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# Potential of wild *Solanum stramonifolium* accesses as rootstock resistant to soilborne pathogens in tomato crops

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## ABSTRACT

Resistant rootstocks is one of the most effective method to control soilborne pathogens in tomato crops. Thus, this study was installed to evaluate the reaction of *Solanum stramonifolium* accesses to *Fusarium oxysporum* f. sp. *lycopersici* (Fol) races 2 and 3 and to *Meloidogyne enterolobii* (Me). The seedlings were grown in trays and inoculated separately with Fol races 2 and 3 at 50 days after planting by immersing the roots in the spore suspension ($1\times10^6$ microconidia $mL^{-1}$). Then, seedlings were transplanted in pots containing sterilized soil and kept in greenhouse conditions. To study the reaction of *S. stramonifolium* accesses to nematodes, we used 27-day old seedlings that were also planted in pots and inoculated with 6,000 eggs and second-stage juveniles in greenhouse conditions. The experiments were evaluated in the 34$^{th}$ day (Fol) and in the 64$^{th}$ day (Me) after inoculation. The experiment consisted of a randomized block design with five replications, where each plot consisted of one pot with three plants (Fol) and one pot with one plant (Me). We observed that the plants used as controls, susceptible to Fol races 2 and 3 and Me, presented 100% of incidence. All accesses were resistant to Fol race 2 and the accesses CNPH-19, CNPH-22, CNPH-23, CNPH-25, CNPH-120, CNPH-122 and CNPH-349 presented multiple resistance to pathogens, indicating great potential for using as resistant rootstock. The CNPH-24, CNPH-119, CNPH-121 and CNPH-336 accesses also presented resistance to nematode. However, they presented slight browning symptoms of vascular tissues when they were inoculated with Fol race 3. This symptom was also observed in the CNPH-21, CNPH-107 and CNPH-117 accesses. All other accesses were resistance to Fol race 3 and susceptible to Me.

**Keywords:** *Meloidogyne enterolobii, Solanum lycopersici*, fusarium wilt, grafting, genetic resistance.

## RESUMO

**Potencial de acessos selvagens de *Solanum stramonifolium* como porta enxertos resistentes para patógenos de solo em cultivos de tomate no Brasil**

O uso de porta-enxertos resistentes é um dos métodos mais efetivos para o controle de patógenos de solo em cultivos de tomateiro. Assim, o objetivo deste estudo foi avaliar a reação de acessos de *Solanum stramonifolium* a *Fusarium oxysporum* f. sp. *lycopersici* (Fol) raças 2 e 3 e a *Meloidogyne enterolobii* (Me). As mudas foram formadas em bandejas e inoculadas separadamente com Fol raças 2 e 3 aos 50 dias após o semeio, mediante imersão das raízes em suspensão de esporos ($1\times10^6$ microconídios $mL^{-1}$). Em seguida, essas mudas foram transplantadas para vasos contendo solo esterilizado. Para a inoculação do nematoide, foram utilizadas plantas com 27 DAS, transplantadas para vasos e inoculadas com 6.000 ovos e juvenis de segundo estádio. As avaliações foram realizadas aos 34 (Fol) e aos 64 (Me) dias após a inoculação. O experimento foi realizado em delineamento de blocos casualizados com cinco repetições, em que cada parcela foi composta por um vaso com três plantas (Fol) e um vaso com uma planta (Me). As testemunhas suscetíveis a Fol raças 2 e 3 e a Me apresentaram 100% de incidência. Todos os acessos foram resistentes a Fol raça 2, enquanto os acessos CNPH-19, CNPH-22, CNPH-23, CNPH-25, CNPH-120, CNPH-122 e CNPH-349 apresentaram resistência múltipla aos patógenos, indicando grande potencial para uso como porta enxertos resistentes. Os acessos CNPH-24, CNPH-119, CNPH-121 e CNPH-336 também apresentaram resistência ao nematoide. Contudo, estes acessos apresentaram leves sintomas de escurecimento nos tecidos vasculares quando inoculados com Fol raça 3. Este sintoma também foi observado nos acessos CNPH-21, CNPH-107 e CNPH-117. Os demais acessos apresentaram resistência a Fol raça 3 e suscetibilidade a Me.

**Palavras-chave:** *Meloidogyne enterolobii*, *Solanum lycopersici*, murcha de fusário, enxertia, resistência genética.

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Tomato crop is highly attacked by pathogens, which can cause significant economic losses. Among these pathogens, we highlight the root-knot nematode (*Meloidogyne* spp.) (Carneiro *et al.*, 2006) and the fungi *Fusarium oxysporum* f. sp. *lycopersici*, whose injuries can become impracticable its cultivation in certain regions or seasons. These pathogens can occur during all over phenological phase of the tomato, but it is more common

during flowering and fruiting periods (Lopes *et al.*, 2005).

