RESEARCH NOTE/NOTA DE INVESTIGACIÓN

RESPONSE OF WILD SOLANACEAE TO *MELOIDOGYNE INCOGNITA* INOCULATION AND ITS GRAFT COMPATIBILITY WITH TREE TOMATO (*SOLANUM BETACEUM*)

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ABSTRACT

Vargas, Y., J. Nicolalde, W. Alcívar, L. Moncayo, C. Caicedo, J. Pico, L. Ron, and W. Viera. 2018. Response of wild Solanaceae to *Meloidogyne incognita* inoculation and its graft compatibility with tree tomato (*Solanum betaceum*). Nematropica 48:126-135.

The Solanaceae includes wild species that have been reported as being resistant to soil borne pathogenic agents. In the Ecuadorian Amazon region, diversity is found among specimens of this plant family. This investigation evaluated the response of seven wild Solanaceae to inoculation with root-knot nematode (*Meloidogyne incognita*). Resistance to the nematode was observed in the Solanaceae collected in Misahuallí (SN5) (Napo Province), which also showed a low total number of galls; whereas the other six showed susceptibility. Regarding compatibility with tree tomato (*Solanum betaceum*), only Solanaceae SN2 and SO7 collected in the localities of San Carlos (Orellana Province) and Curimuyo (Napo Province), respectively, resulted in less than 90% graft survival. Solanaceae S06 collected in the province of Morona Santiago, resulted in the best affinity (1.14) with the fruit species; however, SN5 also resulted in an acceptable value (1.19). SN5 would be recommended to be utilized as rootstock due to the characteristics previously mentioned.

Key words: affinity, graft survivorship, knots, resistance, tolerance

RESUMEN

Vargas, Y., J. Nicolalde, W. Alcívar, L. Moncayo, C. Caicedo, J. Pico, L. Ron, e W. Viera. 2018. Respuesta de solanáceas silvestres a la inoculación de *Meloidogyne incognita* y su compatibilidad de injertación con tomate de árbol (*Solanum betaceum*). Nematropica 48:126-135.

La familia Solánacea abarca especies silvestres que han sido reportadas como resistentes al ataque de agentes patógenos del suelo. En la Amazonía ecuatoriana existe diversidad en especímenes de esta familia de plantas, por lo que esta investigación evaluó la respuesta de 7 Solánaceas silvestres a la inoculación del nematodo agallador del nudo (*Meloidogyne incognita*). Se observó resistencia al nematodo en la Solanácea colectada en Misahullí (SN5) (Provincia de Napo), misma que presentó también un bajo número total de nodulaciones; mientras que el resto resultaron ser susceptibles. En cuanto a compatibilidad con tomate de árbol (*Solanum betaceum*), solamente las Solanáceas SN2 y SO7 colectadas en las localidades de San

Carlos (Provincia de Orellana) y Curimuyo (Provincia de Napo) respectivamente, mostraron porcentajes de prendimiento inferiores a 90%. La Solanácea S06 colectada en la Provincia de Morona Santiago presentó la mejor afinidad (1.14) con la especie frutal; sin embargo SN5 también presentó un valor aceptable (1.19), por lo que éste último se recomendaría para ser utilizado como portainjerto debido a las características mencionadas anteriormente.

Palabras clave: afinidad, nódulos, prendimiento, resistencia, tolerancia

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Tree tomato (*Solanum betaceum* Cav.) is a Solanaceae fruit native to the Ecuadorian and Peruvian Andes (Vasco *et al*., 2009). In Ecuador, this crop is cultivated on about 5,000 ha to meet both national demand and its potential for export (Arahana *et al*., 2010). National yields range from 60 to 80 t/ha/yr although production is low due to susceptibility to pest attack (Viera *et al*., 2016).

