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Short communication

Occurrence of resistance-breaking populations of root-knot nematodes on tomato in Greece

Emmanuel A. Tzortzakakis[1], Mohamed A.M. Adam[2], Vivian C. Blok[2], Charalampos Paraskevopoulos[3], and Kostas Bourtzis[3]
[1]*Nematology Laboratory, Plant Protection Institute, National Agricultural Research Foundation, P.O. BOX 2228, 71003, Heraklion Crete, Greece; (Fax: +30-281-0245858; E-mail: etzortza@her.forthnet.gr);*
[2]*Scottish Crop Research Institute, Plant Pathogen Interactions Program, Invergowrie, DD2 5DA Dundee, UK;* [3]*Department of Environmental and Natural Resources Management, University of Ioannina, 30100, Agrinion, Greece*

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Abstract

Nine populations of *Meloidogyne* spp. from Greece have been identified as *M. javanica* or *M. incognita* using either isozyme phenotypes or the sequence characterized amplified region-polymerase chain reaction (SCAR-PCR) technique. Virulence against the *Mi* resistance gene was assayed by pot experiments in controlled conditions and revealed the ability of five populations of *M. javanica* and one population of *M. incognita* to reproduce on tomato cultivars containing that gene. A resistance-breaking population of *M. incognita* is reported for the first time in the country; the *M. javanica* populations constitute new records for the Greek mainland.

Resistance in commercially grown tomato hybrids to root knot nematodes (RKN, *Meloidogyne* spp.) is conferred by the *Mi* gene which is effective against *M. javanica*, *M. incognita* and *M. arenaria* at moderate soil temperature (Williamson, 1998). However, there are several reports of virulent populations of these *Meloidogyne* species on resistant tomato cultivars occurring either naturally (cited by Williamson, 1998) or after repeated exposure and selection on tomato cultivars with the *Mi* gene (Castagnone-Sereno et al., 1994). Such populations of *M. javanica* have been also found in Crete (Tzortzakakis et al., 1999) and the objective of this study was to characterize further populations from Greece.

Populations were collected from vegetable crops from Preveza, Epirus (10 greenhouses) and Kyparissia, Peloponissos (one outdoor site) on mainland Greece and used to establish cultures on potted tomatoes. Egg masses from these cultures were used to inoculate (six egg masses per plant) seedlings of either the susceptible tomato cv. ACE or the nematode resistant cvs Nikita and Alpado grown in pots. The plants were grown in a controlled environment with a 16 h photoperiod and a soil temperature of 24–26 °C. They were watered and fertilized as required and roots were examined after 7 weeks. If a large number of egg masses were produced on the resistant tomato cultivars, the egg masses were inoculated onto another plant and the process repeated. Four populations from Preveza (*Mj* P1, *Mj* P2, *Mj* P3 and *Mj* P4), which sustained high reproduction rates on resistant tomato cultivars for four successive tests were selected for further study. Two populations from Crete (*Mj* C1 and *Mi* C1) that had been sent to the laboratory

because they were found in association with resistant tomato cultivars were also tested. Their ability to reproduce on cvs Nikita and Alpado was established as described above. One population from Kyparissia (*Mi* K1) and two from Crete (*Mj* C2 and *Mi* C2) were also kept as controls as they did not reproduce on the resistant tomato cultivars. For the molecular identification, females from single egg mass lines of *M. incognita* (A-2) and *M. javanica* (J-2) from Libya that had previously been identified by perineal patterns, isozymes and specific SCAR-PCR were used as controls.

Identifications were based on isozyme phenotypes, IGS-PCR and SCAR-PCR. Esterase and malate dehydrogenase (MDH) isozyme patterns of individual single females from each line were determined (Karssen et al., 1995) (Table 1). Two esterase phenotypes were detected in the Greek populations. The *M. javanica* phenotype J3 was present in six populations (*Mj* P1, *Mj* P2, *Mj* P3, *Mj* P4, *Mj* C1 and *Mj* C2) and the *M. incognita* I1 esterase phenotype was found for *Mi* C1, *Mi* C2 and *Mi* K1. All populations had one MDH phenotype, N1.

