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Pepper Rootstock Graft Compatibility and Response to *Meloidogyne javanica* and *M. incognita*[1]

Yuji Oka,[2] Rivka Offenbach,[3] and Shimon Pivonia[3]

Abstract: Resistance of pepper species (*Capsicum annuum, C. baccatum, C. chinense, C. chacoense,* and *C. frutescens*), cultivars and accessions to the root-knot nematodes *Meloidogyne incognita* race 2 and *M. javanica,* and their graft compatibility with commercial pepper varieties as rootstocks were evaluated in growth chamber and greenhouse experiments. Most of the plants tested were highly resistant to *M. javanica* but susceptible to *M. incognita. Capsicum annuum* AR-96023 and *C. frutescens* accessions as rootstocks showed moderate and relatively high resistance to *M. incognita,* respectively. In *M. incognita*-infested soil in a greenhouse, AR-96023 supported approximately 6-fold less nematode eggs per gram root and produced about 2-fold greater yield compared to a non-grafted commercial variety. The commercial variety grafted on AR-96023 produced a yield as great as the non-grafted variety in the root-knot nematode-free greenhouse. Some resistant varieties and accessions used as rootstocks produced lower yields ($P < 0.01$) than that of the non-grafted variety in the noninfested greenhouse. Use of rootstocks with nematode-resistance and graft compatibility may be effective for control of root-knot nematodes on susceptible pepper.

Key words: Capsicum spp., graft compatibility, *Meloidogyne incognita, Meloidogyne javanica,* nematode control, pepper, resistance, root-knot nematodes, rootstock.

Pepper (*Capsicum annuum*) is one of the most important vegetable crops in Israel. Most common pepper varieties are susceptible to the southern root-knot nematode *Meloidogyne incognita.* This nematode has been managed with soil fumigants, mainly methyl bromide. The mandated elimination of methyl bromide will make nematode control more difficult and require alternative control methods (Noling and Becker, 1994). Also, the trend of reduced use of nematicides due to concerns for the environment and safe food require environmentally friendly nematode control methods, including use of resistant varieties. Unfortunately, no pepper varieties resistant to the root-knot nematodes are available in Israel. Grafting of susceptible commercial varieties onto nematode-resistant rootstocks is an alternative method to developing new resistant varieties. Use of grafted vegetable plants has not been popular in Israel, but restricted availability of fumigant nematicides and increased interest in organic farming likely will increase the use of grafting as a method to manage soilborne diseases, including root-knot nematodes. At present, grafted seedlings of tomato (*Lycopersicon esculentum*), eggplant (*Solanum melongena*), and watermelon (*Citrullus lanatus*) are commercially available in Israel. Ros et al. (2002) indicated that use of pepper rootstocks is an effective method to control *M. incognita.*

In the present study, resistance of accessions of *Capsicum* spp., pepper cultivars, and rootstocks to Israeli populations of *M. javanica* and *M. incognita* host race 2 was evaluated in growth chamber and greenhouse studies, and their compatibility as rootstocks with commercial pepper cultivars also was tested under greenhouse conditions.

Materials and Methods

Nematode inoculum: Eggs of *Meloidogyne incognita* host race 2 and *M. javanica* were extracted from pepper (*C. annuum* cv. Celica) and tomato (*Lycopersicon esculentum* cv. Daniela) roots, respectively (Hussey and Barker, 1973). Second-stage juveniles (J2) that emerged from the eggs on a sieve were collected daily and stored at 15 °C. Only J2 no more than 5 days old were used in experiments.

Pepper genotypes: The *Capsicum* genotypes used in the study are listed in Table 1.

