Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
Available online at www.inia.es/sjar
http://dx.doi.org/10.5424/sjar/2014121-4340

Spanish Journal of Agricultural Research 2014 12(1): 225-232
ISSN: 1695-971-X
eISSN: 2171-9292

Development of virulence to *Meloidogyne incognita* on resistant pepper rootstocks

Caridad Ros-Ibáñez[1]*, Lee Robertson[2], Maria del Carmen Martínez-Lluch[1], Ana Cano-García[3] and Alfredo Lacasa-Plasencia[1]

[1] Departamento de Biotecnología y Protección de Cultivos. IMIDA. C/ Mayor, s/n. 30150 La Alberca (Murcia), Spain. [2] Departamento de Biodiversidad y Biología Evolutiva. Museo Nacional de Ciencias Naturales (MNCN-CSIC). C/ José Gutiérrez Abascal, 2. 28006 Madrid, Spain. [3] SSVV. Consejería de Agricultura y Agua. C/ Mayor, s/n. 30150 La Alberca (Murcia), Spain

Abstract

The root-knot nematode (RKN) *Meloidogyne incognita* is a major soil parasite of pepper crops in greenhouses in Southeast Spain. Due to the limitations of the use of soil fumigants, grafting plants on resistant rootstocks (R-rootstocks) has become an important alternative to chemical nematicides. The repeated use of R-rootstocks can bring about the selection of virulent populations capable of overcoming resistance. We carried out a six-year investigation on resistant rootstocks in a naturally *M. incognita* infested greenhouse, and found that two successive years of growing plants grafted on R-rootstocks Atlante (ATL) were sufficient to overcome resistance (galling index 1.5 and 5.6 in the first and second years respectively). A large variability was observed between several R-rootstocks. Two R-rootstocks (C19 and Snooker) behaved like ATL while two others (Terrano and DRO 8801) were not infected by RKN. Laboratory studies with the same R-rootstocks, inoculated with two nematode isolates (avirulent and virulent against ATL) confirmed the greenhouse results, indicating that some rootstocks may be infested by virulent populations and others may not. It suggests that different R-genes, which are differentially overcome by RKN, have been introgressed into the rootstocks. This may have consequences for the management of resistant rootstocks in the field.

Additional key words: *Capsicum annum;* nematode; pot experiment; greenhouse experiment; grafting.

Introduction

Nematodes of the genus *Meloidogyne* are obligate endoparasites of more than 5,500 plant species (Goodey *et al*., 1965). Some species are responsible for losses estimated at US$ 100,000 million per year^{-1} (Sasser & Freckman, 1987). Those which cause most damage are *M. incognita, M. arenaria, M. hapla* and *M. javanica*, although in recent years *M. enterolobii*, a tropical species, and *M. chitwoodi* and *M. fallax,* associated with temperate climates, have also been considered to be emerging pathogens (Ozarslandan *et al*., 2009; Wesemael *et al*., 2011; Castagnone-Sereno, 2012).

Meloidogyne incognita is distributed throughout temperate and tropical areas and is considered the main plant-parasitic nematode on a global scale (Lamberti, 1981; Trudgill & Blok, 2001) and is, together with *M. javanica* and *M. arenaria,* the predominant species in Spain (Bello *et al*., 2004; Giné *et al*., 2012; Verdejo-Lucas *et al*., 2012). Both *M. incognita* and *M. arenaria* are found on protected and non-protected crops in central and southern Spain (Bello *et al*., 2004; López-Pérez *et al*., 2011), and only on protected crops close to the Atlantic coast. *M. hapla* is found in the northern regions of Spain and *M. javanica* is mainly found in the eastern and south-eastern regions (Verdejo-Lucas *et al*., 2002; Robertson *et al*., 2006).

Meloidogyne incognita seriously affects protected pepper (*Capsicum annum* L.) crops in the south-east of Spain (Bello *et al*., 1997, 2004) where more than 2,000 ha have been occupied for more than 20 years by a pepper monocropping system, grown over a 9-10

* Corresponding author: caridad.ros@carm.es
Received: 10-04-13. Accepted: 21-02-14.

Abbreviations used: ATL (Atlante rootstock); DRO (DRO 8801 rootstock); MB (methyl bromide); SK (Snooker rootstock); TER (Terrano rootstock).

month cycle. The nematode is considered to be one of their major phytopathological soil problems (Ros *et al*., 2004). Fumigant and non-fumigant nematicides are used to control *Meloidogyne* on vegetable crops, but increasing environmental concerns and government regulations have contributed to a greater emphasis on other control measures (Ornat *et al*., 2001). Researchers have been investigating alternative ways to control nematodes and other soil-born pests.

