Resistance sources to root-knot nematode *Meloidogyne enterolobii* in *Solanum* species

Jadir Borges Pinheiro¹, Giovani Olegario da Silva*¹, Jhenef Gomes de Jesus², Danielle Biscia³, Raphael Augusto de Castro e Melo*²

¹Embrapa Vegetables, Brasília-DF, Brazil, ²Universitary Center ICESP, Brasilia-DF

**ABSTRACT**

The objective of this work was to prospect sources of resistance to root-knot nematode *Meloidogyne enterolobii* in *Solanum* species with potential to be used as rootstocks for cultivated Solanaceae. Nine accessions of *Solanum sessiliflorum*, 27 accessions of *S. lycocarpum*, 21 accessions of *S. acanthodes*, 22 accessions of *S. scinericum* and 26 accessions of *S. scuticum* for resistance to *M. enterolobii*. Rutgers and Nemadoro tomatoes were used as susceptible and resistant controls, respectively. The experiment was conducted in a greenhouse at Embrapa Vegetables, Brasilia-DF, Brazil, in a completely randomized design with six replications. The experimental unit was a represented by a single plant grown in a plastic pot containing 3 L of substrate. 4000 eggs and eventual juveniles of second stage *M. enterolobii* were inoculated per pot. At 119 days after inoculation, gall index (Gi), egg mass index (EMI), number of eggs per root gram (NE) and reproduction factor (Fr) were evaluated. Data were subjected to analysis of variance and grouping of treatments by Scott-Knott. It was verified that *S. acanthodes* and *S. lycocarpum* are species with high resistance to *M. enterolobii*, with accessions being classified identified as immune. *S. scuticum* also has great potential, as several resistant accessions were identified, although some accessions were quite susceptible; whereas for *S. subinerme* only 4 resistant accessions were identified, although all others presented a reproduction factor much lower than tomato cv. Nemadoro as control; and all evaluated *S. sessiliflorum* accessions were susceptible.

**Keywords:** Grafting; Host-parasite relationship; *Solanum* species

**INTRODUCTION**

Root-knot nematodes belonging to the Meloidogyne genus are considered the most important in the world due to the significant economic losses caused in crops, with a wide range of hosts (Ntalli et al., 2016; Bernard et al., 2017). The species *Meloidogyne enterolobii* (Yang and Eisenback, 1983) is known to damage hybrids that present resistance genes to other Meloidogyne species (Carneiro et al., 2006; Tigano et al., 2010) for instance Mi-1 and N (Kiewnick et al., 2009; Melo et al., 2011).

In Brazil, *M. enterolobii* (syn. *Meloidogyne mayaguensis*) was originally reported in 2001 in guava (*Psidium guajava* L.) orchards situated in the states of Pernambuco and Bahia (Carneiro et al., 2001). Since then, this nematode spread quickly in the national territory, causing significant damages in several other species, threatening the horticulture productive chain (Melo et al., 2011). Even though the identification of resistance sources to this nematode within the same genus allows 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 technology with the potential of reducing damage.

According to Mendonça et al. (2018) although vegetable grafting is an efficient technique to overcome the appearance of new pathogen species or races, its adoption in the country is evolving gradually due to the high cost of hybrid rootstocks and scions seeds. Alternatively, grafting using native species that are compatible with other cultivated Solanum, with the possibility of seed production by growers, can reduce costs and improve its sustainability.

The graft compatibility of tomato with native Solanaceae species was confirmed by several authors, for instance, Farias et al. (2013), Simões et al. (2014) and Mendonça et al. (2018) with *S. stramonifolium* and Lopes & Mendonça (2014) with *Solanum paniculatum* L. Similarly, Zeist et al. (2017) and...
Guimarães et al. (2019) established the compatibility with *Solanum sessiliflorum*. Although Simões et al. (2014) and Mendonça et al. (2009) verified lesser compatibility with *Solanum lycocarpum* and Pereira et al. (2018b) with *S. acanthodes*.

