Screening for Resistance to *Meloidogyne enterolobii* in *Capsicum annuum* Landraces From Mexico

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Abstract

Meloidogyne enterolobii has become an economically important plant parasitic nematode worldwide because of its high aggressiveness, increasing geographic distribution, wide host range, and pathogenicity in pepper (*Capsicum annuum*) cultivars carrying resistance genes to *Meloidogyne incognita*, *Meloidogyne arenaria*, and *Meloidogyne javanica*. The objectives of this study were to identify landraces of peppers resistant to *M. enterolobii* and analyze the relationship between resistance indicators and the phenotype parameters of plant height, stem width, leaf length, leaf width, relative chlorophyll, and number of flowers. Ninety landraces of *C. annuum* were collected from several states of Mexico and were inoculated with 2,000 eggs of *M. enterolobii*. Eleven resistant landraces were selected and confirmed with a second inoculation experiment. Seventy-five days after inoculation, in both experiments, the resistance of landraces UTC66, UTC90, UTC67, UTC88, and UTC81 to *M. enterolobii* was consistent. Although genotypes UTC24, UTC79,

UTC65, UTC68, UTC69, and UTC25 were susceptible, these landraces had a significantly higher proportion of resistant plants, less root gall, and a lower reproductive index, in comparison with the rest of the 79 genotypes and the susceptible control, which were highly susceptible in both experiments. There was no correlation between resistant indicators and phenotypic parameters, although plant height, relative chlorophyll, and number of flowers were in general significantly affected compared with noninoculated controls, indicating that the nematodes reduce the growth and yield of peppers. Results indicate that all resistant plants from these landraces are promising sources of resistance for the development of pepper cultivars resistant to *M. enterolobii*.

Keywords: *Capsicum annuum*, genetic resistance, *Meloidogyne enterolobii*, root-knot nematodes

Mexico is one of the main producers of peppers (*Capsicum annuum* L.) worldwide, having 193,435 ha cultivated in 2017 and representing 66.1% of the total pepper acreage in the Americas (FAO 2017). Sinaloa State is one of the main producers of peppers in Mexico, where production reached 17,417 ha in 2017 (SAGARPA 2017).

Root-knot nematodes (*Meloidogyne* spp.) are one of the major yield limiting factors of pepper production (Robertson et al. 2006; Sasser and Freckman 1987). Because of the widespread risk of root-knot nematodes, several researchers have evaluated the host status of pepper cultivars, landraces, and the wild relatives of *Capsicum* to this nematode (Brito et al. 2007; Carneiro et al. 2006; Castagnone-Sereno 2012; Djian-Caporalino et al. 2001; Fazari et al. 2012; Goncalves et al. 2014; Sarath Babu et al. 2011; Wang and Bosland, 2006;). Previous reports suggested that six species of root-knot nematode (*Meloidogyne incognita*, *M. arenaria*, *M. javanica*, *M. hapla*, *M. chitwoodi*, and *M. enterolobii*) attack solanaceous crops in Mexico (Carrillo-Fasio et al. 2000, 2019; Cid del Prado Vera et al. 2001; Esbenshade and Triantaphyllou 1985; Martínez-Gallardo et al. 2015; Villar-Luna et al. 2016). Additionally, *M. enterolobii* is the predominant species of *Meloidogyne* in tomato in Sinaloa, Mexico (Carrillo-Fasio et al. 2019).

Genetic resistance is considered the most efficient and highly desirable method of management of plant parasitic nematodes (Khanal

et al. 2018a). Therefore, the search for resistance genotypes is fundamental for the development of any resistance breeding programs aiming to develop cultivars with resistance to plant parasitic nematodes. Resistance to three economically important *Meloidogyne* in Mediterranean and tropical areas (*M. incognita* [Kofoed and White] Chitwood, *M. arenaria* [Neal] Chitwood, and *M. javanica* [Treub]), have been associated with at least nine dominant genes (*N*, *Me1*, *Me2*, *Me3* [Me7], *Me4*, *Me5*, *Me6*, *Mech1*, and *Mech2*) (Djian-Caporalino et al. 2001; Fazari et al. 2012; Sarath Babu et al. 2011; Wang and Bosland 2006). However, over the last two decades, *M. enterolobii* has become more important worldwide because of its high aggressiveness, increasing geographic distribution, wide host range, and pathogenicity in plants carrying genes resistant to *Meloidogyne* species (Brito et al. 2007; Carneiro et al. 2006; Castagnone-Sereno 2012; Karssen et al. 2012).

Mexico is considered the center of domestication for pepper (Carrizo-García et al. 2016; Hernández-Verdugo et al. 1999). Genetic variability in landraces and wild relatives of *C. annuum* in Mexico (Hernández-Verdugo et al. 2001; Pacheco-Olvera et al. 2012; Retes-Manjarrez et al. 2016, 2017, 2018) represent a potential source of resistance to *M. enterolobii*. Several accessions of *Capsicum chinense* and *C. annuum* have been reported as resistant to *M. enterolobii* (Goncalves et al. 2014; Melo et al. 2011). However, pepper cultivars resistant to *M. enterolobii* are not available. Because screening of wild relatives and landraces is critical first step in any resistance breeding program (Pickersgill 1997), screening of pepper germplasm lines can lead to the identification of potential sources of resistance to *M. enterolobii*. The objectives of present study were to identify resistance sources against *M. enterolobii* in landraces of *C. annuum* collected from various regions of Mexico and analyze the relationship between resistance levels and plant phenotype parameters.