Molecular identifications were performed using IGS-PCR and SCAR-PCR. DNA was extracted from individual single females from each population by crushing them in 25 µl of Worm Lysis buffer (Castagnone-Sereno et al., 1995), 50 mM KCl, 10 mM Tris–Cl pH 8.2, 2.5 mM $MgCl_2$, 60 µg ml^{-1} proteinase K (Roche, UK), 0.45% NP40, 0.45% Tween 20 and 0.01% gelatin, centrifuging for 2 min and placing at –70 °C for 10 min. Then two drops of mineral oil were added and incubated for 1 h at 60 °C followed by 15 min at 95 °C. Extracted DNA (0.9 µl) from each single female was used as a template to amplify the IGS between the 5S and 18S ribosomal genes using 194/195 primers (Table 2) as described by Blok et al. (1997), in a 25 µl reaction using QIAGEN Taq PCR Master Mix as described by the manufacturer (Qiagen, Hilden, Germany). PCR amplification products (15 µl) were electrophoresed through a 1% agarose gel in Tris borate (TBE) buffer and visualised with UV illumination after staining with ethidium bromide (Sambrook et al., 1989).

The 720 bp amplification product obtained from primers 194/195 was obtained from all the populations confirming that they belong to the main tropical species of *Meloidogyne* (Table 1). Specific SCAR primers Finc/Rinc and Fjav/Rjav (Table 2; Zijlstra et al., 2000) were used with 0.9 µl of DNA template, following the same PCR reaction conditions but with 45 cycles. A product of 670 bp was obtained from *Mj* P1, *Mj* P2, *Mj* P3, *Mj* P4, *Mj* C1 and *Mj* C2 and the *M. javanica* control (Figure 1); this confirms that these populations are *M. javanica*. A product of 1200 bp was obtained with Finc/Rinc from *Mi* C1, *Mi* C2, *Mi* K1 and the control *M. incognita* line (Figure 2).

As populations were received at different times at the Plant Protection Institute of Heraklion, Crete, a comparative reproduction test was conducted when all populations were identified and successfully established in pot cultures. Three populations from Crete (*Mj* C4/1, *Mj* C6/3 and *Mi* C33) maintained in the laboratory (Tzortzakakis et al., 1999) were included in the study as controls. Egg masses from the susceptible tomato cultivar for each population were put on 90 µm mesh

Table 1. Esterase (Est) and malate dehydrogenase (MDH) phenotypes, sizes of the IGS region (bp) between 5S and 18S ribosomal genes and SCAR-PCR results

Population code	Origin in Greece	Est	MDH	rDNA IGS (bp)	SCAR PCR Fjav/Rjav	SCAR PCR Finc/Rinc	Species
Mj P1	Preveza	J3	N1	720	+	–	*M. javanica*
Mj P2	Preveza	J3	N1	720	+	–	*M. javanica*
Mj P3	Preveza	J3	N1	720	+	–	*M. javanica*
Mj P4	Preveza	J3	N1	720	+	–	*M. javanica*
Mj C1	Crete	J3	N1	720	+	–	*M. javanica*
Mj C2	Crete	J3	N1	720	+	–	*M. javanica*
Mi C1	Crete	I1	N1	720	–	+	*M. incognita*
Mi C2	Crete	I1	N1	720	–	+	*M. incognita*
Mi K1	Kyparissia	I1	N1	720	–	+	*M. incognita*

+ = product of 670 bp for Fjav/Rjav or 1200 bp for Finc/Rinc, – = no product.

Table 2. Nucleotide sequences of the primers used in identification

Primer	Sequence 5′–3′
194	TTAACTTGCCAGATCGGACG
195	TCTAATGAGCCGTACGC
Fjav	GGTGCGCGATTGAACTGAGC
Rjav	CAGGCCCTTCAGTGGAACTATAC
Finc	CTCTGCCCAATGAGCTGTCC
Rinc	CTCTGCC-CTCACATTAGG

sieves sited over water in shallow trays and incubated at 25 °C. The juveniles collected within 4 days were used for inoculation at a rate of c. 300–500 J_2s per plant. The juveniles were pipetted into 300 ml pots with the seedlings of either a resistant tomato cultivar or the susceptible cv. ACE in four replicates. A total of six comparative tests were conducted with the resistant tomato cultivars Carmello (two experiments) and Baez (four experiments). Plants were maintained in a growth room as described before and the number of visible egg masses on roots was assessed under a stereoscope.