Looking for rootstocks resistant to *M. enterolobii*, Pinheiro et al. (2014) evaluated the reaction of *S. stramonifolium* to this nematode, and found that of 17 accessions, 7 were resistant. Likewise, Pereira et al. (2018) evaluated 22 accessions of *S. stramonifolium* to *M. enterolobii*, and found that 11 were resistant.

Thus, the objective of this work was to prospect sources of resistance to the root-knot nematode *Meloidogyne enterolobii* in *Solanum* species to be used as potential rootstocks for cultivated Solanaceae.

**MATERIAL AND METHODS**

An experiment was conducted in a single span greenhouse from January to June, 2017 at Embrapa Vegetables – 996 MASL, 15° 56’ S, and 48° 08’ W – Brasília-DF, Brazil. Nine (9) *Solanum sessiliflorum* accessions; twenty-seven (27) *Solanum lycocarpum* accessions, 21 *Solanum acanthodes* accessions; 22 *Solanum subinermis* accessions and 26 *Solanum scuticum* accessions were evaluated for resistance to *Meloidogyne enterolobii*. The tomato cultivars ‘Rutgers’ and ‘Nemadoro’ were used as susceptible and resistant controls, respectively. The trial was held in a completely randomized design with six replications, with one plant representing the experimental plot. Seedlings were produced in plastic trays with 128 plugs (40 cm³ per plug) using coconut coir and peat moss mix based substrate (Plantmax®, Eucatex, São Paulo, Brazil). Thirty days after sowing (DAS) plants were transplanted in plastic pots containing 1.5 dm³ of a mix: sterilized subsurface soil (a clayey Oxisol, typically encountered in the Cerrado Biome region in Brazil), washed sand, cow manure and carbonized rice husk in the proportion of 1:1:1:1. It was fertilized and corrected with 300 g of 4-30-16 formulation and 300 g of calcined dolomitic lime per 300 kg of this mixture. After transplantation, plants were inoculated with 5,000 eggs and eventual second-stage juveniles (J2) of *M. enterolobii* by means of a 5 ml suspension applied around the plant shoot region.

One hundred and nineteen days after inoculation (119 DAI), the gall index (Gi), egg mass index (EMI), number of eggs per gram of roots (NE) and reproduction factor (Rf) were evaluated according Dickson and Struble (1965). IMO was obtained according to Taylor and Sasser (1978) using a scoring scale from 0 to 5, wherein: 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. Gi was quantified according to Taylor and Sasser (1978) likewise, using the aforementioned scoring scale. Rf was obtained by dividing the initial and final nematode population (Rf=Pf/Pi), considering the initial population (Pi) the one inoculated and the final population (Pf) as the one extracted from the root system using Boneti and Ferraz (1981) recommendations. Plants were considered immune (I) when presented an Rf value = 0, resistant with an Rf value <1 and susceptible (S) with Rf value >1 (Oostenbrink, 1966).

Data were subjected to an analysis of variance (ANOVA) and the means were clustered using the Scott-Knott test at a significance level of 0.05. All computations were performed using Genes software (Cruz, 2013).

**RESULTS AND DISCUSSION**

Significant differences were observed for all the evaluated characters in each species (P<0,005). The coefficient of variation values for NE ranged between 20.00% and 53.35%, being higher than the other evaluated characters. The relation between the genotypic coefficient of variation and the environmental coefficient of variation (CVg/CV) was superior to the unity value for all the characters. This indicates the preponderance of genetic variability when compared to the environmental variability, as well a satisfactory degree of accuracy regarding the obtained results.

Giving the EMI, Gi, and Rf values, conjointly, it can be observed that the resistant *Solanum* species to *M. enterolobii* in a decreasing order were: *S. acanthodes*, *S. lycocarpum*, *S. subinermis* and *S. sessiliflorum*. The control treatments, tomato cultivars ‘Nemadoro’ considered resistant and ‘Rutgers’ considered susceptible to root-knot nematodes, were both susceptible to *M. enterolobii* (Tables 1-5). An important aspect to take into consideration is that the tomato hybrids available in the national market with resistance to *M. incognita* race 1 and *M. javanica* are all susceptible to *M. enterolobii*. This species presents a wide polyphagia and aggressive behavior for most of the cultivated vegetables compared to the aforementioned nematode species that prevail in the country. Other aspect to bear, regarding crop rotation and the necessity of resistant sources, is the ability of *M. enterolobii* to multiply its population in the soybean cultivar ‘Forest’, ‘CDH’ sweetpotato cultivar and ‘Rosso’ tomato cultivar, considered resistant to other *Meloidogyne* species, a case registered in Africa in the late 80’s (Fargette, 1987).

