Detection of *Meloidogyne arenaria* in cucumber in Rio Grande do Sul state, Brazil

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Abstract

Diseased cucumber plants with symptoms of decline and root knots were collected from a crop in the municipality of Faxinal do Soturno, Rio Grande do Sul state, Brazil. Morphological (perineal patterns), biochemical (esterase phenotypes) and molecular (D2–D3 region) studies allowed to identify parasitizing nematodes, such as *Meloidogyne arenaria*. To our knowledge, this is the first report of *Meloidogyne arenaria* on Cucumber in Rio Grande do Sul State, Brazil.

Keywords Occurrence · Identification · Root-knot nematodes · *Cucumis sativus*

Brazil is among the leading countries of cucumber (*Cucumis sativus*) producers but per hectare yield of this vegetable in the country is limited due to parasitism of fungi, viruses, bacteria, and nematodes (Cardoso et al. 2001; Sikora and Fernandez 2005). Of all pathogens, parasitism of root-knot nematodes (*Meloidogyne* spp.) is the most economically important (Mukhtar et al. 2013).

Root-knot nematodes belong to the genus *Meloidogyne*, which are highly-adaptable, obligate, and polyphagous plant parasites. *Meloidogyne* spp. occur worldwide and parasitize most flowering plants, including economically relevant crops (Moens et al. 2009). The pathogen is spread throughout regions of Brazil and is associated with numerous plants. (Moens et al. 2009; Pinheiro and Amaro 2010). In the case of cucurbit, one of the main routes of nematode spread are by infected seedlings. However, despite efforts to restrict or even prevent the entry of the pest into nematode-free areas, there is still a lack of guidance to growers. Seedlings infected with nematodes restricts plant development thus limiting productivity.

Meloidogyne arenaria is one of the most important root-knot nematodes and causes great losses to many crops worldwide. Although other *Meloidogyne* species, including *M. javanica*, *M. incognita*, and *M. enterolobii*, have already been reported infecting cucumber in Brazil (Pinheiro and Amaro 2010), *M. arenaria* has not been detected before.

Cucumber plants collected in the municipality of Faxinal do Soturno, Rio Grande do Sul State, Brazil, showed wilting and stunting leaves symptoms. Roots clearly showed visible galls and egg masses indicative of root-knot nematodes. The aim of this study was to identify the species of *Meloidogyne* involved and here we report the identification of *M. arenaria*.

In January 2016, samples of cucumber plants (cv. Monalisa) (Fig. 1A, B and C) exhibiting many galls on the roots collected in Faxinal do Soturno (29° 34' 12" S; 53° 26' 27" W; 63 m), central region of Rio Grande do Sul state, Brazil. The plants were grown in a commercial field, with sandy soil (125 g kg⁻¹ clay and 505 g kg⁻¹ sand). Roots were washed with tap water and dissected. Adult female nematodes ($n = 40$) were extracted from the dissected roots. After the extraction of adult females, the total nematode population was estimated per gram of cucumber roots (Hussey and Barker 1973).

The nematode species was identified using morphological, biochemical, and molecular characters. The nematode specimens were identified from their perineal patterns (Hartman and Sasser, 1985) and esterase phenotypes of 20 adult females, extracted from dissected roots (Carneiro et al. 2000; Carneiro and Almeida 2001). Microscope slides of perineal patterns were deposited (LB0010) in

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Fig. 1 Cucumber roots showing galls caused by *Meloidogyne arenaria* (A and B)

the Universidade Federal de Santa Maria (UFSM) Collection, Rio Grande do Sul state, Brazil.

Genomic DNA was obtained using the NaOH method (Stanton et al. 1998). Amplification of D2–D3 fragments of 28S rDNA gene was performed using the Kit Taq PCR Master Mix (Promega) and nematode universal primers D2A and

D3B (De Ley et al. 1999). Nematode species identification was confirmed with PCR species-specific sequence characterized amplified region (SCAR), using a primer set Far and Rar (Zijlstra et al. 2000).

DNA sequences were analyzed using BLAST megablast and deposited in GenBank (GenBank accession number

Fig. 2 Perineal pattern (A and B), esterase phenotype (C), and SCAR species-specific (D) of *Meloidogyne arenaria* detected in cucumber in Faxinal do Soturno, Rio Grande do Sul state, Brazil (A2: *Meloidogyne arenaria*; J3*: *Meloidogyne javanica* reference isolate; M: Molecular-weight size marker)

Fig. 3 Phylogenetic tree (Neighbor-Joining) resulting from alignment of the partial sequences of D2/D3 of populations of *Meloidogyne* spp. Bootstrap values were obtained from 1000 replicates. Populations isolated from cucumber plants were indicated by *

KY293688). The D2/D3 sequences were aligned using CLUSTAL W (Tompson et al. 1994) and the tree generated using the Neighbor-Joining (NJ) algorithm in the MEGA 6.06 (Tamura et al. 2013). To test the node support of the generated trees, 1000 bootstrap replicates were performed and only values above 50% were considered.

