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Occurrence of a resistance breaking pathotype of *Meloidogyne javanica* on tomatoes in Crete, Greece

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Summary – The root galling and reproduction of five single egg mass lines of *Meloidogyne javanica* originating from three different field sites of Crete was assessed on fourteen resistant tomato hybrid cultivars (Scala, 7352 Silco, Myrto, Menglo, Rakata, GC785, GC788 Alpado, Bermuda, 7353, 7358, W1964, W1967, W2913, 399) in a controlled environment where soil temperature was 22-24 °C. Two lines originating from a field where the resistant cv. Menglo had been grown for two years were able to infect the resistant cultivars and to reproduce on them as well as on a susceptible cultivar (Carouso). One line isolated from cucumber infected and reproduced only on the susceptible cultivars. Two lines from a field where the susceptible cultivar Carouso was grown showed either slight or no reproduction on resistant cultivars. Under greenhouse conditions (soil temperature 20-42 °C) Mi conferred resistance was nullified and all cultivars became hosts for an avirulent line.

Résumé – ***Observation d'un pathotype de* Meloidogyne javanica *brisant la résistance variétable de la tomate en Crête, Grèce*** – L'index de galle et le taux de reproduction de cinq lignées issues de masses d'œufs de *Meloidogyne javanica* originaires de trois champs différents en Crête ont été évalués sur quatorze cultivars hybrides résistants de tomate (Scala, 7352 Silco, Myrto, Menglo, Rakata, GC785 Alpado, Bermuda, 7353, 7358, W1964, W1967, W2913, 399) en conditions contrôlées avec une température du sol de 22-24 °C. Deux lignées originaires d'un même champ où le cultivar résistant Menglo avait été cultivé pendant deux ans pénétraient et se multipliaient dans les cultivars résistants et dans un cultivar sensible (Carouso). Une lignée isolée des racines de concombre pénétrait et se reproduisait uniquement sur cultivar sensible. Deux lignées originaires d'un même champ où le cultivar sensible Carouso avait été cultivé avaient un taux de reproduction faible à nul sur les cultivars résistants. En serre (température du sol de 20-42 °C), la résistance conférée par le gène Mi avait disparu et tous les cultivars étaient devenus hôte pour une lignée avirulente.

Key-words : *Lycopersicum esculentum,* Mi gene, root knot nematode.

Growing resistant tomatoes has been an effective means to diminish root knot nematode damage in several regions of the world. Resistance is conferred in tomato by the single major gene Mi which is effective against *Meloidogyne incognita, M. javanica* and *M. arenaria,* but not against *M. hapla* (Roberts, 1992). However, infection of resistant tomatoes by virulent populations occurring naturally or selected after continuous exposure of avirulent populations to certain host genotypes has been observed (Jarquin-Barberena *et al.,* 1991; Roberts, 1992; Young, 1992).

The objective of this work was to compare the virulence of *M. javanica* lines taken from a site where resistant tomatoes had been grown for two years with those from two fields where the resistant genotypes had not been grown before. The studies were carried out in pot experiments at the Plant Protection Institute of Heraklion, Crete.

Materials and methods

PLANT MATERIAL

Fourteen tomato hybrid cvs with resistance to root knot nematodes were tested (Table 1). The Mi-conferred resistance was confirmed for eleven of the cvs by the respective seed companies (Rjik Zwaan, De Ruiter Seeds, Enza Zaden BV). Seeds germinated in flats with commercial sterilized compost soil (on a green house bench) and seedlings at the two-leaf stage were transplanted into 250 ml plastic cups (growth room experiment) or 500 ml pots (greenhouse experiment) filled with steam-sterilized sandy loam soil. Plants were allowed to re-establish for 5 to 10 days before nematode inoculation. During the growing period they were watered as required and 20 ml of liquid fertilizer (Complesal 5-8-10 NPK) diluted 200 times in water was applied weekly.