Several authors have described a resistance in some lines and cultivars of *Capsicum* to several different *Meloidogyne* species, and several genes have been identified [the *Me*(s) and *N* genes] which confer specific resistance (Hendy *et al*., 1985; Fery *et al*., 1998; Djian-Caporalino *et al*., 1999, 2004; Castagnone-Sereno *et al*., 2001; Thies & Fery, 2002). The *Me1* gene from PI 201234 of Central America, the *Me3* gene from PI 322719 (India) and the *Me7* gene from CM334 (Mexico) are effective against *M. incognita* (Djian-Caporalino *et al*., 2007, 2011). Previous work by Fazari *et al*. (2012) has demonstrated that *Me7* and *Me3* are the same gene and suggested simplifying the nomenclature and calling *Me3* the resistant allele carried by both PI 322719 and CM334 *Capsicum* lines.

The selection for virulence has lead researchers to investigate alternative control methods for nematodes and other soil-born pathogens which are common in areas of intensive tomato (*Solanum lycopersicum* L.) cultivation, in which many isolates of *M. incognita* and *M. javanica* have overcome the resistance conferred by the *Mi* gene(s). Examples of such populations have been found in some European countries such as France, Greece (Crete) and Spain (Castagnone-Sereno *et al*., 1991, 1999; Tzortzakakis *et al*., 1998; Ornat *et al*., 2001; Jacquet *et al*., 2005; Verdejo-Lucas *et al*., 2009, 2012). The selection of *Meloidogyne* populations virulent to tomato cultivars carrying the *Mi* gene may occur naturally or arise from repeated cultivation of resistant rootstocks over three consecutive years (Verdejo-Lucas *et al*., 2009).

Isolates of *M. incognita* have also been found infecting and reproducing on resistant peppers (Ros *et al*., 2005, 2006; Robertson *et al*., 2006). After repeated cultivation of pepper grafted on rootstocks carrying the resistance genes *Me3* (= *Me7)*, populations of *M. incognita* capable of multiplying on resistant rootstocks have been found in some greenhouses (Ros *et al*., unpublished data).

This paper presents the results of investigations carried out in *M. incognita*-infested greenhouses to study the development of virulence in nematode populations after the repeated cultivation of resistant pepper rootstocks. Pot tests under controlled conditions were carried out to determine the variability of the phenotype response of several pepper rootstocks against virulent and avirulent *M. incognita* isolates. Knowledge of how rootstocks and nematode populations behave will permit the design of novel management strategies based on the use of resistance genes in intensive monoculture or rotation systems.

Material and methods

The six following pepper rootstocks resistant to *M. incognita* were used: Atlante (ATL), C19, C138 (three genetically similar lines from Ramiro Arnedo S.A., Calahorra); DRO 8801 (DRO) (De Ruiter Seeds, Almería); Snooker (SK) (Syngenta Seeds, Almería), Terrano (TER) (Syngenta Seeds Almería). The susceptible pepper cultivars used were: 'Almuden' (Syngenta Seeds, Almería) in the greenhouse experiment and 'Sonar' (Clause Tezier, Almería) in the pot experiment under controlled conditions.

Greenhouse experiment

The experiment was carried out in a greenhouse (X: 685,049.85 m; Y: 4,183,2681.4 m, in "Campo de Cartagena", Murcia, SE Spain). The greenhouse measured 1,000 m^2, and was drip-irrigated (16 mm diameter tube and emitters 0.40 m apart providing 3 L h^{-1}). The clay-loam soil had a pH of 7.8 and 2.0% organic matter content. Before starting the experiment, nematode-susceptible peppers were cultivated in this greenhouse for three consecutive years in non disinfected soil which was naturally infested with *M. incognita,* race 2 (Robertson *et al*., 2006).

The area has a Mediterranean climate. During the pepper growing season (mid-December or beginning of January to the end of July or mid-August), the average soil temperature in the greenhouse ranged from 15°C to 23°C at 30 cm depth and from 17°C to 26°C at 15 cm.

The experiment lasted a total of six years. A randomized block design with three repetitions per treatment was established in the first year (Table 1). Each elemental plot consisted of a row of 47 plants (1 m × 20 m). Plants were spaced 0.40 m apart in the row and there was 1.00 m between two rows (2.5 plants m^{-2}). One

Table 1. Greenhouse experimental design. Distribution of treatments along the six years of the experiment

Treatment	Year					
	1	2	3	4	5	6
T1	MB + NG	MB + NG	MB + NG	MB + NG	MB + NG	MB + NG
T2	NG	NG	NG	NG	NG	NG
T3	ATL	ATL	ATL	ATL	ATL	ATL
T4	ATL	ATL	ATL	DRO	TER	TER
T5	ATL	ATL	ATL	SK	SK	C138
T6	ATL	ATL	ATL	C19	C19	C19

