Investigation note

Identification and distribution of *Meloidogyne* spp. in tomato in Sinaloa Mexico

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Abstract

Worldwide, the most important phytoparasitic nematode genus is *Meloidogyne*, since it affects more than 3 000 plant species and its infection is characterized by the formation of galls in the root of the infected plant. In Sinaloa, the current distribution of *Meloidogyne* is unknown, because the most recent reports are from the year 2000 and 2001, identifying the species *M. incognita*, *M. arenaria*, *M. hapla* and *M. javanica*, distributed in the state. In the present work of investigation, the objectives were to identify morphologically and molecularly the species of the root-knot nematode (*Meloidogyne* spp.), as well as, to determine its distribution in the culture of tomato (*Solanum lycopersicum* L), in Sinaloa, Mexico. Cultivated lots with tomato were sampled in the different horticultural zones of Sinaloa, Mexico, during the agricultural cycles 2013-2014, 2014-2015, 2015-2016 and 2016-2017, in the open field, shade mesh and greenhouses, where samples of soil and roots, to perform morphological and molecular identification. The species identified in the samples collected were *M. enterolobii*, *M. incognita* and *M. arenaria* with 88, 10 and 2% incidence respectively. These results indicate that *M. enterolobii*, *M. incognita* and *M. arenaria* are distributed in the state of Sinaloa in the tomato crop, being *M. enterolobii* the predominant species.

Keywords: *Solanum lycopersicum*, horticulture, root-knot nematode.

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Introduction

Tomato is the main export agro-food product in Mexico, its production in 2013 was 3.2 million tons (SIAP, 2017). In the 2013-2014 season, 47 136 ha of vegetables were grown in Sinaloa, producing around 1 million t of tomato, of which 313 914 t were exported with a value of 303.2 million dollars. (CIDH, 2014). Nematode species of the genus *Meloidogyne* are one of the most harmful phytopathogens in tomato cultivation worldwide, as they severely damage the root system of the crop. *Meloidogyne* is distinguished from other genera by having a wide range of hosts, this has made it classified as the genus of phytoparasitic nematodes of greatest economic importance in the world (Salazar-Antón and Guzmán-Hernández, 2013).

In Mexico, *Meloidogyne* spp., is the most important phytoparasitic nematode genus that attacks tomato cultivation, due to the percentage of losses it causes and in different producing states the presence of four species is reported: *M. incognita*, *M. arenaria*, *M. javanica* and *M. hapla* (Carrillo *et al*., 2000; Cid del Prado *et al*., 2001); however, in the 2012-2013 season, Martínez *et al*. (2015), made the first report of the presence of the species *M. enterolobii* attacking tomato plants carrying the Mi gene (highly resistant to *M. incognita*, *M. javanica* and *M. arenaria*) under shade culture conditions in Culiacán , Sinaloa. Among the reports of Carrillo *et al*. (2000); Cid del Prado *et al*. (2001); Martínez *et al*. (2015), used the polymerase chain reaction (PCR) as a molecular biology tool, since it has provided an alternative and sensitive approach for the detection and identification of root-knot nematodes and many pathogenic organisms present in the soil that was previously difficult their identification (Hu *et al*., 2011).

The objectives of the present study were to identify *Meloidogyne* spp. morphologically and molecularly, as well as to know its distribution in the tomato crop, in Sinaloa, Mexico.

The sampling was carried out during the reproductive stage of 160 lots cultivated with tomato, in open field conditions, shade mesh and greenhouse in four of the main production areas of Sinaloa: (Los Mochis, Culiacán, La Cruz de Elota and Escuinapa), During the agricultural cycles 2013-2014, 2014-2015, 2015-2016 and 2016-2017, each sampling point was georeferenced (Table 1).

Table 1. Location of samples in tomato culture, in Sinaloa.