Table 1. *Tomato hybrid cultivars resistant to root-knot nematodes.*

Name/code number	Seed Company (Holland)
Scala	Rijk Zwaan
7352 Silco	Rijk Zwaan
7353	Rijk Zwaan
7358	Rijk Zwaan
Menglo	De Ruiter Seeds
Myrto	De Ruiter Seeds
Rakata	De Ruiter Seeds
W1964	De Ruiter Seeds
W1967	De Ruiter Seeds
W2913	De Ruiter Seeds
GC 785	Sluis & Groot Sandoz Seeds
GC 788 Alpado	Sluis & Groot Sandoz Seeds
399	Sluis & Groot Sandoz Seeds
Bermuda	Enza Zaden BV

Nematode populations and lines

Three nematode populations were collected from vegetable growing regions on the south coast of Heraklion, Crete Province : *i)* population 1 originates from roots of the cv. Menglo collected in a field previously cultivated for 2 years with the cv. Menglo; *ii)* population 2 originates from roots of cucumber collected in a field previously cultivated for 2 years with cucumber; *iii)* population 3 originates from roots of the cv. Carouso collected in a field previously cultivated for 2 years with susceptible tomato cvs and cucumber. The infected roots were macerated in a kitchen blender to release the eggs, which were collected after washing the slurry through a 38 mm sieve nested below a 150 mm sieve. Stock cultures were established by pipetting egg suspensions into pots planted with the dwarf tomato cv. Tiny Tim. Eggs were also left to hatch in extraction dishes (Southey, 1986) and 1-3 day-old second stage juveniles (J2) were inoculated on pepper (*Capsicum annuum* cv. California Wonder) and peanut (*Arachis hypogaea* cv. Florunner) at rates of 5000 per plant. The morphology of perineal patterns on ten to fifteen females from each population and the absence of galls and egg masses in peanut and pepper indicated that all populations were *M. javanica*. To verify the absence of low populations of other *Meloidogyne* species, ten single egg mass lines were established from each population by inoculating tomato seedlings cv. Tiny Tim with individual egg masses. All lines were identified as *M. javanica* by morphological characteristics (perineal pattern of mature females, tail length and hyaline portion of J2) and by the North Carolina differential host test (Hartman & Sasser, 1985; Jepson, 1987). The same single egg mass lines tested for their pathogenicity on the resistant cv. Menglo and five lines with different pathogenicity characteristics were used in this study.

Preliminary tests indicated that population 1 reproduced equally on susceptible (Carouso) and resistant (Bermuda, Rakata, Menglo) tomato cultivars at soil temperature 25-27 °C while a few females from population 3 developed and reproduced on certain cultivars; population 2 infected only the susceptible tomato (unpub.). An initial discrimination of virulence of the ten single egg mass lines of each population was done by inoculating four seedlings of a susceptible (Dombito) and a resistant cv. (Menglo) with 300 J2 deriving from subcultures on cv. Tiny Tim. The plants grew in a growth room with air temperature adjusted to 20-25 °C and a 16 h photoperiod. Soil temperature in plastic cups was 22-24 °C; at this temperature resistance conferred by the Mi gene is functional (Araujo *et al.*, 1982) and populations infecting resistant cvs could be characterized as resistance – breaking pathotypes. After 45 days, the plants were uprooted and juvenile inoculum was prepared from the egg masses collected from the susceptible cv. Depending on the response of the resistant cv. Menglo to inoculation, the nematode lines were designated as : *i)* virulent lines producing galls and reproducing on resistant cvs which were further discriminated as lines of high virulence (HV) (numbers of galls and egg masses on resistant cultivars similar or higher to those on susceptible cultivar) and lines of low virulence (LV) (numbers of galls and egg masses on resistant cultivars significantly lower than those on the susceptible cultivar); *ii)* avirulent lines (AV) (line which produced galls and reproduced only on susceptible cultivar). The preliminary tests on the resistant cv. Menglo conducted with ten single egg mass lines from each population revealed the following trends of pathogenicity : pop. 1 : all of high virulence (HV); pop. 2 : all avirulent (AV); pop. 3 : 70 % avirulent (AV) and 30 % of low virulence (LV) indicating traces of root galling and reproduction. The following single egg mass lines representing all categories were selected for plant inoculation : two from population 1 (lines 1 HVa and 1HVb); one from population 2 (line 2 AV); two from population 3 (lines 3 AV and 3 LV).

