The African Journal of Plant Science and Biotechnology ©2008 Global Science Books

Biological Characteristics of Two Populations of *Meloidogyne* spp. Virulent to the *Mi* Resistance Gene in Tomato Isolated from South Tunisia

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ABSTRACT

Two populations of the root knot nematode were analysed for their virulence against the single dominant gene *Mi* which controls resistance in commercially grown tomatoes. These populations were obtained from resistant tomato roots in a naturally infested plastics house at two locations in south Tunisia, Gabes and Kebilli. Identification of *Meloidogyne* species by morphological identification and izozyme electrophoresis showed the presence of two species, *M. javanica* and *M. incognita* at a 25%:75% ratio. Several resistant and susceptible tomato cultivars were used. The two populations were able to develop and to reproduce similarly on resistant and susceptible cultivars. The development rate (Pf/Pi) was generally greater on tomato genotypes heterozygous for the *Mi* gene than on homozygous genotypes.

Keywords: development rate, fecundity, *Meloidogyne incognita*, *Meloidogyne javanica*

INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) is the most important horticultural crop in Tunisia. This crop is well adapted to most Tunisian climatic conditions.

Root-knot nematodes (RKN) belonging to the genus *Meloidogyne* cause extensive damage to a wide variety of economically important crops (Sasser 1980). Among them, *M. incognita* (Kofoid & White) Chitwood, *M. javanica* (Treub) Chitwood and *M. arenaria* (Neal) Chitwood, are considered the three major species on the basis of both their worldwide geographical distribution and very large host range (Trudgill and Blok 2001). These nematodes (RKN) are among the main pathogens of tomato (Bleve-Zacheo *et al*. 2007). Several nematicides are used to control *Meloidogyne* on vegetable crops but their increasing environmental risk (Vermis and Robert 1996) has led to a search for other management methods.

The use of resistant cultivars that inhibit the reproduction of the parasite is the most efficient and environmentally safe method of controlling *Meloidogyne* spp.

Many commercial tomato varieties carry a single dominant gene *Mi* which confers resistance to three of the most damaging species of root knot nematodes *M. incognita*, *M. javanica* and *M. arenaria*. However the emergence of a virulent population able to overcome the tomato resistance gene may constitute a severe limitation to such a control strategy.

The occurrence of virulent populations of *Meloidogyne* has been reported in several Mediterranean countries in the last decade or so (Castagnone-Séréno *et al.* 1994; Eddaoudi *et al.* 1997; Ornat *et al.* 2001).

The objectives of this research were to study the biological characteristics of two populations of *Meloidogyne* causing severe damage to tomato roots in two localities in south Tunisia, Gabes and Kebilli.

MATERIALS AND METHODS

Identification of *Meloidogyne* species was established from perineal patterns and isoesterase zymograms (Karssen and Van Aelst 2001). Egg masses were collected and conserved in a 0.3 M NaCl solution.

Biological characteristics (hatching %, diapause, egg mortality, development rate) of *Meloidogyne* populations from Gabes and Kebilli were studied on two different cultivars ('Nemador', 'Rio Grande') and three F1 tomato hybrids ('Amel', 'Bochra', 'Sincara'). Plants were arranged in a randomized complete test design with five replicates for each nematode-plant combination.

Two weeks after germination, seedlings of the different cultivars were transplanted singly to 250 ml pots containing substrate (mixture of black and white peat, clay, 0.5% mineral nitrogen, 90% organic matter and water retention capacity of 60 to 70%). The experiment was carried out in controlled environment at a mean temperature of 27±1°C.

Egg masses collected were washed three times and allowed to hatch in distilled water. At the four-leaf stage, a water suspension containing 700 J2 was pipetted and added to the substrate surface around the stem base of every plant and lightly watered. The number of egg masses and eggs per plant were determined 8 weeks after infestation. The final population was evaluated by using the plant: For every plant inoculated we evaluated the final population (Pf) by counting the number of eggs in each mass formed thus allowing us to evaluate the development rate (Pf/Pi) since the initial population (Pi) (700 J2) is known.

The fecundity of females was evaluated by counting the number of eggs per mass; 30 egg masses were removed from each combination and assayed for the average number of eggs. Hatching was evaluated by counting the number of J2 every 48 hours under a stereoscopic microscope. On the 24th day, and after the end of hatching, egg masses were recuperated and treated with "Bleu of Meldola" for colouring dead eggs, in which those that were non-coloured were considered to be in diapause (de Guiran and Ritter 1979)

The development rate was calculated according to the following ratio: Pf/Pi (Viane and Abawi 1996). Evaluation of Pf/Pi was based on the knowledge of initial population inoculated (Pi) and

Received: 5 February, 2008. Accepted: 25 March, 2008.

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the final population by plant.