Materials and Methods

Plant material. Fruits were collected from about 15 plants of each of the 90 landraces of *C. annuum* used in this study in the states of

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Sinaloa, Nayarit, Jalisco, Colima, Michoacan, Guerrero, Oaxaca, Chiapas, and Tabasco in Mexico (Table 1). All landraces were classified as *C. annuum* according to the descriptors for *Capsicum* (IPGRI et al. 1995). The *C. annuum* cultivar Red Madona Lamuyo type (Harris Moran, Davis, CA) was used as the susceptible control. Seeds were disinfected with sodium triphosphate at 10% for 30 min and dried at room temperature for 3 days before the sowing. Then these seeds were germinated and grown individually in 5-liter plastic pots containing an autoclaved mixture of soil and sand (2:1) in a greenhouse. All experiments were performed with 15-day-old plants under greenhouse conditions with insect-proof netting. Greenhouse temperatures varied between 22 and 34°C during the period of the study.

Inoculum production. A population of *M. enterolobii* collected in Culiacan (Sinaloa State, Mexico) was used in this study because of its pathogenicity on commercial pepper. This population was obtained through the isolation of 50 individual egg masses from infected roots. Each of these egg masses was placed individually in 1-liter plastic

pots containing autoclaved substrate as described above and plants of the tomato cultivar Aguamiel (Vilmorin, Ledenon, France) resistant to *M. incognita*, *M. javanica*, and *M. arenaria*. The identification of species was done through analysis of the perineal pattern of adult females (Riggs 1990) and using endpoint PCR with the specific primers designed by Hu et al. (2011). Nematode inoculum was raised on tomato cultivar Aguamiel resistant to *M. incognita*, *M. javanica*, and *M. arenaria*. Eggs were extracted from infected tomato roots with 0.5% NaOCl (Hussey and Barker 1973). Eggs were enumerated with a light microscope at 40× magnification.

Resistance experiments. Two experiments were conducted, with the first being conducted in March 2017. Individual pepper seedlings were grown in 30-cm top diameter plastic pots having 4 kg of sterilized soil. Each seedling was inoculated with 2,000 eggs of *M. enterolobii* by pipetting the egg suspension into a 1-cm-deep hole around the stem base. The experiment was established as a completely randomized design with 90 treatments, where each treatment was a *C.*

Table 1. Response of 90 landraces plus one susceptible cultivar of *Capsicum annuum* to *Meloidogyne enterolobii* at 75 days after inoculation^z