Regarding *S. sessiliflorum*, all the accessions were susceptible to *M. enterolobii* with an average population 28 times higher than the inoculated, even when compared to the controls – ‘Nemadoro’ and ‘Rutgers’ tomato cultivars, according to...
The Rf values (Table 1). Guimarães et al. (2019) affirm that S. sessiliflorum provided vigor to tomato cultivar ‘Santa Clara’. However, even though it presents such an advantage, due to its susceptibility to M. enterolobii, its adoption as a rootstock should be restricted to areas without the presence of this pathogen, taking advantage of its resistance to bacterial wilt Ralstonia solanacearum (Fernandes and Bentes, 2018).

Some of the accessions of S. lycocarpum were considered resistant, providing a smaller nematode population than the inoculated, with the exception of CNPH 310, which presented EMI and Gi equivalent to the controls. Accessions CNPH 303, CNPH 304 and CNPH 320 presented value of Rf above its unit being considered susceptible. Accessions CNPH 321, CNPH 299, CNPH 307, CNPH 314 and CNPH 329 were immune, that is, although a small amount of eggs (EMI and NE) and galls on the roots (Gi) were observed, no nematodes were found surviving in the samples (Table 2). Mendonça et al. (2005) and Farias et al. (2013), in the conditions of the Cerrado and Amazon Biomes, respectively, recommend S. lycocarpum due to its good compatibility with tomato, resistance to R. solanacearum and adaptability to organic production systems. Thus, in complementary usage to these indications, since they are considered immune to M. enterolobii, the accessions mentioned above are considered suitable rootstock alternatives.

As for S. acanthodes accessions, only two were susceptible - CNPH 171 and CNPH 337. Thirteen accessions were resistant, and accessions CNPH 145, CNPH 147, CNPH 157, CNPH 166 and CNPH 167 were immune (Table 3). However, although the immunity of this accessions, they should be evaluated regarding their influence in the tomato yields, as Pereira et al. (2018b) state that S. acanthodes had a lower response in terms of fruit production than accessions of S. scuticum, S. stramonifolium, S. subinerme and not grafted tomato cv. BRS Kiara.

Most of the accessions of S. subinerme were considered susceptible. However, their average means value of Rf was 2.55, much lower than the controls. Accessions CNPH 126, CNPH 134, CNPH 141 and CNPH 207, were resistant (Table 4).
Table 3: Evaluation of Solanum acanthodes accessions to Meloidogyne enterolobii. Embrapa Vegetables, 2019

| Accessions | EMI | Meloidogyne enterolobii |
|------------|-----|-------------------------|
| CNPH 145   | 1.17^a | 1.17^a | 76.00^a | 0.00^a |
| CNPH 147   | 1.50^b | 1.33^c | 83.62^b | 0.00^b |
| CNPH 154   | 1.33^c | 1.17^d | 93.33^c | 0.00^c |
| CNPH 157   | 1.33^c | 1.33^d | 352.00^c | 0.00^c |
| CNPH 166   | 1.83^a | 1.83^a | 53.33^a | 0.00^a |
| CNPH 167   | 1.00^d | 1.00^d | 96.83^d | 0.00^d |
| CNPH 155   | 1.39^c | 1.19^c | 71.49^c | 0.03^c |
| CNPH 168   | 1.00^d | 1.00^d | 78.33^d | 0.17^d |
| CNPH 151   | 2.50^b | 2.33^c | 177.33^b | 0.17^b |
| CNPH 156   | 1.00^d | 1.00^d | 126.00^d | 0.17^d |
| CNPH 152   | 2.33^b | 2.17^b | 111.83^b | 0.33^b |
| CNPH 158   | 1.83^c | 1.83^c | 261.33^c | 0.33^c |
| CNPH 164   | 1.00^d | 1.00^d | 108.67^d | 0.33^d |
| CNPH 146   | 2.50^b | 2.50^b | 139.50^b | 0.50^b |
| CNPH 153   | 2.67^c | 2.67^c | 70.50^c | 0.50^c |
| CNPH 162   | 1.83^c | 1.50^c | 158.17^c | 0.50^c |
| CNPH 149   | 2.00^c | 1.17^d | 121.50^c | 0.67^c |
| CNPH 150   | 2.17^c | 2.17^c | 203.67^c | 0.83^c |
| CNPH 165   | 2.00^c | 1.33^c | 400.67^c | 0.83^c |
| CNPH 171   | 1.33^c | 1.17^d | 152.17^c | 1.00^c |
| CNPH 337   | 1.00^d | 1.00^d | 424.33^c | 1.50^c |
| Rutgers    | 5.00^e | 5.00^e | 8814.00^e | 5.00^e |
| Nemadouro  | 5.00^e | 5.00^e | 3034.83^e | 15.00^e |
| Means      | 1.94 | 1.82 | 661.28 | 1.23 |
| CV (%)     | 11.00 | 10.00 | 53.35 | 31.38 |
| Cvg/CV     | 1.84 | 2.16 | 2.10 | 1.76 |

