THE RESISTANCE RESPONSE OF *SOLANUM HUAYLASENSE* ACCESSION LA1358 TO *MELOIDOGYNE* SPP.

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ABSTRACT

Cortada, L., P. Manzano, F. J. Sorribas, C. Ornat, and S. Verdejo-Lucas. 2010. The resistance response of *Solanum huaylasense* accession LA1358 to *Meloidogyne* spp. Nematropica 40:31-40.

Pathogenicity tests were performed to determine resistance in the wild tomato *Solanum huaylasense* to four populations of *Meloidogyne* spp. The *S. huaylasense* accession LA1358, the root-knot nematode resistant tomato cultivar 'Anairis' and the susceptible tomato cultivar 'Bodar' were assessed against three *Mi*-avirulent populations of either *Meloidogyne arenaria*, *M. incognita* or *M. javanica*, and to one naturally *Mi*-virulent population of *M. javanica*. The relationship between the final and initial population, calculated as the number of eggs per plant (final population) divided by the initial juvenile inoculum, was used as the dependent variable to determine variability in the reproduction of the four *Meloidogyne* populations tested. Reproduction of *M. arenaria* was similar on *S. huaylasense* accession LA1358 and cultivar 'Anairis'. Reproduction of *M. incognita* on accession LA1358 did not differ statistically from the resistant or susceptible tomato cultivars. Reproduction of the *Mi*-avirulent population of *M. javanica* on accession LA1358 did not differ from the susceptible cultivar 'Bodar', and the *Mi*-virulent *M. javanica* population reproduced similarly on accession LA1358 and both cultivars. This is the first report on a nematode-species specific resistance in the newly described species of *S. huaylasense*. Identification of novel root-knot nematode resistance genes in wild *Solanum* species is the first step for the deployment of new resistance genes in tomato cultivars to preserve durability of plant resistance to root-knot nematodes.

Key words: *Mi* gene, *Mi*-homologues, resistance genes, root-knot nematodes, *Solanum* spp., wild tomatoes.

RESUMEN

Cortada, L., P. Manzano, F. J. Sorribas, C. Ornat, and S. Verdejo-Lucas. 2010. La respuesta de resistencia de la variedad LA1358 de la especie de tomate silvestre *Solanum huaylasense* a *Meloidogyne* spp. Nematropica 40:31-40.

Se realizaron pruebas de patogenicidad con el fin de determinar la resistencia del tomate silvestre *Solanum huaylasense* a cuatro poblaciones de *Meloidogyne* spp. Se evaluó la variedad LA1358 de *S. huaylasense*, el cultivar resistente al nematodo 'Anaris' y el cultivar susceptible 'Bodar' frente a tres poblaciones avirulentas para el gen *Mi* de *Meloidogyne arenaria*, *M. incognita* o *M. javanica* y una población naturalmente virulenta de *M. javanica*. La relación entre la población final y la inicial, calculada como el número de huevos por planta (población final) dividido por el inóculo inicial de juveniles, se empleó como variable dependiente para determinar la variabilidad en la reproducción de las cuatro poblaciones de *Meloidogyne* ensayadas. La reproducción de *M. arenaria* fue similar en la variedad LA1358 de *S. huaylasense* y en el cultivar resistente 'Anairis'. La reproducción de *M. incognita* en la variedad LA1358 no difirió estadísticamente de la alcanzada en los cultivares de tomate resistente o susceptible; la reproducción de la población *Mi*-aviruleta de *M. javanica* en la variedad LA1358 no difirió de la reproducción en el cultivar susceptible 'Bodar' y la población *Mi*-virulenta de *M. javanica* se reprodujo por igual en la variedad LA1358 y en ambos cultivares de tomate. En este trabajo se describe por

primera vez la resistencia especie-específica de *Solanum huaylasense* a *Meloidogyne*. La identificación de nuevos genes de resistencia en tomates silvestres es el primer paso para la agrupación piramidal de dichos genes en cultivares de tomate con la finalidad de preservar la durabilidad de la resistencia frente al nematodo.