Among the most important pests of commercially cultivated Solanaceae, are plant-parasitic nematodes (Castro *et al*., 2011; Orrico *et al*., 2013). In Ecuador, losses caused by nematodes on *S. betaceum* can be up to 90%, resulting in a 50% reduction in the life cycle of this crop (Ramírez *et al*., 2015). *Meloidogyne* sp. is the main genus detected in Solanaceae affected by nematodes. *Meloidogyne* is a sedentary endoparasite of the root, inciting galls that cause severe growth delays (Castro *et al*., 2011; Hussain *et al*., 2016). Yield losses are due to the accumulation of pathogen inoculum as a result of continuous use of a field for the same crop (Pakeerathan *et al*., 2009; Hussain *et al*., 2016).

The use of resistant cultivars as an integrated management component is an option for reducing yield losses by plant-parasitic nematodes. Nematicides, although efficient, are not attractive for use in agriculture due to their high costs and dangerous effects on human health and environment; also nematicides reduce biodiversity of the ecosystem (Castro *et al*., 2011; Hussain *et al*., 2016). The main nematode that has been encountered infesting different vegetables and Solanaceous species is *M. incognita* (Bastidas *et al*., 2004; Gonáles *et al*., 2010). The use of grafted plants in crop production is appropriate as the rootstock protects the plant from soil borne pathogens, helps to improve yield through efficient nutrient assimilation, prolongs the commercial life of clones, allows changes in scion cultivars on plants already established, and hastens reproductive maturity of the plant (Martínez *et al*., 2010; Arizala *et al*., 2011). González *et al*. (2010) and Navarrete *et al*. (2018) showed that different Solanaceous species have different resistance/tolerance responses to this phytopathogenic nematode. It has been demonstrated that Solanaceae plants like *Nicotiana glauca* and *S. auriculatum* can be utilized as tree tomato rootstocks in the Ecuadorian Andean Region because they present resistance/tolerance to *M. incognita* and double production (22 t/ha) in contrast with plants originated by seeds (12 t/ha) (Viteri *et al*., 2010).

This research evaluated the resistance/tolerance response of different wild Solanaceae from the Ecuadorian Amazon Region to inoculation with *M. incognita*, as well as their graft compatibility with *S. betaceum* for possible use as a rootstock of this fruit crop.

Plant material

Wild Solanaceae were obtained in three provinces of the Ecuadorian Amazon: Napo, Morona Santiago, and Orellana (Table 1). Plants with outstanding characteristics (architecture, yield, and nematode absence) were selected. Three physiologically mature fruits from each plant were placed in plastic bags and labeled with origin and GPS coordinates. From each Solanaceae plant, agronomic characteristics such as plant height, leaf blade shape, leaf apex shape, leaf base shape, fruit peel color, fruit pulp color, fruit shape, fruit weight, fruit length, and fruit diameter were recorded (Table 2). Description of materials was carried out following the Bioversity International (2013) descriptors.

Nematode inoculum

As *M. incognita* affects both *S. betaceum* and *S. quitoense* (Bastidas *et al*., 2004), nematodes were extracted from galled naranjilla roots (*S. quitoense*) using a blender method (Hussey and Barker, 1973). Roots were chopped into 5 mm

Table 1. Wild Solanaceae collection locations in three provinces of the Ecuadorian Amazon.

Code	Province	County	Location	Latitude	Longitude	Altitude[z]
SN1	Napo	Tena	Tamiahurco	0°59′37.82′′	77°35′59.88′′	566
SN2	Napo	Archidona	Curimuyo	0°55′39.78′′	77°48′4.62′′	542
SM3	Morona	Palora	Santiago	1°44′22.67′′	77°53′59.93′′	877
SO4	Orellana	Joya de los Sachas	San Carlos	0°21′27.33′′	77°52′25.96′′	298
SN5	Napo	Tena	Misahuallí	1°1′30.37′′	77°39′41.94′′	442
SO6	Orellana	Loreto	Comuna	0°43′58.24′′	77°17′44.36′′	364
SO7	Orellana	Joya de los Sachas	San Carlos	0°21′27.33′′	77°52′25.96′′	298

[z]meters above sea level

length pieces, shaken in sodium hypochlorite for 3 min with constant agitation, washed with water, and filtered through nested 100-µm and 20-µm-pore sieves. Eggs collected on the 20-µm-pore sieve were transferred to a 100-ml Erlenmeyer, diluted with 100 ml water, from which 1 ml aliquots were transferred to a counting dish and the number of eggs determined.

