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Host status of three Solanaceae species from Lasiocarpa Section to root-knot nematode *Meloidogyne incognita*

Capacidad hospedante de tres especies de Solanáceas de la Sección Lasiocarpa al nematodo agallador de la raíz *Meloidogyne incognita*

Patricio Castro-Quezada*[1], Luis Pacheco-Atariguana[1] and Lourdes Díaz-Granda[1]

[1]*Grupo de Biotecnología Agropecuaria, Facultad de Ciencias Agropecuarias, Universidad de Cuenca, Cuenca, Ecuador.*

*Corresponding author: patricio.castro@ucuenca.edu.ec

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Abstract

Root knot nematode *M. incognita* is one of the most dangerous and common species affecting Solanaceae family, including the naranjilla crop (*S. quitoense*). The objective of this work was to evaluate three species of Solanaceas as hosts (*S. sessiliflorum*, *S. hirtum* and *S. quitoense*) for an isolated of *M. incognita* in greenhouse. Plants of three species were planted in pots and each plant was inoculated with 2500 eggs and second stage juveniles (J2). Host suitability was assessed 80 days after inoculation. Initial inoculum was obtained from infested roots of *S. quitoense* plants collected in commercial naranjilla orchards. A completely randomized experimental design was used. The variables evaluated at 80 days after inoculation were: gall index (GI), nematode reproduction factor (RF), dry weight of the foliar area, plant height and stem diameter. All species were galled, but *S. sessiliflorum* and *S. hirtum* showed the least number of root knots with values of 33.73 and 34.73. Both were classified as resistant / hypersensitive with reproduction factors of 0.94 and 0.85 (RF> 1) respectively, while *S. quitoense* was susceptible with a value of 1.56. In terms of foliage yield (dry weight), plant height and stem diameter, *S. sessiliflorum* and *S. hirtum* showed a tolerance response in relation to *S. quitoense*.

Keywords: Host range, hypersensitive, root-knot nematode, *Solanum hirtum*, *Solanum sessiliflorum*.

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Resumen

El nematodo del nudo de la raíz *Meloidogyne incognita* es una de las especies más peligrosas y comunes que afectan a las solanáceas, entre ellas la naranjilla *Solanum quitoense*. El objetivo de este trabajo fue evaluar el potencial reproductivo de un aislamiento de *M. incognita* en tres especies de Solanaceas en invernadero: *Solanum sessiliflorum*, *Solanum hirtum* (reportada anteriormente como resistente) y *S. quitoense* (susceptible). Plantas de las tres especies fueron sembradas en maceta y a las cuatro semanas fueron inoculadas con 2500 huevos más juveniles en estado 2 (J2). El inóculo inicial se obtuvo de raíces infestadas de plantas de *S. quitoense* recolectadas en huertos comerciales de naranjilla. Se utilizó un diseño experimental completamente aleatorizado. Las variables evaluadas a los 80 días después de la inoculación fueron: índice de agallas (GI), factor de reproducción de nematodos (RF), peso seco del área foliar, altura de la planta y diámetro del tallo. Se encontró que las tres especies mostraron agallamiento, pero *S. sessiliflorum* y *S. hirtum* mostraron el menor número de nudos de raíz con valores de 33,73 y 34,73. Además, *S. sessiliflorum* y *S. hirtum* presentaron una categoría de resistente/hipersensitivo con factores de reproducción de 0,94 y 0,85 (RF $>$ 1) respectivamente, mientras que *S. quitoense* fue susceptible con un valor de 1,56. En términos de rendimiento de follaje (peso seco), altura de la planta y diámetro del tallo se observó una respuesta de tolerancia en *S. sessiliflorum* y *S. hirtum* en relación a *S. quitoense.*

Palabras clave: Capacidad hospedante, hipersensibilidad, nematodo agallador, *Solanum hirtum*, *Solanum sessiliflorum*.