In Brazil, the most common *Meloidogyne* species in tomato crops are *M. javanica*, *M. incognita* (races 1, 2, 3 and 4) and *M. arenaria*. However, in 2001, Carneiro *et al.* (2001) reported large losses in tomato crops in São Paulo State due to the attack of *M. enterolobii* (syn. *M. mayaguensis*). Recently, Pinheiro *et al.* (2015) reported the occurrence of this species in Central Region of Brazil, which made unfeasible the tomato cultivation in such areas.

Currently, three physiological races of *F. oxysporum* f. sp. *lycopersici* are known in Brazil, which are able to infect and to cause disease in many tomato host cultivars. Race 3 has been confirmed to be responsible for epidemics outbreaks in tomato crops in the Southeast and Northeast regions of Brazil (Reis *et al.*, 2005; Reis & Boiteux, 2007; Barbosa *et al.*, 2013; Gonçalves *et al.*, 2013), although races 1 and 2 are the most common in tomato producing areas.

The majority of commercial tomato cultivars grown in Brazil have the dominant gene *Mi*, which confers resistance to the prevailing nematode species in the country. However, the gene *Mi* do not provide resistance to *M. enterolobii* in many crops, including tomato (Pinheiro *et al.*, 2011). According to Trudgill (1991), little is known about sources of resistance to nematodes in vegetables, such as *Solanum* species. The majority of tomato cultivars and rootstocks traditionally grown in Brazil are resistant to races 1 and 2 of *F. oxysporum* f. sp. *lycopersici*. However, resistant cultivars and rootstocks to race 3 are scarce, Race 3 is disseminated to the main production areas of tomato in Brazil (Reis & Boiteux, 2007; Gonçalves *et al.*, 2013).

Therefore, the use of resistant tomato rootstocks can be an alternative method to be used in the control of soilborne pathogens responsible for causing diseases in tomato crops (Lopes & Mendonça, 2016). In this sense, plant species of the genus *Solanum*, sub-genre *Leptostemonum* have been evaluated in order to use them as rootstock for tomato. Mendonça *et al.* (2005) evaluated the performance of 'Santa Clara' tomato grafted onto *S. lycocarpum* rootstock on soil infested with *Ralstonia solanacearum* and obtained higher yields in comparison to the non-grafted. These authors also reported 95% of compatibility between *S. lycocarpum* accesses and 'Santa Clara' tomato. Mattos *et al.* (2011) evaluated the reaction of *S. stramonifolium* and *S. asperolanatum* and reported resistance of accesses to *M. incognita* race 1. In addition, they found that accesses of *S. stramonifolium*, *S. paniculatum* and *S. subinerme* showed resistance to *M. enterolobii*. Pinheiro *et al.* (2011) evaluated the reaction of the same accesses of *S. stramonifolium*, *S. paniculatum* and *S. subinerme* evaluated by Mattos *et al.* (2011) to root-knot nematode and they found that these rootstocks were also resistant to *M. incognita* race 1 and *M. javanica*.

Considering the encouraging results reported up to date to wild *Solanum* resistant rootstocks against the main soil pathogens in tomato crops, we developed a study to evaluate the reaction of *S. stramonifolium* accesses to the fungus *F. oxysporum* f. sp. *lycopersici* races 2 and 3 and to the nematode *M. enterolobii*.

## MATERIAL AND METHODS

The assays were conducted in the Laboratory of Plant Pathology and Nematology and in a greenhouse located at Embrapa Hortaliças, Brasília-DF, Brazil, during the period of September to December 2014.

Twenty-two accesses of *S. stramonifolium* were evaluated separately regarding the reaction to *F. oxysporum* f. sp. *lycopersici* race 2 and 3 and to *M. enterolobii*, CNPH-19, CNPH-21, CNPH-22, CNPH-23, CNPH-24, CNPH-25, CNPH-107, CNPH-108, CNPH-109, CNPH-110, CNPH-111, CNPH-113, CNPH-114, CNPH-116, CNPH-117, CNPH-118, CNPH-119, CNPH-120, CNPH-121, CNPH-122, CNPH-336 and CNPH-349. In the experiments of *F. oxysporum* f. sp. *lycopersici* race 2 and 3 the tomato cv. Santa Clara was used as susceptible control to pathogen, while in the reaction experiments to nematodes, we used tomato plants cv. Nemadoro and cv. Rutgers, which are resistant and susceptible control to pathogen, respectively.