Growth chamber experiments: One-month-old seedlings of pepper varieties Nivla, Charleston Hot, Carolina Cayenne, Carolina Wonder, and Charleston Belle and the accessions, 7470 of *C. baccatum,* 7494 of *C. chacoense,* 7472 of *C. chinense,* and 7475 and 7493 of *C. frutescens* were planted singly in 250-ml plastic pots filled with dune sand (pH 7.8) and inoculated with 1,500 J2 of *M. incognita* or *M. javanica* 2 days after planting. The plants were maintained at 27 ± 2 °C in a growth chamber with 13-hour days and fertilized with 25 ml of a 0.1% solution of fertilizer (20-20-20, N-P-K) every 2 weeks. Five weeks later, galling indexes (GI) were assessed according to a 0-to-5 scale (0 = no infection, 1 = 1–20% of roots galled, 2 = 21–40%, 3 = 41–60%, 4 = 61–80%, and 5 = 81–100%), and number of eggs per root system was counted. A second experiment was conducted with cv. Ohad and the rootstocks AR-96023, AR-96025, RS-27, and RS-28. Plants were inoculated with 1,500 J2 of *M. javanica* or *M. incognita* and maintained under the same conditions as described above. In a third experiment, Celica and the rootstocks AX 592, AX 568, EV 359, AR-96023, AR-96025, Nun 9435, and Snooker were inoculated with 1,500 J2 of *M. javanica* or *M. incognita* and

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[1] Supported by the Chief Scientist's Fund of the Ministry of Agriculture and Rural Development.
[2] Research Nematologist, Nematology Unit, Gilat Research Center, Agricultural Research Organization, M. P. Negev 85280, Israel.
[3] Extension Agronomist and Research Plant Pathologist, Arava Research and Development, Yair Experiment Station, M. P. Arava 86825, Israel.
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E-mail: okayuji@volcani.agri.gov.il
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TABLE 1. *Capsicum* genotypes tested for resistance to *Meloidogyne incognita* and *M. javanica.*

Capsicum species	Genotype	Use[a]	Host status[b]	Origin
C. baccatum	7470			A. Levi, Bet-Dagan, Israel
C. chacoense	7494			A. Levi, Bet-Dagan, Israel
C. chinense	7472			A. Levi, Bet-Dagan, Israel
C. frutescens	7475			A. Levi, Bet-Dagan, Israel
C. frutescens	7493			A. Levi, Bet-Dagan, Israel
C. annuum	Charleston Hot	F	R	R. L. Fery, USDA, Charleston, SC, USA
C. annuum	Carolina Cayenne	F	R	R. L. Fery, USDA, Charleston, SC, USA
C. annuum	Carolina Wonder	F	R	R. L. Fery, USDA, Charleston, SC, USA
C. annuum	Charleston Belle	F	R	R. L. Fery, USDA, Charleston, SC, USA
C. annuum	Celica	F	S	Enza Zaden, Enkhuizen, Netherlands
C. annuum	Nivla	F	S	De Ruiter Seeds, Bergschenhoek, Netherlands
C. annuum	Parker	F	S	De Ruiter Seeds, Bergschenhoek, Netherlands
C. annuum	Ohad	F	S	Hazera, Brurim, Israel
C annuum	AR96023	RS		Milchan Bros., Ramat-Gan Israel
C. annuum	AR96025	RS		Milchan Bros., Ramat-Gan Israel
C. annuum	RS-27	RS		Milchan Bros., Ramat-Gan Israel
C. annuum	RS-28	RS		Milchan Bros., Ramat-Gan Israel
C. annuum	RS-29	RS		Milchan Bros., Ramat-Gan Israel
C. annuum	RS-50	RS		Milchan Bros., Ramat-Gan Israel
C. annuum	AX 592	RS	T	Tarsis, Beer-Sheva, Israel
C. annuum	AX568	RS	T	Tarsis, Beer-Sheva, Israel
C. annuum	EV 359	RS	T/R	Nirit Seeds, Hadar, Israel
C. annuum	Snooker	RS		Syngenta Seeds, Westeinde, Netherlands
Capsicum sp.	Nun9453	RS	R	Nunhems Zaden, Haelen, Netherlands

[a] Commercial use: fruit production (F), rootstock (RS).
[b] Host status indicated by the supplier: resistant (R), tolerant (T), susceptible (S).

maintained under the same conditions described above. All experiments were performed with 5 replications/genotype and nematode species.