Palabras clave: gen *Mi*; genes de resistencia; homólogos *Mi*, *Solanum* spp., nematodos agalladores, tomates silvestres.

INTRODUCTION

Root-knot nematodes, *Meloidogyne* spp., are important pathogens that cause millions of dollars in economic losses in agriculture worldwide (Sasser and Freckman, 1987). In tomato (*Solanum lycopersicum* Mill.), the *Mi-1* gene confers resistance to *Meloidogyne arenaria* (Neal) Chitwood, *M. incognita* (Kafoid and White) Chitwood and *M. javanica* (Treub) Chitwood (Roberts and Thomason, 1989). This gene was first identified in *Solanum peruvianum* L. accession PI 128657 in the 1940s and introduced through embryo rescue in the commercial *S. lycopersicum* (Smith, 1944). Many commercial cultivars possess the *Mi-1* gene which is referred to as *Mi* in this text. The *Mi* mediated resistance is phenotypically expressed only when soil temperature is below 28°C (Dropkin, 1969). The resistance conferred by the *Mi* gene reduces nematode reproduction; however, variability in the efficiency of the resistant cultivars to reduce reproduction by various *Meloidogyne* populations has been reported (Netscher, 1976; Roberts and Thomason, 1989; Tzortzakakis *et al.*, 1998). Despite these limitations, plants carrying the *Mi* gene are considered a reliable, economical and environmentally friendly method to control *Meloidogyne* spp. in infested fields (Sorribas *et al.*, 2005). Moreover, specific rotation sequences with resistant tomato cultivars have been suggested to prevent the emergence of virulent nematode populations (Talavera *et al.*, 2009).

The use of a single genetic source of resistance (*R*-genes) in monoculture can lead to the defeating of valuable resistance genes (Pedersen and Leath, 1988). Race-specific resistance associated to monogenic genes that provide a hypersensitive response has proved to be non-durable (Lindhout, 2002). Despite the fact that *Mi*-mediated resistance in tomato has remained durable for a long time (Roberts, 1995; Castagnone-Sereno, 2002) virulent root-knot nematode populations has been reported after repeated exposure to the *Mi* gene in agricultural fields (Kaloshian *et al.*, 1996; Eddaoudi *et al.*, 1997; Xu *et al.*, 2001; Tzorzakakis *et al.*, 2005; Verdejo-Lucas *et al.*, 2009). In this sense, gene pyramiding has been proposed as a strategy to reduce the chances of appearance of virulent populations and to preserve the durability of plant resistance (Pink, 2002). Simultaneous deployment of *R*-genes has been implemented to control nematodes (Sacks and Robinson, 2009), bacteria (Kousik and Ritchie, 1999; Singh *et al.*, 2001), virus (Pérez de Castro *et al.*, 2008; Vidavsky *et al.*, 2008) or fungi (Richardson *et al.*, 2006).

Wild *Solanum* species have been widely explored as a source of new *R*-genes to control root-knot nematodes in tomato. Several *Mi*-homologues (from *Mi-2* to *Mi-9*), that present resistance either to virulent nematode populations or to high temperatures have been found in accessions of the species that integrate the *Solanum peruvianum* Marañón complex (Ammati *et al.*, 1986; Cap *et al.*, 1993; Yaghoobi *et al.*, 1995;

Veremis and Roberts 1996a, 1996b, 2000; Jablonska *et al.*, 2007). This complex has been recently divided into four new species (Peralta *et al.*, 2005; Zuriaga *et al.*, 2008): *S. arcanum* Peralta (formerly *S. peruvianum*), *S. corneliomulleri* J. F. Macbr. (formerly *S. glandulosum*), *S. huaylasense* Peralta, and *S. peruvianum* L. s.str. Both, *S. peruvianum* and *S. arcanum* have provided most of the *R*-genes to root-knot nematodes (Ammati *et al.*, 1986; Veremis and Roberts, 1996a, 1996b). However, no information is available about the spectrum of resistance of *S. cornelioulleri* and *S. hualyasense* to root-knot nematodes. Only resistance to the fungi *Alternaria solani* Sorauer and *A. tomatophila* Simmons has been described in *S. huaylasense* (Foolad *et al.*, 2007). Therefore, identification and characterization of new nematode *R*-genes would be the first step towards the use of "pyramided" cultivars, with the aim to preserve plant resistance on a long term basis. The objective of the present work was to determine the response of the wild tomato *S. huaylasense* accession LA1358 to three *Mi*-avirulent populations of *M. arenaria, M. incognita* and *M. javanica* and one naturally *Mi*-virulent population of *M. javanica.*

