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Virulence of root-knot nematodes, *Meloidogyne* spp., on tomato bearing the *Mi* gene for resistance

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Summary. Three species of root-knot nematodes, *Meloidogyne javanica*, *M. incognita* races 1 and 2, and *M. arenaria* race 2 occur in Jordan. These species and their races were identified using morphological characters, the North Carolina differential host test, and SCAR-PCR. The virulence of 83 isolates belonging to *Meloidogyne* species and races was assayed. The virulence assay was based on the isolate reproduction rate on a resistant tomato cultivar Betterboy bearing the *Mi* gene for resistance, and was compared with that on the susceptible tomato cultivar Rutgers. Three *M. javanica* isolates were highly virulent on the resistant cv. Betterboy as indicated by their high root gall index (4.73) and high reproduction factor (3.73). The horticultural parameters (shoot and root fresh weights and root dry weight) were negatively correlated with the reproduction factor.

Key words: race, reproduction, susceptibility, SCAR-PCR.

Introduction

The genus *Meloidogyne* comprises a widely distributed group of plant-parasitic nematode species that attack a wide range of plant species including vegetable crops and fruit trees. In Jordan, average annual losses of irrigated vegetable crops cultivated in the Jordan Valley due to root-knot nematodes (RKNs) are estimated at nearly 15% (Abu-Gharbieh, 1994)

The *Mi* gene conifers resistance to *Meloidogyne arenaria* (Neal) Chitwood, *M. incognita* (Kofoid and White) Chitwood, and *M. javanica* (Treub) Chitwood is present in several commercial tomato cultivars. However, the occurrence of resistance-breaking was noticed in *M. incognita*, *M. arenaria* and *M. javanica* isolates (Riggs and Winstead, 1959; Haroon *et al*., 1993; Tzortakakis *et al*., 1998; Xu *et al*., 2001). In addition to the development of virulence under selective conditions, naturally resistance-breaking field populations have been found even when they had not previously been exposed to resistant cultivars (Roberts, 1992).

A limited number of resistant tomato cultivars with the *Mi* gene have been introduced to Jordan. The aim of this study is to investigate the occurrence of *Meloidogyne* populations virulent to a RKN-resistant tomato cultivar.

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Materials and methods

Collection of samples

Eighty-three soil and galled root samples were

collected from infected vegetable crops and fruit trees from May 2002 to August 2003. The survey covered most of the irrigated agricultural areas in Jordan with a range of climatic conditions: Southern Ghors, Jordan Valley, the elevated uplands, and the eastern desert plains.

Isolate identification

Isolates derived from a single egg-mass were obtained by inoculating individual egg-masses on the susceptible tomato cv. GS 12. The identification of the 83 *Meloidogyne* isolates was based on the perineal pattern morphology on the North Carolina (NC) differential host (Hartman and Sasser, 1985), and on a DNA fingerprinting assay, the sequence-characterized amplified regions-polymerase chain reaction (SCAR-PCR). The primer pairs Far/Rar, Finc/Rinc, and Fjav/Rjav, reported to be specific for amplifying the DNA of *M. arenaria*, *M. incognita*, and *M. javanica*, respectively (Fig. 1), were developed by Zijlstra *et al.* (2000) and synthesized by Alpha DNA (Montreal, Canada). Nematode genomic DNA was extracted according to the minipreparation procedure reported by Cenis (1993) and Swain *et al.* (1999) modified by increasing the concentration of ß-mercaptoethanol from 1 to 2.5%.

