RESEARCH/INVESTIGACIÓN

EVALUATION OF SOURCES OF RESISTANCE TO *MELOIDOGYNE ENTEROLOBII* IN *SOLANUM STRAMONIFOLIUM* AND *S. SCUTICUM* AS POTENTIAL ROOTSTOCKS FOR CULTIVATED SOLANACEAE

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ABSTRACT

Pinheiro J. B., G. O. da Silva, A. G. Macedo, D. Biscaia, and R. A. de Castro e Melo. 2020. Evaluation of sources of resistance to *Meloidogyne enterolobii* in *Solanum stramonifolium* and *S. scuticum* as potential rootstocks for cultivated Solanaceae. Nematropica 50:144-150.

Root-knot nematodes (*Meloidogyne* spp.) are responsible for economic losses in various crops across the world. *Meloidogyne enterolobii* was first found in Brazil on guava in 2001 and spread rapidly, causing loses in many other plant species as well as vegetables such as tomatoes, *Capsicum* species, lettuce, and sweet potato. *Meloidogyne enterolobii* is also known to affect plant hybrids that contain resistance genes to other major *Meloidogyne* species. The objective of this research was to identify sources of resistance to *M. enterolobii* in *Solanum stramonifolium* and *S. scuticum* with the potential to be used as rootstocks for cultivated crops in the Solanaceae family. Twenty-nine accessions of *S. scuticum* and seven of *S. stramonifolium* were evaluated. Tomato cultivars 'Rutgers' and 'Nemadoro' (standard for *M. javanica*, *M. incognita,* and *M. arenaria*) were used as susceptible and resistant controls, respectively. The experiment was conducted in a greenhouse in a completely randomized design with six replications. Plants were grown in plastic pots containing 1.5 L of substrate and then inoculated with 5,000 eggs and second-stage juveniles of *M. enterolobii*. After 74 days incubation, gall index (GI), egg mass index (EMI), number of eggs per gram of root (NOGR), and reproduction factor (RF) were determined. *Solanum stramonifolium* was the best source of resistance to *M. enterolobii*, with the accessions CNPH 349 and CNPH 24 being highly resistant. *Solanum scuticum* had a lower frequency of resistance compared with *S. stramonifolium*. However, it was possible to identify some *S. scuticum* accessions, CNPH 07, CNPH 39, and CNPH 9, that were resistant to *M. enterolobii*.

Key words: *Meloidogyne enterolobii*, resistance, rootstock, *Solanum* species

RESUMO

Pinheiro J. B., G. O. da Silva, A. G. Macedo, D. Biscaia, and R. A. de Castro e Melo. 2020. Avaliação de fontes de resistência a *Meloidogyne enterolobii* em *Solanum stramonifolium* e *S. scuticum* como potencial porta enxertos para Solanaceae cultivadas. Nematropica 50:144-150.

Os nematoides das galhas são responsáveis por perdas econômicas em várias culturas em todo o

mundo. A espécie *Meloidogyne enterolobii* foi encontrada no Brasil em cultivos de goiaba no ano de 2001, e se espalhou rapidamente, causando perdas em muitas espécies vegetais tais como hortaliças - tomate, Capsicum spp., alface e batata-doce. É conhecida por afetar até híbridos que contêm genes de resistência a outras espécies principais de *Meloidogyne*. O objetivo deste trabalho foi avaliar fontes de resistência a *M. enterolobii* em *Solanum stramonifolium* e *S. scuticum* com potencial para serem utilizadas como porta-enxertos em culturas cultivadas na família Solanaceae. Foram avaliados 29 acessos de *S. scuticum* e sete de *S. stramonifolium*. As cultivares de tomate 'Rutgers' e 'Nemadoro' (padrão para *M. javanica*, *M. incognita* e *M. arenaria*) foram usadas como controles suscetíveis e resistentes, respectivamente. O experimento foi conduzido em cultivo protegido, em delineamento inteiramente casualizado, com seis repetições. As plantas foram cultivadas em vasos de plástico contendo 1,5 L de substrato e inoculadas com 5.000 ovos e juvenis de *M. enterolobii* no segundo estágio. Após 74 dias de inoculados foram avaliados o índice de galhas (GI), o índice de massa de ovos (EMI), o número de ovos por grama de raiz (NOGR) e o fator de reprodução (Rf). *Solanum stramonifolium* é a melhor fonte de resistência a *M. enterolobii*, principalmente os acessos CNPH 349 e CNPH 24. S. scuticum teve uma menor frequência de resistência quando comparado com Solanum stramonifolium. No entanto, foi possível identificar alguns acessos de S. scuticum, CNPH 07, CNPH 39 e CNPH 97 que foram considerados resistentes ao *M. enterolobii*.