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Orcid IDs:

Patricio Castro-Quezada: http://orcid.org/0000-0002-2366-2256
Luis Pacheco-Atariguana: http://orcid.org/0000-0003-1308-0495
Lourdes Díaz-Granda: http://orcid.org/0000-0003-0983-723X

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1 Introduction

Naranjilla (*S. quitoense*), known as "lulo" in Colombia, is an important fruit crop in Ecuador. It is a perennial herb plant belonging to the Solanaceae family. It is considered the "golden fruit" of the Andes by its color, whose flavor is acid and exotic (Ramírez et al., 2018). According to Whalen and Caruso (1983) solanaceae belonging to the Lasiocarpa family is mainly found in northeast South America, and it is said that naranjilla tres are native from Colombia and Ecuador (Morton, 1987; Council, 1989). The main cultivated variety is *S. quitoense* Var. *Quitoense* without thorns. Naranjilla is a commonly underused crop for manufacturing juices, ice cream, jellies and other sweets. However, this crop has economic potential (Heiser, 1993).

One of the greatest challenges for producers is the susceptibility of naranjilla to the root knot nematode *M. incognita* and the fungus *Fusarium oxysporum* (Mosquera-Espinosa, 2016). Nematodes are biotrophic parasites capable of infecting more than 2000 plant species. These parasites infect plant roots and induce the formation of giant feeding cells that reduce plant nutrition and water absorption. As a result, plants may show several symptoms such as wilting, stunting, and significantly reduced yields (Barbary et al., 2015; Ralmi et al., 2016; El Sappah et al., 2019).

To fight againts *M. incognita*, farmers in Ecuador crop naranjilla in recently dismantled areas, free of nematodes or use nematicides directly to the soil (Ramírez et al., 2018). However, these products can generate resistance, affect human health and the environment. In addition, many countries, especially within the European Union, have now banned the use of chemicals such as carbofuran for controlling nematodes (Caromel et al., 2005; Barbary et al., 2015).

The use of resistant cultivars may be the cheapest and best environmental method for controlling root knot nematodes (Barbary et al., 2016). In the case of naranjilla, hybridization or the use of grafts of other nematode-resistant, compatible solanaceous species could be the key to improving plant performance (Anderson et al., 2005; Heiser, 1985). There are several wild species related to naranjilla such as: *Solanum tequilense*, *Solanum vestissirmum*, *Solanum lasiocarpum*, *Solanum arboretum*, *S. sessiliflorum* and *S. hirtum* (Ramírez et al., 2018). Among these, *S. hirtum* and *S. arboretum* have been used as patterns for the Baeza lulo variety (*S. quitoense* var. *quitoense*) developed by the National Autonomous Institute of Agricultural Research (INIAP) (Sowell and Shively, 2012; Clements et al., 2017).

In some cases, hybridization between *S. quitoense* and wild relatives has resulted in viable plants such as *S. quitoense* × *S. hirtum* (Heiser, 1972). These hybrids have resulted in segregation plants with some resistance to root knot nematodes (Heiser, 1993; Ramírez et al., 2018). However, fruits of these hybrids have a bad taste (Heiser, 1972; Ramírez et al., 2018) and roots are infected by the nematode present in the development of nodules caused by the penetration of the nematode, indicating absence of immunity (Navarrete et al., 2018). Additionally, the fact of classifying *S. hirtum* as resistant to its host capacity includes the reproductive factor and excludes the galled index, as proposed by Canto-Saenz modified scheme (De Almeida et al., 1997; Sasser et al., 1984).

Regarding *S. sessiliflorum* in Ecuador, Puyo and INIAP Palora hybrids have been cropped. The commercial hybrid of Puyo results from a cross between "coccona" (*S. sessiliflorum*) × naranjilla var., Agria (*S. quitoense* Var. *quitoense*). The hybrid INIAP Palora results from the crossing between naranjilla var. Baeza (*S. quitoense* Var. *quitoense*), used as male progenitor and "coccona" (*S. sessiliflorum*) as female progenitor (Revelo et al., 2010). However, there are no studies on host capacity or reproductive potential of *S. sessiliflorum* species for the galled nematode. Thus, the aim of this study was to determine the host capacity of three species of Solanaceae belonging to the Lasiocarpa section for an isolate of *M. incognita*.