O	G	NRP/TTP	GS	RS	RI	DR	O	G	NRP/TTP	GS	RS	RI	DR
N/A	Red Madona	0/54 (0%)	8.5 a	7–9	100.0 a	S	Jalisco	UTC40	0/18 (0%)	8.5 ab	7–9	99.6 a	S
Sonora	UTC6	0/18 (0%)	9.0 a	9	100.3 a	S	Colima	UTC43	0/18 (0%)	8.5 ab	7–9	99.2 a	S
Sinaloa	UTC13	0/18 (0%)	9.0 a	7–9	98.5 a	S	Michoacán	UTC55	0/18 (0%)	8.5 ab	7–9	98.8 a	S
Sinaloa	UTC14	0/18 (0%)	9.0 a	7–9	98.7 a	S	Oaxaca	UTC70	0/18 (0%)	8.5 ab	7–9	99.1 a	S
Nayarit	UTC22	0/18 (0%)	9.0 a	9	104.2 a	S	Michoacán	UTC52	0/18 (0%)	8.4 ab	7–9	98.9 a	S
Nayarit	UTC23	0/18 (0%)	9.0 a	9	101.6 a	S	Michoacán	UTC53	0/18 (0%)	8.4 ab	7–9	99.0 a	S
Nayarit	UTC28	0/18 (0%)	9.0 a	9	100.4 a	S	Michoacán	UTC54	0/18 (0%)	8.4 ab	7–9	99.1 a	S
Nayarit	UTC29	0/18 (0%)	9.0 a	9	99.0 a	S	Oaxaca	UTC71	0/18 (0%)	8.4 ab	7–9	98.7 a	S
Nayarit	UTC30	0/18 (0%)	9.0 a	9	98.9 a	S	Sonora	UTC2	0/18 (0%)	8.3 ab	7–9	109.0 a	S
Jalisco	UTC38	0/18 (0%)	9.0 a	9	105.4 a	S	Sinaloa	UTC15	0/18 (0%)	8.3 ab	7–9	100.2 a	S
Colima	UTC44	0/18 (0%)	9.0 a	9	98.9 a	S	Sinaloa	UTC16	0/18 (0%)	8.3 ab	7–9	100.5 a	S
Colima	UTC45	0/18 (0%)	9.0 a	9	98.9 a	S	Nayarit	UTC20	0/18 (0%)	8.3 ab	7–9	98.9 a	S
Michoacán	UTC46	0/18 (0%)	9.0 a	9	99.5 a	S	Jalisco	UTC41	0/18 (0%)	8.3 ab	7–9	100.1 a	S
Michoacán	UTC47	0/18 (0%)	9.0 a	9	99.2 a	S	Jalisco	UTC42	0/18 (0%)	8.3 ab	7–9	99.9 a	S
Michoacán	UTC48	0/18 (0%)	9.0 a	9	99.6 a	S	Sonora	UTC1	0/18 (0%)	8.2 ab	7–9	98.5 a	S
Michoacán	UTC49	0/18 (0%)	9.0 a	9	100.1 a	S	Sonora	UTC3	0/18 (0%)	8.0 ab	7–9	98.7 a	S
Guerrero	UTC60	0/18 (0%)	9.0 a	9	99.0 a	S	Sinaloa	UTC17	0/18 (0%)	8.0 ab	7–9	100.5 a	S
Guerrero	UTC61	0/18 (0%)	9.0 a	9	99.6 a	S	Jalisco	UTC39	0/18 (0%)	8.0 ab	7–9	106.6 a	S
Guerrero	UTC62	0/18 (0%)	9.0 a	9	99.3 a	S	Michoacán	UTC56	0/18 (0%)	8.0 ab	7–9	98.7 a	S
Guerrero	UTC64	0/18 (0%)	9.0 a	9	99.1 a	S	Michoacán	UTC57	0/18 (0%)	8.0 ab	7–9	99.2 a	S
Oaxaca	UTC76	0/18 (0%)	9.0 a	9	123.5 a	S	Michoacán	UTC58	0/18 (0%)	8.0 ab	7–9	99.2 a	S
Chiapas	UTC77	0/18 (0%)	9.0 a	9	113.2 a	S	Sonora	UTC10	0/18 (0%)	7.8 abc	7–9	99.7 a	S
Chiapas	UTC82	0/18 (0%)	9.0 a	9	106.5 a	S	Michoacán	UTC59	0/18 (0%)	7.5 bcd	5–9	70.6 bc	S
Chiapas	UTC83	0/18 (0%)	9.0 a	9	110.2 a	S	Oaxaca	UTC74	0/18 (0%)	7.4 bcd	5–9	78.4 bc	S
Chiapas	UTC85	0/18 (0%)	9.0 a	9	138.2 a	S	Nayarit	UTC26	0/18 (0%)	7.3 bcd	5–9	69.8 bc	S
Chiapas	UTC86	0/18 (0%)	9.0 a	9	144.1 a	S	Nayarit	UTC21	0/18 (0%)	7.1 bcd	5–9	64.9 bc	S
Chiapas	UTC87	0/18 (0%)	9.0 a	9	136.1 a	S	Nayarit	UTC27	0/18 (0%)	7.0 bcd	5–9	108.8 a	S
Sonora	UTC5	0/18 (0%)	8.9 a	7–9	98.8 a	S	Jalisco	UTC37	0/18 (0%)	7.0 bcd	5–9	77.6 bc	S
Jalisco	UTC36	0/18 (0%)	8.9 a	7–9	106.6 a	S	Oaxaca	UTC75	0/18 (0%)	6.9 bcde	5–9	63.8 bc	S
Sonora	UTC4	0/18 (0%)	8.8 a	7–9	98.5 a	S	Nayarit	UTC31	0/18 (0%)	6.8 bcde	5–9	72.3 bc	S
Sonora	UTC7	0/18 (0%)	8.8 a	7–9	98.8 a	S	Chiapas	UTC84	0/18 (0%)	6.8 bcde	5–9	73.6 bc	S
Sinaloa	UTC18	0/18 (0%)	8.8 a	7–9	98.9 a	S	Guerrero	UTC63	0/18 (0%)	6.6 bcde	5–9	109.8 a	S
Jalisco	UTC35	0/18 (0%)	8.8 a	7–9	104.8 a	S	Chiapas	UTC80	0/18 (0%)	6.5 cde	5–9	169.6 a	S
Oaxaca	UTC72	0/18 (0%)	8.8 a	7–9	98.9 a	S	Chiapas	UTC78	0/18 (0%)	6.5 cde	5–9	160.7 a	S
Oaxaca	UTC73	0/18 (0%)	8.8 a	7–9	99.0 a	S	Nayarit	UTC25	2/18 (11%)	6.4 cde	1–9	72.3 bc	S
Tabasco	UTC89	0/18 (0%)	8.8 a	7–9	125.2 a	S	Oaxaca	UTC69	4/18 (22%)	6.4 def	1–9	70.0 bc	S
Sonora	UTC8	0/18 (0%)	8.7 a	7–9	99.5 a	S	Oaxaca	UTC68	6/18 (33%)	6.3 def	1–9	95.8 a	S
Sinaloa	UTC19	0/18 (0%)	8.7 a	7–9	98.8 a	S	Guerrero	UTC65	3/18 (17%)	6.1 def	3–9	88.1 a	S
Michoacán	UTC50	0/18 (0%)	8.7 a	7–9	100.3 a	S	Chiapas	UTC79	4/18 (22%)	5.8 ef	1–9	91.9 a	S
Michoacán	UTC51	0/18 (0%)	8.7 a	7–9	99.9 a	S	Nayarit	UTC24	4/18 (22%)	5.7 ef	3–7	81.9 a	S
Sinaloa	UTC12	0/18 (0%)	8.6 a	7–9	99.0 a	S	Chiapas	UTC81	6/18 (33%)	5.4 ef	1–9	8.1 d	R
Sonora	UTC9	0/18 (0%)	8.5 ab	7–9	99.1 a	S	Chiapas	UTC88	9/18 (50%)	4.9 ef	1–9	9.1 d	R
Sonora	UTC11	0/18 (0%)	8.5 ab	7–9	98.9 a	S	Oaxaca	UTC67	10/18 (56%)	4.3 fg	1–9	6.2 d	R
Jalisco	UTC32	0/18 (0%)	8.5 ab	7–9	99.0 a	S	Tabasco	UTC90	12/18 (67%)	3.5 fg	1–9	5.6 d	R
Jalisco	UTC33	0/18 (0%)	8.5 ab	7–9	104.7 a	S	Oaxaca	UTC66	13/18 (72%)	3.3 g	1–7	7.3 d	R
Jalisco	UTC34	0/18 (0%)	8.5 ab	7–9	103.8 a	S							