Testing procedures

Second stage juveniles were obtained by incubating egg masses collected from roots of the susceptible cultivar. Dombito used in the previous test in extraction dishes at 25-28 °C (Southey, 1986). The juveniles that appeared during the first 24 h were discarded because they might have hatched before the egg masses were placed in the dish. Those collected within the 3 follow-

ing days were used for inoculation. To confirm virulence on a range of resistant cultivars, 300 J2 from the five lines (1 HVa, 1 HVb, 2 AV, 3 AV and 3 LV) were inoculated onto the hybrids listed in Table 1 with the susceptible cultivar, cv. Carouso serving as control. After a 56-day growing period under the previously described conditions, the plants were uprooted and the number of galls and visible egg masses were recorded for all combinations. The number of egg masses was estimated under a dissecting microscope after staining in a solution of phloxine B (Hartman & Sasser, 1985). The experiment was run in two different growing rooms under the same conditions testing separately six and eight cultivars on all nematode lines. Each group had its own controls and there were four replicates per treatment. After recording data the experiment was repeated in the same way.

The pathogenicity of a line from each category was also tested on the same cvs in the greenhouse. The plants grew in pots on a bench without artificial heating/cooling or illumination. Air temperature ranged from 15 to 48 °C while soil temperature in pots was 20-42 °C. High temperatures were usually recorded from late morning until late afternoon. The experiment was run once and results were again recorded after 35 days.

Results from the two experiments in the growth rooms are presented separately. In the cases where root galling and nematode reproduction were slight or zero, results were not analyzed. Otherwise data were subjected to ANOVA and the least significant differences ($P < 0.05$ and $P < 0.01$) among the treatment means were calculated in order to estimate differences. Data were transformed to square roots in cases where the value for the standard error of a mean was higher than the mean value (Mead & Curnow, 1990).

Results

The preliminary characterization of lines according to their virulence on the resistant cv. Menglo was consistently verified on the fourteen tested cvs under controlled conditions. In all inoculations with lines 1 HVa and 1 HVb, the number of galls and egg masses on resistant cultivars did not differ significantly from, or exceed, those on the susceptible cultivars (Tables 2, 3). The line 3 LV also produced slight root galling and reproduced on resistant cultivars, while lines 2 AV and 3 AV did not infect or reproduce on any cultivar.

The virulence of lines 1 HVb, 3 AV, and 3 LV was also assessed in the greenhouse. Results revealed that resistance against avirulent lines conferred by the Mi gene may decrease in certain growing conditions associated with high soil temperature, and that line 3 AV infected and reproduced on the resistant cultivars. Infection and reproduction rates for lines 3 AV and 3 LV on resistant cultivars were similar or significantly lower than those on susceptible cultivars (Tables 4-5).

Discussions

Reproduction of *Meloidogyne* on nematode resistant cultivars has been demonstrated with different populations and under different experimental conditions. These resistance breaking populations of *Meloidogyne* can occur naturally without previous exposure to these cultivars, or arise from non-virulent populations after repeated selection by certain tomato genotypes (Sikora *et al.,* 1973; Philis & Vakis, 1977; Viglierchio, 1978; Netscher & Taylor, 1979; Bost & Triantaphyllou, 1982; Hadisoeganda & Sasser, 1982; Prot, 1984; Roberts & Thomason, 1986; Roberts *et al.,* 1990; Jarquin-Barberena *et al.,* 1991). In this study the lines 1 HV overcame completely the effect of Mi gene. Variability in number of galls and egg masses was recorded with some resistant cvs which supported greater numbers than the susceptible cultivar. The culture of the population in the laboratory and the increase of the lines on susceptible cvs took a period of four generations before it was inoculated on resistant cvs, but this did not alter its pathogenicity. This indicates that the population has a compatibility mechanism (s) overcomming the resistance response in the certain cultivars which may be explained by a gene-for-gene system (Roberts *et al.,* 1990). It is not known whether this population has been selected from the 2-year period of associatioon in the field with the resistant cultivar or whether its pathogenic ability is spontaneous. The previous cultivation history of the field is unknown and there is no information on any ancestral association of nematodes with cultivars bearing the Mi gene.

Line 3 LV which had never been previously exposed to the Mi gene produced few galls and egg masses on the resistant cultivars. Jarquin-Barberena *et al.* (1991) showed that isolates of *M. incognita* originating from a single juvenile may be progressively selected for virulence against the Mi gene. The selected single lines were able to overcome resistance by progressively increasing the proportion of invading juveniles and egg producing females. Those differed from homologous avirulent lines originating from the same female by a spot revealed by dimensional gel electrophoresis of soluble proteins (Dalmasso *et al.,* 1991).