Data analysis. An analysis of variance (one-way ANOVA) and Duncan's multiple range test was performed to separate the mean number of egg masses, the fecundity and the development rate. All statistical analysis were conducted according the values of the tested variables means ($P \leq 0.05$) using SPSS 10.0 procedures.

RESULTS

Forty females of each population were identified, 20 according to the perineal patterns and the others according to isoesterase proteingrams. *M. incognita* and *M. javanica* were 75% and 25% present in the two localities, respectively.

Variation in egg mass number

The emergence of white egg masses on the outside of root tissues indicate that the biological cycle was completed. Females of *Meloidogyne* spp. of Gabes's population produced more eggs on susceptible tomato cultivar ('Rio Grande') than on resistant cultivars ('Nemador', 'Bochra', 'Sincara'). Concerning Kebilli's population, the number of egg masses was significantly higher on resistant cultivars 'Bochra' and 'Nemador' (**Fig. 1**).

Fecundity of two populations studied on different tomato cultivars

The fecundity of Gabes's population was significantly lower on 'Rio Grande' and 'Amel' than the other tomato cultivars ('Bochra', 'Nemador' and 'Sincara'). On the other hand, a significant difference was found between the fecundity of Kebilli's population on the resistant cultivar 'Nemador' and the other F1 tomato hybrids ('Bochra', 'Amel' and 'Sincara') and the susceptible cultivar ('Rio Grande') (**Fig. 2**).

Comparison of the fecundity of the two south populations showed that there is no difference between 'Amel', 'Bochra' and 'Sincara', however a significant difference was found for the others cultivars.

Variation of development rate of *Meloidogyne* spp. populations in different tomato cultivars

Development rate (Pf/Pi) varied according to the cultivar. In Gabes's population there was no significant difference in the Pf/Pi ratio between resistant and susceptible cultivars but there was a lower development rate than the Kebilli population on the different cultivars tested except for resistant cultivar 'Nemador' (**Fig. 3**).

DISCUSSION

Many studies showed that *M. incognita*, *M. javanica* and *M. arenaria* are the most widespread species of *Meloidogyne* in Mediterranean countries (Bleve-Zacheo *et al*. 2007).

We showed in this study the presence of *M. incognita* and *M. javanica*, two species which had been reported by B'Chir and Horrigue-Raouani (1991) and Horrigue-Raouani (2003) in other Tunisian populations and which focussed on other aspects such as histology and the plant-nematode interaction.

Tomato cultivars resistant to *Meloidogyne* have been used since 1980 in several Mediterranean countries and are useful to reduce the initial soil population (Philis 1990; Horrigue 1983) but the occurrence of virulent populations of *Meloidogyne* spp. able to overcome the resistant *Mi* gene have also been reported in several countries (USA, Morocco, Spain, Jordan, etc.) (B'Chir and Horrigue-Raouani 1991; Kaloshian *et al.* 1996; Eddaoudi *et al*. 1997; Ornat *et al.* 2001; Karajeh *et al*. 2005).

Two mechanisms may be proposed to explain such virulence. The first hypothesis involves a modification in the genotype of avirulent nematode in response to the selection pressure of resistance (Castagnone-Séréno *et al.* 1993;

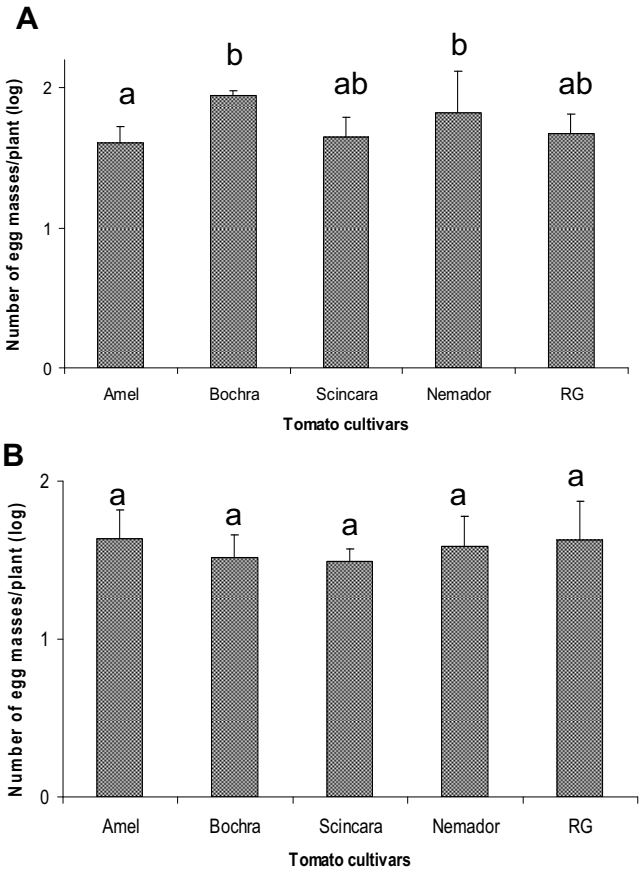

Fig. 1 Variation of the number of egg masses by plant in the localities of Kébilli (A) and Gabes (B).