^z O, Origin; G, genotype; NRP/TTP, disease incidence: number of resistant plants and total tested plants; GS, average of gall symptoms; RS, range of symptoms; RI, reproductive index; DR, degree of resistance at 75 days after inoculation; N/A, not applicable; S, susceptible; R, resistant. Means comparison made with Dunn test ($P \leq 0.0001$). Means with the same letter in columns indicate nonsignificant differences.

annuum landrace. Each treatment had three replicates of six plants per treatment (18 in total each). Twelve plants of each landrace received only water and served as the noninoculated control. Basal application of fertilizer was carried out on the pots every 2 weeks after inoculation, using NPK (17:17:17) (triple 17). At 75 days after inoculation, individual plant root systems were rinsed with tap water and evaluated. To determine the degree of resistance, roots were evaluated individually for gall symptoms and reproductive index (RI). Galling was rated on a scale of 1 to 9, where 1 = 0% of roots with galls, 3 = 25% of roots with small galls, 5 = 50% of roots with small galls, 7 = 75% of roots with some large galls, and 9 = 100% of roots with large galls, roots decaying (Carrillo-Fasio et al. 2019). RI was calculated as $100 \times \text{number of eggs per plant for a landrace} / \text{average number of eggs per plant for the susceptible cultivar Red Madona}$. Host status was determined based on RI, classified as susceptible (S), >50%; moderately resistant (MR), 26 to 50%; highly resistant (HR), 1 to 25%; and immune (I), 0% (Taylor 1967).

The second experiment was conducted in March 2018 with 11 landraces that showed at least one resistant plant in the first experiment to corroborate their level of resistance. The same method of inoculation with eggs of *M. enterolobii*, experimental design, and criteria for degrees of resistance used in the first experiment was used in this experiment.

Plant growth parameters. Quantitative evaluations in both experiments, including plant height, stem width (at soil level), leaf length, leaf width (first leaf expanded from the top of the plant), relative chlorophyll as measured by a chlorophyll meter (Soil-Plant Analyses Development [SPAD] units of the same leaf selected to measure leaf length and width), and number of flowers were recorded 75 days after inoculation.

Data analysis. To discriminate between levels of resistance (resistance indicators) to *M. enterolobii*, data obtained from the two experiments were subjected to nonparametric Kruskal–Wallis and Dunn median tests to determine the significance of differences between landraces ($P \leq 0.05$).

To analyze the relationship between resistance indicators and plant growth parameters, a separate analysis was conducted combining data from the landraces that were used in both experiments. The data were combined because there were no significant differences between the two experiments for all plant growth parameters in landraces. The same statistical nonparametric test used for the resistance indicators was used to determine the significance of differences between genotypes ($P \leq 0.05$) for the resistance parameters, and data of the plant phenotype indicators were assessed via parametric variance analysis and Tukey test to determine the significance of differences between genotypes ($P \leq 0.05$). The Pearson correlation analysis (r) was used to

assess the galling, RI, and the plant growth parameters mentioned above. All statistical analyses were performed with the SAS statistical package (ver. 8.00; SAS Institute, Cary, NC).

Results

In the first experiment, the majority of the landraces (85 of 90 landraces) were susceptible to *M. enterolobii* at 75 dpi (Table 1). However, the percentage of resistant plants, root gall severity, and RI varied significantly between the landraces of pepper ($P < 0.0001$) (Table 1). Landraces UTC66, UTC90, UTC67, UTC88, and UTC81 were resistant to *M. enterolobii*, with a significantly higher proportion of resistant plants of 33 to 72%, less galling of 3.3 to 5.4, and a lower RI of 5.6 to 9.1, than most of the landraces evaluated (Table 1 and Supplementary Table S1). Although genotypes UTC24, UTC79, UTC65, UTC68, UTC69, and UTC25 were susceptible, they had 11 to 33% of resistant plants and showed significantly less galling and lower RI in comparison with most of the landraces evaluated (Table 1). The remaining 79 landraces were fully susceptible, with severe galling and RI similar to that of the susceptible control (Table 1). Landraces with at least one resistant plant from the 90

Table 2. Response of 11 promising accessions plus one susceptible cultivar of *Capsicum annuum*, inoculated with 2,000 eggs of *Meloidogyne enterolobii* per plant^a

O	G	NRP/TTP	GS	RS	RI	DR
N/A	Red Madona	0/18 (0%)	8.7 a	7–9	100.0 a	S
Nayarit	UTC25	3/18 (17%)	6.2 b	1–9	81.1 a	S
Guerrero	UTC65	2/18 (11%)	5.7 bc	3–9	75.7 ab	S
Oaxaca	UTC69	5/18 (28%)	5.6 bc	1–9	59.7 b	S
Nayarit	UTC24	3/18 (17%)	5.2 bcd	1–7	70.2 ab	S
Oaxaca	UTC68	5/18 (28%)	5.0 bcd	1–9	84.0 a	S
Chiapas	UTC79	5/18 (28%)	4.9 bcd	1–9	79.1 ab	S
Chiapas	UTC81	6/18 (33%)	4.9 cd	1–7	4.9 c	R
Chiapas	UTC88	8/18 (44%)	4.4 de	1–7	5.9 c	R
Oaxaca	UTC67	11/18 (61%)	3.7 ef	1–7	3.3 c	R
Oaxaca	UTC66	15/18 (83%)	2.9 f	1–7	4.3 c	R
Tabasco	UTC90	11/18 (61%)	2.8 f	1–7	4.9 c	R

^a O, Origin; G, genotype; NRP/TTP, disease incidence: number of resistant plants and total tested plants; GS, average of gall symptoms; RS, range of symptoms; RI, reproductive index; DR, degree of resistance at 75 days after inoculation; N/A, not applicable; S, susceptible; R, resistant. Means comparison made with Dunn test ($P \leq 0.0001$). Means with the same letter in columns indicate nonsignificant differences.