Municipality	Latitude	Longitude	Altitude (m)
Los Mochis	25°43’51.56”	108°45’42.67”	19
Los Mochis	25°55’43.13”	108°50’11.68”	30
Los Mochis	25°51’16.28”	108°54’36.75”	22
Los Mochis	25°47’17.75”	108°44’32.27”	24
Los Mochis	25°39’55.11”	108°45’37.99”	12
Culiacán	24°53’22.86”	107°39’54.45”	26
Culiacán	24°52’27.97”	107°41’41.41”	18
Culiacán	24°48’53.58”	107°48’11.44”	7
Culiacán	24°43’31.98”	107°36’26.59”	14

Culiacán	24°39’07.41”	107°28’37.93”	15
Culiacán	24°38’45.56”	107°30’18.54”	15
Culiacán	24°36’19.72”	107°34’37.05”	7
Culiacán	24°45’54.16”	107°31’03.34”	27
Culiacán	24°56’41.70”	107°28’13.01”	109
Culiacán	24°55’59.57”	107°26’36.05”	72
Culiacán	24°32’49.77”	107°26’12.75”	17
Culiacán	24°32’19.36”	107°26’18.25”	15
Culiacán	24°31’18.36”	107°27’45.30”	10
Elota	24°01’55.38”	107°00’32.72”	7
Elota	24°00’23.13”	107°01’27.80”	10
Elota	24°00’20.38”	106°59’41.17”	7
Elota	23°57’24.13”	106°52’37.48”	54
Elota	23°57’17.58”	106°51’42.45”	67
Elota	23°54’04.78”	106°53’53.99”	10
Elota	23°54’10.31”	106°52’26.60”	15
Elota	23°53’40.45”	106°52’29.58”	20
Escuinapa	23°06’49.21”	106°01’54.69”	91
Escuinapa	23°01’21.33”	105°55’10.96”	31
Escuinapa	22°55’55.85”	106°06’31.24”	7
Escuinapa	24°44’25.53”	105°50’22.38”	5
Escuinapa	22°43’40.57”	105°50’16.49”	4
Escuinapa	22°40’49.13”	105°47’57.83”	5

The soil sample was taken between 5 and 30 cm deep, close to the root growth zone (rhizosphere) of the plants, because it is where the highest population density of phytoparasitic nematodes is found. Also included was the collection of gilled roots for their respective analysis. Each soil sample consisted of 2 kg (8 to 10 subsamples) and 5 roots of galls (taken completely at random), labeled and stored at 4 °C until nematode extraction.

The samples composed of soil and roots of each sample were analyzed in the nematology laboratory of CIAD, Culiacán. The identification of the specimens was made based on their morphological characteristics (cephalic region, type of stylet, type of basal nodules and the distance of the mouth of the dorsal esophageal gland [DGED]) and perineal patterns of the females, supported by keys taxonomic studies of Eisenback *et al*. (1981) and EPPO (2011).

To confirm the identity of *Meloidogyne* at the species level, the roots were then washed with distilled water to remove the soil, individual galls were selected, where 50 females were removed with a dissecting needle and placed in a microcentrifuge tube 1.5 mL, subsequently, an aliquot of 45 µL of lysis buffer (50 mM NaOH) was added, it was subjected to heat lysis at 95 °C for 10 min, an aliquot of 45 µL of Tris-HCl (pH 8) was added, and centrifuged for 3 min

at 10 000 rpm (Hu *et al*., 2011), the supernatant was recovered, to proceed with PCR using the specific primers Me-F and Me-R (*Meloidogyne enterolobii*), F-jav and R -jav (*Meloidogyne javanica*), Ma-F and Ma-R (*Meloidogyne arenaria*), Mi-F and Mi-R (*Meloidogyne incognita*), Mha-F and Mha-R (*Meloidogyne hapla*), which code for the 28S rRNA region (Table 2) (Hu *et al*., 2011).

Table 2. Sequence of specific Meloidogyne primers.

Primer	Sequence of primer (5'-3')	Species specific
F: Me	AACTTTTGTGAAAGTGCCGCTG	*M. enterolobii*
R: Me	TCAGTTCAGGCAGGATCAACC	
F: Jav	GGTGCGCGATTGAACTGAGC	*M. javanica*
R: Jav	CAGGCCCTTCAGTGGAACTATAC	
F: Ma	TCGAGGGCATCTAATAAAGG	*M. arenaria*
R: Ma	GGGCTGAATATTCAAAGGAA	
F: Mi	GTGAGGATTCAGCTCCCCAG	*M. incognita*
R: Mi	ACGAGGAACATACTTCTCCGTCC	
F: Mha	TCGAGGGCATCTAATAAAGG	*M. hapla*
R: Mha	GGGCTGAATATTCAAAGGAA	

PCR reactions were performed using the core Systems 1 PCR system (Promega). The total volume of the reaction mixture was 25 µL for all reactions. The content of the reaction mixture was: 10 ng of genomic DNA, 5 µL of 10x PCR buffer, 3 µL of $MgCl_2$ (25 mM), 0.5 µL of each dNTP (10 µM), 1 µL of each primer, 0.2 µL of *Taq* polymerase (5u μL^{-1}) and the rest of sterile nanopure water. The amplification of the DNA was carried out in a thermal cycler (BIO-RAD T100), under the following amplification conditions: 94 °C for 2 min, 35 cycles of 94 °C for 30 s, 64 °C for 30 s, 68 °C for 1 min, followed by a final extension at 72 °C for 5 min.

