First report of southern root-knot nematode, *Meloidogyne incognita*, infecting pomegranate, *Punica granatum*, in Peru

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Pomegranate (*Punica granatum* L.) is an exotic fruit in Peru that has unique pharmacological characteristics including several bioactive compounds. Its cultivation is intended for ornamentation, fruit production for fresh consumption, or processed products, such as juices, syrups, and jellies (Saroj et al., 2008), among others.

Plants can be attacked by pests, diseases, and plant-parasitic nematodes, which can qualitatively and quantitatively impair production (Dias-Arieira et al., 2010; Sikora et al., 2018). Among the plant-parasitic nematodes, the most important the genus is *Meloidogyne* Göldi, 1887, which causes damage in the form of root galls and reduction in the number of roots, and predisposition to fungal and bacterial diseases causing losses in crop yields (Karssen, 2002; Sikora et al., 2018). Furthermore, root-knot nematodes often thrive and cause damage on perennial hosts for many years preventing them from reaching their full yield potential. The root-knot nematodes, *Meloidogyne incognita* (Kofoid and White, 1919; Chitwood, 1949) and *M. javanica* (Treub, 1885; Chitwood, 1949), the economically important parasites of pomegranate cultivars in the world (Singh et al., 2019).

In a six-year-old pomegranate (cv. Wonderful) plantation aged six years old in Majes, Arequipa, Peru (16°19´37.0˝S; 72°13’08.0˝W), plants after pruning were slow to develop new shoots (Figure 1A) and roots with distinct galls (Figure 1B-D) were collected on September, 2019. In order to identify the plant-parasitic nematode species, a combination of morphological, biochemical, and molecular analyses were performed.

This population of root-knot nematode was identified to species with esterase phenotypes (*n* = 36 females) (Carneiro and Almeida, 2001); morphology and morphometrics of second-stage juveniles (J2) (*n* = 30) and females (*n* = 10), and perineal patterns (*n* = 15); and molecular characterization of the mitochondrial DNA region between the cytochrome oxidase subunit II and 16S rRNA genes (mtDNA) using the primers C2F3 (5´-GGTCAATGTTCAGAAATTTGTGG-3´) and 1108 (5´-TACCTTTGACCAATCAGCT-3´) (Powers and Harris, 1993) along with PCR species-specific sequence characterized amplified region (SCAR) for confirmation, using a primer set composed of inc-K14-F (5´-GGGATGTGTAAATGCTCCTG-3´) and inc-K14-R(5´-CCCGCTACACCCTCAACTTC-3´) (Randig et al., 2002).
The nematode population density was 1,500 second-stage juveniles (J2)/g of root. Morphometric study showed the following results; J2s: length (L) = 350.5 ± 25.7 (315-490) μm, a = 23.0 ± 4.5 (20.1-26.5), c = 8.9 ± 0.9 (5.0-10.5), stylet length = 11.5 ± 0.5 (9.2-12.4) μm, dorsal esophageal gland orifice to base of stylet (DGO) = 2.4 ± 0.3 (1.8-2.9) μm, tail length = 40.5 ± 1.0 (39.0-48.5) μm and hyaline tail terminus = 10.3 ± 0.8 (10.1-11.2) μm. Morphometrics of females: L = 645.5 ± 30.0 (544.5-705.5) μm, stylet length = 14.2 ± 0.5 (12.4-15.7) μm, and DGO = 3.6 ± 0.2 (2.9-4.1) μm. The perineal pattern of the female included a high and square dorsal arch with wavy striae bending toward the lateral lines and the absence of distinct lateral line incisures (Figure 2A-C). The overall morphology and morphometrics of this population appears similar to that of *M. incognita* (Hunt and Handoo, 2009).

The polymorphisms of the esterase bands by electrophoresis revealed the phenotype I2 (Rm = 1.05 and 1.10) typical of *M. incognita* (Carneiro et al., 1996). The mtDNA sequence (1,638 bp) was submitted to GenBank with Accession No. MT066217.1. Searches on BLAST showed a 99% identity with sequences of *M. incognita* isolates from Brazil (GenBank MK861920.1), USA (GenBank KP001567.1 and KF993635.1), and China (GenBank MH152335.1 and MH152333.1). The PCR amplification using SCAR technique produced a specific fragment of expected size (~399 bp) for *M. incognita* (Randig et al., 2002).