MATERIALS AND METHODS

Nematode Screens

Seeds of the tomato cultivars were germinated in seedling trays filled with the organic planting Germination Mix #3 (Sun Gro Horticulture; Bellevue, WA, USA). Tomato cultivars used as controls were 'Anairis' (De Ruiter Seeds, highly resistant to *M. arenaria, M. incognita*, and *M. javanica* according to the seed company) and the susceptible 'Bodar' (Seminis Royal Sluis). Cuttings from the wild tomato *S. huaylasense* accession LA1358 were treated with Inavarplant-IV growing hormone (3-indolbutiric acid at 0.4%; 1-naphthaleneacetic acid at 0.4%; Captan 15%; Inbar) and rooted in seedling trays containing vermiculite. Cuttings were maintained in a growth chamber at 25 ± 1.5°C until new leaves were produced. Five-week-old rooted cuttings and four-week-old plantlets of the tomato cultivars at the three-true-leaves stage were transplanted singly into 500 cm^3 pots containing a mixture of steam-sterilized river sand and peat (v/v) and used for nematode assays a week later.

Four *Meloidogyne* populations were used for nematode screening: *Mi*-avirulent population of *M. arenaria* (MA-68), *M. incognita* (MI-CROSS) and *M. javanica* (MJ-05), and one *Mi*-virulent population of *M. javanica* (MJ-27). The *Mi* (a)virulent condition of each population had been reported (Ornat *et al.*, 2001; Cortada *et al.*, 2009). Species identification of each *Meloidogyne* population was confirmed by SCAR-PCR reaction (Ziljstra *et al.*, 2000). Nematode inoculum consisted of eggs obtained from infected tomato plants 'Roma' maintained in a glasshouse. Roots were macerated by grinding in a food blender with a 0.5% NaOCl solution for 30 sec (Bonetti and Ferraz, 1981), and the suspension was left to settle for 5 min. Macerated roots were passed through a 74 µm sieve to remove root debris, and the dispersed eggs were collected on a 25 µm sieve. Infective second-stage juveniles (J2) were obtained from hatched eggs as described by Martínez de Ilarduya and Kaloshian (2001) and 72-hour-old J2 used as inoculum. Each plant was inoculated with 130 J2. Every cultivar-nematode population combination was replicated 12 times whereas the accession LA1358 was replicated 10 times, due to the insufficient number of size-homogeneous cuttings. Pots were distributed at random in a growth chamber and maintained at 25 ± 1.5°C; temperatures were registered daily at 30 min intervals by placing temperature

probes (Testostor® 171-4, TESTO, Spain) into the potted soil. The mean daily soil temperature was calculated as the maximum plus the minimum temperatures divided by two. The number of degree-days accumulated by *M. javanica* was calculated using a base temperature of 13°C and 343°C as the minimum thermal time requirement for one generation (Tzortzakakis and Trudgill, 1996). At the end of the tests, the nematode had accumulated 486° DD which indicated the completion of its life cycle. A slow release coated fertilizer (NPK 17+ 11+ 10 + 2MgO + TE, Osmocote® Pro 3-4M, The Scotts Company, The Netherlands) was added to the surface of the potted soil at about 3 g per plant after transplanting. At the end of the experiment, plants were cut at ground level and the root systems washed free of soil and weighed. Eggs were extracted from the entire root system as described previously and they were quantified under a compound microscope at 40X. The number of eggs per plant represented the final nematode population (Pf). The Pf/Pi relationship was then calculated as the number of eggs per plant (Pf) divided by the initial J2 inoculum (Pi).