Continuous planting of resistant cvs in the field where population 3 LV occurs will probably increase its compatibility to certain cvs resulting in a build up over the non virulent population 3 AV. In heavily infested fields, it is logical to assume that the roots will be invaded by higher proportions of such populations. In these cases Netscher and Taylor (1979) recommended the previous reduction of nematode densities by chemical or physical means before planting resistant plants.

Previous work (Tzortzakakis, 1993) and observations of infected roots routinely examined in the Nematology Laboratory of Plant Protection Institute revealed that *M. javanica* is the dominant species in the most impor-

Table 2. *Number of galls (G) and egg masses (EM) produced on one susceptible (C) and eight resistant tomato cultivars by five single egg mass lines of* M. javanica (two growth chamber experiments).

Cultivars	Line 1HVa		Line 1HVb		Line 2AV		Line 3AV		Line 3LV	
	G	EM	G	EM	G	EM	G	EM	G	EM
EXPERIMENT 1										
Carouso (C)	30.3	17	39.5	28	44.5	29	61.5	40	58	43
Scala	39	25.3	27.5	18.5	0	0	0	0	3.5	2
7352 Silco	40.3	29	55	31.5	0	0	0	0	1	1
Myrto	28.5	15.5	43.5	25	0	0	0	0	1.5	1
Menglo	28.5	14.5	27	21.5	0	0	0	0	2.5	2
Rakata	44	27	40.5	21	0	0	0	0	2.5	1.5
GC 785	64.5	47	34.5	21.5	0	0	0	0	3	2.5
GC788 Alpado	66	49	44.8	26	0	0	0	0	1	0.5
Bermuda	72.5	55.5	39.5	14	0	0	0	0	2.5	1.5
LSD 5 %	30.5	27.5	34.1	19.5	–	–	–	–	–	–
LSD 1 %	43.8	39.6	49.1	28	–	–	–	–	–	–
EXPERIMENT 2										
Carouso (C)	43.3	24	59.5	28	50	30	52	29	59	43.8
Scala	59	38.3	32	17.5	0	0	0	0	1	0.5
7352 Silco	34.5	18	20.8	17.5	0	0	0	0	1.5	1
Myrto	46	23	64	19	0	0	0	0	0.5	0.5
Menglo	30.5	12.5	76	28.7	0	0	0	0	1.5	1
Rakata	44	30.5	30	15.5	0	0	0	0	0.5	0
GC 785	58.5	44	54.5	37.5	0	0	0	0	8	5
GC788 Alpado	47	28	36.8	22.5	0	0	0	0	0.5	0
Bermuda	40.5	16.5	37	15.5	0	0	0	0	1	1
LSD 5 %	48.5	29.3	31.5	14.7	–	–	–	–	–	–
LSD 1 %	69.8	42.1	45.3	21.2	–	–	–	–	–	–

Average of four replicates.

tant vegetable growing areas of Crete where it occurs alone or with a small proportion of *M. incognita*. Although preliminary tests indicated that resistance conferred by the Mi gene was functional on two single egg mass lines of *M. incognita* from different fields when soil temperature was 22-24 °C (unpubl.), the pathogenicity of the species and its possible variability requires to be studied in detail.

While line 3 AV was unable to break resistance of the tested tomato cvs at the low temperature regimes of the growth room, it infected and reproduced under greenhouse conditions at high soil temperature. Thus planting resistant cvs in growing areas of Crete should be avoided during hot season since the resistance response probably decreases with high soil temperature (Araujo *et al.*, 1982). However, resistance decrease varied between cvs and the number of galls and egg masses were the same or significantly lower than those on control. Variations between nematode lines and cvs recorded in experiments probably result from the existence in different genotypes of different resistance genes from the Mi gene base (Roberts & Thomason, 1986).

Interest in the use of resistant cvs for managing root-knot infestations in Crete will increase if the use of methyl bromide is restricted or banned and nematicide application discouraged. The success in implementation of the resistant cultivars in management systems should include preliminary tests of virulence of nematode populations present in a certain growing region before use in large scale plantings. This has been suggested by Ro-

Table 3. *Number of galls (G) and egg masses (EM) produced on one susceptible (C) and six resistant tomato cultivars by five single egg mass lines of* M. javanica (two growth chamber experiments).