Palavras chave: Meloidogyne enterolobii, resistência, enxertia, espécies de *Solanum*

INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp.) are considered important nematode pests globally due to the significant economic yield losses they cause in a wide range of crops (Ntalli *et al*., 2016). *Meloidogyne enterolobii* (Yang and Eiseinback, 1983) is known to damage *Solanum* cultivars such as tomato (*Solanum lycopersicum*) cultivars 'Rossol', 'Andréa' and 'Débora' and sweet pepper (*Capsicum annuum*) 'Silver' (Carneiro *et al*., 2006; Tigano *et al*., 2010; Pinheiro *et al*., 2015) that contain resistance genes to other *Meloidogyne* species. Genes involved in plant resistance to *Meloidogyne* spp. include Mi-1, Me-3/Me-7 and N (Kiewnick *et al*., 2009; Melo *et al*., 2011).

In Brazil, *M. enterolobii* (syn. *M. mayaguensis*) was originally reported in 2001 in guava (*Psidium guajava*) orchards in the states of Pernambuco and Bahia (Carneiro *et al.*, 2001). Since this time, *M. enterolobii* has quickly spread in Brazil, causing damage to several other crops such as tomatoes, bean, lettuce, sweet potato and *Capsicum* (Melo *et al*., 2011). Even though the identification of sources of resistance to this nematode within the same genus allows for the utilization of cell biology and cisgenesis in order to isolate or transfer beneficial alleles of interest into the recipient plant (Michereff-Filho *et al*., 2012), grafting is a simpler technique with the potential to reduce nematode damage (Mendonça *et al*., 2018). Solanaceae is a plant family that consists of approximately 96 genera and 2,300 species, with the genus *Solanum* comprised of almost 1,000 species (Cuevas-Arias *et al*., 2008). Most of the species can be used as rootstocks for the production of fruiting vegetables and for the control of soilborne pathogens (Goto *et al*., 2003; Cohen *et al*., 2017).

Although vegetable grafting is an efficient technique to overcome the pressures of new pathogen species or races (Mendonça *et al*., 2018), its adoption in Brazil is only gradually increasing because of the high cost of hybrid rootstocks and scion seed. Alternatively, grafting using native species that are compatible with other cultivated *Solanum*, with the possibility of seed production by growers, may reduce costs and improve adoption. Tomato is graft-compatible with *S. stramonifolium* (Farias *et al*., 2013; Simões *et al*., 2014; Mendonça *et al*., 2018), a native plant from Brazil and other countries in the north region of the Amazon forest (Mendonça and Lopes, 2019). *Solanum scuticum*, is also a native plant from Brazil and other countries from the tropical American region (Mendonça and Lopes, 2019) and could be used as a rootstock for grafting with other Solanaceous crops (Pinheiro *et al*., 2014a; Lopes and Mendonça, 2016). The objective of this work was to evaluate *S. stramonifolium* and *S. scuticum* as sources of resistance to *M. enterolobii* to be used as potential rootstocks for cultivated crops such as eggplant,

tomato, scarlet eggplant, and other compatible species.

MATERIALS AND METHODS

The *M. enterolobii* population used in this study was first identified affecting *Capsicum* rootstocks in the Central region of Brazil (Pinheiro *et al*., 2015). The population was identified *as M. enterolobii* by morphological examination of the perineal region of adult females and comparison with taxonomic descriptions and keys (Yang and Eisenback, 1983; Rammah and Hirschmann, 1988; Eisenback and Triantaphyllou, 1991). Additionally, phenotypic analysis of the esterase enzyme was conducted (Carneiro and Almeida, 2001). The population was cultured on tomato and maintained in a greenhouse. To obtain inoculum for experiments, galled tomato roots were chopped into 5-mm long pieces using a blender, then shaken in sodium hypochlorite for 3 min with constant agitation, washed with water, and passed through nested 100- and 20-µm-pore sieves. Eggs collected on the 20-µm-pore sieve were transferred to a 100-ml Erlenmeyer flask and diluted with 100 ml water, from which 1 ml aliquots were transferred to a counting dish and the number of eggs determined (Hussey and Barker, 1973; Boneti and Ferraz, 1981).