Statistical analysis

Statistical analyses were performed using Statistica software (StatSoft, Inc., 2004). Fresh root weight of *S. huaylasense* accession LA1358 and of the cultivars 'Anairis' and 'Bodar' were compared by analysis of variance (factors: nematode population and tomato variety) to detect differences between *S. huaylasense* cuttings and the cultivars. The Pf/Pi was fourth root transformed to comply with test assumptions and then subjected to factorial ANOVA, where nematode population and tomato variety (accession and cultivars) were included as factors. For each nematode population, the post-hoc Tukey test procedure was used to determine differences in nematode reproduction on *S. huaylasense* accession LA1358 and on the resistant or susceptible tomato cultivars. The reproduction index (RI) was calculated as the number of eggs per plant on the *S. huaylasense* accession LA1358 or on the resistant cultivar divided by the number of eggs per plant on the susceptible cultivar x100; the level of resistance was categorized as highly resistant (RI< 10%), intermediate resistant (10% ≤ RI < 25%) or moderately resistant (25% ≤ RI < 50%) (Hadisoeganda and Sasser, 1982).

RESULTS

The fresh root weight of *S. huaylasense* accession LA1358 differed from that of both tomato cultivars ($F_{(6, 121)} = 5.384$; $P < 0.001$) (Fig. 1). The accession and the tomato cultivars showed a differential reproduction across the four different nematode populations tested ($F_{(6,121)} = 3.468$; $P = 0.003$) (Fig. 2). The four popula-

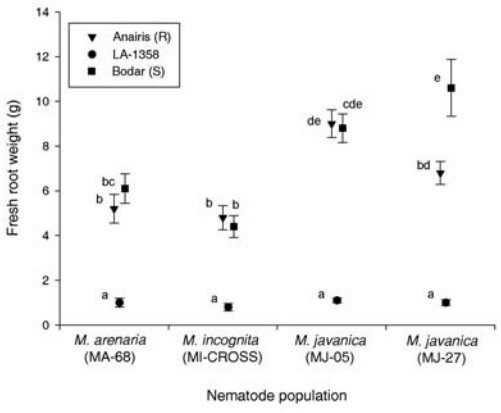

Fig. 1. Mean fresh root weight (g) of *Solanum huaylasense* accession LA1358, resistant tomato cultivar 'Anairis' and susceptible tomato cultivar 'Bodar' six weeks after nematode inoculation with four *Meloidogyne* populations. Different letters indicate significant differences (HSD test). Error bars depict the standard error of the mean.

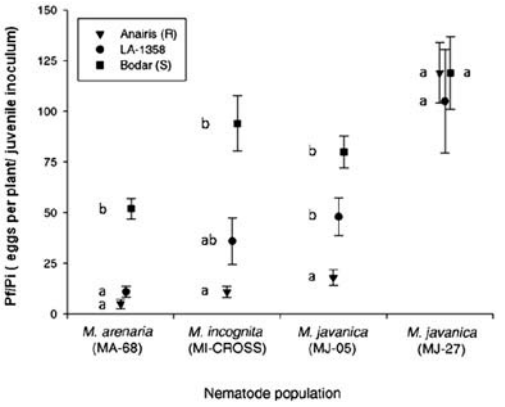

Fig. 2. Pf/Pi values (number of egg per plant/ initial juvenile inoculum) for four *Meloidogyne* populations on *Solanum huaylasense* accession LA1358, resistant tomato cultivar 'Anairis' and susceptible tomato cultivar 'Bodar' six weeks after nematode inoculation. For each nematode species, values in the same column followed by different lower-case letters are significantly different according to Tukey's studentized range test ($P < 0.05$). Error bars depict the standard error of the mean.