### Methods for obtaining pathogen isolates and inoculum preparation

#### *Fusarium oxysporum* f. sp. *lycopersici* races 2 and 3

Pathogens were isolated from adult tomato plants presenting symptoms of the disease. After isolation, the races were identified based on the reaction of differential cultivars, by using 'IPA-5' tomato plants (resistant to race 1), 'BHRS-2,3' (resistant to races 1, 2 and 3), 'Floradade' (resistant to races 1 and 2) and 'Ponderosa' (susceptible to all races) (Reis & Boiteux, 2007).

For inoculum preparation, we used three discs (5 mm diameter) removed of pure pathogen colonies. Then, the discs were transferred to Erlenmeyer flasks containing 250 mL of culture medium made of potato dextrose broth (BD) and maintained at temperature of 23 to 27°C under constant agitation (90 rpm). After seven days of incubation, the liquid culture medium containing the fungus microconidia was filtered on sterile gauze, and its concentration was measured by hemocytometer, according to Santos (1997). In order to identify races and accesses in all reaction experiments, we used the same concentration of the inoculum suspension ($1\times10^6$ microconidia $mL^{-1}$).

#### *Meloidogyne enterolobii*

The nematode species were obtained from tomato plants presenting gall symptoms on roots. The identification process was done by exam of the perineal cuts of adult females extracted from galls and confirmed by standard isoenzymes (Carneiro & Almeida, 2001).

Eggs and any second stage juveniles (J2) were collected of *M. enterolobii* females previously obtained and inoculated in tomato cv. Rutgers plants for multiplication of inoculum. These plants were grown in 3.0 L pots containing sterilized substrate and maintained in greenhouse. Fifty days after inoculation, eggs and J2

were extracted from tomato roots [Hussey & Barker (1973) modified by Bonetti & Ferraz (1981)] and quantified under microscope stereoscope. For the experiment, the inoculum suspension was adjusted to 6,000 eggs and J2 per plant, and distributed in 5 mL of suspension.

**Experiments conduction**

All tomato and *S. stramonifolium* accesses were planted in 72-cell trays containing substrate, which was composed of vermiculite and carbonized pine bark. Seedlings were daily irrigated according to necessity and kept in greenhouse throughout the experimental period.

***Fusarium oxysporum* f. sp. *lycopersici* races 2 and 3**

Seedlings of *S. stramonifolium* accesses and tomato plants were inoculated separately with the races of the pathogen after 50 days in case of *S. stramonifolium* and 25 days for tomatoes (Santos, 1997). After that, seedlings were removed from the trays, and roots washed in tap water in order to remove the substrate. Roots were cut about 4 cm from the stalk by using a sterile scissor. Then, roots were completely immersed (2 min.) in the inoculum suspension ($1 \times 10^6$ microconidia $mL^{-1}$) and transplanted to 1.5 L pots, filled with autoclaved substrate, which was a mixture of 85% of sifted "cerrado" underground, 5% of dry rice husk and 10% carbonized rice husk (v:v). The substrate was enriched with 100 g of dolomite lime, 200 g of superphosphate and 60 g of ammonium sulfate.

The experiment consisted of randomized block design with five replications where each plot consisted of one pot containing three plants.

***Meloidogyne enterolobii***

The 27-day old seedlings of *S. stramonifolium* accesses and tomato plants used as controls were also transplanted into pots (4.5 L) filled with same substrate described in the previous experiment.

The accesses of *S. stramonifolium* and controls were inoculated, distributing 5 mL of the suspension (6,000 eggs and J2) per plant, around the neck of the seedlings, with 2 cm range and depth approximately.

The experiment consisted in a randomized block design with five replications, where each plot consisted of one pot with one plant.

**Experimental assessments**

***Fusarium oxysporum* f. sp. *lycopersici* races 2 and 3**

The disease symptoms were evaluated 50 days after inoculation, based on a scale of notes, wherein: 0= no symptoms, 1= plants without wilting or yellowing symptoms but with vascular browning, 2= plants with intense vascular browning and beginning to wilt or with yellow leaves, 3= plants with intense wilting associated with yellowing and leaf drop, 4= dead plants (Aguiar *et al.*, 2013).

Based on the notes, we were able to determine the disease index (DI), that was calculated by using the formula DI (%) = $100.\Sigma$ [(fv) / (nx)], where f= is the number of plants with same note, v= observed note, n= total number of evaluated plants and x= maximum rating scale plants (McKinney, 1923). It is important to highlight that only the genotypes with note zero were considered as resistant.