2 Materials and methods

The host capacity of the species *S. sessiliflorum*, *S. hirtum* for *S. quitoense* para *M. incognita* was evaluated in this research. This study was carried out in a greenhouse of the Faculty of Agricultural Sciences of University of Cuenca, province of Azuay, at an altitude of 2567 meters above sea level, at the coordinates UTM 2°55′12.564″, 79°1′30.6122″. The

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average greenhouse temperature during the experiment varied between 12 to 32°C and the relative humidity between 60 and 80%.

The propagation of plants of the three species was made from seeds collected in the province of Morona Santiago (eastern Ecuador). Seeds were planted in germination trays with sterile substrate (peat). After two months, seedlings of the three species were planted in 2 kg pots with sterile substrate composed of loamy soil (Vertisol order), black hill soil and bocashi (4:4:2).

The inoculum of *M. incognita* was obtained from naranjilla plants collected in the province of Morona Santiago. The specific MI-F initiators or primers GTGAGGATTCAGCTCCCG and MI-R ACGAGATACTTCTCCGTCC (Martínez-Gallardo et al., 2019) were used to identify the nematode species. Amplification reactions were performed on an Eppendorf Mastercycler NexusGSX1 thermal cycler, at a final volume of 25 μl which contained 2.5 μl buffer (10X) (Invitrogen), 0.5 μl of each dNTP (10 mM), 1.5 μl of $MgCl_2$ (50 mM), 2.5 μl of each primer (100 μm), 0.2 μl of Taq polymerase (Invitrogen, 5 or μl^{-1}) and 1 μl of DNA (10 ng/μl). For PCR, an initial denaturation was performed at 94°*C* for 2 min, followed by 35 cycles of 94°*C* for 30s, 64°*C* for 30s and 68°*C* for 1 min, followed by a final extension at 72°*C* for 5 min. Samples were analyzed in 1% of agarose gel with ethidium bromide (0.25 $\mu g\ ml^{-1}$) and visualized in a BioRad UV transilluminator. A molecular marker of 1 kpb (Invitrogen) was used as a molecular standard.

The population of *M. incognita* was multiplied in tomato plants var. Sheila in greenhouse conditions in pots of 2 kg with sterile substrate and was kept in the greenhouse of the Department of Phytopathology of the University of Cuenca. Tomato seedlings were inoculated with 20 mL of the extract obtained from naranjilla infected roots. Inoculation was made in four 3 cm deep holes in the tomato seedling. After 60 days, infected roots were processed according to Hussey and Baker's extraction methodology (Hussey, 1973) using 0.5% sodium hypochlorite (NaClO). For the extraction of eggs, an optical stereoscope was used to identify *M. incognita* eggs and larvae embedded in the oval masses. The masses were separated from the plant tissue with sieves of 150 and 25 μm pores and placed in Petri dishes that remained for 72 hours at a temperature of 28°C, to allow the hatching of nematode eggs and emergence of juvenile nematodes in the J2 state.

This inoculum was used to infest 4-week seedlings of the species *S. sessiliflorum*, *S. hirtum* and *S. quitoense*. Each plant was inoculated with 2500 younger eggs in state 2 (J2). Inoculation was done by placing 2 mL of the inoculum suspension in four 3-cm deep holes around the base of each plant. Then holes were filled with soil. Sterile water was placed in the control plants instead of the inoculum.

After 80 days of inoculation, the roots of the three species were washed individually with running water. Excess water was removed with paper towels and stained with Floxine B to facilitate the counting of egg masses (Taylor and Sasser, 1978). The root gall index (GI) was obtained using a rating scale of 0-5 (0 = no galls, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls, 5 = > 100 galls) (Taylor and Sasser, 1978). Eggs were extracted from each root with 0.5% of sodium hypochlorite (NaClO) (Hussey, 1973) and were counted to determine the final population density (Pf) (Oostenbrink, 1966). The density of the final population of nematodes (Pf) was estimated as the total number of J2 and eggs extracted from the roots of each plant and the reproduction factor RF= Pf/π was calculated (Bybd et al., 1983). The state of the host was determined by the gall index (GI) and the reproductive factor according to the modified Canto-Saenz scheme (De Almeida et al., 1997; Sasser et al., 1984). Plants with $GI > 2$ are defined as susceptible ($RF > 1$) or resistant/hypersensitive ($RF \geq 1$); plants with $GI \leq 2$ are defined as resistant ($RF \geq 1$) or tolerant ($RF > 1$) (Sasser et al., 1984; De Almeida and De A Santos, 2002; Maleita et al., 2011).