Gall index (Gi) and egg mass index (EMI) - Taylor and Sasser (1978); (NE) - number of eggs per gram of roots; Rf - reproduction factor = initial/ final nematode population (Rf=Pf/Pi) (5000 eggs and J2); Resistance reactions according to Oostenbrink (1966): immune (I) when presented with a Rf value = 0, resistant with a Rf value <1 and susceptible (S) with Rf value >1; Means followed by the same lowercase letters in the columns and capital letters in the lines do not differ by Scott-Knott clustering test at 5% probability; CV: coefficient of variation; Cvg/CV: relation between the genotypic and environmental coefficient of variation.

S. scuticum was the Solanum species with the greatest variation regarding the Rf values, with 14 resistant accessions and 12 susceptible accessions. Among the susceptible, 4 were extremely susceptible, grouped together with EMI values above 3.50, NE values above 3,000 eggs per gram of roots; and Rf greater than 17 (Table 5). Of the accessions of S. scuticum considered resistant in the present study (Table 5), Lopes and Mendonça (2016) found that the vast majority are also resistant to R. solanacearum; the only access that was resistant in the present study and which was susceptible to R. solanacearum was CNPH 84, while the CNPH 64 access was not characterized in that study. These results further emphasize the importance of this species for potential use as rootstocks.

González et al. (2010) evaluated the reaction of wild Solanaceae to M. incognita race 2 and M. arenaria, and found that Datura stramonium L. was immune, and S. mammosum L. was highly resistant to both nematode species; while S. stramonium and S. erianthum were immune to M. incognita race 2 and highly resistant to M. arenaria. Navarete et al. (2018) classified accessions of S. birtum and S. arboresum, as resistant to M. incognita, whereas the accessions of S. auriculatum, S. hispidum, S. quitusense, S. betaceum were susceptible. Cardoso et al. (2019) evaluated the reaction of wild Solanaceae species to M. javanica and found that the species S. capsicioides, S. palmaeacanthum were resistant; while S. viarum was susceptible.

Looking for rootstocks resistant to M. enterolobii, Pinheiro et al. (2014) evaluated the reaction of S. stramonifolium to this nematode, and found that of 17 accessions, 7 were resistant. Likewise, Pereira et al. (2018) evaluated 22 accessions of S. stramonifolium to M. enterolobii, and found that 11 were resistant.

Thus, Solanum acanthodes and S. hyoecarpum are species with a high degree of resistance to M. enterolobii, with...
accessions were quite susceptible; whereas for *S. subinerme* only 4 resistant accessions were identified, although all others presented a reproduction factor much lower than tomato cv. Nemadoro as control; and all evaluated *S. sessiliflorum* accessions were susceptible.

