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Reaction of wild Solanaceae rootstocks to the parasitism of *Meloidogyne javanica*

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ABSTRACT

Intensive production of vegetables in greenhouses can increase the amount of inoculum of soil-borne pathogens, such as the root-knot nematode. Thus, in this study we aimed to evaluate the potential of Solanaceae as rootstocks resistant to *Meloidogyne javanica* nematodes as an alternative to tomato grafting. The experiment consisted of seven treatments: wild species joá-vermelho (*Solanum capsicoides*), joá-bagudo (*Solanum palinacanthum*), joá-bravo (*Solanum viarum*), jurubeba (*Solanum* spp.) and the commercial tomato cultivars Santa Cruz Kada, Batalha and Guardião. The analyzed variables were gall index; egg mass index; final nematode population; reproduction factor (FR) and reaction: susceptibility, resistance and immunity; fresh shoot and root mass and number of eggs per gram of roots. The wild species joá-vermelho, joá-bagudo and jurubeba showed resistance, with the lowest indexes of galls, egg mass, final population of nematodes and number of eggs per gram of root, not differing from the resistant control treatment (hybrid rootstock Guardião), with a reproduction factor less than 1, showing potential to be used as a resistant rootstock to *M. javanica*. Joá-bravo species showed susceptibility to the root-knot nematode, with a FR>1, not differing from the susceptible tomato Santa Cruz Kada. These results confirm the resistance of wild species to nematode parasitism, which can prove the viability of use as possible alternative rootstocks, and reinforce the idea that more studies should be carried out aiming to provide more viable options for farmers and plantlet producers.

Keywords: *Solanum lycopersicum*, root-knot nematode, grafting, resistance.

RESUMO

Reação de porta-enxertos de Solanáceas silvestres ao parasitismo de *Meloidogyne javanica*

A produção intensiva de hortaliças em ambiente protegido pode aumentar a quantidade de inóculo de patógenos habitantes do solo, a exemplo dos nematoides das galhas. Dessa forma, objetivou-se avaliar o potencial de solanáceas como porta-enxertos resistentes ao nematoide *Meloidogyne javanica* como alternativa à enxertia em tomateiro. O experimento foi constituído de sete tratamentos: as espécies silvestres joá-vermelho (*Solanum capsicoides*), joá-bagudo (*Solanum palinacanthum*), joá-bravo (*Solanum viarum*), jurubeba (*Solanum* spp.) e os tomateiros comerciais Santa Cruz Kada, Batalha e Guardião. As variáveis analisadas foram índice de galhas; índice de massas de ovos; população final de nematoides; fator de reprodução e reação: de suscetibilidade, resistência e imunidade; matéria da parte aérea fresca, matéria de raiz fresca e número de ovos por grama de raiz. As espécies joá-vermelho, joá-bagudo e jurubeba foram resistentes, apresentando os menores índices de galhas, massa de ovos, população final de nematoides e número de ovos por grama de raiz, não diferindo do tratamento testemunha resistente (porta-enxerto híbrido Guardião), com um fator de reprodução menor que 1, indicando potencial de utilização como porta-enxerto resistente a *M. javanica*. A espécie joá-bravo mostrou-se suscetível ao nematoide das galhas, com um fator de reprodução maior que 1, não diferindo do tomateiro suscetível Santa Cruz Kada. Tais resultados confirmam a resistência de espécies silvestres ao parasitismo de nematoides, o que pode comprovar a viabilidade de uso como possíveis porta-enxertos alternativos, reforçando a ideia de que mais estudos devem ser realizados com esse enfoque, visando fornecer opções viáveis para o agricultor, bem como aos produtores de mudas.

Palavras-chave: *Solanum lycopersicum*, nematoide das galhas, enxertia, resistência.

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One of the main challenges faced by horticulture these days is to meet the growing demand for food due to global population growth (Rouphael *et al*., 2018), as well as to produce fresh, high-quality and free-chemical-residue food in order to meet the demands of consumers (Bisbis *et al*., 2018).

Vegetable production in the state of Parana showed an increase of 77% from 2,000 to 2,016, due to an increase in crop productivity, as a result of the producer organization and investment in technology (Salvador, 2017). According to Instituto Paranaense de Assistência Técnica e Extensão Rural, these vegetables are grown essentially by family farmers, on average areas of approximately 3 hectares, in about 13% of family properties in the state (EMATER, 2016).

In order to meet market demand, farmers use intensive production in protected environment, reducing, partially, the negative effects provided by harsh climatic conditions (Araújo *et al*., 2009; King *et al*., 2010; Bisbis *et* *al.*, 2018). However, intensive vegetable cultivation under greenhouses may increase inoculums of soil pathogen, due to the fact that, when the producer specializes in growing one vegetable species, performs successive cultivation in this environment, limiting crop rotation (King *et al.*, 2010; Rosa *et al.*, 2013; Pinheiro *et al.*, 2014).