Fig. 1. Picture of the second experiment for screening plants of *Capsicum annuum* landraces for resistance to *Meloidogyne enterolobii*. Susceptible control plant of the Red Madona cultivar with severe damage (left) and *C. annuum* resistant plant of the UTC66 landrace collected in Oaxaca, Mexico (right) inoculated with *M. enterolobii*. The picture was taken at 75 days after inoculation.

tested in the first experiment were used in the second experiment to corroborate resistance results.

Most of the landraces had a narrow range of gall symptoms of 7 to 9 under *M. enterolobii* inoculation. However, landraces UTC66, UTC90, UTC67, UTC81, UTC24, UTC79, UTC65, UTC68, UTC69, and UTC25 showed the widest range of symptoms in response to the inoculation with this nematode, with a range of gall symptoms of 1 to 9 in most of these landraces in this experiment (Table 1 and Supplementary Table S1).

The second experiment had results similar to those of the first experiment. Landraces UTC90, UTC66, UTC67, UTC88, and UTC81 were the only landraces resistant to *M. enterolobii*, with a significantly higher proportion of resistant plants (33 to 83%), less severe root galling (2.8 to 4.9), and a lower RI (3.3 to 5.9) (Table 2 and Supplementary Table S1) (Fig. 1). Landraces UTC79, UTC68, UTC24, UTC69, UTC65, and UTC25 were susceptible, although these landraces had 11 to 28% resistant plants, significantly less severe root galling, and lower RI in comparison with the susceptible control Red Madona (Table 2). The commercial cultivar Red Madona was fully susceptible, as in the first experiment (Table 2).

Most of the landraces showed a wide range of root galling symptoms in response to *M. enterolobii* infestation. The landraces UTC24, UTC81, UTC88, UTC67, UTC66, and UTC90 had a gall rating from 1 to 7, whereas a gall rating of 1 to 9 was observed in UTC69, UTC68, and UTC79 (Table 2 and Supplementary Table S1). The commercial cultivars had a narrow range of gall rating of 7 to 9 (Table 2 and Supplementary Table S1).

Plant growth parameters. There was no significant correlation between the resistance indicators (root gall symptoms and RI) and the plant growth parameters measured in experiment 1 and experiment 2 for all the landraces.

Plant height, relative chlorophyll content, and number of flowers were significantly affected by the nematode in most landraces in combined results for experiments 1 and 2 ($P \leq 0.01$) (Fig. 2A, E, and F). There were no significant differences in plant height between noninoculated and inoculated landraces UTC67, UTC68, and UTC88. The remaining landraces that were inoculated with the root-knot nematode had significantly lower plant height ($P \leq 0.01$) (Fig. 2A). In combined results for experiments 1 and 2, the relative chlorophyll content (SPAD units) of noninoculated plants of Red Madona, UTC24, UTC25, UTC65, UTC68, and UTC81 were

Fig. 2. The average effect of inoculation with *Meloidogyne enterolobii* on phenotypic parameters of five resistant *Capsicum annuum* landraces for experiments 1 and 2. **A**, Plant height. **B**, Stem width. **C**, Leaf length. **D**, Leaf width. **E**, Chlorophyll. **F**, Number of flowers per plant. An asterisk indicates a significant difference between inoculated and noninoculated controls based on single-degree contrast at $P \leq 0.01$. Data were taken 70 to 75 days after inoculation.

significantly greater than those of the inoculated plants. The inoculated and noninoculated genotypes UTC66, UTC67, UTC69, UTC79, UTC88, and UTC90 did not differ significantly ($P \leq 0.01$) for relative chlorophyll content (Fig. 2E). The number of flowers were not significantly different in noninoculated and inoculated plants of genotypes UTC68, UTC69, and UTC81. However, the number of flowers was significantly lower in inoculated plants in genotypes Red Madona, UTC24, UTC25, UTC65, UTC66, UTC67, UTC79, UTC88, and UTC90 ($P \leq 0.01$) (Fig. 2F). Other measured phenotypic parameters were generally not affected by the nematode, although one or more parameters were affected in a few genotypes (Fig. 2B to D).

Discussion

M. enterolobii is one of the most challenging species of root-knot nematodes to manage in agricultural crops (Brito et al. 2007; Carneiro et al. 2006; Castagnone-Sereno 2012). The use of fumigant nematicides has been gradually restricted worldwide for environmental reasons and because of undesirable effects on health (Zasada et al. 2010). Several agroecological and biologically based methods, such as sanitation, soil tillage, organic amendments, green manure, antagonistic organisms, and heat-based methods, are known to affect *M. enterolobii* populations (Collange et al. 2014). Each of these techniques is only partially effective, necessitating their combinations, which further complicates their adoption in farms where space is limited (Collange et al. 2014). An effective and widely accepted method of management of this nematode is the use of resistant genotypes (King et al. 2010). Therefore, to manage *M. enterolobii* in pepper-producing regions of Mexico, the resistance breeding program must be implemented.

Mexico is a potential center of origin for domesticated *Capsicum* species (Carrizo-García et al. 2016; Hernández-Verdugo et al. 1999). Plant centers of origin often possess the highest genetic diversity and are considered potential sources of resistant genotypes against plant diseases and insect pests. We hypothesize that *Capsicum* landraces from Mexico must have different levels of resistance to *M. enterolobii*.