Authors’ contributions

All authors participated in the planning of this work and also contributed to the written. Jadir Borges Pinheiro, Danielle Bicaia and Jhenef Gomes de Jesus performed the evaluations in the laboratory and greenhouses. The authors Giovani Olegario da Silva and Raphael Augusto de Castro and Melo helped in all stages of the work development, they just did not participate in the greenhouse and laboratory evaluations.

REFERENCES

Bernard, G. C., M. Egnin and C. Bonsi. 2017. The impact of plant-parasitic nematodes on agriculture and methods of control. In: Shah, M. M. and M. Mahamood, (Eds.), Nematology: Concepts, Diagnosis and Control, Books on Demand, Germany, pp. 121-151.

Cardoso, J., L. Tonelli, T. S. Kutz, F. D. Brandeiero, T. O. Vargas and R. Dallemole-Giaretta. 2019. Reaction of wild *Solanaceae* rootstocks to the parasitism of *Meloidogyne javanica*. Hortic. Bras. 37: 17-21.

Carneiro, R. M. D., W. A. Moreira, M. R. A. Almeida and A. C. M. Gomes. 2001. Primeiro registro de *Meloidogyne mayaguensis* em goiabeira no Brasil. Nem. Bras. 25: 223-228.

Cruz, C. D. 2013. Genes: A software package for analysis in experimental statistics and quantitative genetics. Acta Sci. Agron. 35: 271-276.

Dickson, D. W. and F. B. Struble. 1965. A sieving-staining technique for extraction of egg mass of *Meloidogyne incognita* from soil. Phytopathology. 55: 497.

Farias, E. A. P., R. L. F. Ferreira, S. E. de Araújo Neto, F. C. Costa and D. S. Nascimento. 2013. Organic production of tomatoes in the amazon region by plants grafted on wild *Solanum* rootstocks. Cienc. Agrotec. 37: 323-329.

Fargette, M. 1987. Use of esterase phenotype in the taxonomy of the genus *Meloidogyne*. 2. Esterase phenotypes observed in West African populations and their characterization. Rev. Nematol. 10: 45-56.

Fernandes, B. S. and J. L. S. Bentos. 2018. Enxertia de tomateiro em solâncées silvestres no controle da murcha bacteriana. Rev. Agr. Acad. 1: 26-32.

González, F. M., L. Gómez, M. G. Rodríguez, M. Piñón, A. Casanova, O. Gómez and Y. Rodríguez. 2010. Respuesta de genotipos de solanáceas frente a *Meloidogyne incognita* (Kofoid y White) Chitwood raza 2 y M. arenaria (Neal) chitwood. Rev. Prot. Veg. 25: 51-57.

Guimarães, M. A., M. F. N. Garcia, J. P. J. Tello, H. S. Lemos Neto, B. P. Lima Neto and J. S. Rabelo. 2019. Tomato grafting on rootstock of jilo, cocona and jurubeba. Hortic. Bras. 37: 138-145.

Kiewnick, S., M. Dessimoz and L. Franck. 2009. Effects of the Mi-1 and the N root-knot nematode-resistance gene on infection and reproduction of *Meloidogyne enterolobii* on tomato and pepper cultivars. J. Nematol. 41: 134-139.

Lopes, C. A. and J. L. Mendonça. 2014. Enxertia em Tomateiro Para

CONCLUSIONS

*S. acañthodes* and *S. Lycoearpum* are species with high resistance to *M. enterolobii*, with accessions being classified identified as immune. *S. scuticum* also has great potential, as several resistant accessions were identified, although some
o Controle da Murcha-bacteriana, Circular Técnica No. 131. Embrapa Hortaliças, Brazil, p. 8.

Lopes, C. A. and J. L. Mendonça. 2016. Reação de acessos de jurubeba à murcha bacteriana para uso como porta-enxerto em tomateiro. Hortic. Bras. 34: 356-360.

Melo, O. D., W. R. Maluf, R. J. de Sousa Gonçalves, Á. C. G. Neto, L. A. A. Gomes and R. C. Carvalho. 2011. Triagem de genótipos de hortaliças para resistência a Meloidogyne enterolobii. Pesqui. Agropecu. Bras. 46: 829-835.