Immediately after evaluation, the plants with symptoms of the pathogen were identified and sent to the laboratory for isolation of the fungus in potato-dextrose-agar (PDA) culture medium. Conidia suspensions of each isolate were prepared from pure cultures of the pathogen, which were inoculated on susceptible tomato plants cv. Santa Clara. Then, the pathogenicity of these isolates was confirmed in all *S. stramonifolium* plants, which present darkened vascular bundles symptoms.

***Meloidogyne enterolobii***

The evaluation of *M. enterolobii* was performed 64 days after inoculation, where plants were removed from the pots and identified. Roots were washed thoroughly in tap water and processed by using the technique developed by Hussey & Barker (1973) and modified by Bonetti & Ferraz (1981). Then, the final population of eggs and J2 obtained from each root system was quantified under microscope stereoscope.

The reproduction factor (RF) was obtained by the ratio between the final densities (Pf) and initial (Pi) of the nematodes, according to the formula: RF = Pf / Pi (Oostenbrink, 1966). Pi was considered the inoculum distributed at the time of inoculation, where, 6,000 eggs and J2 per pot. Pants with RF= 0 were considered as immune, those resistant to RF≤1.0 and those susceptible to RF>1.0.

The reproduction factor data (RF) were transformed to $\sqrt{(x+1)}$, submitted to the analysis of variance in statistical software Sisvar® (v. 4.5). Averages were grouped by the Scott-Knott test ($p \leq 0.05$).

## RESULTS AND DISCUSSION

All 22 accesses of *S. stramonifolium* showed resistance to *F. oxysporum* f. sp. *lycopersici* race 2 (Table 1), without expressing any symptoms of the disease. However, seven accesses of *S. stramonifolium* (CNPH-21, CNPH-24, CNPH-107, CNPH-117, CNPH-119, CNPH-121 and CNPH-336) presented mild symptoms of vascular browning when inoculated with race 3 of the pathogen. These accesses presented disease index (DI) of 3.10, 6.30, 6.30, 9.40, 12.50, 15.60 and 18.80, respectively, and they were considered susceptible. The tomato cv. Santa Clara, used as susceptible control for both pathogen races, showed the symptom of disease in 100% of the plants and presented DI= 82.30% to race 2 and 71.90% to race 3.

Regarding the resistance of *S. stramonifolium* to the nematode *M. enterolobii*, we observed that there was great variability among the accesses (Table 1). Eleven accesses (50.0%) (CNPH-19, CNPH-22, CNPH-23, CNPH-25, CNPH-120, CNPH-122, CNPH-349, CNPH-119, CNPH-24, CNPH-121 and CNPH-336) showed complete resistance to the nematode, with reproductive factors (RF) lesser

**Table 1.** Reaction of *Solanum stramonifolium* accesses to soilborne pathogens *Fusarium oxysporum* f. sp. *lycopersici* races 2 and 3 and to *Meloidogyne enterolobii*. Brasília, Embrapa Hortaliças, 2014.

| Accesses | *Fusarium oxysporum* f. sp. *lycopersici* race 2 | | *Fusarium oxysporum* f. sp. *lycopersici* race 3 | | *Meloidogyne enterolobii* | |
|---|---|---|---|---|---|---|
| | DI (%)[1] | Reaction[3] | DI (%)[1] | Reaction[3] | RF[2] | Reaction[3] |
| CNPH-19 | 0.00 | R | 0.00 | R | 0.05 a | R |
| CNPH-22 | 0.00 | R | 0.00 | R | 0.86 b | R |
| CNPH-23 | 0.00 | R | 0.00 | R | 0.26 a | R |
| CNPH-25 | 0.00 | R | 0.00 | R | 0.22 a | R |
| CNPH-120 | 0.00 | R | 0.00 | R | 0.44 a | R |
| CNPH-122 | 0.00 | R | 0.00 | R | 0.97 b | R |
| CNPH-349 | 0.00 | R | 0.00 | R | 0.02 a | R |
| CNPH-108 | 0.00 | R | 0.00 | R | 1.71 c | S |
| CNPH-109 | 0.00 | R | 0.00 | R | 1.92 c | S |
| CNPH-110 | 0.00 | R | 0.00 | R | 5.00 d | S |
| CNPH-111 | 0.00 | R | 0.00 | R | 1.24 b | S |
| CNPH-113 | 0.00 | R | 0.00 | R | 3.24 c | S |
| CNPH-114 | 0.00 | R | 0.00 | R | 2.67 c | S |
| CNPH-116 | 0.00 | R | 0.00 | R | 2.50 c | S |
| CNPH-118 | 0.00 | R | 0.00 | R | 2.58 c | S |
| CNPH-119 | 0.00 | R | 3.10 | S | 0.33 a | R |
| CNPH-24 | 0.00 | R | 6.30 | S | 0.01 a | R |
| CNPH-107 | 0.00 | R | 6.30 | S | 2.46 c | S |
| CNPH-121 | 0.00 | R | 9.40 | S | 0.44 a | R |
| CNPH-336 | 0.00 | R | 12.50 | S | 0.41 a | R |
| CNPH-117 | 0.00 | R | 15.60 | S | 1.34 b | S |
| CNPH-21 | 0.00 | R | 18.80 | S | 1.44 b | S |
| 'Santa Clara' | 82.30 | S | 71.90 | S | - | - |
| 'Nemadoro' | - | - | - | - | 0.03 a | R |
| 'Rutgers' | - | - | - | - | 4.48 d | S |
| CV (%) | - | - | - | - | 15.75 | - |
| Means | 3.58 | - | 6.26 | - | 1.44 | - |