MB: methyl bromide disinfected. NG: Pepper cv. 'Almuden' non grafted. ATL, C19, DRO, C138, SK and TER: Pepper cv. 'Almuden' grafted on pepper rootstocks Atlante, C19, DRO 8801, C138, Snooker and Terrano, respectively.

row of susceptible ungrafted plants separated each plot. The plot of the first treatment was disinfected with 30 g m^{-2} of methyl bromide (MB) 98:2 and covered with virtually impermeable (VIF) plastic film in November each year; and the nematode-susceptible pepper cv. 'Almuden' was planted. The other plots were not disinfected. In these plots, the same cultivar 'Almuden' was used, but ungrafted (T2) or grafted (T3 to T6) on different resistant rootstocks. Each year of the experiment, 38 day old plants were planted in the first week of January and removed in the first week of August. During the interim between test years the soil was left fallow. Each plot was tilled independently from the others, longitudinally to avoid mixing the soils. Drip irrigation was used and the soil received no phytosanitary treatment. For the first three years, the rootstock ATL was used (Table 1). In the fourth year, the plants were grafted on different resistant rootstocks in T4 (DRO), T5 (SK) and T6 (C19) (planted in same rows where ATL grafted plants had been grown for the first three years). For T4, the plants were grafted on TER in the fifth year. For T5, plants grafted on SK were changed onto C138 in the sixth year.

In the first and fourth years, two weeks before planting, the population density of *M. incognita* in the disinfected and non-disinfected plots was estimated by taking 5 samples from 10 to 30 cm depth in 5 cores for each sample of fumigated and non-fumigated treatment, extracting the nematodes on Baermann trays and counting the juveniles (J2) (Flegg, 1967). In the first year of the experiment, the population density for the whole greenhouse of *M. incognita* was 274.7 ± 82.0 J2 per 100 cm^3 of soil. In the fourth year and before disinfection with MB, the density was 246.7 ± 159.3 J2 per 100 cm^3 of soil. Before planting, the density in the non-disinfected plots averaged 3,778.7 ± 1,672.4 J2 per 100 cm^3 of soil, while in the plots disinfected with MB no juveniles were found.

Each year when the crop was harvested, the plants were removed and ten random plants from each row were taken to the laboratory, where the roots were washed and examined. The percentage of infected plants was calculated and the gall index was evaluated following the 0-10 scale developed by Bridge & Page (1980). Data was checked for normality and homogeneity of variances and transformed, where required, to arcsin $\sqrt{x}$ (x being = percentage of infested plants), or $\log_{10}(x+1)$ (x being = the gall index). An LSD test at 95% was used to compare averages.

Pot experiment in controlled conditions

We used an avirulent isolate (MI-E) and a virulent isolate (MI-CH) of *M. incognita*, originally collected from infested greenhouses, where resistant rootstocks had been cultivated. MI-CH was isolated from resistant ATL (grown in the greenhouse of our investigation) and MI-E from susceptible pepper (grown in a greenhouse where ATL was not infected). Both isolates were identified at the reference laboratory of the Consejo Superior de Investigaciones Científicas (CSIC, Madrid) as being *M. incognita* race 2 "Capino pepper" (Robertson *et al.,* 2006) in differential host tests (Hartman & Sasser, 1985). Both isolates were maintained and multiplied on tomato Marmande Claudia plants (Clause-Tezier, Almería), a cultivar susceptible to *Meloidogyne*.

Seedlings of rootstocks and susceptible cultivars were grown individually in 200 mL pots containing

steam-sterilized substrate composed of a 1:1 (v:v) mixture of perlite and horticultural organic peat. Experiments were conducted in a climatic chamber maintained at 23-24°C with a 14-h light cycle and relative humidity 45-60% (light period) and 100% (dark period). For each isolate (MI-CH and MI-E) plants with 4-6 true leaves were inoculated with a water suspension of 2,000 eggs and J2s (Hussey & Barker, 1973). Three replications of 8 pots for each cultivar and isolate were tested in each experiment. The plants were irrigated three times per week and given a complete nutritive solution once a week.

Eight weeks after inoculation, the plant roots were individually washed with tap-water, and stained with 50 mg L^{-1} erioglaucine (Omwega *et al*., 1988) in order to specifically stain the egg masses green. The roots were rinsed and examined under a magnifying glass to calculate the galling index and the number of egg masses per plant. The experiment was repeated three times using the same design. The percentage of infected plants was calculated, the number of egg masses per plant was counted, and the gall index was evaluated as previously indicated.