Mendonça, J. L., C. A. Lopes and I. Lüdke. 2018. Enxertia de tomateiro em baquicha (Solanum stramonifolium var inerme (Dunal) Whalen) para controle de doenças de solo. Embrapa Hortaliças. Bras. Circ. Técnica. 163: 15.

Mendonça, J. L., C. A. Lopes, L. S. Boiteux, A. W. Moita and A. R. Oliveira. 2009. Compatibilidade de Enxertia de Tomateiro e Jurubeba (S. stramonifolium e S. asperolanatum). Proceedings of 3º Congresso Brasileiro de Tomate Industrial and 1º Seminário Nacional de Tomate de Mesa, Goiânia-GO (CD-ROM).

Mendonça, J. L., C. A. Lopes, R. J. Andrade and L. B. Giordano. 2005. Avaliação da lobeira (Solanum lycocarpum St Hill.) e do tomateiro CNPH 1048 como porta-enxerto para cultivares de tomateiro em solo infestado com RS (R. solanacearum). Hortic. Bras. 23: 370.

Michereff-Filho, M., W. D. B. Machini, J. L. Mendonça, M. E. N. Fonseca, N. A. N. Fernandes-Acioli and L. S. Boiteux. 2012. Resposta à mosca-branca (Bemisia tabaci) e ao Tomato severe rugose virus de acessos de Solanum subgênero Leptostemonum. Hortic. Bras. 30: 440-445.

Navarrete, X., L. Ron, P. Viteri and W. Viera. 2018. Parasitism of the root knot nematode Meloidogyne incognita (Kofoid and White) chitwood in five wild Solanaceae species. Rev. Fac. Nac. Agron. Medellin. 71: 8367-8373.

Ntalli, N., M. Ratajczak, C. Oplos, U. Menkissoglu-Spiroudi and Z. Adamski. 2016. Acetic acid, 2-undecanone, and (e)-2-decenal ultrastructural malformations on Meloidogyne incognita. J. Nematol. 48: 248-260.

Oostenbrink, M. 1966. Major characteristics of the relation between nematodes and plants. Meded. Landb. 66: 1-46.

Pereira, R. B., J. B. Pinheiro, T. B. Torres, J. L. Mendonça, G. C. Lucas and J. A. Guimarães. 2018. Potential of wild Solanum stramonifolium accesses as rootstock resistant to soilborne pathogens in tomato crops. Hortic. Bras. 36: 235-239.

Pereira, R. B., J. Silva, A. C. Sousa and J. R. Oliveira. 2018b. Compatibilidade de porta-enxertos de solanum silvestres com o tomateiro BRS Kiara. In: Boletim de pesquisa e desenvolvimento. Vol. 171. Embrapa Hortaliças, Brasília, DF, p. 22.

Pinheiro, J. B., J. L. Mendonça, C. S. Rodrigues, R. B. Pereira and F. A. Suinaga. 2014. Avaliação de Solanum stramonifolium para reação a Meloidogyne enterolobii. In: Boletim de Pesquisa e Desenvolvimento. Vol. 124. Embrapa Hortaliças, Brasília, p. 16.

Simões, A. C., G. E. B. Alves, R. Ferreira, S. E. Araújo Neto and J. Rocha. 2014. Compatibilidade de tomateiro sob diferentes porta-enxertos e métodos de enxertia em sistema orgânico. Enciclopédia Biosf. 10: 961-972.

Taylor, A. L. and J. N. Sasser. 1978. Biology, Identification and Control of Root-knot Nematodes (Meloidogyne species). Department of Plant Pathology, North Carolina State University Graphics, Raleigh, p. 111.

Tigano, M., K. Siqueira, P. Castagnone-Sereno, K. Mulet, P. Queiroz, M. dos Santos, C. Teixeira, M. Almeida, J. Silva and R. Carneiro. 2010. Genetic diversity of the root-knot nematode Meloidogyne enterolobii and development of a SCAR marker for this guava-damaging species. Plant Pathol. 59: 1054-1061.

Zeil, A. R., J. T. V. Resende, C. L. Giacobbo, C. Faria, M. D. Rios and D. M. Dias. 2017. Graft takes of tomato on other solanaceous plants. Rev. Caat. 30: 513-520.