The experiment was conducted from July to September 2013 at Embrapa Vegetables, Brasília - DF, Brazil. Twenty-nine *S. scuticum* from the central (Distrito Federal and Goiás) southeast (Minas Gerais) and northern (Acre) regions of Brazil and seven *S. stramonifolium* from the northern states (Acre, Amazonas, Maranhão, Pará e Roraima) of Brazil were obtained from Embrapa Vegetables germplasm repository (Mendonça and Lopes, 2019). Tomato cultivars 'Rutgers' and 'Nemadoro' were used as susceptible and resistant controls, respectively. Tomato 'Nemadoro' is a standard with resistance to *M. javanica*, *M. incognita* and *M. arenaria* because of the Mi gene; there is not a tomato standard with resistance to *M. enterolobii* available. The experiment was conducted in a greenhouse in a completely randomized design with six replications for each accession, with one plant representing one replicate. Seedlings were produced in 128-cell plastic trays (40 cm^3 per cell) containing a substrate mix of coconut coir and peat moss mix (Plantmax, Eucatex, São Paulo, Brazil). Thirty days after sowing, plants were transplanted into plastic pots containing 1.5 L of a soil mix. The soil was a 1:1:1:1 subsurface soil (a clay Oxisol, typically encountered in the Cerrado Biome region in Brazil):washed sand:cow manure:carbonized rice husk mix autoclaved at 121°C for 60 min. Three hundred grams of N-P-K (4-30-16) fertilizer and 3,000 g of calcined dolomitic lime were added per 300 kg of this mixture.

After transplanting, plants were inoculated with 5,000 eggs and second-stage juveniles (J2) of *M. enterolobii*. Nematode inoculum was added to plants by pouring 5 ml of a suspension containing nematodes around the base of the plant, followed by irrigation. Seventy-four days after inoculation, the following parameters were assessed: gall index (GI), egg mass index (EMI), number of eggs per gram of roots (NOGR), and reproduction factor (Rf). Root systems were washed free of soil, weighed, and then stained by immersion in a Phloxine B solution (0.5 g/L of water) for 15 min, to observe and count the egg masses. Subsequently, roots were chopped into 5 mm long pieces using a blender, then shaken in sodium hypochlorite for 3 min with constant agitation, washed with water, and passed through nested 100- and 20-µm-pore sieves. Eggs collected on the 20-µm-pore sieve were transferred to a 100-ml Erlenmeyer flask and diluted with 100 ml water, from which 1 ml aliquots were transferred to a counting dish (Hussey and Barker, 1973; Boneti and Ferraz, 1981) and the NOGR was counted using a microscope (Dickson and Struble, 1965). EMI and GI were obtained according to Taylor and Sasser (1978) using a scoring scale of 0 to 5, (0 = roots without egg masses; 1 = presence of 1 to 2 galls or egg masses; 2 = presence of 3 to 10 galls or egg masses; 3 = presence of 11 to 30 galls or egg masses; 4 = presence of 31 to 100 galls or egg masses; and, 5 = presence of more than 100 galls or egg masses). Rf was calculated by dividing the final nematode population density (Pf) by the initial nematode population density (Pi) (Rf = Pf/Pi), with a Pi of 5,000 eggs and J2 of *M. enterolobii* and Pf as eggs extracted from the root system (Boneti and Ferraz, 1981). Plants were considered highly resistant (HR) with Rf = 0, resistant (R) with an Rf <1, and susceptible (S) with an Rf >1 (Oostenbrink, 1966).

Data were subjected to a one-way analysis of variance (ANOVA), and calculations of the environmental coefficient of variation (CV), and

the relationship between the genotypic coefficient of variation and the environmental coefficient of variation (CVg/CV), according with (Cruz and Regazzi, 2001), using Genes software (Cruz, 2013). Means were grouped using the Scott-Knott clustering test at a significance level of 0.05.

RESULTS AND DISCUSSION

Significant differences were observed for all the evaluated characters in each *Solanum* species ($P < 0.005$). The CV was higher for NOGR in *S. stramonifolium* and *S. scuticum* with 28.58% and 26.49%, respectively, compared to the other characters. The relationship between the genotypic CV and the CVg/CV was superior to the unity value for all the characters (Tables 1 and 2). This superiority of the CVg/CV (genetic) compared to CV (environmental), indicates a satisfactory degree of accuracy regarding the results obtained (Cruz and Regazzi, 2001).