Greenhouse resistance experiments: Grafted seedlings were prepared with Charleston Hot, Carolina Cayenne, AR-96023, AR-96025, RS-27, RS-28, and RS-50 as rootstocks and cv. Celica as the scion by tube grafting (Lee and Oda, 2003). Ungrafted Celica seedlings served as controls. The grafted and non-grafted seedlings were planted in sandy soil (pH 7.4) in a greenhouse ground bed infested with *M. incognita* host race 2. Mean preplant nematode density in the top 20 cm of soil, estimated from 32 samples each consisting of three subsamples of 200 cm^3 soil, was 13.5 ± 15.8 J2/50 g. The planting area was divided into 32 plots of 2.25 m^2 (1.5 × 1.5 m) arranged in a randomized complete block design with four replicates. Eight plants were planted on 13 September 2001 in each plot. Galling index (0–5) and number of nematode eggs per g roots were recorded 42 days after planting. In the second experiment, conducted the following year, seedlings of non-grafted and grafted Celica on AR-96023 and Snooker were planted on 4 September 2002 in the same greenhouse. The experimental unit was 20 plants in two lines on a bed of 4.0 × 1.5 m with four replicates. Mean pre-plant nematode density in the top 20 cm of soil, estimated from 12 samples each consisting of four subsamples of 200 cm^3 soil, was 17.7 ± 24.5 J2/50 g. Standard drip-irrigation, fertilization, insect disease, and foliar disease control methods for pepper in the region were performed. The plants were harvested from 25 December 2002 to 2 April 2003, and GI (0-10) and number of nematode eggs per g roots were recorded at the end of the experiment (Bridge and Page, 1980).

Greenhouse compatibility experiments: Seedlings of non-grafted and grafted Celica on rootstocks AR-96023, AR-96025, RS-27, RS-28, RS-29, and RS-50 and on cultivars Charleston Hot, Carolina Cayenne, Carolina Wonder, and Charleston Belle were planted in a non-infested greenhouse on 5 September 2001. The soil was treated with methyl bromide (600 kg/ha) 21 days before planting to control fungal diseases and weeds. The experimental unit was 20 plants in two lines on a bed of 4.0 × 1.5 m with two replicates, where means and SD of yield were calculated. The pepper plants were harvested from 9 December 2001 to 19 May 2002. In the second experiment, conducted the following year, seedlings of non-grafted and grafted Celica on Snooker, RA-96023, *C. chacoense* accession 7494, and *C. frutescens* accessions 7475 and 7493 as well as seedlings of non-grafted and grafted Parker on AR-96023 were planted on 4 September 2002 in another non-infested greenhouse 21 days after treatment with methyl bromide (600 kg/ha). The experimental unit was 20 plants in two lines on a bed of 4.0 × 1.5 m with four replicates. Pepper plants were harvested from 18 December 2002 to 12 May 2003. Standard drip irrigation, fertilization, insect disease, and foliar disease control methods for pepper in the region were performed in the two experiments.

Data analysis: Data were subjected to analysis of variance, and means were separated according to Tukey-Kramer HSD test (α = 0.05). All calculations were performed with JMP (SAS Institute, Cary, NC).

Results

Growth chamber experiments: The GI and number of *M. javanica* eggs on most of the accessions, pepper cultivars, and rootstocks were low in experiment 1, except the numbers of eggs on Nivla, *C. chinense* 7472, and *C. chacoense* 7494 (Table 2). Among the plants inoculated with *M. incognita,* Carolina Cayenne and the *C. frutescens* accessions had lower GI and numbers of eggs than those of the control Nivla (Table 2). The nematode-resistant cultivars Charleston Belle and Carolina Wonder were susceptible to the *M. incognita* race 2 isolate. Among the rootstocks tested in experiments 2 and 3, only AR-96023 had lower GI ($P < 0.001$) and numbers of *M. incognita* eggs ($P < 0.01$) on the roots than those of the susceptible cultivars (Table 2). The rootstock Snooker had intermediate resistance to *M. incognita.* On some rootstocks in experiment 3, *M. javanica* reproduced at low levels (Table 2).