Of the 90 *C. annuum* landraces used in this study, 79 were susceptible and five were highly resistant to *M. enterolobii*. Seven other landraces were partially susceptible but had between 14 and 25% resistant individuals in both experiments. These results indicated that these genotypes are a promising source of resistance to *M. enterolobii*. Our results are similar to those reported by Melo et al. (2011), who found moderate resistance to *M. enterolobii* in different accessions of *C. chinense* and *C. annuum*, and those reported by Gonçalves et al. (2014), who found immunity to this nematode in one accession (UENF1730) of *C. chinense*, from *Capsicum* accessions collected in Brazil. Based on these results, these resistant landraces could also be used in further studies for developing molecular markers and for elucidating the genetic base of this resistant trait, which would support pepper breeders in designing the best genetic model for the introgression of this resistant trait into commercial genetic background.

Pepper landraces used in this study had wide variation in *M. enterolobii* resistance levels. The 11 landraces that had resistant plants in both experiments and developed lower levels of symptoms showed high variation in root galling, with a gall rating score of 1 to 9. This variation in resistance made it possible to select plants with fewer root galls within each landrace, and these results were corroborated in the second experiment. This high variation in resistance coincides with the high variation observed and estimated with molecular markers RAPDs (random amplified polymorphic DNA), and microsatellites between and within the population of wild peppers and landraces (Hernández-Verdugo et al. 2001; Pacheco-Olivera et al. 2012) and those reported by Retes-Manjarrez et al. (2016, 2017, 2018) for the Pepper huasteco yellow vein virus resistance trait in wild peppers from the northwest of Mexico. On the other hand, this high variation in gall symptoms expression in these pepper genotypes could be also attributed to the variability in virulence of the nematode population used in this study (Khanal et al. 2016, 2018b, 2019; Kularathna et al. 2019).

There was no correlation between gall symptoms and RI. Results corroborate the importance of including RI in addition to root galling to determine the different levels of resistance. Furthermore, our results suggested that the resistant peppers are not immune to *M. enterolobii* infection, but they effectively limit reproduction of the nematode. These results indicated that the resistant pepper landraces probably possess defense mechanisms that diminish the nematode reproduction. García et al. (1996) and Roberts et al. (2008) reported that root galling and nematode reproduction are not always coupled in root-knot nematode–host plant interactions, and genes that mediate reduced root galling but do not affect nematode reproduction have been identified.

Generally, plant height, chlorophyll content (SPAD units), and the number of flowers were reduced by *M. enterolobii*, whereas stem width, leaf length, and leaf width were not reduced. These results suggest that *M. enterolobii* could reduce the pepper yield because plant growth, photosynthesis, and number of flowers affect the productivity of peppers (Ayala-Tafoya et al. 2015; Daramola et al. 2015).

Although the use of chemicals plus crop rotation with nonhost plants could be effective for controlling nematodes, genetic resistance remains the preferred strategy because it is more sustainable and enables intensive cultivation (Khanal et al. 2018a; Louws et al. 2010). In addition, resistance is routinely incorporated into commercial cultivars, genetically or via resistant rootstocks. The availability of resistant varieties is extremely valuable to growers, because it is a means of sustainable production and mitigates the costs of production (Marin et al. 2017).

The resistant landraces identified in this study are valuable source of resistance that could be used in *M. enterolobii* management program to develop resistant pepper cultivars. The similar outcome of the resistant sources in both experiments indicates that these resistant sources are relatively stable for *M. enterolobii* resistance.

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Literature Cited

- Ayala-Tafoya, F., Sánchez-Madrid, R., Partida-Ruvalcaba, L., Yáñez-Juárez, M. G., Ruiz-Espinoza, F. H., Velázquez-Alcaráz, T. J., Valenzuela-López, M., and Parra-Delgado, J. M. 2015. Producción de pimiento morrón con mallas sombra de colores. *Rev. Fitotec. Mex.* 38:93-99.
- Sarath Babu, B., Pandravada, S. R., Prasada Rao, R. D. V. J., Anitha, K., Chakrabarty, S. K., and Varaprasad, K. S. 2011. Global sources of pepper genetic resources against arthropods, nematodes and pathogens. *Crop Prot.* 30:389-400.
- Brito, J. A., Stanley, J. D., Mendes, M. L., Cetintas, R., and Dickson, D. W. 2007. Host status of selected cultivated plants to *Meloidogyne mayaguensis* in Florida. *Nematropica* 37:65-71.
- Carneiro, R. M. D. G., Almeida, M. R. A., Braga, R. S., Almeida, C. A., and Gioria, R. 2006. Primeiro Registro de *Meloidogyne mayaguensis* parasitando plantas de tomate e pimentão resistentes à meloidoginose no estado de São Paulo. *Nematol. Bras.* 30:81-86.
- Carrillo-Fasío, J. A., García-Estrada, R. S., Allende-Molar, R., Márquez-Zequera, I., and Cruz-Ortega, J. E. 2000. Identificación y distribución de especies del nematodo nodulador (*Meloidogyne* spp.) en hortalizas en Sinaloa, México. *Rev. Mex. Fitopatol.* 18:115-119.
- Carrillo-Fasío, J. A., Martínez-Gallardo, J. A., Allende-Molar, R., Velarde-Félix, S., Romero-Higareda, C. E., and Retes-Manjarrez, J. E. 2019. Distribution of *Meloidogyne* species (Tylenchida: Meloidogynidae) in tomato crop in Sinaloa Mexico. *Nematropica* 49:71-82.
- Carrizo-García, C., Barfus, M. H. J., Serh, E. M., Barbosa, G. E., Samuel, R., Moscone, E. A., and Ehrendorfer, F. 2016. Phylogenetic relationships, diversification and expansion of chili peppers (*Capsicum*, Solanaceae). *Ann. Bot. (Lond.)* 118:35-51.
- Castagnone-Sereno, P. 2012. *Meloidogyne enterolobii* (= *M. mayaguensis*): profile of an emerging, highly pathogenic, root-knot nematode species. *Nematology* 14:133-138.
- Cid del Prado Vera, I., Hernández, J. A., and Tovar-Soto, A. 2001. Distribución de especies y razas de *Meloidogyne* en México. *Rev. Mex. Fitopatol.* 19:32-39.
- Collange, B., Navarrete, M., Montfort, F., Mateille, T., Tavoillot, J., Martiny, B., and Tchamitchian, M. 2014. Alternative cropping systems can have contrasting effects on various soil-borne diseases: Relevance of a systemic analysis in vegetable cropping systems. *Crop Prot.* 55:7-15.
- Daramola, F. Y., Popoola, J. O., Eni, A. O., and Sulaiman, O. 2015. Characterization of root-knot nematodes (*Meloidogyne* spp.) associated with