[1]DI= disease index (Mckinney, 1923); [2]RF= reproduction fator; [3]Reaction: R= resistant, S= susceptible. Means followed by same letter correspond to the same group by Scott-Knott test ($p \leq 0.05$); Data transformed to $\sqrt{(x+1)}$.

than 0.99. All other accesses presented RF higher than 1.00 and consequently were considered susceptible. Tomato cultivars Rutgers (susceptible control) and Nemadoro (resistant control) presented RF= 4.48 and RF= 0.03, respectively.

Accesses of *S. stramonifolium* were grouped based on RF value, according to Scott-Knott test ($p \leq 0.05$). The resistant accesses CNPH-19, CNPH-23, CNPH-25, CNPH-120, CNPH-349, CNPH-119, CNPH-24, CNPH-121, CNPH-336 and cv. Nemadoro did not differ from each other and showed RF between 0.01 and 0.44, followed by CNPH-21, CNPH-22, CNPH-111, CNPH-117 and CNPH-122, with RF between 0.86 to 1.44. The accesses CNPH-107, CNPH-108, CNPH-109, CNPH-113, CNPH-114, CNPH-116 and CNPH-118 presented higher multiplication ratio of the nematode, with RF values between 1.71 and 3.24, followed by CNPH-110 and 'Rutgers' tomato, which presented RF 5.00 and 4.48, respectively.

These results highlight the potential use of accesses of *S. stramonifolium* as resistant rootstocks against *F. oxysporum* f. sp. *lycopersici* races 2 and 3 in tomato crops. Similar results were found by Pinheiro *et al.* (2011), where the selected accesses of *S. stramonifolium* and other wild *Solanum* species were resistant to *M. incognita* race 1 and to *M. javanica*. In addition, Mendonça *et al.* (2005) verified higher yield in tomato cv. Santa Clara grafted onto *S. lycocarpum* rootstocks in soils contaminated with *R. solanacearum* when compared to non-grafted tomato. Subsequently, Amorim *et al.* (2012) observed in a greenhouse experiment that the tomato seedlings 'Santa Clara' grafted onto *S. stramonifolium* in soil contaminated with *R. solanacearum* did not show symptoms of bacterial wilt in comparison to non-grafted tomato

plants. The compatibility of tomato cv. IPA-6 (Farias *et al*., 2013) and cv. Santa Adélia (Simões *et al.*, 2014) grafted onto *S. stramonifolium* and *S. lycocarpum* rootstocks was evaluated and higher yield and graft compatibility with tomato cultivars was obtained.

Therefore, according to literature some accesses of wild *Solanum* species presented resistance to multiple soil pathogens, not interfering negatively on the tomato production. In the present work, we could find similar results, where seven accesses of *S. stramonifolium* showed multiple resistance, CNPH-19, CNPH-22, CNPH-23, CNPH-25, CNPH-120, CNPH-122 and CNPH-349, indicating that these accesses possess high potential to be used as resistant rootstocks to diseases in tomato grown in infested areas. According to Farias *et al.* (2013) it is important to keep focusing on studies such as the present one, in order to select new tomato cultivars, compatible with wild Solanaceae rootstocks, which will help to control soil pathogens and increase tomato productivity. However, the knowledge about the genes involved in the resistance reactions and in the defense mechanisms involved in the interactions between accessions of *S. stramonifolium* and soil pathogens need to be elucidated.

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