These variables were also registered: number of knots per root, dry weight of the leaf area (g), plant height (cm) and stem diameter (cm). For the variable dry weight of the foliar area for each seedling, it was placed on paper covers and dried in a Nabertherm furnace at 60°C for 72 hours. It was then weighed with a Boeco BWL 61 digital scale.

A completely randomized experimental design was used. The normal distribution of observations for each species was determined by the modified Shapiro Wilk test with P significance levels $< 0{,}05$

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and the homogeneity of variances by the Levene test. The data were statistically analyzed by analysis of variance with means separated by Duncan's multiple-range test at 5% significance level with the Infostat statistical package (Di Rienzo et al., 2008).

3 Results

The results of the experiment showed that the species *S. sessiliflorum* and *S. hirtum* varied in their host capacity in relation to *S. quitoense* for *M. incognita*. The *M. incognita* species used as an inoculum was verified by PCR, with specific initiators for the species. A visible band of approximately 1000 bp was visualized, which was expected for the 28S rNRA region of the nematode in the three species (Hu et al., 2011; Martínez-Gallardo et al., 2019).

The three infected species allowed the development of the nematode. However, a different degree of gall and reproduction was presented for the three species analyzed. For the reproduction factor (RF), significant differences between *S. sessiliflorum* and *S. hirtum* were found in relation to the control species *S. quitoense* (Table 1). A difference in reproduction was observed for *S. quitoense* with 1.56 ($RF > 1$), while RF values varied between 0.85 for *S. hirtum* and 0.94 for *S. sessiliflorum*. Even though *S. sessiliflorum* favored the multiplication of nematodes, it was not statistically different from the species reported as resistant *S. hirtum* (Navarrete et al., 2018).

Table 1. Host status of three Solanaceae species for *M. incognita* evaluated 80 days after inoculation with 2500 eggs + juvenile (J2) per plant.

Species	GI[1]	Pf[2]	Average number of galls	RF[3]	Hosting capacity[4]
S. sessiliflorum	3.4	1888.5 ± 259.1 a	33.73 ± 13.89 a	0.94	R^H
S. hirtum	3.4	1702.3 ± 252.9 a	34.73 ± 18.09 a	0.85	R^H
S. quitoense	4.9	3125.9 ± 259.1 b	167.87 ± 34.68 b	1.56	S

[1] GI = Gall index (0-5): 0= without galls or egg masses; 1= 1 to 2 galls or egg masses; 2= 3 to 10 galls or egg masses; 3= 11 to 30 galls or egg masses; 4= 31 to 100 galls or egg masses; 5= >100 galls or egg masses per root.
[2]Pf= final population density (J2+eggs). Pf data are averages of three replicates ± standard deviation (mean values followed by the same letter do not differ according to Duncan's 5% probability test).
[3]RF Reproduction Factor.
[4]Host Status Categories R^H = Resistant/Hypersensitive (RF≤1 and GI>2) and S=susceptibleSensitive (RF>1 and GI>2).

In terms of the gall index (GI) for the three species, a lower level of gall was also observed for *S. sessiliflorum* and *S. hirtum* compared to *S. quitoense*. The gall index values had a value of 3.4 for the species *S. sessiliflorum* (galls=33.73) and *S. hirtum* (galls=34.73), with no statistical difference; whereas *S. quitoense* used as control had a value of 4.9 (galls= 167.87), confirming the viability of the inoculum. *S. sessiliflorum* and *S. hirtum* were categorized as resistant/hypersensitive according to the classification proposed by Canto-Saenz (De Almeida et al., 1997; Sasser et al., 1984). For the dry weight variables of the leaf area, plant height and stem diameter (Table 2), tolerance to the nematodes was observed for *S. sessiliflorum* and *S. hirtum*, with respect to *S. quitoense*, in which $p-values$ showed significant differences at the level of 1% between inoculated plants and non-inoculated plants. Thus, for the variable dry weight of the leaf area, a value of 8.44 vr 10.57 was obtained. For plant height 16.33 vr 18.20 and stem diameter 0.91 vr 1.08, respectively.