Results were similar between the resistant tomato cultivars and experiments ($P > 0.05$) and were pooled together. These results confirmed the previous avirulent/virulent characterizations of *Mj* C4/1, *Mj* C6/3 and *Mi* C33, and six newly-discovered virulent populations are reported. With five populations of *M. javanica* (*Mj* P1-4 and *Mj* C1) and one of *M. incognita* (*Mi* C1), the number of egg masses on both resistant tomato cultivars did not differ significantly ($P > 0.05$) to that on the susceptible cultivar. This was also observed with the virulent *M. javanica* population *Mj* C4/1 used as a control. Two populations of *M. javanica* (*Mj* C2 and *Mj* C6/3) and three of *M. incognita* (*Mi* K1, *Mi* C2 and *Mi* C33) did not reproduce on the resistant tomato cultivars in all tests (Figure 3). Including results of previous work (Tzortzakakis et al., 1999), eight resistance-breaking populations of *M. javanica* (four from Preveza and four from Crete) and one of *M. incognita* (from Crete) are reported

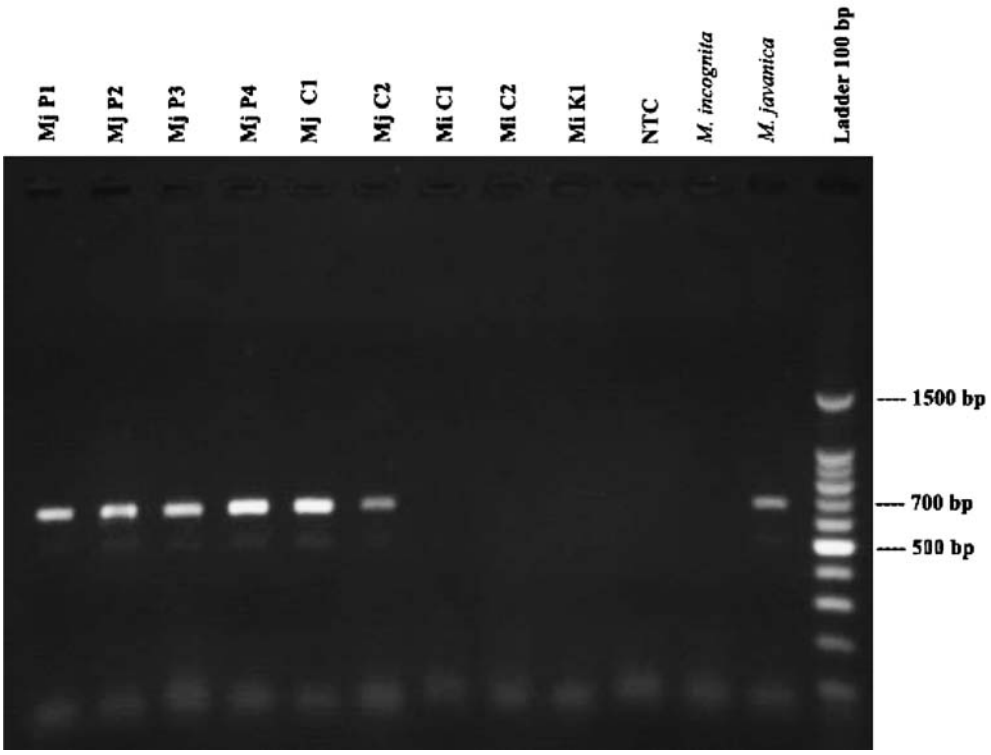

Figure 1. Amplification products from reactions using Fjav/Rjav (*M. javanica* specific SCAR primers) with DNA extracted from single females. *Mj* = *M. javanica* *Mi* = *M. incognita*. NTC is no template control. Origin of populations: P = Preveza, C = Crete, K = Kyparissia. Marker is 100 bp ladder from Promega, UK.