An aliquot of the PCR product was visualized, in a 1% agarose gel, stained with 1 µL of ethidium bromide (10 mg mL^{-1}), in a transilluminator (Benchtop UV). A visible band of ±250 bp was considered as a positive response. (*M. enterolobii*), ±750 bp. (*M. javanica*), ±950 bp. (*M. arenaria*), ±1 000 bp. (*M. incognita*) and ±1 500 bp. (*M. hapla*).

Of all the populations obtained, when analyzing their morphological, morphometric and molecular characterization, a frequency of 88% was recorded for *M. enterolobii*, 10% for *M. incognita* and 2% for *M. arenaria* (Figure 1).

According to reported by EPPO (2011) and Ramírez-Suárez *et al*. (2014; 2016), taking into consideration the perineal patterns of the females collected in the present investigation, the characteristics of the cephalic region, type of stylet, type of basal nodes and the distance of the DGED samples were identified as *M. enterolobii*: ringed females with white lateral fields and of piriform shape, of variable size, the relationship between the distance from the head to the short excretory pore, located at the level of the metacorpus.

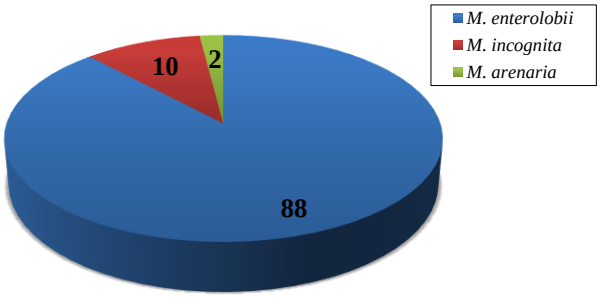

Figure 1. Population percentage of *Meloidogyne* species in the tomato crop in Sinaloa.

Sturdy stylet and perineal patterns were ovoid to rounded, with moderately high and rounded arch. *M. incognita* presented two rings in the ring-shaped cephalic region and the anterior part of the stylet in the form of 'rowing' with blunt tip, rounded basal nodules and the distance from the base of the nodules to the very short DGED (1.8-3.3 µm), in addition, in the perineal sections they presented the high dorsal arch formed by grooves that varied from smooth to wavy, without clearly visible lateral lines. *M. arenaria* had the characteristic of three rings in the cephalic region and the long DGED (3.2-4.9 µm); in addition, in the perineal models, it showed the presence of the dorsal arch with 'shoulder pads', formed by pronounced undulations of the dorsal striae (Figure 2).