Propagation of the wild Solanaceae

For propagation of the wild Solanaceae, soil was sieved and sterilized in an autoclave (121°C) for 30 min. In the greenhouse, seeds of the seven wild Solanaceae as well as commercial *S. betaceum* were sown in propagation trays (24 cells) with 250 g soil/cell. In the *M. incognita* inoculation response study, 60 days after sowing when plants were 15 to 20 cm in height, seedlings were transplanted into pots with 5,000 g soil (for nematode inoculation) or into black polyethylene bags (23 cm long × 15 cm wide) with 1,200 g soil (for graft compatibility). Each plant was fertilized with 10 g/plant of fertilizer 15-15-15.

Inoculation of M. incognita

Twenty-four days after transplanting, four equidistant holes (5 cm^3) 5-cm distance from the plant stem were inoculated with 20,000 eggs of *M. incognita* (4,000 eggs/soil kg) suspended in sterile distilled water (20 ml per plant). Plants were watered with distilled water until field capacity during the experiment.

Host response to the inoculation of M. incognita

A randomized complete block design (RCBD) with three replications (each replication consisted of 3 plants) was utilized for this evaluation. The study of host response to the infection by *M. incognita* was implemented under controlled conditions (greenhouse) in the Hatum Sumaku, canton of Archidona, Napo province (longitude 0°40′30′′ and latitude 77°45′40′′) at 1042 m.s.l., with an average temperature 28°C and 63% relative humidity. The resistant or susceptibility reaction was recorded as nematode final population (Fp) and reproductive factor (Rf = maximum value of the confidential interval/initial nematode population). For Fp, eggs were extracted from 20 g of symptomatic roots using the blender method (Hussey and Barker, 1973). In order to estimate the initial nematode population, a Poisson model with a confidence interval at 95% was used. The Rf value was used to determine the resistance or susceptibility of each Solanaceae evaluted. When Rf > 1, the Solanaceae was considered resistant. When the Rf < 1, the plant was considered susceptible (Oostenbrink, 1966).

To determine tolerance, foliage fresh weight was used. Foliage weight was recorded 107 days after nematode inoculation. A mean contrast (“t” test at 5%) between the non-inoculated and the inoculated plant was conducted in order to determine differences. The wild Solanaceae was considered tolerant when the foliage fresh weight from the inoculated plant was equal to or greater than the weight of the non-inoculated plant. The wild Solanaceae was intolerant when the foliage fresh weight of the non-inoculated plant was greater than the inoculated plant weight.

Finally, in order to determine differences in the degree of damage on each Solanaceae, the total number of roots galls per Solanaceae root system was recorded. Galls on the roots were counted prior to nematode egg extraction. Differences between plants were determined using an analysis of variance (ANOVA) and means were separated by Tukey’s test at 5%.

A RCBD with three replications consisting of 20 plants was utilized for the evaluation. Graft

Table 2. Fruit agronomic characteristics of wild Solanaceae collected in three Ecuadorian Amazon provinces.