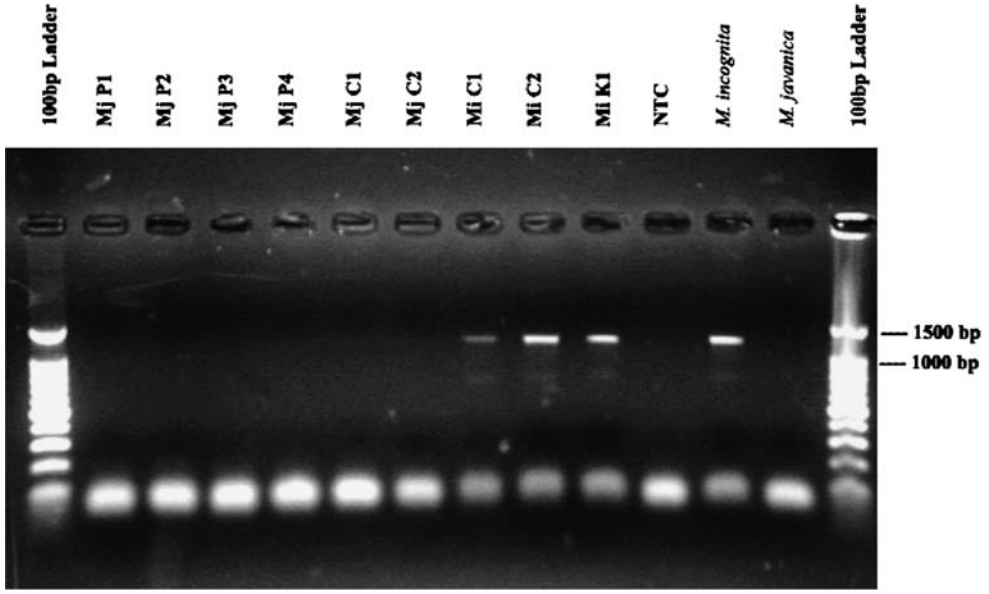

Figure 2. Amplification products from PCR reactions using Finc/Rinc (*M. incognita* specific SCAR primers) with DNA extracted from single females. *Mj* = *M. javanica* *Mi* = *M. incognita*. NTC is no template control. Origin of populations: P = Preveza, C = Crete, K = Kyparissia. Marker is 100 bp ladder from Promega, UK.

to date from the limited surveys conducted in Greece. The populations from Preveza came from sites with susceptible hosts and as far as could be determined, had not been previously exposed to resistant tomato cultivars; this suggests a natural virulence rather than host selection.

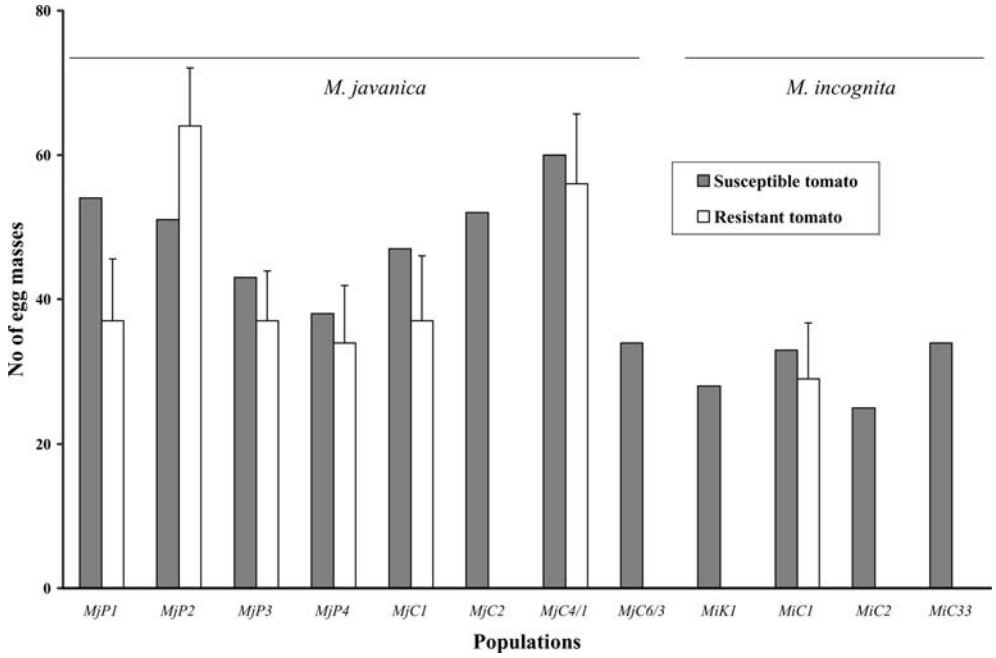

Figure 3. Number of egg masses produced on roots of either susceptible (cv. ACE) or resistant (pooled results from cvs Carmelo and Baez) tomatoes by 12 populations of *Meloidogyne* from Greece (average of six experiments). Bars indicate standard errors of mean comparisons. *Mj* = *M. javanica* *Mi* = *M. incognita*. Origin of populations: P = Preveza, C = Crete, K = Kyparissia.

The occurrence of populations able to overcome the *Mi* resistance gene in tomato cultivars in major greenhouse production areas of Epirus and Crete, Greece may be a serious threat to the successful employment of resistant tomato cultivars in integrated control strategies. In the Mediterranean area, resistance-breaking populations of *M. javanica* have been reported in Cyprus, Crete, Morocco, Tunisia and Spain (Philis and Vakis, 1977; Tzortzakakis and Gowen, 1996; Eddaoudi et al., 1997; Molinari and Miacola, 1997; Ornat et al., 2001) while for *M. incognita* only one population has been found in France (Jarquin-Barberena et al., 1991; Castagnone-Sereno et al., 1993). Our results constitute additional records on the occurrence of such virulent populations in Greece.

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