tions of *Meloidogyne* reproduced at a similar level on the susceptible cultivar 'Bodar'. The *S. huaylasense* accession LA1358 was considered resistant when the Pf/Pi values did not differ statistically from the resistant cultivar 'Anairis'. The Pf/Pi values were significantly lower on the resistant cultivar 'Anairis' than on the susceptible 'Bodar' for *M. arenaria* MA-68 ($P < 0.001$), *M. incognita* MI-CROSS ($P < 0.01$) and *M. javanica* MJ-05 ($P < 0.01$) (Fig. 2), thus confirming the avirulent character of these populations to *Mi* gene. The Pf/Pi value of *M. arenaria* MA-68 on *S. huaylasense* accession LA1358 was similar ($P = 0.07$) to that on the resistant cultivar 'Anairis', and lower ($P < 0.001$) than on the susceptible 'Bodar' (Fig. 2). Reproduction of *M. incognita* MI-CROSS did not differ statistically from the resistant 'Anairis' ($P = 0.70$) or the susceptible 'Bodar' ($P = 0.13$) (Fig. 2). Reproduction of *M. javanica* MJ-05 was higher ($P = 0.02$) on the *S. huaylasense* accession LA1358 than on 'Anairis', but did not differ statistically from the reproduction on 'Bodar' ($P = 0.07$) (Fig. 2). As expected, the *Mi* virulent population *M. javanica* MJ-27 reproduced similarly on the *S. huaylasense* accession LA1358 and both tomato cultivars ($P > 0.60$) (Fig. 2). According to the reproduction index, *S. huaylasense* accession LA1358 expressed intermediate resistance to *M. arenaria* MA-68 (RI= 20%), moderate resistance to *M. incognita* MI-CROSS (RI = 38%), and susceptibility to both *M. javanica* populations (RI > 50%) (Table 1). The resistant control 'Anairis' was highly resistant (RI = 10%) to *M. arenaria* MA-68, and expressed intermediate resistant to *M. incognita* MI-CROSS (RI = 12%) and *M. javanica* MJ-05 (RI= 22%) (Table 1).

DISCUSSION

The four *Meloidogyne* populations produced similar Pf/Pi values on the susceptible cultivar 'Bodar' indicating that the tested populations had comparable reproduction ability in absence of the *Mi* gene. Resistance to root-knot nematodes in tomato has been generally characterized as the failure of the nematodes to induce a feeding site in the host after infection, develop and reproduce successfully (Williamson and Kumar, 2006). As the Pf/Pi value for *M. arenaria* MA-68 on accession LA1358 did not differ statistically from the one on the resistant 'Anairis' but was lower than on the susceptible 'Bodar', it appears that the accession LA1358 has at least one of the mechanisms involved in the *Mi* gene mediated resistant response. A similar situation was observed with *M. incognita* MI-CROSS but to a lesser degree. Reproduction of the *Mi*-virulent *M. javanica* MJ-27 was similar on the accession and both cultivars, confirming that when a compatible interaction occurs between a *Mi* virulent

Table 1. Reproduction index of *Meloidogyne arenaria*, *M. incognita* and *M. javanica* on *Solanum huaylasense* accession LA1358 and on the resistant tomato cultivar 'Anairis' with respect to the susceptible tomato cultivar 'Bodar' six weeks after nematode inoculation.

Population	*Mi* gene virulence status	Tomato	Reproduction index [z]	Response
M. arenaria (MA-68)	Avirulent	LA-1358	20 ± 16	Intermediate resistant
		'Anairis' (R)	10 ± 15	Highly resistant
		'Bodar' (S)	100 ± 34	
M. incognita (MI-CROSS)	Avirulent	LA-1358	38 ± 39	Moderately resistant
		'Anairis' (R)	12 ± 10	Intermediate resistant
		'Bodar' (S)	100 ± 44	
M. javanica (MJ-05)	Avirulent	LA-1358	60 ± 37	Susceptible
		'Anairis' (R)	22 ± 17	Intermediate resistant
		'Bodar' (S)	100 ± 34	
M. javanica (MJ-27)	Virulent	LA-1358	88 ± 61	Susceptible
		'Anairis' (R)	100 ± 44	Susceptible
		'Bodar' (S)	100 ± 52	

[z]number of eggs per plant on the *S. huaylasense* accession LA1358 or the resistant cultivar divided by the number of eggs per plant on the susceptible cultivar x100. For each nematode-plant combination, valid n = 12, except for *S. huaylasense* accession LA1358, where valid n = 10.

nematode and a resistant plant, no differences in nematode reproduction occurs between resistant and susceptible cultivars (Ornat *et al.*, 2001).

