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Identification of sources of resistance to *Meloidogyne* spp. nematodes in striped Jalapeño pepper (*Capsicum annuum* L.)

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ABSTRACT

Objective. To identify striped Jalapeño pepper plants (*Capsicum annuum* L.) as a source of genotypes resistant to *Meloidogyne enterolobii* and *Meloidogyne incognita* that enable genetic diversification in the development of improved crops within genetic improvement programs.

Design/Methodology/Approach. The resistance of 15 accessions of striped Jalapeño peppers native to northern Hidalgo (Mexico) to *Meloidogyne enterolobii* (Me), and of 12 accessions to *Meloidogyne incognita* (Mi) were evaluated. The experimental unit was one plant per replicate, with four replicates per accession. Each plant was inoculated with 1000 J2 of Me and Mi obtained through monoxenic selection. The California Wonder (CW) variety was used as a susceptible control against both *Meloidogyne* species, and the CM-334 line was used as a resistant control against Mi. Resistance was evaluated using the variables number of galls, number of egg masses, number of eggs per gram of root, and reproduction index. Evaluations were performed 34 days-post inoculation (dpi) for Me and 42 dpi for Mi. A randomized complete block design was used in R Studio©.

Results: Accessions CP922, CP933, CP937, CP938, CP939, and CP946 showed resistance to both species. Whereas accessions CP932, CP903, and CP942 showed specific resistance to Mi, and accessions CP941 and CP935 showed resistance to Me. In particular, the striped Jalapeño accessions CP922, CP933, CP937, CP938, CP939, and CP946 showed evidence of a response range from moderately resistant to highly resistant to both *Meloidogyne* species.

Limitations/Implications of the study. This research is an important starting point for identifying intraspecific variation leading to selection for resistance to root-knot nematodes in Jalapeño peppers. At the same time, it constitutes an initial exploratory phase, in which the characterization of this variation does not yet allow for the identification of the physiological, biochemical, or molecular mechanisms that explain the observed resistance. Further research is therefore suggested.

Findings/Conclusions. The identified accessions represent a promising alternative for use in genetic improvement programs for *Capsicum* spp., toward resistance to the root-knot nematodes *Meloidogyne enterolobii* and *Meloidogyne incognita*. However, for the development of appropriate integrated pest management strategies, it is recommended to incorporate molecular biology, transcriptomics, or methabolomics tools to validate the strategic use of the identified variation.

Keywords: reproduction index, *Meloidogyne enterolobii*, *Meloidogyne incognita*, genetic improvement.

INTRODUCTION

Nematodes of the genus *Meloidogyne* —present in various agricultural areas of Mexico— affect vegetables, fruit trees, ornamentals, and staple crops. Although more than 100 species of this genus have been described (Moreira *et al.*, 2018), studies have focused primarily on *Meloidogyne incognita*, *M. hapla*, *M. javanica*, and *M. arenaria*, due to their polyphagous nature and the serious damage they cause worldwide. However, in recent years, *M. enterolobii* has gained greater relevance due to its increasing geographic distribution, wide host range, and high aggressiveness and virulence (Sikandar *et al.*, 2021). Losses caused by these nematodes in agricultural production have driven the search for new control alternatives. Among those, one of the most promising strategies is the identification of resistant genotypes for the development of genetic improvement programs aimed at obtaining crops tolerant or resistant to these pathogens (Devran *et al.*, 2023).

The first source of genetic resistance to *Meloidogyne* spp. was identified in wild forms of tomato [*Lycopersicon peruvianum* L.] (Arias *et al.*, 2009). However, it is recognized that native crops constitute a fundamental reservoir of genes associated with resistance to diseases and adverse environmental conditions (Després *et al.*, 2007). Mexico represents a center of genetic diversity for *Capsicum annuum* L., distributed throughout virtually the entire territory. Plants with genetic resistance to *M. incognita* have been identified in Serrano and Huacle chili pepper lines (Gómez-Rodríguez *et al.*, 2017), as well as in native peppers from different Mexican states with resistance to *M. enterolobii* (Carrillo *et al.*, 2020).

However, to date, there are not commercially available chili pepper varieties with proven resistance to *M. enterolobii*. This highlights the need to continue exploring materials with potential resistance to Me. In this regard, researchers from the Genetics Program at Colegio de Postgraduados (Mexico) have collected various chili pepper varieties from different states in Mexico, including striped Jalapeño pepper accessions from the state of Hidalgo, which have not been evaluated for their resistance to plant parasitic nematodes.