Results

Greenhouse experiments

In the first year (Table 2) plants grafted on Atlante rootstock (T3-T6) were less infected and showed less damage in the roots than ungrafted plants in disinfected soil with BM (T1) and ungrafted plants in non-desinfected soil (T2). In the second year, no difference was observed between the ungrafted plants and those grafted on ATL (Table 2), although disinfecting the soil with MB for the second consecutive year reduced the incidence of the nematode. In the third year, the incidence of nematodes in the MB disinfected plots was even lower. However, the level of infestation and gall index did not differ between the ungrafted plants and plants grafted on ATL.

In the fourth year (Table 3), plants grafted on DRO, C19 and SK, which were grown in the rows where plants grafted on ATL rootstock had been grown for the previous three years, showed a lower (in the case of C19 and DRO) or similar (SK) gall index to that of ungrafted plants cultivated in soil disinfected with MB. The gall index and the percentage of plants infested in the plants grafted on ATL rootstock, was similar to the ungrafted plants cultivated in non-disinfected soil, and both treatments, T2 and T3, showed higher values than those with plants grafted on DRO, SK and C19.

In the fifth year (Table 3), the plants grafted on TER rootstock were not infected when cultivated in the rows where DRO plants had been grown in the fourth year. The percentage of infected plants increased significantly in plants grafted on C19 rootstock, and the gall index was similar to the plants grafted on ATL and similar to that of the ungrafted plants grown in the soil disinfected with MB. Growing the plants on SK rootstock in the same soil significantly increased the incidence of nematodes, and the gall index and percentage of infected plants were similar to those obtained for the plants on ATL rootstock and in the ungrafted plants grown in the soil disinfected with MB.

In the sixth year (Table 3), some plants grafted on TER had a few galls on the roots but no egg masses were observed. The incidence of nematodes in plants grafted on C19 was less than that in plants grafted on ATL, despite being the third year in which they had been cultivated in the same soil. Galls were also found on some plants grafted on C138 and cultivated in the same soil where plants had been cultivated on SK for two previous years, and on which viable egg masses had been observed. The incidence of nematode infection on C138 was less than on ATL, which showed similar percentages of infected plants and a similar gall

Table 2. Percentage of pepper plants infested (mean ± standard deviation) by *Meloidogyne incognita* and gall index (mean ± standard deviation) during the first three years of the greenhouse experiment in thirty observations per treatment

Treatment	First year		Second year		Third year	
	Gall index[1]	% infested plants[2]	Gall index[1]	% infested plants[2]	Gall index[1]	% infested plants[2]
T1	4.3 ± 2.5^{b}	80.0 ± 34.6^{ab}	1.2 ± 1.9^{a}	40.0 ± 40.0^{a}	0.8 ± 1.8^{a}	20.0 ± 20.0^{a}
T2	6.9 ± 1.5^{c}	100.0 ± 0.0^{b}	7.3 ± 1.1^{b}	100.0 ± 0.0^{b}	6.5 ± 2.7^{b}	93.3 ± 11.5^{b}
T3-T6	1.3 ± 1.9^{a}	53.3 ± 1.5^{a}	5.6 ± 1.6^{b}	100.0 ± 0.0^{b}	6.1 ± 1.8^{b}	100.0 ± 0.0^{b}

T1-T6: see Table 1. Values with the same letter in a column are not significantly different ($p < 0.05$) using ANOVA [[1] LSD test, $y = \log_{10}(x+1)$; [2] LSD test, $y = \arcsin\sqrt{x}$)].

Table 3. Mean percentage of pepper plants infected by *Meloidogyne incognita* and gall index during the last three years of the greenhouse experiment in thirty observations per treatment

Treatment	Fourth year			Fifth year			Sixth year		
	Rootstock	Gall index[1]	% infested plants[2]	Rootstock	Gall index[1]	% infested plants[2]	Rootstock	Gall index[1]	% infested plants[2]
T1	NG	1.6 ± 2.0^{b}	46.7^{ab}	NG	1.9 ± 2.5^{b}	46.7^{b}	NG	2.4 ± 2.0^{a}	60.0^{abc}
T2	NG	6.8 ± 1.5^{c}	100.0^{d}	NG	8.4 ± 0.9^{d}	100.0^{c}	NG	6.4 ± 0.9^{b}	100.0^{c}
T3	ATL	4.8 ± 2.8^{c}	80.0^{cd}	ATL	4.2 ± 2.2^{c}	83.3^{c}	ATL	5.1 ± 1.9^{b}	$93.3b^{c}$
T4	DRO	0.3 ± 0.8^{a}	13.3^{a}	TER	0.0 ± 0.0^{a}	0.0^{a}	TER	0.7 ± 1.2^{a}	26.7^{a}
T5	SK	1.0 ± 1.1^{ab}	53.3^{bc}	SK	3.7 ± 2.5^{bc}	80.0^{bc}	C138	1.8 ± 1.3^{a}	80.0^{bc}
T6	C19	0.3 ± 0.8^{a}	13.3^{a}	C19	2.4 ± 1.8^{bc}	76.7^{bc}	C19	1.5 ± 1.6^{a}	60.0^{ab}

Values with the same letter in a column are not significantly different ($p < 0.05$) using ANOVA [[1] LSD test, $y = \log_{10}(x+1)$; [2] LSD test, $y = \arcsin\sqrt{x}$)].

index to susceptible plants grown in the non-disinfected soil.