Among the species, grown in protected environment, the tomato crop stands out due to its high sensitivity to inclement weather (Gusmão *et al.*, 2006; Bergougnoux *et al.*, 2014; Çelik *et al.*, 2017) and susceptibility to several pests and diseases, which makes this cultivation difficult, with economic and environmental risk factors, caused by excessive use of chemical products (Luz *et al.*, 2007; Loos *et al.*, 2008).

Considering soil pathogens, the root-knot nematode belonging to the genus *Meloidogyne* is of great economic importance for tomato crop (Carvalho *et al.*, 1999; Podestá *et al.*, 2016). Its popular name derives from the main symptom presented by plants when affected by these pathogens, which consists of nourishing cells (gall nematodes), which act as a permanent feeding place, providing nutrients for the nematode development and interfering negatively in nutritional and water absorption by plants (Jones *et al.*, 2013; Karssen *et al.*, 2013; Kepenekci *et al.*, 2018).

The reaction of plants to the root-knot nematode can vary according to species and cultivar, being that the crop rotation, duration of the development cycle, planting season, population density and kind of soil also interfere in damage severity (Jones *et al.*, 2013). Generally, these observed symptoms include reduced fruit yield and quality, slow growth, yellowish leaves, wilting in the hottest hours of the day, and decreased resistance to other biotic and abiotic factors (Jones *et al.*, 2013; Podestá *et al.*, 2016; Kepenekci *et al.*, 2018).

The root-knot nematodes are mandatory endoparasites, which may be native or inserted into new production regions, through contaminated soils, root remnants or infected plants (Louws *et al.*, 2010). Although crop rotation with non-host species and soil fumigation are used to control these pathogens, resistance is still the most employed and sustainable strategy (Louws *et al.*, 2010).

The chemical nematicides generally used to control these pathogens are of high costs and present potential risks for beneficial soil microorganisms (Huang *et al.*, 2014), environment and human health (Fournet *et al.*, 2012; Jones *et al.*, 2013; Becker, 2014; Onkendi *et al.*, 2014; Kyriacou *et al.*, 2017). Moreover, the increasing market for organic tomatoes does not allow the use of agrochemicals (López-Pérez *et al.*, 2006; Lee *et al.*, 2010).

Giving up growing tomato in areas infested with root-knot nematode is not a viable option for the farmer, especially when this area is the only option for cultivation. Thus, more sustainable strategies are necessary to make production in areas infested with these pathogens viable (Huang *et al.*, 2016).

Grafting can be a technique used in an integrated management system aiming to minimize damage caused by root-knot nematode (Peil, 2003; Krumbein & Schwarz, 2013; Verdejo-Lucas *et al.*, 2013). This practice improves the crop quality, with more efficient water and nutritional use, increase in plant vigor and harvest time, making the tomato plant more tolerant to pathogen attack, such as phytonematods (Rizzo, *et al.*, 2004; Lee *et al.*, 2010; Kyriacou *et al.*, 2017).

Although the genetic basis of rootstocks for tomato crop seems to be wide, considering the great quantity of species related to the crop which could be used, the current genetic basis is limited (King *et al.*, 2010). Most commercially available rootstocks are high-cost hybrids, produced by specialized companies (King *et al.*, 2010; Lopes *et al.*, 2015).

These rootstocks make the production more expensive for farmers and that is why identifying alternative rootstocks, resistant or tolerant, as an alternative to commercial hybrids, is essential. Wild *Solanum* species are sources of resistance characteristics to various soil pathogens, fruit quality and other important characteristics to be considered in order to improve crops, being possible alternative rootstocks for tomato crop (Keatinge *et al.*, 2014; Migicovsky & Myles, 2017).

Some studies on wild Solanum species as rootstocks, to control *Fusarium oxysporum* f. sp., *Lycopersici, Verticillium dahliae*, *Ralstonia solanacearum*, and root-knot nematode, have been carried out at Embrapa Hortaliças. These studies showed promising results (Pinheiro *et al.*, 2009, 2011, 2014; Pereira *et al.*, 2014; Lopes & Mendonça, 2016). In nematodes, the potential of wild *Solanum* species had been already proved in relation to parasitism of *M. incognita* (González *et al.*, 2010; Dhivya *et al.*, 2014), *M. arenaria* (González *et al.*, 2010) and *M. javanica* (Boiteux & Charchar, 1996).

In this context, the aim of this study was to evaluate the reaction of rootstocks of wild species joá-vermelho (*Solanum capsicoides*), joá-bagudo (*Solanum palinacanthum*), joá-bravo (*Solanum viarum*) and jurubeba (*Solanum* spp.) to parasitism of *Meloidogyne javanica*.