- Abelmoschus esculentus*, *Celosia argentea* and *Corchorus olitorius*. Asian J. Biol. Sci. 8:42-50.
- Djian-Caporalino, C., Pijarowski, L., Fazari, A., Samson, M., Gaveau, L., O'Byrne, C., Lefebvre, V., Caranta, C., Palloix, A., and Abad, P. 2001. High-resolution genetic mapping of the pepper (*Capsicum annuum* L.) resistance loci Me3 and Me4 conferring heat-stable resistance to root-knot nematodes (*Meloidogyne* spp.). Theor. Appl. Genet. 103:592-600.
- Esbenshade, P. R., and Triantaphyllou, A. C. 1985. Use of enzyme phenotype for identification of *Meloidogyne* species. J. Nematol. 17:6-20.
- Fazari, A., Palloix, A., Wang, L., Hua, M. Y., Palloix, A. M. S., Zhang, B. X., and Djian-Caporalino, C. 2012. The root-knot nematode resistance N-gene co-localizes in the Me-genes cluster on the pepper (*Capsicum annuum* L.) P9 chromosome. Plant Breed. 131:665-673.
- Food and Agriculture Organization of the United Nations (FAO). Statistics Division (2017 onwards). Production/Crops. <http://faostat3.fao.org/download/Q/QC/E>
- Garcia, G. M., Stalker, H. T., Shroeder, E., and Kochert, G. 1996. Identification of RAPD, SCAR, and AFLP markers tightly linked to nematode resistance genes introgressed from *Arachis cardenasii* into *Arachis hypogaea*. Genome 39: 836-845.
- Goncalves, L. S. A., Gomes, V. M., Robaina, R. R., Valim, R. H., Rodrigues, R., and Aranha, F. M. 2014. Resistance to root-knot nematode (*Meloidogyne enterolobii*) in *Capsicum* spp. accessions. Revista Brasileira de Ciencias Agrarias. 9:49-52.
- Hernández-Verdugo, S., Davila-Aranda, P., and Oyama, K. 1999. Síntesis del conocimiento taxonómico, origen y domesticación del género *Capsicum*. Bol. Soc. Bot. Mex. 64:65-84.
- Hernández-Verdugo, S., Guevara-González, R. G., Rivera-Bustamante, R. F., and Oyama, K. 2001. Los parientes silvestres del chile (*Capsicum* spp.) como recursos genéticos. Bol. Soc. Bot. Mex. 62:171-181.
- Hu, M., Zhuo, K., and Liao, J. 2011. Multiplex PCR for the simultaneous identification and detection of *Meloidogyne incognita*, *M. enterolobii* and *M. javanica* using DNA extracted directly from individual galls. Phytopathology 101:1270-1277.
- Hussey, R. S., and Barker, K. R. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Dis. 57:1025-1028.
- International Plant Genetic Resources Institute, Asian Vegetable Research and Development Center, and Centro Agronómico Tropical de Investigación y Enseñanza. 1995. Descriptors for *Capsicum* (*Capsicum* spp.). International Plant Genetic Resources Institute, Rome, Italy; the Asian Vegetable Research and Development Center, Taipei, Taiwan, and the Centro Agronómico Tropical de Investigación y Enseñanza, Turrialba, Costa Rica.
- Karssen, G., Liao, J. L., Kan, Z., van Heese, E., and den Nijs, L. 2012. On the species status of the root-knot nematode *Meloidogyne mayaguensis* Rammah & Hirschmann, 1988. ZooKeys 181:67-77.
- Khanal, C., Kularathna, M. T., Stetina, S. R., Ray, J. D., McGawley, E. C., and Overstreet, C. 2019. Single nucleotide polymorphism analysis using KASP assay reveals genetic variability in *Rotylenchulus reniformis*. Plant Dis. 103: 1835-1842.
- Khanal, C., McGawley, E. C., Overstreet, C., and Stetina, S. R. 2018a. The elusive search for reniform nematode resistance in cotton. Phytopathology 108:532-541.
- Khanal, C., McGawley, E. C., Overstreet, C., Stetina, S. R., Myers, G. O., Kularathna, M. T., McInnes, B., and Godoy, F. M. C. 2018b. Reproduction and pathogenicity of endemic populations of *Rotylenchulus reniformis* on cotton. Nematropica 48:68-81.
- Khanal, C., Robbins, R. T., Fiske, T. R., Szalanski, A. L., McGawley, E. C., and Overstreet, C. 2016. Identification and haplotype designation of *Meloidogyne* spp. of Arkansas using molecular diagnostics. Nematropica 46:261-270.
- King, S. R., Davis, A. R., Zhang, X., and Crosby, K. 2010. Genetics, breeding and selection of rootstocks for Solanaceae and Cucurbitaceae. Sci. Hortic. (Amsterdam) 127:106-111.
- Kularathna, M. T., Overstreet, C., McGawley, E. C., Stetina, S. R., Khanal, C., Godoy, F. M. C., and McInnes, B. K. 2019. Pathogenicity and reproduction of isolates of reniform nematode, *Rotylenchulus reniformis*, from Louisiana on soybean. Nematropica 49:31-41.