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Table 2. Response for the variables dry weight of leaf area, stem diameter and plant height in three Solanaceae species inoculated with *M. incognita*.

Species	Inoculated Plants	Non inoculated Plants	Response
Dry weight of the leaf area (g)			
S. sessiliflorum	6.12 ± 0.58 a	5.73 ± 0.12 a	Tolerant
S. hirtum	8.18 ± 0.52 a	8.30 ± 0.56 a	Tolerant
S. quitoense	8.44 ± 0.46 a	10.53 ± 0.25 b	Non tolerant
Height of the plant (cm)			
S. sessiliflorum	8.15 ± 0.74 a	9.33 ± 0.58 a	Tolerant
S. hirtum	15.92 ± 1.13 a	15.33 ± 1.04 a	Tolerant.
S. quitoense	16.33 ± 0.94 a	18.20 ± 0.56 b	Non tolerant
Stem diameter (cm)			
S. sessiliflorum	0.89 ± 0.035 a	0.91 ± 0.03 a	Tolerant
S. hirtum	0.87 ± 0.052 a	0.81 ± 0.051 a	Tolerant
S. quitoense	0.91 ± 0.051 a	1.08 ± 0.045 b	Non tolerant

Means with different letters between inoculated plants and non-inoculated plants indicate highly significant differences (P<0,001).
The values in the table correspond to the averages and their standard deviation.

4 Discussion

In Ecuador, naranjilla or lulo has a high incidence of the galled nematode. *M. incognita* is a pathogen of many crops, including Solanaceae in 5 continents. The nematode causes damage to the plant at the root level, reducing yield and even leading to plant death over time (Ramírez et al., 2018). Within the Lasiocarpa section, different species related to naranjilla have been reported that could present resistance to the galled root nematode (Heiser, 1972, 1993; Ramírez et al., 2018).

The host capacity of three species is reported to *M. incognita*: *S. sessiliflorum*, *S. hirtum* and *S. quitoense*. The nematode species was verified by PCR amplification with specific initiators. The BLAST analysis (Basic Local Alignment Search Tool) of the MF and MR initiator sequences showed that they are specific to *M. incognita*, with 100% coincidences to the sequences available in the National Center for Biotechnology Information (NCBI). These results indicate that the initiators are sensitive to the studied plants (Hu et al., 2011; Martínez-Gallardo et al., 2019); and that PCR conditions are ideal for the rapid and reliable detection of the pathogen in galls for different solanaceae species, as shown by Navarrete et al. (2018).

Resistance to the galled nematode is defined as the capacity of a plant to suppress the development or reproduction (Sasser et al., 1984; Roberts, 2002; Dong et al., 2007) or the ability of a host to suppress the disease (Sasser et al., 1984; Roberts, 2002). In general, nematologists and geneticists have evaluated resistance to the galled nematode in different cultures based on the root gall index and/or mass production of eggs (Holbrook et al., 2000; Timper et al., 2000) or egg counts (Choi et al., 1999). Other authors have also used the gall count to evaluate resistance to the gall nematode in plants (Harris et al., 2003; Navarrete et al., 2018; Correia et al., 2019). The number of galls and the galling degree can be used to reflect a plant's ability to decrease or overcome the attack of the galled nematode (Dong et al., 2007).

Regarding naranjilla, there are not many studies on resistance to *M. incognita*. Navarrete et al. (2018) assessed resistance in different solanaceae but based on reproductive factor. This investigation points *S. quitoense* as sensitive and *S. hirtum* as resistant to *M. incognita* under greenhouse conditions. Thus, *S. hirtum* did not present immunity to the galled nematode by allowing the penetration of the nematodes and the presence of nodules or galls in the root. However, it does have an impact on the reproductive capacity of the pathogen. In this study, *S. hirtum* and *S. quitoense* were evaluated, but *S. sessiliflorum* was not included to assess the resistance level of the galled nematode.