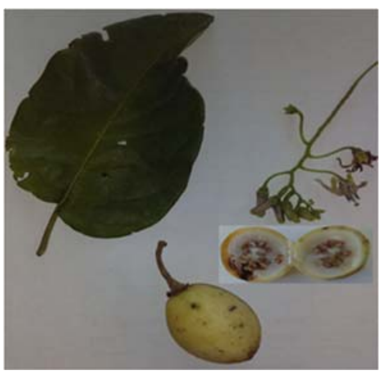
Code	Wild Solanaceae	Characteristics[Z]
SN1		Leaf blade shape: Cordate Leaf apex shape: Apiculate Leaf base shape: Cordate Leaf margin: Entire Fruit peel color: Orange 25-D Fruit pulp color: Orange 28-B Fruit shape: Elongated oval Fruit weight (g): 30.0 Fruit length(mm): 41.6 Fruit diameter (mm): 37.5
SN2		Leaf blade shape: Oblique Leaf apex shape: Accuminate Leaf base shape: Oblique Leaf margin: Entire Fruit peel color: Yellow 4-D Fruit pulp color: White NN 155-D Fruit shape: Elongated oval Fruit weight (g): 25.3 Fruit length(mm): 34.7 Fruit diameter (mm): 39.1
SM3		Leaf blade shape: Oblique Leaf apex shape: Acute Leaf base shape: Oblique Leaf margin: Entire Fruit peel color: Yellow 11-D Fruit pulp color: White NN 155 – C Fruit shape: Ovoid Fruit weight (g): 40.7 Fruit length(mm): 48.1 Fruit diameter (mm): 35.8
SO4		Leaf blade shape: Oblique Leaf apex shape: Apiculate Leaf base shape: Cuneate Leaf margin: Crenate Fruit peel color: Yellow 6-D Fruit pulp color: White 155-B Fruit shape: Elongated oval Fruit weight (g): 30.2 Fruit length(mm): 42.5 Fruit diameter (mm): 34.5

Table 2. Continued

Code	Wild Solanaceae	Characteristics[Z]
SN5		Leaf blade shape: Elliptic Leaf apex shape: Apiculate Leaf base shape: Oblique Leaf margin: Entire Fruit peel color: Yellow 10-C Fruit pulp color: White NN-155C Fruit shape: Elongated oval Fruit weight (g): 45.1 Fruit length(mm): 49.7 Fruit diameter (mm): 42.5
SO6		Leaf blade shape: Ovate/Palmate Leaf apex shape: Apiculate Leaf base shape: Oblique Leaf margin: Entire Fruit peel color: Yellow 10-C Fruit pulp color: White 155-D Fruit shape: Round Fruit weight (g): 90.6 Fruit length(mm): 59.6 Fruit diameter (mm): 51.5
SO7		Leaf blade shape: Oblique Leaf apex shape: Apiculate Leaf base shape: Oblique Leaf margin: Lobate Fruit peel color: Green N 138-C Fruit pulp color: Yellow green 152-D Fruit shape: Round Fruit weight (g): 90.1 Fruit length(mm): 49.7 Fruit diameter (mm): 55.2

[z]Weight, length, and diameter values are the average of three fruits.

compatibility was carried out in a nursery in the Amazonian Central Experimental Station (EECA) of the National Institute of Agricultural Investigations (INIAP), located in the canton Joya de los Sachas, Province of Orellana (longitude 76°52´40.1´´ and latitude 0°21´31.2´´) at 282 m.s.l., with an average temperature of 30°C and 69% relative humidity,

Thirty days after transplanting, Solanaceae plants were grafted by the cleft graft method when the wild Solanaceae were 30 to 40 cm high and had a stem diameter of 8 mm (Arizala *et al*., 2011). The rootstock consisted of each wild Solanaceae cut at 10 cm from stem base and grafted with a 10 cm length scion of *S. betaceum*. Watering was at field capacity during the whole experiment. Graft compatibility was evaluated through survival rate and the ratio between stem diameter above and below the graft union. Thirty days after grafting, the number of living plants was counted for each treatment and survivorship was calculated as the number of sprouted plants divided by the total number of grafted plants. Ninety days after grafting the scion (*S. betaceum*) and the rootstock (wild Solanaceae), the stem diameter was measured at five centimeters above and below the graft union point. The scion stem diameter was divided by the rootstock stem diameter to determine the compatibility ratio. A compatibility ratio equal to 1 showed compatibility and ratios less than 1 demonstrated incompatibility (López *et al*., 2008). Data were analyzed of variance and means separated by Tukey's test at 5%. All data were analyzed using the statistical package R version 3.3.2.