For three consecutive years (Table 3), the incidence of nematode infection in plants grafted on C19 rootstock was similar to that in ungrafted plants cultivated in MB-disinfected soils, although there were significant variations between years both in ungrafted plants grown in the non-disinfected soil and in the MB-disinfected soil, or plants grafted on a different rootstock.

Pot experiment under controlled conditions

With the virulent MI-CH isolate, collected from resistant ATL, no galling was found on TER plants (Table 4). DRO behaved more or less similarly to TER. Nevertheless some non-viable egg masses were found on DRO (these egg masses were inoculated under controlled conditions in other DRO plants and these plants did not show any gall). C19 was moderately infested; significant differences being found between C19 and ATL in both galling index and egg mass number, but not in the percentage of plants infested. The R-rootstocks SK, C138 and ATL were highly infected, ATL behaving similarly to the susceptible cv. 'Sonar' as regards the gall index, percentage of infested plants and the number of egg masses per plant. The MI-CH isolate was virulent to ATL, which explained this result.

The MI-E isolate infested only the susceptible cv. 'Sonar' (Table 4) and was less aggressive than the virulent MI-CH isolate, which was responsible for a higher galling index and greater number of egg masses per plant.

Discussion

M. incognita has a great capacity to respond to environmental selection, even overcoming the known ge-

Table 4. Mean percentage of infected plants, gall index and mean number of egg masses per plant in six resistant pepper rootstocks and the susceptible cv. 'Sonar' inoculated with 2000 J2 plant^{-1} of the avirulent (MI-E) or virulent (MI-CH) *M. incognita* population, 8 weeks after inoculation in 24 observations per combination

Plant material	MI-CH			MI-E		
	Gall index[1]	% infested plants[2]	N° egg masses/plant[2]	Gall index[1]	% a infested plants[2]	N° egg masses/plant[2]
TER	0.0 ± 0.0^{a}	0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0^{a}	0.0 ± 0.0^{a}
DRO	0.3 ± 0.8^{b}	16.6^{b}	0.7 ± 1.7^{b}	0.0 ± 0.0^{a}	0.0^{a}	0.0 ± 0.0^{a}
C19	1.1 ± 0.9^{c}	72.2^{c}	1.4 ± 1.7^{c}	0.0 ± 0.0^{a}	0.0^{a}	0.0 ± 0.0^{a}
C138	4.2 ± 0.7^{d}	100.0^{a}	60.5 ± 20.6^{d}	0.0 ± 0.0^{a}	0.0^{a}	0.0 ± 0.0^{a}
SK	4.8 ± 2.9^{de}	100.0^{a}	65.2 ± 53.7^{d}	0.0 ± 0.0^{a}	0.0^{a}	0.0 ± 0.0^{a}
ATL	6.3 ± 0.8^{f}	100.0^{a}	92.3 ± 19.9^{e}	0.0 ± 0.0^{a}	0.0^{a}	$0.0 \pm \pm 0.0^{a}$
Sonar	6.0 ± 1.0^{ef}	100.0^{a}	103.0 ± 39.7^{e}	4.2 ± 1.5^{b}	100.0^{b}	46.7 ± 26.3^{b}

[1] LSD test, $y = \log_{10}(x+1)$; [2] LSD test, $y = \arcsin\sqrt{x}$). Values with the same letter in a column are not significantly different ($p < 0.05$) using ANOVA.

netic resistance of some hosts (Castagnone-Sereno, 2006). The resistance response of pepper to *M. incognita* is expressed as cell necrosis and pitted tissues once juveniles have established in the roots, or as the partial or total inhibition of their multiplication once installed (Bleve-Zacheo *et al*., 1998; Pegard *et al*., 2005; Castagnone-Sereno, 2006).

The selection of populations that overcome resistance under natural conditions is favoured by the repeated monoculture of cultivars carrying resistance genes. This situation was replicated in the laboratory by infecting the roots of plants carrying the *Me3* gene; under the continuous pressure of the resistant cultivar, resistance was overcome after 5 to 15 generations of the nematode (Castagnone-Sereno *et al*., 1996, 2001). Moreover, the response of the pepper cultivars carrying the gene *Me3* seemed to depend on the *Meloidogyne* population. This was confirmed with several laboratory-selected and natural *M. incognita* virulent isolates (Djian-Caporalino *et al*., 2011).