MATERIAL AND METHODS

The experiment was carried out in a greenhouse at Centro de Biotecnologia Agropecuária of Paraná State (26°11'43"S, 52°41'12"W). In this study, the authors tested the wild species joá-vermelho (*S. capsicoides*), joá-bagudo (*S. palinacanthum*), joá-bravo (*S. viarum*), jurubeba (*Solanum* spp.) and tomato Santa Cruz Kada (root-knot-susceptible variety), Batalha (susceptible hybrid) and Guardião (resistant-hybrid tomato rootstock) commercial tomatoes. Each experimental unit consisted of a pot with one plant, in completely randomized experimental design, with 10 replications per treatment.

The inoculum of root knot nematode *M. javanica* used in the experiment was obtained from a pure population kept in tomato plants, grown in a greenhouse. The nematode eggs were obtained according to the methodology described by Hussey & Barker (1973), modified by Bonetti & Ferraz (1981). The seedlings of all wild and commercial species were produced in a greenhouse, in polystyrene trays with 128 cells. Plantlets were transplanted 30 days after emergence, in 1-kg-capacity plastic containers, with substrate, soil and sand in the ratio 2:1 (v:v), autoclaved in advance for 1 h at 120˚C, 1 atm, (Ito *et al*., 2014). One week after seedling transplant, the nematode was inoculated using 2 mL of suspension containing 5,000 eggs of *M. javanica* through 4 equidistant holes open in soil, close to the plant stem.

Sixty days after nematode inoculation, gall index (IG), egg mass index (IMO), final nematode population (Pf); reproduction factor (FR) and reaction of: susceptibility (S), resistance (R) and immunity (I); fresh shoot mass (MPAF); fresh root mass (MRF); number of eggs per gram of root (NOGR) were evaluated.

In order to determine gall index (IG), the scale proposed by Charchar *et al*. (2003) was used: after being washed, the roots were graded from 1 to 5 (1= without galls; 2= up to 10 small galls; 3= up to 50 small galls; 4= more than 50 small galls and up to 10 big galls; 5= roots with more than 50 small galls and more than 10 big galls).

The egg mass index (IMO) was calculated following the scale proposed by Huang *et al*. (1986), considering a grading scale from 1 to 5 (1= without egg masses; 2= 1 to 5 egg masses; 3= 6 to 15 egg masses; 4= 16 to 30 egg masses; 5= more than 30 egg masses). Coloration methodology was performed according to Rocha *et al*. (2005).

Final nematode population per root (Pf) was obtained according to the methodology described by Hussey & Barker (1973), modified by Bonetti & Ferraz (1981).

Reproduction factor (FR) was calculated using the ratio between final nematode population (Pf) and initial population (Pi), and classified according to Oostenbrink (1966), expressed as follows: FR= 0: immune; FR<1: resistant; FR≥ 1: susceptible.

Variable data were submitted to analysis of variance using F test, comparing the treatment averages by Scott-Knott test ($p<0.05$). For reproduction factor (FR), the averages of treatments using wild Solanaceae were compared to controls (commercial cultivars) by Dunnett test ($p<0.05$). Analyses were performed using Assistat software (Silva & Azevedo, 2002).

RESULTS AND DISCUSSION

The wild species joá-bagudo and jurubeba showed the lowest gall index values (IG). For egg mass (IMO), jurubeba showed the lowest value, followed by joá-bagudo and joá-vermelho. On the other hand, joá-bravo showed the highest indexes for both variables (Table 1).

Final nematode population (Pf) and number of eggs per gram of root (NOGR) of joá-vermelho, joá-bagudo and jurubeba showed lower values (Table 1), in comparison to the other species, proving the lowest root-knot nematode multiplication rate. However, the species joá-bravo showed the highest value for both variables, due to high ratio of Pf/MRF, proving the high susceptibility of this species to nematode *M. javanica*. Silva *et al*. (2013) found similar values for Pf in another species of the wild Solanaceae, Maria-pretinha (*Solanum americanum*), which showed to be susceptible with a final population of 34,720 eggs.

Variable MPAF showed lower value for wild Solanaceae joá-bravo*,* for its high susceptibility to nematode *M. javanica*, followed by jurubeba. On the other hand, although the species joa-bagudo presented a higher value when compared to the other wild Solanaceae species, did not differ statistically from joá-vermelho.

The lowest development of shoot area of the plants is related to the highest number of galls which cause interferences on plant roots, which interfere on efficient absorption of nutrients and consequently on plant development (Jones *et al*., 2013; Karssen *et al*., 2013; Kepenekci *et al*., 2018). Therefore, plants showing lower MPAF generally show higher susceptibility to *M. javanica*.

