

Diseases Caused by Nematodes

First Report of *Meloidogyne javanica* Parasitizing Ridge Gourd (*Luffa acutangula*) Roots in Sinaloa, Mexico

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The ridge gourd or Chinese okra (*Luffa acutangula* [L.] Roxb.) is a valuable vegetable in markets in China, India, and other Asian nations. Root-knot nematode species (*Meloidogyne* spp.) have been reported parasitizing key crops grown in Sinaloa, Mexico, including tomato, pepper, and cucumber. At harvest in January 2023, ridge gourd plants (cultivar not provided by the farmer) in 10% of open-field commercial cultivation (24.253030°N, 107.184044°W) exhibited wilt symptoms. The roots showed typical root-knot nematode galls. Nematodes were isolated from soil and root tissues. Second-stage juveniles (J2) of *Meloidogyne* were extracted from 10 soil samples (200 cm³) using the sieve-funnel technique (Hooper et al. 2005). Adult females ($n = 20$) were dissected from galls. The morphometrics (body length and width) of females and J2s corresponded with *Meloidogyne* spp. (Hunt and Handoo 2009). Molecular identification was conducted on the females with the universal primers D2a/D3b for *Meloidogyne* spp. and species-specific primers for *M. incognita* (Finc/Rinc, 399 bp), *M. hapla* (Fhap/Rhap, 610 bp), *Meloidogyne javanica* (Fjav/Rjav, 517 bp), *M. arenaria* (Fare/Rare, 420 bp), and *M. enterolobii* (Fent/Rent, 322 bp) (Hu et al. 2011). All the PCR reactions amplified the correct products to identify *M. javanica*. All other primer sets lacked amplification in the PCR reactions. A single egg mass was isolated and reproduced on a susceptible tomato plant (cv. Moneymaker) for 50 days. For pathogenicity tests, ridge gourd seedlings ($n = 18$) were inoculated with 1,000 eggs of *Meloidogyne* (suspended in 1.0 ml of sterilized tap water). Inoculum was pipetted on the roots of each seedling in the germination tray, and seedlings were immediately transplanted in 1,000-cm³ pots containing clay loam soil. Control plants

received 1.0 ml of sterilized tap water. Pots were set in a randomized complete block design in a greenhouse for 35 days (25 to 30°C) after inoculation. Then, the root tissue was examined to assess the galling level (using a scaling of 0 to 100 based on the percentage of the roots with galls), followed by weighing and stirring in NaOCl to extract the eggs (Gómez-González et al. 2021). Pathogenicity tests were conducted two times, and the data were combined and analyzed using PROC UNIVARIATE (SAS version 9.1; SAS Institute, Cary, NC). The galling level was 35%, the eggs per gram of root were 210, and the reproduction factor (pf/pi) was 3.2. After the pathogenicity tests, molecular identification was conducted on one individual female from each pot ($n = 18$) (Hu et al. 2011). The species-specific primers Fjav/Rjav (SCAR markers) and the primers D2a/D3b (28S rRNA) were used in PCR reactions (Nunn 1992; Zijlstra et al. 2000). After PCR amplification, representative fragments of both, the partial rRNA 28S gene D2/D3 ($\approx 1,000$ bp) domain and SCAR marker (≈ 700 bp), were purified and sequenced for BLAST and phylogenetic analysis. The first 100 data resulting from BLAST (NCBI) of 28S were then subjected to an alignment with default parameters in the MAFFT version 7.487 server. Two *Pratylenchus* homolog sequences were used as outgroups (OQ587943.1 and JN244270.1). A phylogenetic tree was constructed after removing redundant sequences (Kuraku et al. 2013). The constructed tree was visualized with Archaeopteryx 0.9921 (Han and Zmasek 2009). Sequences were submitted to GenBank. The highest identity percentage with the sequence from species-specific primers (accession no. OR122724) showed 99.68% identity to the *M. javanica* sequence KP411880. The D2/D3 region (OR135246) was also located in the same clade with *M. javanica* (KP901084.1). To our knowledge, this is the first report of *M. javanica* affecting ridge gourd in Sinaloa, Mexico. These results will help farmers in developing management programs for root-knot nematodes.

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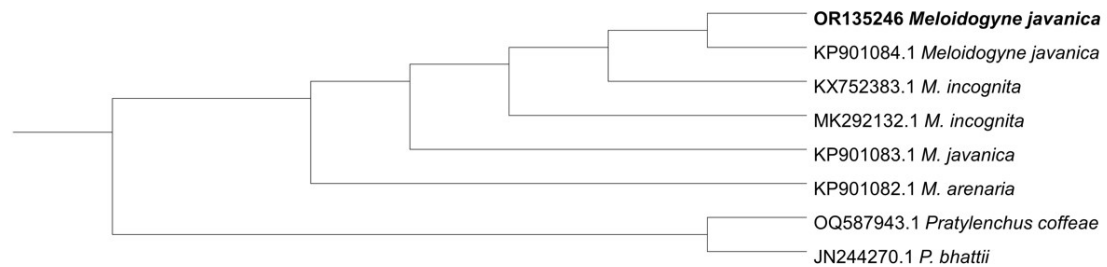


Fig S1. Neighbour-joining tree generated from the partial rDNA 28S gene D2/D3 domain (688 sites) of 8 *Meloidogyne* spp. *Pratylenchus* sequences were added as an outgroup clade.