Therefore, the objective of this study was to identify striped Jalapeño pepper plants (*Capsicum annuum* L.) as a source of genotypes resistant to *Meloidogyne enterolobii* and *Meloidogyne incognita* that enable genetic diversification in the development of improved crops within genetic improvement programs

MATERIALS AND METHODS

We evaluated the resistance of 15 accessions of striped Jalapeño pepper native from the north of the state of Hidalgo (CP903, CP907, CP908, CP922, CP932, CP933, CP934, CP935, CP937, CP938, CP939, CP941, CP942, CP945 and CP946) against *Meloidogyne enterolobii* (Me), and of 12 accessions (CP903, CP907, CP922, CP932, CP933, CP935, CP937, CP938, CP939, CP941, CP942 and CP946) against *Meloidogyne incognita* (Mi). California Wonder (CW) chili pepper was used as a susceptible control against both nematode species, whereas the CM-334 line was used as a resistant control against *M. incognita*.

Seeds of the accessions were provided by the Genetics Program of Colegio de Postgraduados (CP, colpos.mx) and preconditioned for *in vitro* germination (De la Rosa *et al.*, 2012). They were then placed in plastic containers with absorbent paper moistened with sterile distilled water and kept in a controlled growth chamber (APT.line™ KBWF, E5.1 model) at 28 °C, with cycles of 23 °C per 6 h for 5 days and subsequently at 28 °C for 15 days.

Once germinated, the seedlings were individually transplanted into 25 cm^3 beakers filled with sterile fine sand. Plants were maintained in a controlled growth chamber of the Botany Program CP under conditions of 26±2 °C, a photoperiod of 14 h light and 10 h dark, 80% relative humidity, and daily watering. Sixty plants were evaluated per nematode species. When the seedlings reached three pairs of true leaves, they were inoculated with 1000 second-stage juveniles (J2) per plant apiece of both *Meloidogyne* species (Filialuna *et al.*, 2022). The classification of resistance to *Meloidogyne* was based on the reproduction index (RI%) used by Hadisoeganda and Sasser (1982) and defined by;

$$RI = \left(Nh\,accesion \;/\; Nh\,control \right) \times 100$$

where: *RI*= reproduction index (%); *Nh accession*= number of eggs per g of root of each accession evaluated; *Nh control*= number of eggs per g of root of the susceptible control × 100 (Table 1).

The experimental unit consisted of one plant per replicate, with four replicates per accession. The trial was established using a randomized complete block design. Variables evaluated were number of egg masses, number of galls, and number of eggs per gram of root; at 34 days-post inoculation (dpi) for *Meloidogyne enterolobii* (Me) and at 42 dpi for *M. incognita* (Mi) (Castagnone-Sereno, 2012). The data obtained for each variable were subjected to an analysis of variance at a 95% confidence level. Statistical differences between means were determined using Tukey's test ($p \leq 0.05$), and the open-source statistical software R Studio© was used. Before analyses, the data were transformed to logarithmic form using the log10 (x+1) function to normalize their distribution.

RESULTS AND DISCUSSION

The 15 striped Jalapeño pepper accessions evaluated showed significant differences ($p \leq 0.05$) in the response variables to infection by *Meloidogyne enterolobii* compared to the susceptible control California Wonder (CW) (Table 2).

Table 1. Classification of the resistance response to *Meloidogyne* spp. (Hadisoeganda and Sasser, 1982).

Category	Reproduction index (RI%)
Highly resistant	RI<1%
Very resistant	1%≤RI<10%
Intermediate resistant	10%≤RI<25%
Moderately resistant	25%≤RI<50%
Susceptible	RI≥50%

Table 2. Response of 15 striped Jalapeño pepper accessions to infection by *Meloidogyne enterolobii*, and resistance classification based on the reproduction index (Hadisoeganda and Sasser, 1982).