Under our experimental conditions, three successive years of growing plants grafted on the R-rootstock ATL were sufficient for the nematodes to overcome the resistance (Table 2). Starting with medium-high population densities in the soil, together with an 8 month growing period in the greenhouse, allowed several nematode generations to be produced. In previous experiments carried out under controlled conditions, Robertson *et al*. (2006) found that the "Capino pepper" population of *M. incognita* present in Campo of Cartagena is virulent to ATL as is the population MI-CH.

Five R-rootstocks (the six used in this experiment except ATL), showed a significant reduction of the number of galls on their root systems compared to non grafted peppers, after one year of greenhouse experiment in a soil where a population virulent towards ATL multiplied. TER, DRO and C19 showed the greatest possibility of being suppressed. However, differences were noticed among the five rootstocks after a second year of cultivation: SK and C19 allowed the multiplication of the virulent MI-CH population, while TER remained resistant. Under controlled conditions, the virulent population that multiplied on ATL was able to multiply easily on SK and C138.

Previous work by Castagnone-Sereno *et al.* (2001) and Djian-Caporalino *et al.* (2011) demonstrated that selection of virulence was highly specific, as naturally occurring and laboratory selected *Mi*-virulent nematodes to resistant tomato were unable to multiply on resistant peppers carrying the *Me3* (= *Me7*) resistance gene and viceversa. The *Me1* gene showed itself to be robust, as none of the virulent populations against *Mi*, and *Me3* (= *Me7*) multiplied on peppers containing the *Me1* resistance gene.

Based on this specificity of virulence, the results here suggest that (i) TER carries a robust RKN R-gene(s), different from that carried by ATL, and (ii) C19, SK, C138 and ATL carry the same R-gene which can be overcome. DRO could be nearly as robust as TER, and different from ATL R-gene, considering the laboratory experiment, but it was used during only one year in greenhouse experiment. The variability observed among C19, SK, C138 and ATL in the infection and reproduction of the virulent RKN population could be attributed to the pepper genetic backgrounds, because they did not originate from the same progenitors even if the R-gene in these plants was the same. It was suggested by Jacquet *et al*. (2005) when the *Mi* R-gene was introgressed into different tomato genetic backgrounds: the varying genetic backgrounds had a major effect on the variations observed in nematode reproduction. Nevertheless, to confirm this hypothesis, the progenitors of the pepper rootstocks should be defined in order to determine which R-gene has been introgressed.

For the proper management of resistance in controlling *M. incognita* for the cultivation of pepper, the frequency of distribution of the virulent populations towards R-genes and the time or number of successive crops that is necessary for the selection of virulent populations with an incidence above acceptable levels must be known, since not all of the natural populations of *M. incognita* in greenhouses in south-east Spain are equally susceptible to virulence selection. In tomato protected crops, Talavera *et al*. (2009) proposed crop rotation with *Mi* gene resistant and susceptible cultivars as a strategy for the management of root-knot nematodes.

The results of this research on rootstocks challenged with different nematode populations should allow the design of novel management strategies such as the alternation of R-genes based on the specificity of virulence. By avoiding the successive use of the same R-gene, the occurrence of virulent population of nematodes could be prevented.

Acknowledgements

The study was supported by projects INIA RTA2005-00209 and RTA2009-0058. The authors are

grateful to the Plant Pathology Group of IMIDA, to the Federation of Agrarian Cooperatives of Murcia and the Murcia Regional Department of Agriculture and Water for collaboration in the greenhouse experiments.

References

Bello A, Escuer M, Sanz R, López-Pérez JA, Guirao P, 1997. Biofumigación, nematodos y bromuro de metilo en el cultivo de pimiento. In: Posibilidad de alternativas viables al bromuro de metilo en pimiento de invernadero (López A, Mora JA, eds). Consejería de Medioambiente, Agricultura y Agua, Murcia, España. pp: 67-108.

Bello A, López-Pérez JA, García Álvarez A, Arcos, SC, Ros C, Guerrero MM, Guirao P, Lacasa A, 2004. Biofumigación con solarización para el control de nematodos en cultivo de pimiento. In: Desinfección de suelos en invernaderos de pimiento (Lacasa A, Guerrero MM, Oncina M, Mora JA, eds). Consejería de Agricultura, Agua y Medio Ambiente. Región de Murcia, España. pp: 129-208.l

Bleve-Zacheo T, Bongiovanni M, Melillo MT, Castagnone-Sereno P, 1998. The pepper resistance genes *Me1* and *Me3* induce differential penetration rates and temporal sequences of root cell ultrastructural changes upon nematode infection. Plant Sci 133: 79-90.