Response to M. incognita

The only wild Solanaceae with nematode resistance was SN5 collected in the locality Misahuallí (Table 1). SN5 reduced nematode population and had an Rf = 0.64 in contrast with the other Solanaceae with Rfs greater than 1 (Table 3).

All the wild Solanaceae had a tolerance response similar to *S. betaceum*. The foliage yield was not superior (P>0.05) to the inoculated plants (Table 4). The same response has been observed in the tree tomato cultivar 'Anaranjado Puntón', where low nematode reproduction indexes were found and little reduction in plant growth was observed resulting in the cultivar being susceptible-tolerant (Guamán, 1996). Our results might be due to the relatively short evaluation period of 107 days. Evaluations over longer periods of time could result in a nematode-intolerant response, especially in *S. betaceum*, since more root development could result in greater nematode population densities due to a larger food supply allowing for increased root and plant damage.

SN5 was also among the plants showing fewer number of galls (Table 5). SN5 could be considered as a promising material to be used as rootstock. *Solanum betaceum* showed a low number of galls at the 107-day evaluation; however, it is known that nematode infection and the number of galls rapidly increase as tree tomato roots develop.

Graft compatibility

The wild Solanaceae rootstocks and *S. betaceum* scion interacted. The wild Solanaceae differed in the percentage of survival of the grafts (P<0.05). Solanaceae SO4, SN1, SN5, SM3, and SO6 had graft survivorship percentages that varied between 90 and 100%; whereas SO7 and SN2 resulted in graft survivorship rates less than 80% (Table 6). Ninety days after grafting, the wild Solanaceae differed in compatibility with the scion *S. betaceum*. Solanaceae SO6, SM3, SN5 (collected from Orellana, Morona Santiago, and Napo) were compatible, whereas SO7 and SN2 were incompatible (Table 6). The wild Solanaceae that showed good compatibility between rootstock

Table 3. Response of wild Solanaceae (resistance or susceptibility) based on confidence limit values of the final population of *Meloidogyne incognita*.

Wild Solanaceae	Nematode/ 20 g fresh root[z]	Confidence interval (Nematode/ 20 g fresh roots)	Reproduction factor	Response
SN1	43,679	43,333 – 44,027	2.20	Susceptible
SN2	105,922	105,206 – 106,641	5.33	Susceptible
SM3	37,756	37,536 – 37,976	1.90	Susceptible
SO4	110,292	109,520 – 111,038	5.55	Susceptible
SN5	12,737	12,590 – 12,886	0.64	Resistant
SO6	107,691	106,964 – 108,422	5.42	Susceptible
SO7	31,569	31,317 – 31,823	1.59	Susceptible
Solanum betaceum	95,711	95,055 – 96,372	4.82	Susceptible

[z] Average value of 9 plants

Table 4. Response (tolerance and intolerance) of wild Solanaceae to *Meloidogyne incognita*.

Wild Solanaceae	Foliage fresh weight (g)		*P*-value (0.05)	Response
	Inoculated[z]	Non-inoculated [z]		
SN1	30.26	36.66	0.19	Tolerant
SN2	47.23	57.80	0.62	Tolerant
SM3	30.83	62.23	0.13	Tolerant
SO4	65.00	76.66	0.45	Tolerant
SN5	52.33	71.10	0.06	Tolerant
SO6	69.46	102.20	0.07	Tolerant
SO7	93.35	77.50	0.41	Tolerant
Solanum betaceum	55.00	58.33	0.86	Tolerant

[z]Average of 9 plants

Table 5. Total number of root knots on wild Solanaceae inoculated with *Meloidogyne incognita*.

Wild Solanaceae	Number of knots[z]	Confidence interval
SN1	715 ab	668 – 766
SN2	1,814 b	1,714 – 1,931
SM3	347 a	424 – 471
SO4	815 ab	763 – 871
SN5	652 ab	609 – 699
SO6	1243 ab	1,171 – 1,327
SO7	949 ab	892 – 1,010
Solanum betaceum	416 a	385 – 449

[z]Average from 9 plants

Table 6. Graft survivorship and compatibility of different wild Solanaceae utilized as rootstock for *Solanum betaceum*.