Greenhouse resistance experiments: In the *M. incognita*-infested greenhouse, the rootstock AR-96023 and Charleston Hot and Charleston Cayenne had lower GI ($P < 0.001$) and numbers of nematode eggs ($P < 0.01$) than those of the susceptible control Celica in the first experiment (Table 3). In the second experiment, the rootstock AR-96023 had lower GI than the susceptible cultivars ($P < 0.001$) (Table 4). The yield of Celica grafted on AR-96023 was more than 2-fold that of non-grafted Celica (Table 4). The rootstock Snooker, which was heavily galled, did not increase the yield of the cultivar, though the number of nematode eggs was lower ($P = 0.03$) than that on Celica roots (Table 4).

Greenhouse compatibility experiments: In the non-infested greenhouse, yields of Celica grafted on the rootstocks were the same or slightly lower than that of the non-grafted Celica plants in the first experiment (Table 3). In contrast, the yield of Celica grafted on Charleston Hot or Charleston Belle was lower than that of the non-grafted plants. In the second experiment, Celica grafted on the rootstock AR-96023 had similar yield to the non-grafted Celica, but the yield and percentage of exportation grade fruit of Parker grafted on the rootstock were lower ($P = 0.005$) than those of non-grafted Parker (Table 4). The yield and percentage of exportation grade fruit of Celica grafted on the acces-

Table 2. Root galling index (GI) and number of eggs per root system on pepper genotypes inoculated with *Meloidogyne javanica* or *M. incognita* in pots in a growth chamber.

	M. javanica		*M. incognita*	
Pepper genotypes	GI (0–5)	Eggs/root system[a]	GI (0–5)	Eggs/root system[a]
		Experiment 1		
C. annuum cultivar				
Nivla	1.3 a	19,650 a	3.7 a	104,625 a
Carolina Wonder	0 b	0 b	2.5 bc	74,813 ab
Charleston Belle	0 b	0 b	2.8 abc	90,000 a
Carolina Cayenne	0 b	0 b	0.8 d	33,188 b
C. frutescens accession				
7475	0 b	0 b	0.3 d	8,265 bc
7493	0 b	0 b	1.4 d	4,125 c
C. chinense				
7472	0.8 a	24,563 a	2.1 c	62,813 ab
C. chacoense				
7494	0.9 a	16,219 a	0.4 d	16,875 abc
C. baccatum				
7470	0 b	0 b	3.2 ab	87,000 a
		Experiment 2		
C. annuum cultivar				
Ohad	0.5 a	16,867 a	2.5 a	109,710 a
C. annuum rootstock				
AR-96023	0 b	0 b	0.8 c	38,563 b
AR-96025	0 b	0 b	2.0 ab	108,790 a
RS-27	n.t.[b]	n.t.	1.8 b	68,157 a
RS-28	0 b	0 b	1.7 b	84,103 a
		Experiment 3		
C. annuum cultivar				
Celica	0.7 ab	9,500 a	3.3 ab	112,800 ab
Capsicum rootstock				
AX 592	1.0 a	18,750 a	3.2 ab	139,600 a
AX 568	0 c	0 c	2.9 b	146,600 a
AR-96023	0 c	0 c	0.8 c	43,800 c
AR-96025	0 c	0 c	2.9 b	127,875 ab
EV 359	0.5 abc	6,000 a	3.0 b	133,800 ab
Snooker	0.3 bc	1,625 b	1.3 c	85,200 bc
Nun 9453	0 c	0 c	3.3 ab	139,250 ab

Data are means of five replicates. Means within a column followed by the same letter in each experiment are not different according to Tukey-Kramer HSD test (α = 0.05).
[a] Statistical analysis of eggs/root system data was done after $\log_{10}(x + 1)$ transformation.
[b] n.t. = not tested.

Table 3. Root galling index (GI) and number of eggs per gram of root on non-grafted and grafted pepper cultivars and rootstocks grown in a greenhouse infested with *Meloidogyne incognita,* and yield in uninfested soil.