Bridge JS, Page LJ, 1980. Estimation of root-knot nematodes infestation levels on roots using a rating chart. Trop Pest Manage 26: 296-298.

Castagnone-Sereno P, 1999. Limites de l'utilisation de la résistance aux nématodes à galles chez la tomate. Des risques nouveaux liés au développement de populations virulentes du parasite. Phytoma 522: 61-63.

Castagnone-Sereno P, 2006. Genetic variability and adaptive in parthenogenetic root-knot nematodes. Heredity 96: 282-289.

Castagnone-Sereno P, 2012. *Meloidogyne enterolobii* (= *M. mayaguensis*): profile of an emerging, highly pathogenic, root-knot nematode species. Nematology 14: 133-138.

Castagnone-Sereno P, Piotte C, Abad P, Bongiovanni M, Dalmasso A, 1991. Isolation of a repeated DNA probe showing polymorphism among *Meloidogyne incognita* populations. J Nematol 23(3): 316-320.

Castagnone-Sereno P, Bongiovanni M, Palloix A, Dalmasso A, 1996. Selection for *Meloidogyne incognita* virulence against resistance genes from tomato and pepper and specificity of the virulence/resistance determinants. Eur J Plant Pathol 102: 585-590.

Castagnone-Sereno P, Bongiovanni M, Dijan-Caporalino C, 2001. New data of the root-knot nematode resistance genes *Me1* and *Me3* in pepper. Plant Breeding 120: 429-433.

Djian-Caporalino C, Pijarowski L, Januel A, Lefebvre V, Daubeze A, Palloix A, Dalmasso A, Abad P, 1999. Spectrum of resistance to root-knot nematodes and inheritance of heat-stable resistance in pepper (*Capsicum annuum* L.). Theor Appl Genet 99: 496-502.

Djian-Caporalino C, Berthou F, Fazari A, Lefebvre A, Palloix A, Pergard A, Pijarowski, L, 2004. Genetic, cytological and molecular bases of the resistance to root-knot nematodes (*Meloidogyne spp*.) in pepper (*Capsicum annum* L). Proc 12th Eucarpia Meeting on Genetics and Breeding of Capsicum and Eggplants, 17-19 May, Noordwijkerhout, The Netherlands. p: 180.

Djian-Caporalino C, Fazari A, Arguel MJ, Vemie T, Vande-Casteele C, Faure I, Brunoud G, Pijarowski L, Palloix A, Lefebvre V, Abad P, 2007. Root-knot nematode (*Meloidogyne* spp.) *Me* resistance genes in pepper (*Capsicum annum* L.) are clusted on P9 chromosome. Theor Appl Genet 114: 473-486.

Djian-Caporalino C, Molinari S, Palloix A, Ciancio A, Fazari A, Marteu,N, Ris N, Castagnone-Sereno P, 2011. The reproductive potential of the root-knot nematode *Meloidogyne incognita* is affected by selection for virulence against major resistance genes from tomato and pepper. Eur J Plant Pathol 31: 431-440.

Fazari A, Palloix A, Wang LW, Hua MY, Sage-Palloix AM, Zhang BX, Djian-Caporalino C, 2012. The root-knot nematode resistance N-gene co-localizes in the Me-genes cluster on the pepper (*Capsicum annuum* L.) P9 chromosome. Plant Breeding 131 (5): 665-673.

Fery RL, Dukes PD, Thies JA, 1998. "Carolina Wonder" and "Charleston Belle": Southern root-knot nematode resistant bell peppers. HortScience 33: 900-902.

Flegg JJM, 1967. Extraction of *Xiphinema* and *Longidorus* species from soil by modification of Cobb's decanting sieving technique. Ann Appl Biol 60: 429-437.

Giné A, Bonmatí M, Sarro A, Stchiegel M, Valero J, Ornat C, Fernández C, Sorribas FJ, 2013. Natural occurrence of fungal egg parasites of root-knot nematodes, *Meloidogyne* spp. in organic and integrated vegetable production systems in Spain. BioControl 58: 407-416.

Goodey JB, Franklin MT, Hooper DJ, 1965. The nematode parasites of plant catalogued under their host. Commonwealth Agricultural Bureaux, Farnham Royal, UK. 214 pp.

Hartman KM, Sasser JN, 1985. Identification of *Meloidogyne* species on the basis of differential host test and perineal pattern morphology. In: Advanced treatise on Meloidogyne, Vol II, Methodology (Barker KR, Carter CC, Sasser, JN, eds). North Carolina State University Graphics, Raleigh, NC, USA. pp: 69-77.