Cultivars	Line 1HVa		Line 1HVb		Line 2AV		Line 3AV		Line 3LV	
	G	EM	G	EM	G	EM	G	EM	G	EM
EXPERIMENT 1										
Carouso (C)	71	47.3	61.5	44	54.5	40.5	59.5	53.5	68	55
7353	82.5	60	113.5	71.3	0	0	0	0	0.5	0
7358	73.3	37.5	68.3	54.5	0	0	0	0	2.5	1.5
W1964	60.5	43	65.5	40.5	0	0	0	0	0.5	0.5
W1967	57	51.5	108	93	0	0	0	0	1	0.5
W2913	54.5	27.8	65	50	0	0	0	0	1.5	1
399	85.5	67	76	51.5	0	0	0	0	1.5	0.5
LSD 5 %	36.7	31	82.7	31	–	–	–	–	–	–
LSD 1 %	54.4	46	122.6	46	–	–	–	–	–	–
EXPERIMENT 2										
Carouso (C)	63	45.3	65	49.5	39	26	59	46.5	61.5	43.5
7353	93	67	81.5	64.3	0	0	0	0	1.5	1
7358	57.8	30	78.8	44	0	0	0	0	2.5	2
W1964	58	42	56.5	26.5	0	0	0	0	1	0.5
W1967	50	31.5	104	77.8	0	0	0	0	1	1
W2913	51.3	35.3	57.5	33.5	0	0	0	0	2	1.5
399	77.5	51.5	62.5	42.5	0	0	0	0	0.5	0.5
LSD 5 %	28	20.7	44.9	41.4	–	–	–	–	–	–
LSD 1 %	41.6	30.7	66.7	61.4	–	–	–	–	–	–

Average of four replicates.

Table 4. *Number of galls (G) and egg masses (EM) produced on one susceptible (C) and eight resistant tomato cultivars by three single egg mass lines of* M. javanica *(glasshouse experiment).*

Cultivars	Line 1HVb		Line 3AV*		Line 3LV*	
	G	EM	G	EM	G	EM
Carouso (C)	36.3	15.5	7.79	4.74	6.66	4.93
Scala	42.8	22	4.28	3.31	5.6	3.38
7352 Silco	52	22.5	4.22	3.46	5.14	4.34
Myrto	32.8	12	3.46	2.69	2.02	1.82
Menglo	37.5	20.5	4.78	2.98	3.5	2.64
Rakata	29	11.3	2.44	1.57	1.57	1.2
GC 785	29.5	17	5.69	4.83	2.15	1.61
GC788 Alpado	33.5	13.8	2.94	2.08	2.95	2.41
Bermuda	28.5	12.5	2.59	2.2	3.25	2.5
LSD 5 %	25	12.9	3.88	1.58	1.76	1.42
LSD 1 %	35.1	18.6	5.59	2.27	2.53	2.04

Average of four replicates; * square root transformed data.

Table 5. *Number of galls (G) and egg masses (EM) produced on one susceptible (C) and eight resistant tomato cultivars by three single egg mass lines of* M. javanica *(glasshouse experiment).*

Cultivars	Line 1HVb		Line 3AV		Line 3LV	
	G	EM	G	EM	G	EM
Carouso (C)	56.8	35.8	44.5	25.5	52.8	30
7353	63.5	45.3	39.3	29	30.3	21.8
7358	54.5	40	28.3	19	28	21
W1964	51.3	31.5	27.3	17.8	25.5	18.8
W1967	43	27.3	26	18.8	24.3	18
W2913	48.8	25.5	31	18.3	29	19.3
399	50.8	35	23	15.8	33.8	21.5
LSD 5 %	14.9	13.1	12.1	10.8	15.2	10
LSD 1 %	20.7	19.4	16.8	14.9	21.1	15

Average of four replicates.

berts and Thomason (1986) for the tomato growing areas in California. Rotating resistant cultivars with susceptible cultivars and non host plants may reduce selection pressure on the target nematode and delay the selection of resistant breaking pathotypes. Producers should be convinced that it will be in their long term economic interest to manage those potential problems instead of taking only short term financial gains provided by growing resistant tomato cultivars (Young, 1992).

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