Wild Solanaceae	Graft survivorship (%)	Compatibility ratio
SO6	100.00 a	1.14 a
SM3	98.33 a	1.18 ab
SN5	92.50 ab	1.19 ab
SN1	90.00 ab	1.22 b
SO4	90.00 ab	1.23 b
SO7	80.00 b	1.32 c
SN2	76.67 b	1.37 c

and scion had compatibility ratios close to 1 (Martínez *et al*., 2010; Arizala *et al*., 2011). SO6, SM3, SN5 had good tissue binding between the rootstock and the scion.

Previous studies have shown that in Ecuador the area of tree tomato production has increased; however, its yield has decreased (Viteri *et al*., 2010). One of the main factors contributing to yield reduction was the damage caused by *M. incognita*. One effective and safe alternative for control of this disease is the use of grafts with rootstock coming from wild Solanaceae that show major resistance and/or tolerance to this nematode.

SN5, with its resistant response, could be considered as a source of genes for resistance to *M. incognita*. González *et al*. (2010) and Gelpud *et al*. (2011) mentioned this resistance is possibly produced by the Mi gene that gives resistance to three of the most devastating nematode species *M. incognita*, *M. arenaria*, and *M. javanica*. This resistance is phenotypically characterized by a hypersensitivity response in the zone where nematodes seek to feed. Gelpud *et al*. (2011) mention that response mechanisms to *Meloidogyne* spp., either constitutive or inductive, are expressed in different ways depending on the interaction between nematode and host. This factor determines activation or absence of expression of these resistance mechanisms. Dhivya *et al*. (2014) reported that *S. sisymbrifolium*, *Physalis peruviana*, and *Solanum torvum* presented high resistance; whereas *S. incanum* and *S. aethiopicum* presented moderate resistance to *M. incognita*.

They also mentioned that these plant materials could be considered promising to use as tree tomato rootstock in root-knot nematode infested areas. Rahman *et al*. (2002) determined that *S. torvum* and *S. sisymbriifolium* presented resistance to *M. incognita*, suggesting its utilization as rootstock. Also, Rodríguez *et al.* (2009) showed that *S. torvum* and *S. lycopersicum* were resistance to *M. incognita* race 2.

Salazar and Guamán (2013) mention that for each nematode at transplanting time there occurs a nematode increment of 1.94 at the end of the crop cycle. It is inferred that after 6 months of plant life, there would be an increase in the number of knots in control plant.

In other Solanaceae crops such as naranjilla, 50% average graft survival has been reported when utilizing wild Solanaceae as rootstocks (Pakeerathan *et al*., 2009). On the other hand, grafting eggplant on *S. torvum* resulted in 95% graft survivorship, whereas on *S. sisymbriifolium,* graft survivorship was only 85% (Rahman *et al*., 2002). In our study, some wild Solanaceae rootstocks resulted in graft survivorship greater than 90%. SN5, collected in Misahualli, showed a resistance response to root-knot nematode infection and was graft compatible with *S. betaceum.* SN5 has potential for use as rootstock for *S. betaceum* in tropical zones.

Fruit species belonging to the Solanaceae family are susceptible to *M. incognita* attack. Although most of the Solanaceae evaluated showed graft compatibility with tree tomato, not all of the accessions were resistant to the nematode. In this study, wild Solanaceae SN5 was resistant to *M. incognita* and had good compatibility with *S. betaceum* resulting in an ecologically favorable alternative for nematode control. Use of SN5 as a rootstock will minimize yield loss in tree tomato from *M. incognita* and reduce the use of nematicides in tree tomato plantations. However, additional investigations are required in order to evaluate this Solanaceae as rootstock in the field to determine its effect on the development and yield of tree tomato. Likewise, the botanical identification of the selected wild specimen is necessary.

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