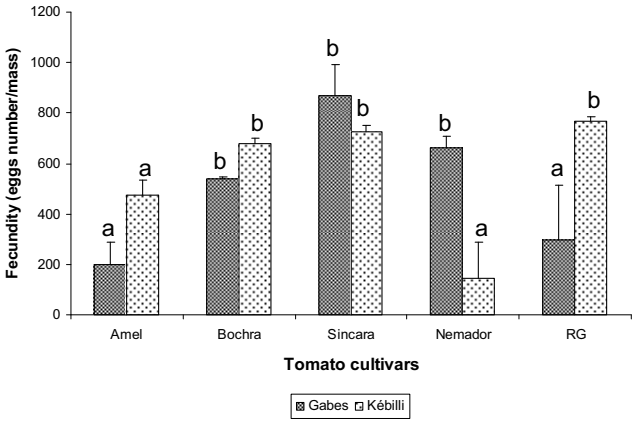

Fig. 2 Comparison of fecundity (eggs number/mass) of *Meloidogyne* spp. on different tomato cultivars.

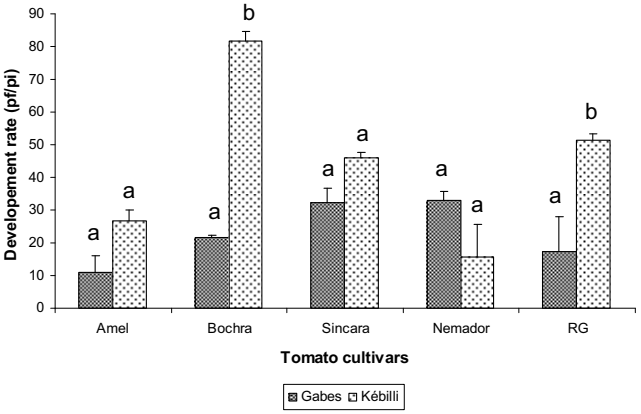

Fig. 3 Comparison of the development rate (Pf /Pi) of *Meloidogyne* spp. on different tomato cultivars.

Eddaoudi *et al.* 1997); the second is related to a gene mutation (Dalmasso *et al.* 1991; Kaloshian *et al.* 1996).

A negative correlation was observed between fecundity and egg mass number. The Kebilli population showed a consistently higher reproductive potential on the F1 tomato hybrid 'Bochra' and the resistant cultivar 'Nemador' than those from Gabes. These results are similar to those of Castagnone-Séréno *et al.* (1993) in that their study showed that the naturally virulent *Meloidogyne* population showed a higher rate of multiplication when maintained on the resistant rather than on the susceptible tomato cultivar. The virulent populations were able to reproduce on a susceptible cultivar 'Saint Pierre' but they showed the lowest development rate, although it was not quantified in that study.

M. incognita and *M. javanica* reproduce similarly or even higher on the resistant F1 tomato hybrid than on the resistant cultivar 'Nemador'. Virulent root knot population of *M. incognita* and *M. javanica* produces more galling and egg masses on heterozygous plants than on homozygous ones (Tzortzakakis *et al.* 1998; Jaquet *et al.* 2005). According to Jaquet *et al.* (2005) the tomato genetic background is a major influence in this variation. As a large proportion of modern tomato cultivar are F1 hybrids, with *Mi* being heterozygous, this could promote the selection of a virulent *Meloidogyne* biotype in field conditions.

The *Mi* resistant gene was efficient in the most agronomic situations but the emergence of virulent biotypes able to overcome the plant resistance gene may constitute a threat to the durability of plant resistance gene (Castagnone-Séréno 2002) and it appears quite obvious that additional management alternatives are needed especially the use of biological agents.

CONCLUSION

The two populations of *Meloidogyne* spp. which were isolated from severely affected resistant tomato cultivars, were able to break the resistance of the four resistant tomato cultivars tested. At a constant temperature of 27°C these populations could reproduce in four resistant cultivars ('Amel', 'Bochra', 'Sincara', 'Nemador') as well as on the susceptible cultivar 'Rio Grande'. It has been shown that a dosage effect of the *Mi* gene can occur (Jaquet *et al.* 2005) and, consequently, that some nematodes can reproduce on heterozygous tomato genotypes more than on homozygous ones. The results obtained are important for the planning and the design of other management alternatives integrating new genes of resistance to regulate populations and retard the development of virulence in field populations.

ACKNOWLEDGEMENTS

The authors thank the research unit 04 AGRO 4 (Invertebrates, Microorganisms, Weeds: alternative methods of control) for its financial contribution, as well as Wassila Hlaoua and Mejda Daami-Remadi for help and suggestions.

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