	Infested soil[a]		Fruit yield in uninfested soil[c]	
Cultivar and rootstock	GI (0–5)	Eggs/g roots[b]	Total (kg)[d]	Exp. (%)[e]
Non-grafted cv. Celica	4.7 a	29,947 a	72.3 ± 3.5	74.5
Grafted cv. Celica on				
AR-96023	0.7 c	4,117 b	75.0 ± 0.7	73.0
AR-96025	4.8 a	29,002 a	72.1 ± 3.8	76.0
RS-27	3.8 ab	27,506 a	61.9 ± 38.3	76.0
RS-28	3.1 b	10,192 ab	68.2 ± 2.1	73.0
RS-29	4.0 ab	18,168 ab	69.3 ± 1.7	75.5
RS-50	4.7 a	24,570 a	66.3 ± 2.8	71.0
Charleston Hot	0.2 c	1,800 b	47.9 ± 0.4	63.5
Charleston Belle	4.4 a	20,193 ab	47.4 ± 13.0	71.0
Charleston Cayenne	0.7 c	2,295 b	59.5 ± 11.6	69.5
Charleston Wonder	n.t.[f]	n.t.	65.0 ± 0.4	71.5

[a] Data are means of four replicates. Means within a column followed by the same letter are not different according to Tukey-Kramer HSD test (α = 0.05).
[b] Statistical analysis of eggs/g root data was done after $\log_{10}(x + 1)$ transformation of six replicates.
[c] Data are means and SD of two replicates.
[d] Means ± SD of two experimental units, each containing 20 plants.
[e] Percentages of exportation grade fruit.
[f] n.t. = not tested.

TABLE 4. Root galling index (GI) and number of eggs per gram of root on pepper cultivars and grafted rootstocks and accessions grown in a greenhouse infested with *Meloidogyne incognita,* and fruit yield of the non-grafted and grafted cultivars on the rootstocks grown in uninfested soil.

	Infested soil					
			Fruit yield[c]		Fruit yield in uninfested soil[c]	
Cultivar, rootstock, and accession	GI (0–10)[a]	Eggs/g roots[b]	Total (kg)	Exp. (%)[d]	Total (kg)	Exp. (%)[d]
Parker						
Non-grafted	n.t.[e]	n.t.	n.t.	n.t.	54.4 a	65.3 a
Grafted on AR-96023	n.t.	n.t.	n.t.	n.t.	47.9 b	50.0 b
Celica						
Non-grafted	7.8 a	46,800 a	20.2 b	29.3 ab	70.3 a	63.8 a
Grafted on						
AR-96023	0.7 b	8,343 c	43.8 a	37.3 a	68.6 a	53.5 ab
Snooker	6.5 a	29,400 b	26.8 b	15.3 b	53.0 a	57.0 ab
C. chacoense 7494	n.t.	n.t.	n.t.	n.t.	44.8 a	53.5 ab
C. frutescens 7475	n.t.	n.t.	n.t.	n.t.	6.3 b	25.0 b
C. frutescens 7493	n.t.	n.t.	n.t.	n.t.	49.4 a	44.0 ab

[a] Data are means of four experimental units containing 20 plants each. Means within a column followed by the same letter in each cultivar are not different according to Tukey-Kramer HSD test ($\alpha = 0.05$).
[b] Statistical analysis was done after $\log_{10}(x + 1)$ transformation of four replicates.
[c] Means of four experimental units, each containing 20 plants.
[d] Percentages of exportation grade fruit.
[e] n.t. = not tested.

sion 7475 of *C. frutescens* were lower ($P < 0.001$) than that of non-grafted Celica plants. No root galls were found on ungrafted Celica roots at the end of the two experiments.