Virulence assays

The 83 isolates were assayed for their virulence toward the resistant gene *Mi* based on their relative reproduction rate on the resistant tomato cv. Betterboy and isolate virulence was compared with virulence on a susceptible tomato cv. Rutgers. Tomato seeds were sown in nursery polystyrene trays filled with a pasteurized mixture of peatmoss, perlite and clay soil (1:2:1, v:v:v) in the greenhouse. Each replication (1 plant/200-ml pot) of 4 tomato seedlings was inoculated with 3000 eggs of each RKN isolate. Non-inoculated tomato plants were used as controls. The tomato plants were transferred to a controlled growth room (25±3°C air temperature and a 16-h day). All pots were arranged in a randomized complete block design. Sixty days after inoculation, plants were removed from the pots and the roots were washed to remove soil particles. Shoot and root fresh weight and dry root weight were recorded. The gall and egg-mass in-

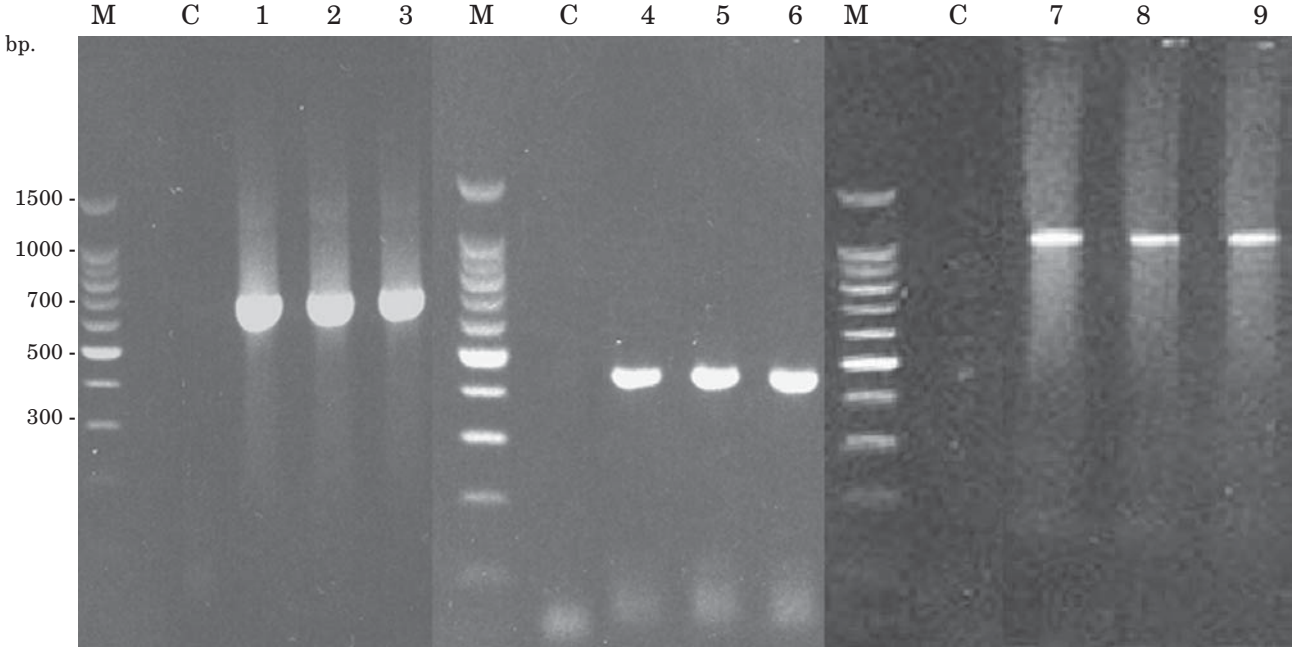

Fig. 1. DNA amplification products (670, 420, and 1200 bp) of *Meloidogyne javanica*, *M. arenaria*, and *M. incognita* using species-specific SCAR-PCR primer pairs (Fjav/Rjav, Far/Rar, and Finc/Rinc respectively). Lanes 1–3, *M. javanica* isolates (4–6); lanes 4–6, *M. arenaria* isolates (26–28); lane 7–9, *M. incognita* populations (15–17); lane C, water control; lane M, 100 bp-DNA marker.

dex was evaluated according to the scale: 0, no galls or egg-masses; 1, 1–2 galls or egg-masses; 2, 3–10; 3, 11–30; 4, 31–100; and 5, over 100 galls (Hartman and Sasser, 1985).