Hendy H, Pochard E, Damalso A, 1985. Transmission héréditaire de la résistance aux nématodes *Meloidogyne* Chitwood (Tylenchida) portée par 2 lignés de *Capsicum Nahum* L.: étude de descendances homozygotes sigues d'androgenése. Agronomie 5(2): 93-100.

Hussey RS, Barker KR, 1973. A comparison of methods of collecting inocula on *Meloidogyne spp*. including a new technique. Plant Dis Rep 57: 1025-1028.

Jacquet M, Bongiovanni M, Martínez M, Verschave P, Wajnberg E, Castagnone-Sereno P, 2005. Variation in resistance to the root-knot nematode *Meloidogyne incognita* in tomato genotypes bearing *Mi* gene. Plant Pathol 54: 93-99.

Lamberti F, 1981. Plant nematode problems in the Mediterranean region. Helmintol Abstr Ser B Plant Nematol CAB 50: 145-166.

López-Pérez JA, Escuer M, Díez-Rojo M, Robertson L, Piedra-Buena A, López-Cepero J, Bello A, 2011. Host range of *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (nematoda: Meloidogynae) in Spain. Nematropica 41(1): 130-139.

Omwega C O, Thomason IJ, Roberts PA, 1988. A nondestructive technique for screening bean germ plasm for resistance to *Meloidogyne incognita.* Plant Dis 72: 970-972.

Ornat C, Verdejo-Lucas S, Sorribas FJ, 2001. A population of *Meloidogyne javanica* in Spain virulent to the *Mi* resistance gene in tomato. Plant Dis 85(3): 271-276.

Ozarslandan A, Devran Z , Mutlu N, 2009. First report of Columbia root-knot nematode (*Meloidogyne chitwoodi*) in potato in Turkey. Plant Dis 93: 3.

Pegard A, Brizzard G, Fazari A, Soucaze O, Abad P, Djian-Caporalino C, 2005. Histological characterization of resistance to different root-knot nematode species related to phenolics accumulation in *Capsicum annuum.* Phytopathology 95: 158-165.

Robertson L, López-Pérez JA, Bello A, Díez-Rojo MA, Escuer M, Piedra-Buena A, Ros C, Martínez C, 2006. Characterization of *Meloidogyne incognita, M. arenaria* and *M hapla* populations from Spain and Uruguay parasitizing pepper (*Capsicum annuum*). Crop Prot 25: 440-445.

Ros C, Guerrero MM, Lacasa A, Guirao P, González A, Bello A, López-Pérez JA, Martínez MA, 2004. El injerto en pimiento. Comportamiento de patrones frente a hongos y nematodos. In: Desinfección de suelos en invernaderos de pimiento (Lacasa A, Guerrero MM, Oncina M, Mora JA, eds). Consejería de Agricultura, Agua y Medio Ambiente. Región de Murcia, España. pp: 279-312.

Ros C, Guerrero MM, Martínez MA, Barceló N, Martínez MC, Rodríguez I, Guirao P, Bello A, Lacasa A, 2005. Resistant sweet pepper rootstocks integrated into the management of soilborne pathogens in greenhouse. Acta Hortic 698: 305-310.

Ros C, Guerrero MM, Martínez MA, Lacasa A, Bello A, 2006. Integrated management of *Meloidogyne* resistance in sweet pepper in greenhouses. Bull IOBC-WPRS 29(4): 319-324.

Sasser JN, Freckman DW, 1987. A world perspective on Nematology: the role of society. In: Vistas on Nematology (Veech JA, Dickerson DW, eds). Society of Nematologists, Hyattsville, MD, USA. pp: 7-14.

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.

Thies JA, Fery RL, 2002. Heat stability of resistance to souther root-knot in nematode in bell pepper genotypes homozygous and heterozygous for the N gene. J Am Soc Hortic Sci 127(3): 371-375.

Trudgill DL, Blok VC, 2001. Apomictic, polyphagous root-knot nematodes: exceptionally successful and damaging biotrophic root pathogens. Ann Rev Phytopathol 39: 53-77.

Tzortzakakis EA, Trudgill DL, Phillips MS, 1998. Evidence for a dosage effect of the *Mi* gene on partially virulent isolates of *Meloidogyne javanica.* J Nematol 30(1): 76-80.

Verdejo-Lucas S, Ornat C, Sorribas FJ, Stchiegel A, 2002. Species of rootknot nematodes and fungal egg parasites recovered from vegetables in Almería and Barcelona. J Nematol 34: 405-408.

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.

Verdejo-Lucas S, Talavera M, Andrés MF, 2012. Virulence response to the *Mi 1* gene of *Meloidogyne* populations from tomato in greenhouses. Crop Prot 39: 97-105.

Wesemael WML, Viaene N , Moens M, 2011. Root-knot nematodes (*Meloidogyne* spp.) in Europe. Nematology 13: 3-16.