Accession	Number of galls	Number of masses	Eggs per gram of root	Resistance
CP934	3.59±1.66 b	6.40±1.40 ab	162.83±14.44 ab	SC
CP942	5.12±1.81 ab	9.19±4.07 ab	187.01±31.16 ab	SC
CP933	4.19±1.38 ab	4.11±3.23 b	70.70±32.70 d	IR
CP932	5.99±1.56 ab	10.05±1.81 ab	172.01±21.99 ab	SC
CP935	5.95±0.63 ab	10.24±2.04 ab	143.55±15.82 abc	MR
CP937	5.26±1.03 ab	7.76±1.99 ab	128.62±8.52 bc	MR
CP938	4.90±1.81 ab	8.71±4.55 ab	141.89±22.55 abc	MR
CP903	4.92±1.39 ab	8.90±2.70 ab	175.74±39.65 ab	SC
CP939	3.71±1.61 b	6.00±2.69 ab	78.36±27.21 d	IR
CP946	4.03±1.51 ab	4.77±2.92 b	84.65±48.47 d	IR
CP922	4.63±1.02 ab	7.67±0.63 ab	137.80±18.59 abc	MR
CP945	4.40±0.63 ab	8.23±0.58 ab	173.71±21.83 ab	SC
CP941	3.92±1.76 b	6.82±2.06 ab	122.29±13.38 bc	MR
CP908	4.51±0.71 ab	8.88±1.02 ab	189.57±47.84 ab	SC
CP907	5.07±1.17 ab	8.05±2.27 ab	192.72±50.34 ab	SC
CM-334	5.32±0.73 ab	8.97±0.39 ab	114.77±20.41 cd	MR
CW	7.65±1.97 a	11.46±1.96 a	207.14±25.06 a	SC

SC: Susceptible, MR: Moderately resistant, IR: Intermediate resistant, VR: Very resistant, HR: Highly resistant. Means with different letters in the same column indicate a statistically significant difference (Tukey, $p \leq 0.05$).

Among them, accessions CP934, CP939, and CP941 had the fewest galls, while CP933 and CP946 had the fewest egg masses. Similarly, accessions CP933, CP939, and CP946 had the fewest eggs. Although CP934 had the fewest galls, its reproduction index (RI) reached 61.48%, thus indicating a susceptible response.

This result suggests that gall formation is not an essential requirement for nematode development, as noted by Cook and Starr (2006). In the case of tomato, Navarro *et al*. (2009) reported a similar phenomenon when analyzing the behavior of the FA 572-Katerine and LT-M12 genotypes against *M. incognita*. Additionally, Roberts *et al*. (2008) demonstrated in *Phaseolus lunatus* L. that nematode reproduction and root galling can be controlled by independent genes (*Mir-1*, *Mig-1*, and *Mjg-1*) in species of the genus *Meloidogyne*.

According to the resistance classification used in this study, accessions CP933, CP939, and CP946 were categorized as intermediate resistant, while CP935, CP937, CP938, CP922, and CP941 were moderately resistant, as did the CM-334 pepper line against *M. enterolobii*. In contrast, accessions CP334, CP942, CP332, CP903, CP945, CP908, and CP907 showed a behavior similar to the susceptible control CW.

Among the accessions qualified as intermediate resistant, CP933 and CP946 had significantly ($p \leq 0.05$) the lowest number of eggs per gram of root and egg masses, while

CP939 recorded in turn the lowest number of eggs and galls, reaching a RI between 10 and 25%.

In general, of the more than 100 chili pepper varieties evaluated worldwide against *M. enterolobii*, only a few are reported as resistant (Gonçalves *et al.*, 2014; Carrillo *et al.*, 2020). In this study, accessions CP933, CP939, and CP946 were classified as intermediate resistant ($10\% \leq RI < 25\%$). On the other hand, accessions CP935, CP937, CP938, CP922 and CP941, along with CM-334, showed a moderately resistant response ($25\% \leq RI < 50\%$). Although, since the latter is reported as susceptible (Villar-Luna *et al.*, 2016), these accessions can be considered borderline resistant.

In the evaluations of the accessions against *Meloidogyne incognita*, significant differences ($p \leq 0.05$) were detected in all the variables analyzed (Table 3). Accessions CP946, CP939, CP938, CP903, and CP942 showed very resistant response; CP932, CP922, and CM-334 were classified as highly resistant; CP933 presented moderate resistance, and CP937 intermediate resistance. Among these, accessions CP903, CP922, and CM-334 recorded the lowest number of galls, egg masses, and eggs, while CP946, CP939, CP938, CP932, and CP942 presented a low number of galls and egg masses. In particular, accession CP903 showed as Very resistant ($1\% \leq RI < 10\%$), in contrast to CM-334 line, which exhibited High resistance ($RI < 1\%$). However, no significant differences were observed between the two in the number of galls, egg masses, and eggs (Table 3).