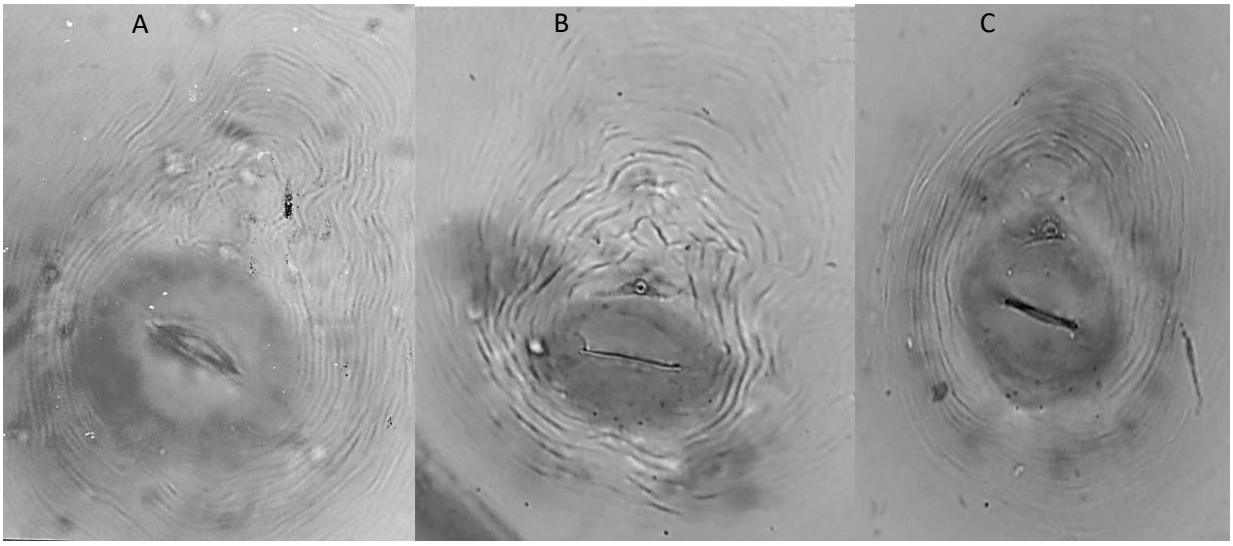

Figure 2. Perineal patterns of: A) *M. incognita*, B) *M. arenaria* and C) *M. enterolobii*, obtained from females of roots of tomato crop of Sinaloa.

The PCR amplified fragments of ±250 bp. (*M. enterolobii*), ±950 bp. (*M. arenaria*) and ±1 000 bp. (*M. incognita*) respectively, which confirms the results obtained by morphology and molecular biology.

The species *M. enterolobii* was identified in the four production zones of Sinaloa, while *M. arenaria* was presented in Los Mochis, La Cruz de Elota and Escuinapa, and *M. incognita* was only found in the areas of Culiacan and La Cruz de Elota (Figure 3).

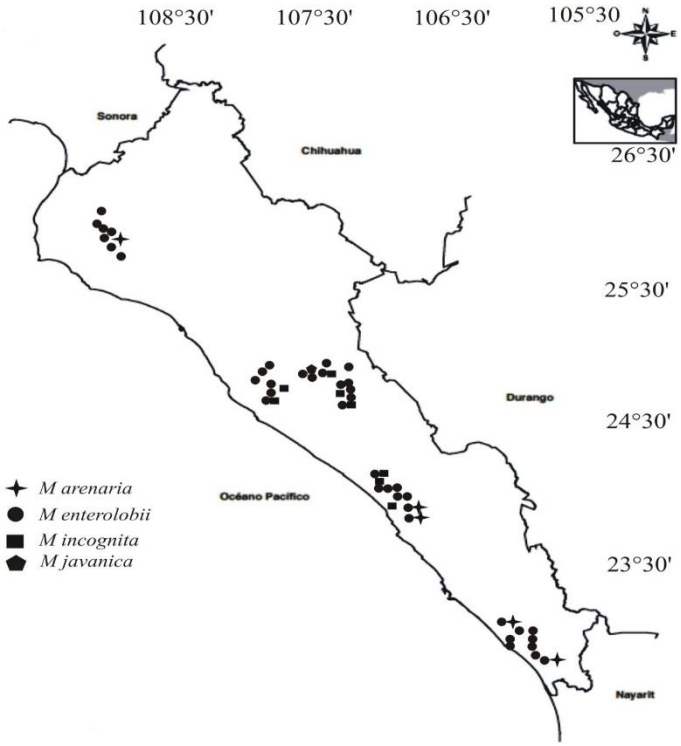

Figure 3. Distribution of *Meloidogyne* species in tomato crops in Sinaloa.

In five sampling lots (3% of the samples) mixed species were found, one presented *M. enterolobii* and *M. incognita* and in four the population mixture of *M. arenaria* and *M. enterolobii* was found.

The results of the identification of *Meloidogyne* species and their relationship with georeferencing coincide with the reports of other researchers (Castro *et al*., 1990; Cid del Prado *et al*., 1998; Carrillo *et al*., 2000; Cid del Prado *et al*., 2001; Martínez *et al*., 2015), since they report that the distribution intervals are based on the ranges of variation of each of the species.

The present study contributes to the knowledge of the current distribution of *Meloidogyne* spp. in Sinaloa and is considered a basis for future control tools.

Conclusions

The root-knot nematode (*Meloidogyne*), is distributed in all areas of tomato production in Sinaloa analyzed in the present study, being the species *M. enterolobii* which was found with greater frequency and distribution in the four areas sampled, *M. incognita* it was found only in two, whereas *M. arenaria* was found in three of the four sampled areas.

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