2005) Use of *Cucumis metuliferus* as a rootstock for melon to manage *Meloidogyne incognita*. *J Nematol* 37:276-280.
- Sikora RA, Bridge J, Starr JL (2005) Management Practices: an overview of integrated nematode management technologies. In: Luc M, Sikora RA, Bridge J (eds), Plant parasitic nematodes in subtropical and tropical agriculture, CABI Publishing, Wallingford, UK.

- Sorribas FJ, Ornat C, Verdejo-Lucas S, Galeano M, Valero J (2005) Effectiveness and profitability of the *Mi*-resistant tomatoes to control root-knot nematodes. *Eur J Plant Pathol* 111:29–38.
- Talavera M, Verdejo-Lucas S, Ornat C, Torres J, Vela MD, Macias FJ, Cortada L, Arias DJ, Valero J, Sorribas FJ (2009) Crop rotations with *Mi* gene resistant and susceptible tomato cultivars for management of root-knot nematodes in plastic houses. *Crop Prot* 28:662–667.
- Tzortzakakis EA, Adam MAM, Blok VC, Paraskevopoulos C, Bourtzis K (2005) Occurrence of resistance-breaking populations of root-knot nematodes on tomato in Greece. *Eur J Plant Pathol* 113:101–105.
- Verdejo-Lucas S, Sorribas FJ (2008) Resistance response of the tomato rootstock SC 6301 to *Meloidogyne javanica* in a plastic house. *Eur J Plant Pathol* 121:103-107.
- Verdejo-Lucas S, Cortada L, Sorribas FJ, Ornat C (2009) Selection of virulent populations of *Meloidogyne javanica* by repeated cultivation of *Mi* resistance gene tomato rootstocks under field conditions. *Plant Pathol* 58:990-998.
- Williamson VM (1998) Root-knot nematode resistance genes in tomato and their potential for future use. *Annu Rev Phytopathol* 36:277-293.