Solanum stramonifolium had EMI values ranging from 1.5 to 4.8, GI from 0.5 to 4.0, and Rf from 0.0 to 6.4 (Table 1). Values for *S. stramonifolium*, in general, were lower compared to those for *S. scuticum* (Table 2). Tomato 'Nemadoro' (considered resistant) and 'Rutgers' (considered susceptible) to *Meloidogyne* spp., were susceptible to *M. enterolobii*. Two accessions of *S. stramonifolium* were identified as highly resistant (CNPH 349 and CNPH 24); while lower EMI, NOGR and GI were observed, none of the accessions supported *M. enterolobii* repreoduction, with a Rf = 0.0. The *S. stramonifolium* accessions CNPH 336, CNPH 23 and CNPH 120 were considered resistant, with an Rf < 1. The *S. stramonifolium* accession CNPH 21 had an Rf > 1, and even though it did not differ significantly from the resistant accessions CNPH 336, CNPH 23, and CNPH 120, it exhibited the highest NOGR. The *S. stramonifolium* accession CNPH 110 was found to be the most susceptible accession with the highest EMI, GI, and Rf (Table 1). Pinheiro *et al*. (2014b) reported that 7 out of 17 *S. stramonifolium* accessions were resistant to *M. enterolobii*. An additional 11 *S. stramonifolium* accessions were also identified as resistant to *M. enterolobii* (Pereira *et al*., 2018). *Solanum stramonifolium* has been reported to be resistant to other pests and pathogens including Tomato Severe Rugose virus, whitefly (*Bemisia tabaci* biotype B), and isolates of *Ralstonia solanacearum* (Michereff-Filho *et al.*, 2002; Lopes and Mendonça, 2016). These results demonstrate the potential of several accessions of *S. stramonifolium* to be used as rootstocks that have multiple resistance and/or tolerance to *M.*

Table 1. Evaluation of *Solanum stramonifolium* accessions for resistance to *Meloidogyne enterolobii*.

Accessions	EMI[t]	GI[u]	NOGR[v]	Rf [w]	Reaction[x]
CNPH 110	4.8 a[y]	4.0 a	5480.5 b	6.4 a	S
CNPH 336	2.2 b	1.8 b	732.9 c	0.4 b	R
CNPH 349	1.0 c	1.0 c	41.7 c	0.0 b	HR
CNPH 21	2.2 b	2.2 b	36211.1 a	1.4 b	S
CNPH 23	1.5 b	1.5 b	443.5 c	0.3 b	R
CNPH 24	0.5 d	0.5 c	7. 5 c	0.0 b	HR
CNPH 120	1.5 b	1.7 b	873.0 c	0.5 b	R
'Rutgers'	2.3 a	2.2 a	64.9 c	2.8 a	S
'Nemadoro'	1.4 c	1.3 c	8.6 d	0.7 b	R
CV (%)[z]	11.0	13.6	28.6	19.1	-
CVg/CV	2.3	2.0	2.1	2.0	-

[t]Egg mass index
[u]Gall index (GI)
[v]Number of eggs per gram of root
[w]Rf: reproduction factor = final/initial nematode population (Rf=Pf/Pi) (5,000 eggs and J2)
[x]Resistance reactions according to Oostenbrink (1966): highly resistant (HR) when presented a Rf value = 0, resistant with a Rf value <1 and susceptible (S) with Rf value >1
[y]Means followed by the same letters in the columns do not differ by Scott-Knott clustering test at 5% probability
[z]CV: coefficient of variation; CVg/CV: relation between the genotypic and environmental coefficient of variation

Table 2. Evaluation of *Solanum scuticum* accessions for resistance to *Meloidogyne enterolobii*.