This response could be associated with what was described by Pegard *et al.* (2005) in CM-334, who pointed out the existence of two resistance mechanisms against *Meloidogyne incognita*, *M. arenaria* and *M. javanica*: i) a preventive resistance based on physical barriers

Table 3. Response of 12 striped Jalapeño pepper accessions to infection by *Meloidogyne incognita* and resistance classification based on the reproduction index (Hadisoeganda and Sasser, 1982).

Accession	Number of galls	Number of masses	Eggs per gram of root	Resistance
CP922	1±0 c	1.65±1.13 e	1.13±0.23 b	HR
CP932	1±0 c	4.22±0.45 e	2.69±0.27 ab	HR
CP938	1±0 c	11.78±1.58 e	2.20±0.45 ab	VR
CP939	1±0 c	15.65±6.69 e	2.20±0.45 ab	VR
CP942	1±0.23 c	19.15±4.61 e	2.92±0.82 ab	VR
CP946	1±0 c	25.53±0.85 de	4.19±0.24 ab	VR
CP903	1.48±0.83 bc	12.22±10.48 e	1.13±0.23 b	VR
CP907	5.11±0.46 a	194.42±35.53 a	7.94±2.29 ab	SC
CP941	4.67±0.45 ab	112.07±10.31 b	8.87±1.12 a	SC
CP933	3.87±2.63 abc	59.64±13.33 cd	6.82±5.67 ab	MR
CP935	2.46±1.53 abc	91.73±2.24 bc	5.62±5.39 ab	SC
CP937	2.65±1.56 abc	37.91±21.37 de	4.15±1.46 ab	IR
CM-334	5.11±0.46 bc	7.29±0.78 e	1.52±0.18 b	HR
CW	4.91±0.84 a	105.09±8.38 b	6.89±2.02 ab	SC

SC: Susceptible, MR: Moderately resistant, IR: Intermediate resistant, VR: Very resistant, HR: Highly resistant. Means with different letters in the same column indicate a statistically significant difference (Tukey, $p \leq 0.05$).

and exudation of nematostatic compounds that limit the penetration of J2 juveniles, and ii) an induced resistance mediated by a hypersensitivity reaction (HR) and the accumulation of chlorogenic acid, which inhibit the formation of galls and egg masses by blocking the migration, development and reproduction of the nematode.

According to Roberts *et al.* (2008), nematodes of the genus *Meloidogyne* often induce gall formation in compatible interactions, since the main resistance genes suppress both gall formation and nematode reproduction, primarily affecting pathogen multiplication in the later stages of infection. In addition, Villar-Luna *et al.* (2015) noted that in the incompatible interaction *Mi-CM-334* an overexpression of genes (*EAS*, *HMG2*, *WRKY-a*, *PR-1*, and *POX*) associated with plant defense is observed. As well as the accumulation of the phytoalexin capsidiol, a compound linked to restricting nematode establishment and reproduction.

The results obtained in accessions CP903 and CP922, classified as very resistant and highly resistant, coincide with those reported by Gómez-Rodríguez *et al.* (2017). Those authors described in Serrano and Huacle pepper lines, a low number of galls and eggs of *M. incognita*. In particular, they highlighted the Serrano pepper lines 41-1 and 41-2, in which no galls or eggs were recorded, a behavior similar to that observed in the Morelos native chili pepper line CM-334.

Results of this research show that plants of native chili pepper responded differentially to *Meloidogyne incognita* and *M. enterolobii*. This variation supports the findings of Després *et al.* (2007), who indicated that defense levels can vary within the same species depending on the genotype and the environment. Also, that native populations constitute a valuable reservoir of genes associated with resistance. These genetic resources are key to identify new sources of resistance, and to support breeding programs that prioritize genetic resistance through the evaluation, selection, and validation of promising materials under contrasting conditions, with their eventual incorporation as resistant genotypes.

CONCLUSIONS

In this study, striped Jalapeño peppers from northern Hidalgo demonstrated differential resistance to *Meloidogyne enterolobii* and *M. incognita*. This characteristic placed these accessions evaluated as a potential alternative for inclusion in genetic improvement strategies for chili pepper.

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