Each plant-root system was finely chopped and blended in a 0.5% NaOCl solution to extract eggs and 2nd stage juveniles (J2) (Hussey and Barker, 1973). The solution was poured directly through a 200-mesh (75 μm) sieve nested on a 1-l beaker, then into a 500-mesh (26 μm) sieve. The eggs+J2 collected on the 500-mesh sieve, per plant were counted and the reproduction factor (Rf) was then calculated according to the formula Rf = Pf /Pi, where Pi represents the initial population (3000 eggs), and Pf the final population of eggs+J2 recovered at the end of the experiment.

Isolates producing similar numbers of egg-masses and a similar gall index on both resistant and susceptible cultivars were classed as virulent, while isolates producing no or very few egg-masses (Rf≤1) and a gall index ≤2.0 on the resistant cultivar were avirulent (Khan and Khan, 1991).

Data analysis

Data were statistically analysed using the general linear model (GLM) procedure of the system of analytical statistics (SAS). Duncan's multiple range test (DMRT) was used for means separation at 0.05 probability. A simple correlation procedure was conducted to assess the interrelationships among the characters and was tested for significance using LSD at the 0.05 probability level (Steel and Torrie, 1980).

Results

Seventy isolates (84%) were identified as *M. javanica*, 5 (6%) as *M. incognita* race 1, 3 (4%) as *M. incognita* race 2, and 5 (6%) as *M. arenaria* race 2.

Responses of the susceptible (Rutgers) and the *Mi*-resistant (Betterboy) tomato cultivars to the 83 RKN isolates in terms of their root gall index and Rf are shown in Table 1. As expected, all *Meloidogyne* isolates reproduced on the susceptible tomato cv. Rutgers with the Rf averaging between 4.71 and 6.64. Rates of nematode reproduction on the resistant cv. Betterboy separated three isolates of *M. javanica* which were virulent against the *Mi* gene (Rf>1) from the other isolates which were avirulent (Rf≤1). The virulent *M. javanica* isolates

Table 1. Virulence of *Meloidogyne* isolates against a susceptible (Rutgers) and a resistant (Betterboy) tomato cultivar.

Cultivar	Parameter	*M. javanica*		*M. incognita* race 1	*M. incognita* race 2	*M. arenaria* race2
		(3) [a]	(67) [a]	(5) [a]	(3) [a]	(5) [a]
Rutgers	Shoot f wt[b]	13.36a ± 4.52 [c]	13.60a ± 4.55	11.32a ± 3.24	12.50a ± 3.98	12.00a ± 4.24
	Root f wt	3.17a[d] ± 0.90	3.58a ± 1.23	2.76a ± 0.93	3.63a ± 0.76	2.92a ± 1.27
	Root d wt	1.57a ± 0.46	1.71a ± 0.64	1.32a ± 0.48	1.77a ± 0.47	1.28a ± 0.63
	GI [e]	5.00a ± 0.00	5.00a ± 0.00	5.00a ± 0.00	5.00a ± 0.00	5.00a ± 0.00
	Rf [f]	4.90a ± 1.55	4.71a ± 1.14	6.64a ± 0.80	5.30a ± 1.00	5.28a ± 0.77
	Rating	Virulent	Virulent	Virulent	Virulent	Virulent
Betterboy	Shoot f wt	12.33a ± 0.81	18.03b ± 2.60	17.30b ± 3.59	18.53b ± 3.97	18.24b ± 4.89
	Root fresh weight	3.47a ± 0.85	9.61b ± 1.47	9.36b ± 1.23	10.33b ± 1.29	9.78b ± 1.77
	Root d wt	1.03a ± 0.71	3.90b ± 0.64	3.70b ± 0.72	4.50b ± 1.13	3.92b ± 0.91
	GI[e]	4.37b ± 0.46	0.12a ± 0.40	0.00 a	0.00 a	0.00 a
	Rf [f]	3.73b ± 0.76	0.01a ± 0.05	0.00 a	0.00 a	0.00 a
	Rating	Virulent	Avirulent	Avirulent	Avirulent	Avirulent