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In the present study, the results corroborate that *S. quitoense* was considered sensitive (RF close to 5.0) and a good host (RF = 1.1-5.0) according to De Almeida and De A Santos (2002) and Ibrahim et al. (1993), respectively, as previously stated by Navarrete et al. (2018). For the reproduction factor, *S. quitoense* exceeded the initial population (3125 eggs plus J2). In contrast, according to the results of the present study, *S. hirtum* and *S. sessiliflorum* were considered resistant/hypersensitive (if RF and GI are considered according to Canto Saenz´s classification) or poor hosts ($RF < 1$) (Maleita et al., 2011).

The estimated RF for the final population variable of nematodes indicates that there was a hypersensitive resistance response in *S. sessiliflorum* and *S. hirtum*, because the average number of eggs plus J2 (1888 and 1702, respectively) was lower than the initial population of the nematode (2500 eggs plus J2) and the reproduction factor was less than 1. Both *S. hirtum* and *S. sessiliflorum* showed no significant statistical differences in the resistance reaction. It has been stated that *S. hirtum* could be used as a pattern of *S. quitoense*, sensitive to nematodes (Navarrete et al., 2018). The results of this study suggest that the species *S. sessiliflorum*, having the same level of hypersensitive resistance as *S. hirtum*, could also be considered to be used as a pattern or in a selection program for naranjilla versus nematode.

GI of *M. incognita* attributed to the species under evaluation showed a relationship with the RF of the nematode. In the experiment for *S. sessiliflorum* and *S. hirtum*, GI presented intermediate values. Low or intermediate GI values demonstrate the difficulty of nematodes in establishing parasitism in the roots of these species or cultivars, as obtained from lettuce cultivars (Correia et al., 2019).

In terms of the variables dry weight of the leaf area, height of the plant and stem diameter, significant differences were found between *S. sessiliflorum* and *S. hirtum* in relation to *S. quitoense*. Navarrete et al. (2018) found no significant difference for the dry weight variable of the foliar area of *S. hirtum* between inoculated and non-inoculated plants, classifying it as tolerant according to Cook (1974), while *S. quitoense* was classified as non-tolerant because there were differences between inoculated plants and non-inoculated plants. In this paper, differences in plant height and stem diameter have also been found between the species *S. sessiliflorum* and *S. hirtum* in relation to *S. quitoense*, variables that are possibly related to tolerance response. However, additional research will be needed to observe these results in terms of field performance, as proposed by Navarrete et al. (2018).

From the point of view of improvement or use as patterns, it would be interesting to have a resistance response similar to immunity, which is characterized by the total absence of galls in the roots and absence of giant cells in the vascular cylinder. These are induced by the attack of nematodes that restrict water flow and adequate nutrient absorption in sensitive plants (Correia et al., 2019). In the case of naranjilla, no species has been found to prevent the penetration of the nematode or the development of galls in the roots of plants, even in wild species with no commercial value (Heiser, 1972, 1993; Navarrete et al., 2018; Ramírez et al., 2018). However, despite showing galling between *S. sessiliflorum* and *S. hirtum*, they could be used as patterns for naranjilla growing. The variability observed in PF and RF values for these species may reflect an influence of plant genetic background that could be used in *S. quitoense* breeding programs against root knot nematodes.

5 Conclusion

Knowing the host capacity of *S. sessiliflorum* and *S. hirtum* based on RF and GI is useful for continuing a genetic improvement plan in naranjilla and observing the behavior of species used as patterns in areas heavily infested by galled nematode. *S. sessiliflorum* and *S. hirtum* had different host capacity compared to *S. quitoense* for the *M. incognita* nematode and both species could be used as an alternative in an integrated pest management program, since repeated exposure of *S. quitoense* in the field could lead to a selection of virulent nematode isolates.

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