- Louws, F. J., Rivard, C. L., and Kubota, C. 2010. Grafting fruiting vegetables to manage soilborne pathogens, foliar pathogens, arthropods and weeds. Sci. Hortic. (Amsterdam) 127:127-146.
- Marin, V. C., Santos, L. S., Gaion, L. A., Hudson, R., Franco, C. A., Diniz, G. M., Silva, E. H. C., and Braz, L. 2017. Selection of resistant rootstocks to *Meloidogyne enterolobii* and *M. incognita* for okra (*Abelmoschus esculentus* L. Moench). Chil. J. Agric. Res. 77:58-64.
- Martínez-Gallardo, J. A., Díaz-Valdez, T., Allende-Molar, R., García-Estrada, R. S., and Carrillo-Fasio, J. A. 2015. Primer reporte de *Meloidogyne enterolobii* parasitando tomate en Culiacán, Sinaloa México. Rev. Mex. Cienc. Agric. 11: 2165-2168.
- Melo, O. D., Maluf, W. R., Gonçalves, R. J. S., Gonçalves Neto, A. C., Gomes, L. A. A., and Carvalho, R. C. 2011. Triagem de genótipos de hortaliças para resistência a *Meloidogyne enterolobii*. Pesqui. Agropecu. Bras. 46:829-835.
- Pacheco-Olvera, A., Hernández-Verdugo, S., Rocha-Ramírez, R. V., González-Rodríguez, R. A., and Oyama, K. 2012. Genetic diversity and structure of pepper (*Capsicum annuum* L.) from northwestern Mexico analyzed by microsatellite markers. Crop Sci. 52:231-241.
- Pickersgill, B. 1997. Genetic resources and breeding of *Capsicum* spp. Euphytica 96:129-133.
- Retes-Manjarrez, J. E., Hernández-Verdugo, S., Evrard, A., and Garzón-Tiznado, J. A. 2017. Heritability of the resistance to pepper huasteco yellow vein virus in wild genotypes of *Capsicum annuum*. Euphytica 213:275.
- Retes-Manjarrez, J. E., Hernández-Verdugo, S., Pariaud, B., Hernández-Espinal, L. A., Parra-Terraza, S., Trejo-Saavedra, D. L., Rivera-Bustamante, R. F., and Garzón-Tiznado, J. A. 2018. Resistance to Pepper huasteco yellow vein virus and its heritability in wild genotypes of *Capsicum annuum*. Bot. Sci. 96:52-62.
- Retes-Manjarrez, J. E., Hernández-Verdugo, S., Pariaud, B., Melgoza-Villagómez, C. M., Pacheco-Olvera, A., Parra-Terraza, S., and Garzón-Tiznado, J. A. 2016. Detección de resistencia al Virus huasteco vena amarilla del chile y su heredabilidad en genótipos silvestres de *Capsicum annuum* L. Interciencia 41:541-547.
- Riggs, R. D. 1990. Making perineal patterns of root-knot nematodes and vulval cones of cyst nematodes. Pages 103-106 in: Plant Nematology Laboratory Manual. B. M. Zuckerman, W. F. Mai, and L. R. Krusberg, eds. University of Massachusetts Agricultural Experiment Station, Amherst, MA.
- Roberts, P. A., Matthews, W. C., Ehlers, J. D., and Helms, D. 2008. Genetic determinants of differential resistance to root-knot nematode reproduction and galling in lima bean. Crop Sci. 48:553-561.
- Robertson, L., Lopez-Pérez, J. A., Bello, A., Díez-Rojo, M. A., Escuer, M., Piedra-Buena, A., Ros, C., and Martínez, C. 2006. Characterization of *Meloidogyne incognita*, *M. arenaria* and *M. hapla* populations from Spain and Uruguay parasitizing pepper (*Capsicum annuum* L.). Crop Prot. 25:440-445.
- Sasser, J. N., and Freckman, D. W. 1987. A world perspective on nematology: The role of society. Pages 7-14 in: Vistas on Nematology. J. A. Veech, and D. W. Dickson, eds. Society of Nematologists, Hyattsville, MD.
- SAGARPA (Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación), Servicio de Información Agroalimentaria y Pesquera, México, 2017. <https://www.gob.mx/siap>.
- Taylor, A. L. 1967. Page 133 in: Introduction to Research on Plant Nematology: An FAO Guide to the Study and Control of the Plant-Parasitic Nematodes. Food and Agricultural Organization of the United Nations, Rome.
- Villar-Luna, E., Gómez-Rodríguez, O., Rojas-Martínez, R. I., and Zavaleta-Mejía, E. 2016. Presence of *Meloidogyne enterolobii* on jalapeño pepper (*Capsicum annuum* L.) in Sinaloa, Mexico. Helminthologia 53:155-160.
- Wang, D., and Bosland, P. W. 2006. The genes of *Capsicum*. HortScience 41: 1169-1187.
- Zasada, I. A., Halbrendt, J. M., Kokalis-Burelle, N., LaMondia, J., McKenry, M. V., and Noling, J. W. 2010. Managing nematodes without methyl bromide. Annu. Rev. Phytopathol. 48:311-328.