Discussion

Use of resistant rootstocks is an effective control method for plant-parasitic nematodes. Fruit trees such as *Prunus* spp., *Citrus* spp., *Vitis* spp. are usually grafted onto rootstocks possessing resistance to soilborne pathogens and pests, including nematodes. Rootstocks are also used to improve plant growth. Use of grafted vegetable seedlings, especially watermelon, melon, cucumber, tomato, and eggplant, has been popular in some countries, such as Japan (Lee and Oda, 2003). Withdrawal of effective nematicides and adverse effects of the use of pesticides have promoted development and use of rootstocks in vegetable crops. In the present study, *Capsicum* spp. accessions, rootstocks, and cultivars were tested for resistance to *M. incognita* and *M. javanica* as well as their graft compatibility to commercial pepper cultivars. All the plants tested in the experiments were highly to moderately resistant to *M. javanica.* Carolina Wonder and Charleston Belle, which have been reported to be resistant to *M. incognita* race 3 (Fery et al., 1998), were not resistant to race 2 of the nematode in our study. The difference in our results from those reported by other authors may be due to the nematode races and populations, and also the temperature in our study. The accessions of *C. frutescens* were highly resistant to both *M. javanica* and *M. incognita.* The accession of *C. chacoense* was also resistant to *M. incognita,* but it was not immune to *M. javanica.* Several accessions of *Capsicum* spp. have been tested for resistance to four *Meloidogyne* species, and different levels of resistance to the nematodes were found (Di Vito et al., 1991). Resistance of *C. chinense* lines has also been reported (Fery and Thies, 1997; Thies and Fery, 2000). The rootstock AR-96023, the lines of *C. frutescens,* and hot pepper cv. Charleston Hot and Carolina Cayenne showed resistance to *M. incognita* in both growth chamber and greenhouse experiments.

Soil temperature may be an important factor for nematode resistance in pepper plants, similar to resistance to *Meloidogyne* species in tomato based on the *Mi* gene (Holtzmann, 1965). The resistance to *M. incognita* of one line each of *C. annuum, C. chacoense* and *C. frutescens* and three lines of *C. chinense* was reported to be stable at a soil temperature of 38 °C (Di Vito et al., 1995), but in other reports the resistance was broken by temperatures higher than 32 °C (Thies and Fery, 1998, 2000). In our greenhouse experiments, soil temperature (10-cm depth) rose above 32 °C early in the growing season; however, AR-96023 roots had far fewer galls than the susceptible cultivar and the rootstock Snooker. This result suggested that AR-96023 is likely to be a heat-stable resistant rootstock. Use of resistant rootstocks, in addition to reduction of plant damage caused by nematodes, may reduce nematode populations in soil and may serve as a rotation crop (Thies et al., 1998; Thies and Fery, 2002). However, increased nematode incidence and population aggressiveness were reported after using the same rootstocks in the same soil (Ros et al., 2002).

Another important feature of rootstocks is their compatibility with commercial varieties used as scions. An incompatible rootstock may result in a lower yield of a scion cultivar than that of the ungrafted cultivar even in the absence of nematodes. The accessions of *C. chacoense* and *C. frutescens* and Charleston Hot and Carolina Cayenne showed high resistance to *M. incognita;* however, the cultivar Celica grafted on most of these rootstocks gave lower yields than that of the ungrafted cultivar in non-infested greenhouse experiments. Grafting of two *Capsicum* spp. may not always result in low compatibility. Grafting between different species (inter-specific) and even genera (inter-generic) is well accepted in vegetable crops grown from grafted seedlings (Lee and Oda, 2003).

A problem of using grafted seedlings is their relatively high cost, although their use would offset the cost for pest management, particularly that of soil disinfestations. Cost of a grafted vegetable seedling is as much as 5-fold higher than that of a non-grafted seedling in Israel. Development of improved grafting technology and horticultural practices in the future may reduce the cost of the seedlings and the number of plants per area.

The results of the present study suggest that use of nematode-resistant pepper rootstocks can be an effective control method, especially when nematode-resistant cultivars are not available or nematicides cannot be used, such as in organic farming systems. Rootstocks possessing resistance to *M. incognita* races and good graft compatibility with commercial varieties can be developed by careful selection for the resistance and compatibility.

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