In greenhouse tests, *P. granatum* (cv. Wonderful) plantlets were maintained in pots with 5,000 dm³ sterilized soil. In total, eight replicates were inoculated with 5,000 eggs and J2s from the original population of *M. incognita*, in addition to a non-inoculated control. Plants were well maintained under greenhouse conditions.
conditions at 25±3°C. After 120 days, the inoculated plants exhibited galled root systems similar to plants observed in the field, with a nematode reproduction factor (final population/initial population) of 18.5. The non-inoculated plants did not exhibit any galls. The morphological and molecular characterization of this re-isolated root-knot nematode were identical those of *M. incognita*.

This is the first report of *M. incognita* parasitizing pomegranate plants in Peru. This finding has great importance for the fruit, and nursery industry in Peru, since this nematode may damage pomegranate plants and become more widespread and a significant problem for this crop.

**References**

Carneiro, R. M. D. G. and Almeida, M. R. A. 2001. Técnica de eletroforese usada no estudo de enzimas dos nematoides de galhas para identificação de espécies. Nematologia Brasileira 25:555–60.

Carneiro, R. M. D. G., Almeida, M. R. A. and Carneiro, R. G. 1996. Enzyme phenotypes of Brazilian populations of *Meloidogyne* spp. Fundamental & Applied Nematology 3:555–60.

Chitwood, B. G. 1949. Root-knot nematodes – part I. A revision of the genus *Meloidogyne* Göldi, 1887. Proceedings of the Helminthological Society of Washington 16:90–104.

Dias-Arieira, C. R., Furlanetto, C., Santana, S. M., Barizão, D. A. O., Ribeiro, R. C. F. and Formentini, H. M. 2010. Fitonematoides associados a frutíferas na região noroeste do Paraná, Brasil. Revista Brasileira de Fruticultura 32:1064–71.

Göldi, E. A. 1887. Relatório sôobre a molestia do cafeeiro na provincial da Rio de Janeiro. Arquivos do Museu Nacional do Rio de Janeiro 8:7–123.

Hunt, D. J. and Handoo, Z. A. 2009. “Taxonomy, identification and principal species”, in Perry, R. N., Moens, M. and Starr, J. (Eds), Root-knot Nematodes CABI Publishing, Wallingford, pp. 55–118.

Karsen, G. 2002. The Plant-Parasitic Nematode Genus *Meloidogyne* Goldi, 1892 (Tylenchida) in Europe Brill, Leiden.

Kofoid, C. A. and White, W. A. 1919. A new nematode infection of man. Journal of the American Medical Association 72:567–69.

Powers, T. O. and Harris, T. S. 1993. A polymerase chain reaction method for identification of five major *Meloidogyne* species. Journal of Nematology 25:1–6.

Randig, O., Bongiovanni, M., Carneiro, R. M. and Castagnone-Sereno, P. 2002. Genetic diversity of root-knot nematodes from Brazil and development of SCAR marker specific for the coffee damaging species. Genome 45:622–70.

Saroj, P. L., Awasthi, O. P., Bhargava, R. and Singh, U. V. 2008. Standardization of pomegranate propagation by cutting under mist system in hot arid region. Indian Journal Horticulture, Bikaner 65:25–30.

Sikora, R., Coyne, D., Hallmann, J. and Timper, P. 2018. Plant Parasitic Nematodes in Subtropical and Tropical Agriculture CABI Publishing, Wallingford.

Singh, T., Prajapati, A., Maru, A. K., Chaudhary, R. and Patel, D. J. 2019. Root-knot nematodes (*Meloidogyne* spp.) infecting pomegranate: a Review. Agricultural Reviews 40:309–13.

Treub, M. 1885. Onderzoekingen over Sereh-Ziek Suikerriet gedaan in s Lands Plantentium te Buiten-zorg. Mededeelingen uit’s Lands Plantentium, Batavia 2:1–39.