In relation to MRF, the species joá-bravo showed the lowest value (in relation to the other wild Solanaceae species) and did not differ statistically from jurubeba. These results were obtained due to the slow initial growth of jurubeba, which results in a lower development, considering that this species showed reduced number of galls.

The species joá-bagudo, jurubeba and joá-vermelho showed to be resistant to *M. javanica*, when compared to the susceptible commercial controls (Batalha and Santa Cruz Kada) and similar to resistant-hybrid rootstock Guardião (Table 2), showing FR<1 according to Oostenbrink (1966). However, the species joá-bravo showed high susceptibility showing FR>1, not differing from susceptible tomato Santa Cruz Kada, which proved viability of the inoculum used in the present study, showing FR>1.

The results found in this study corroborate the ones found by Pinheiro *et*

Table 1. Average values of gall index (IG), egg mass index (IMO), final nematode population (Pf), fresh shoot mass (MPAF), fresh root mass (MRF) and number of eggs per gram of roots (NOGR) of *Meloidogyne javanica* on alternative and commercial rootstocks. Pato Branco, UTFPR, 2015.

Treatment	IG	IMO	Pf	MPAF (g)	MRF (g)	NOGR
Batalha	4.8 a	4.5 a	66,029.0 a	12.9 b	7.0 a	9,344.0 b
Santa Cruz Kada	4.9 a	5.0 a	32,555.0 b	6.1 c	4.2 b	7,865.5 b
joá-bravo	4.9 a	4.3 a	32,081.0 b	1.7 e	2.5 b	13,450.1 a
joá-vermelho	3.4 b	2.4 b	258.0 c	6.3 c	7.9 a	33.2 c
joá-bagudo	1.5 c	1.7 c	889.3 c	7.8 c	8.0 a	126.2 c
jurubeba	1.5 c	1.0 d	691.9 c	4.5 d	4.2 b	295.3 c
Guardião	1.0 c	1.0 d	133.7 c	15.7 a	7.9 a	16.9 c
CV (%)	15.4	26.9	25.5	30.4	87.9	79.2

Averages followed by same lowercase letter in the column do not differ statistically by Scott-Knott test ($p<0.05$).

Table 2. Average values of reproduction factor (FR) and rootstock reaction of wild Solanaceae species submitted to *Meloidogyne javanica* parasitism and comparison with susceptible and resistant controls. Pato Branco, UTFPR, 2015.

Treatments	Reproduction factor	Reaction
Batalha	13.2	S
Santa Cruz Kada	6.5	S
Guardião	0.0	R
joá-bravo	6.4(1.3)	S
joá-vermelho	0.1(1.2)	R
joá-bagudo	0.2(1.2)	R
jurubeba	0.1(1.2)	R
CV(%)	79.2	-

Averages followed by 1, 2 or 3 differ from the controls: Batalha, Santa Cruz Kada and Guardião respectively, by Dunnett test ($p<0.05$). S= susceptible; R= resistant.

al. (2009), who observed that the species *S. asperolanatum*, *S. stramonifolium* and *Solanum* spp., showed to be resistant to *Meloidogyne incognita* race 1, whereas *S. straminifolium, S. paniculatum*, and *S. subinerme* were resistant to *Meloidogyne enterolobii*, Sin.: *M. mayaguensis*. Some studies evaluating different accessions of *Solanum stramonifolium* in relation to parasitism of nematode *M. enterolobii*, Pinheiro *et al*. (2014) found 6 accessions of Solanaceae resistant to 17 studied species, being suitable rootstocks for Solanaceae in areas infested with this pathogen. Besides, Dhivya *et al*. (2014), evaluating rootstocks of wild Solanaceae resistant to *M. incognita*, obtained similar values for joá-bravo with FR>1, showing to be susceptible, however five from the other evaluated Solanaceae showed to be resistant or moderately resistant to the pathogen.

These results confirm the resistance of wild species (belonging to this family) to nematode parasitism, which can prove the viability of using them as alternative rootstocks, and reinforce the idea that further studies on grafting should be carried out, aiming to provide more viable options for farmers.

Although the wild Solanaceae species (joá-bagudo, joá-vermelho and jurubeba), evaluated in the present study, showed resistance to the nematode *M. javanica*, the compatibility of these species is another important factor to be taken into account. In this context, Simões (2014) obtained compatibility of jiló and jurubeba species as rootstocks for tomato cultivar IPA-6. Farias *et al*. (2013) obtained *S. gilo, S. lycocarpum* and *S. stramonifolium* as alternative rootstocks compatible with tomato and suitable for conditions in Brazilian Amazon Region. This fact demonstrates feasibility of grafting and the importance of further studies aiming to show the efficiency of these species as alternative rootstocks for tomato crop.

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