Accessions	EMI[t]	GI[u]	NOGR[v]	Rf[w]	Reaction[x]
CNPH 01	4.5 a[y]	3.7 b	3423.9 c	11.9 b	S
CNPH 02	5.0 a	4.7 a	2338.6 c	13.1 b	S
CNPH 03	5.0 a	4.5 a	4481.1 c	18.5 a	S
CNPH 04	5.0 a	4.8 a	1075.3 d	9.6 b	S
CNPH 05	4.2 a	4.2 a	1124.1 d	8.0 c	S
CNPH 07	2.0 c	2.0 b	72.7 d	0. 5 d	R
CNPH 08	3.5 b	3.2 b	306.2 d	1.6 d	S
CNPH 09	5.0 a	4.5 a	5202.0 b	7.5 c	S
CNPH 10	3.5 b	3.3 b	468.3 d	4.0 d	S
CNPH 11	1.5 c	1.5 d	507.0 d	1.3 d	S
CNPH 12	3.3 b	2.7 b	699.6 d	5.2 c	S
CNPH 13	4.5 a	4.5 a	3672.0 c	23.1 a	S
CNPH 14	5.0 a	4.7 a	2940.6 c	18.6 a	S
CNPH 15	4.3 a	4.3 a	2066.8 c	15.1 b	S
CNPH 16	2.0 c	1.8 d	242.9 d	1.0 d	S
CNPH 17	5.0 a	4.5 a	2048.8 c	5.9 c	S
CNPH 18	5.0 a	4.5 a	3191.5 c	11.5 b	S
CNPH 39	3.2 b	2.8 c	159.8 d	0.7 d	R
CNPH 93	3.8 a	3.5 b	714.4 d	4.2 d	S
CNPH 94	4.5 a	3.3 b	900.3 d	3.2 d	S
CNPH 95	2.5 c	2.5 c	1098.6 d	2.9 d	S
CNPH 96	3.3 b	3.3 b	1241.4 d	8.2 c	S
CNPH 97	2.5 c	2.3 c	143.6 c	0.7 d	R
CNPH 98	4.5 a	4.2 a	1738.8 c	7.8 c	S
CNPH 99	4.8 a	4.3 a	4243.1 c	13.8 b	S
CNPH 101	3.7 b	3.7 b	1192.2 d	5.8 c	S
CNPH 102	4.5 a	3.7 b	12598.2 a	19.2 a	S
CNPH 103	4.8 a	4.5 a	4928.6 c	10.7 b	S
CNPH 104	3.5 b	3.3 b	2786.9 c	11.0 b	S
'Rutgers'	2.3 a	2.2 c	64.9 a	2.8 d	S
'Nemadoro'	1.4 d	1.3 d	8.6 d	0.7 d	R
CV (%)[z]	12.15	13.01	21.7	26.49	-
CVg/CV	1.13	1.03	1.28	1.38	-

[t]Egg mass index
[u]Gall index (GI)
[v]Number of eggs per gram of root
[w]Rf: reproduction factor = final/initial nematode population (Rf=Pf/Pi) (5,000 eggs and J2)
[x]Resistance reactions according to Oostenbrink (1966): highly resistant (HR) when presented a Rf value = 0, resistant with a Rf value <1 and susceptible (S) with Rf value >1
[y]Means followed by the same letters in the columns do not differ by Scott-Knott clustering test at 5% probability
[z]CV: coefficient of variation; CVg/CV: relation between the genotypic and environmental coefficient of variation

enterolobii and to other important diseases/pests.

Of the 29 *S. scuticum* accessions evaluated, only CNPH 07, CNPH 39, and CNPH 97 had Rf values < 1 and were, therefore, classified as resistant to *M. enterolobii*. Significant differences in Rf values for CNPH 07, CNPH 39 and CNPH 97 and the other 7 accessions (CNPH 08, CNPH 10, CNPH 11, CNPH 16, CNPH 93, CNPH 94, and CNPH 95) were not observed (Table 2). Pinheiro *et al*. (2014a) observed that 14 out of 26 accessions of *S. scuticum* were resistant to *M. enterolobii*. Similar to *S. stramonifolium*, *S. scuticum* accessions were also resistant to other pathogens, including *R. solanacearum* and *Fusarium oxysporum* races 2 and 3 (Lopes and Mendonça, 2016; Pereira *et al*., 2018).

The use of grafts with wild Solanaceae rootstock that have resistance and/or tolerance to plant-parasitic nematodes is considered an integrated management option for reducing yield losses (Vargas *et al*., 2019). Thus, additional investigations are required in order to determine graft compatibility of *S. stramonifolium* and *S. scuticum* with tomato cultivars. Additionally, the effects of rootstocks on the development and yield of tomato cultivars suited to different locations

where *M. enterolobii* occurs, especially in the Amazon region, should be investigated. Knowledge about the genes involved in the resistance reactions and in the defense mechanisms involved in the interactions between these accessions and soilborne pathogens also needs to be elucidated.

Meloidogyne enterolobii has the ability to overcome resistance in tomatoes carrying the Mi-1 gene, making it difficult to manage *Meloidogyne* species, particularly in organic farming systems (Kiewnick *et al*., 2009). In Brazil, mainly in the Amazonian states due to the association of harsh climatic conditions for tomato cultivation and the natural occurrence of soilborne pathogens (Farias *et al*., 2013), the availability of resistant rootstocks can be a significant change to the status of this crop production/market in the region. *Solanum stramonifolium* was the best source of resistance to *M. enterolobii*, mainly the CNPH 349 and CNPH 24 accessions. Although *S. scuticum* had a lower frequency of resistance to *M. enterolobii*, some *S. scuticum* accessions such as CNPH 07, CNPH 39, and CNPH 97 were also considered resistant.

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