Differences in fresh root weight between the accession LA1358 and the cultivars can be mostly attributed to the wild origin of *S. huaylasense* that showed poor vigor and a herbaceous nature (Peralta *et al.*, 2005) in contrast with the F1 hybrid commercial tomato cultivars used here that provided vigorous plants. The wild tomato accession originates from rocky slopes at a 800 m altitude in a very dry area of north Peru (web site; http://tgrc.ucdavis.edu). Tomato is usually propagated by seeds but we used rooted cuttings of the accession LA 1358 because of the scarcity and low germinations rate of these seeds. Although cuttings provided lower root biomass than the seeds of the cultivars, they allowed multiplication of the *Mi*-virulent population of *M. javanica* MJ-27 at levels similar to those reached on the resistant and susceptible cultivars.

From the species formerly included in the *S. peruvianum* Marañón complex, *S. huaylasense* is the closest to *S. peruvianum* according to molecular data (Moyle, 2008). The fact that both species derive from an original *Solanum* ancestor, supports the hypothesis that all of them share a common pool of *Mi*-homologues (Seah *et al.*, 2007) and hence, it would not be surprising to find a resistant *Mi*-homolog in *S. huaylasense*. Specificity in the resistance response of some *Mi*-homologues has been reported in other wild tomato species from the *Solanum* genus, whose *Mi*-homologues were phenotypically variable according to

the *Meloidogyne* isolate tested (Roberts *et al.*, 1990; Huang *et al.*, 2004). Similar results have been reported for the tomato hybrid rootstocks Beaufort and Maxifort (Cortada *et al.*, 2009). This mechanism has also been observed for other nematode resistance genes (*Nem-R* genes) that can be found on soybean [*Glycine max* (L.) Merr.] (Luzzi *et al.*, 1987), tobacco (*Nicotiana tabacum* L.) (Bowman and Tart, 1990; Noe, 1992), or pepper (*Capsicum annum* L.) (Castagnone-Sereno *et al.*, 2001), and for other families of *R*-genes for resistance to pathogens different from nematodes (i.e. *Ty*-homologues) (Pérez de Castro *et al.*, 2008). Even within tomato cultivars, the resistance response of the *Mi* gene is phenotypically variable (Roberts and Thomason, 1989; Jaquet *et al.*, 2005). Understanding this phenomenon will be necessary for the successful transfer of new root-knot nematode *R*-genes into cultivated tomato although the main obstacle to obtain new resistant hybrids is the incompatibility between the germplasms of wild *Solanum* species and cultivated tomato (Veremis and Roberts, 2000; Ammiraju *et al.*, 2003). Transference of the *Mi*-resistance gene to susceptible tomato plants has been achieved using transgenic techniques (Goggin *et al.*, 2006; Williamson and Kumar, 2006). So far, it seems that the most feasible way to overcome germplasm incompatibility to put new resistance genes from wild *Solanum* species at disposal of new tomato cultivars is the use of tomato hybrid rootstocks (*S. lycopersicum* × *Solanum* spp.) (Santos *et al.*, 2004).

The identification of new root-knot nematode *R*-genes in tomato opens a door to preserve the efficiency of the *Mi*-mediated resistance through combination of different *Mi*-homologues into one single genotype. This is the first step towards the identification of *S. huaylasense* as a new source of root-knot nematode *R*-genes. Since only one *Mi*-avirulent population of *M. arenaria*, *M. incognita* and *M. javanica* was tested, it cannot be asserted that the resistance response of accession LA1358 is nematode-isolate specific. Although all races of *Meloidogyne* reproduce on tomato, it will be necessary to determine the resistant response of *S. huaylasense* to the different races of the nematode to ascertain that the broad resistance detected in this study is or is not race-specific. This information is also important for rotational purposes. In addition, populations from several geographic origins and their response under different agronomic and environmental conditions (i.e. high temperatures) should be tested. Comprehension of the species-specific resistance response of the *S. huaylasense* accession LA1358 will also provide insights into host mechanisms underlying specific plant-nematode interactions.

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