[a] Number of isolates.
[b] Shoot and root fresh weigth (f wt) is given in g.
[c] Average and standard deviation of isolate means (each mean of four plants).
[d] Means followed by the same letter within rows are not significantly different according to DMRT (P≤0.05).
[e] GI, gall index: 0, no galls or egg-masses; 1,1–2 galls or egg-masses; 2, 3–10; 3, 11–30; 4, 31–100; and 5, over 100 galls or egg-masses per root system.
[f] Rf, reproduction factor = Pf (final population)/Pi (initial population).

had high values in the gall index (average 4.37) and Rf (average 3.73). The galls produced on *Mi*-resistant plants resembled those produced on susceptible plants. Most of the isolates were avirulent with a low gall index (<2) and a Rf averaging between 0.00 and 0.01.

Horticultural characteristics (shoot and root fresh weight, root fresh weight and root dry weight) of tomato plants were significantly lower in plants that had been subjected to the virulent *Meloidogyne* isolates. In both tomato cultivars, all three horticultural parameters were negatively correlated with Rf (Table 2).

Discussion

The three primer pairs Fjav/Rjav, Finc/Rinc, and Far/Rar directly amplified single fragments of a specific size from the target DNA of *M. javanica*, *M. incognita*, and *M. arenaria* respectively. The size and specificity of the amplified DNA products agreed with Zijlstra *et al*. (2000). The results confirmed that SCAR-PCR is a rapid and efficient method for identification of populations of these economically important RKN species and is fundamental for plant protection. In this work, genetic diversity (based on virulence) was studied among isolates of RKNs from Jordan. Of the 83 isolates, only three *M. javanica* isolates had a high frequency of individuals that reproduced on a *Mi* gene bearing cultivar. Resistance-breaking field populations of RKN may occur when there has been prolonged exposure to tomato cultivars bearing the *Mi* gene, or when populations are selected in controlled experiments by re-inoculation on resistant cultivars (Roberts and Thomason, 1989). Whether the isolates here tested were naturally virulent or whether their virulence was selected for by introduced tomato cultivars having the *Mi* gene, remains uncertain. The parasitic ability of *M. javanica* was greater than that of the other nematode species on this cultivar. None of the isolates of *M. arenaria* race 2 or of the two host races of *M. incognita* reproduced on cv. Betterboy. The selection of *M. incognita*-virulent lines from wild-type avirulent isolates under laboratory-controlled conditions by repeated exposure to resistant tomato cultivars carrying the *Mi* gene was reported by Jarquin-Barberena *et al*. (1991). Selection for the virulence mutant also occurs under agronomic conditions after repeated planting of resistant tomato (Semblat *et al*., 2000). The occurrence of three virulent *M. javanica* isolates in separate agricultural areas in Jordan did not significantly affect the essential utility and benefits achieved by introducing resistant tomato cultivars having the *Mi* resistance gene into Jordan since the loss of nematode resistance was in most cases due to selection within the present field population rather than to actual mutation processes. Further research is required to determine the effectiveness of *Mi* gene resistance in managing RKN in tomato growing areas of Jordan.

Table 2. Coefficients of correlation among the parameters of the virulence test in a susceptible (Rutgers) and a resistant (Betterboy) tomato cultivar.

		cv. Rutgers				cv. Betterboy			
	Parameter[a]	Shoot f wt	Root f wt	Root d wt	Rf[b]	Shoot f wt	Root f wt	Root d wt	Rf
cv. Rutgers	Shoot f wt	1							
	Root f wt	0.76[c]	1						
	Root d wt	0.15	0.33[c]	1					
	Rf	0.05	-0.02	0.02	1				
cv. Betterboy	Shoot f wt	-0.15	-0.04	0.16	-0.23[c]	1			
	Shoot f wt	-0.09	-0.04	0.12	-0.08	0.86	1		
	Root d wt	0.12	-0.09	0.19	-0.06	0.78	0.90[c]	1	
	Rf	-0.01	-0.06	-0.04	0.02	-0.49[c]	-0.63[c]	-0.61[c]	1

[a] Shoot and root fresh weigth (f wt) is given in g.
[b] Rf, see Table 1.
[c] Significant at 0.05 probability level using the LSD procedure.

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