The estimated population density of the root samples was an average of 2350 s-stage juveniles + eggs per gram of cucumber roots. The females showed perineal patterns typical of *M. arenaria*, with characteristic low dorsal arch, which is indented near the lateral field and with irregular forks. Striae were smooth and slightly wavy (Fig. 2A). The morphology of perineal patterns is considered the most important morphological feature for species identification in *Meloidogyne* (Eisenback 1985; Karssen and van Aelst 2001).

The esterase activity showed a pattern with two bands ($R_m = 1.20$, and 1.30) (Fig. 2B), which corresponds to A2 phenotype of *M. arenaria* and is an important characteristic as it differentiates *M. arenaria* from other *Meloidogyne* species (Carneiro et al. 2000). The PCR-SCAR product was approximately 420 bp, identical to previous reports for *M. arenaria*. (Zijlstra et al. 2000) (Fig. 2C).

The 28S rDNA sequence, amplicon of approximately 750 bp fragments obtained in this study (GenBank KY293688) corresponds to known sequences of *M. arenaria* from the United States (GenBank EU364889), South Africa (GenBank KC287191, KC287192 and JX987332), and Brazil (GenBank KX151138). Phylogenetic analyses of the sequences using the Neighbor-Joining algorithm placed the *Meloidogyne* isolate from our study in a clade with *M. arenaria* sequences from the GenBank database, thereby confirming it as *M. arenaria* (Fig. 3).

To the best of our knowledge, this is the first report on *M. arenaria* parasitizing cucumber in Rio Grande do Sul state, Brazil. Generally, cucurbitaceous plants are suitable hosts for major species of *Meloidogyne* and should therefore be listed

as a new concern for growers and farm advisors. Hence, further studies are necessary to investigate the damage it can cause to crops, such as cucumber, grown in tropical, subtropical, and temperate regions.

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References

- Cardoso MO, Boher B, Avila AC, Assis LAG (2001) Doenças das Cucurbitáceas no Estado do Amazonas. Embrapa Amazônia Ocidental. Circular Técnica 9:2–14
- Carneiro RMDG, Almeida MRA (2001) Técnica de eletroforese usada no estudo de enzimas dos nematóides de galhas para identificação de espécies. Nematologia Brasileira 25:555–560
- Carneiro RMDG, Almeida MRA, Quénéhervé P (2000) Enzyme phenotypes of *Meloidogyne* spp. populations. Nematology 2:645–654. <https://doi.org/10.1163/156854100509510>
- De Ley P, Félix MA, Frisse LM, Nadler SA, Sternberg PW, Thomas WK (1999) Molecular and morphological characterization of two reproductively isolated species with mirror-image anatomy (Nematoda: Cephalobidae). Nematology 1:519–612. <https://doi.org/10.1163/156854199508559>
- Eisenback JD (1985) Diagnostic characters useful in the identification of the four most common species of root-knot nematodes (*Meloidogyne* spp.). In: Sasser JN, Carter CC (eds) An advanced treatise on Meloidogyne, vol. I, biology and control. Raleigh: North Carolina State University graphics, pp. 95–112
- Hartman KM, Sasser JN (1985) Identification of *Meloidogyne* species on the basis of differential host and perineal pattern morphology. In: Barker KR, Carter CC, Sasser JN (eds). An advanced treatise on Meloidogyne. Volume II methodology Raleigh: North Carolina State University graphics, pp. 115–123
- Hussey RS, Barker KR (1973) Comparison of methods for collecting inocula of *Meloidogyne* spp., including a new technique. Plant Dis 57:1025–1028

- Karssen G, Van Aelst AC (2001) Root-knot nematode perineal pattern development: a reconsideration. *Nematology* 3:95–111. <https://doi.org/10.1163/156854101750236231>
- Moens M, Perry RN, Starr J (2009) *Meloidogyne* species - a diverse group of novel and important plant parasites. In: Perry RN, Moens M, Starr JL (eds) Root-knot nematodes. CABI Publishing, CAB International, Wallingford, pp 1–17
- Mukhtar T, Kayani MZ, Hussain MA (2013) Response of selected cucumber cultivars to *Meloidogyne incognita*. *Crop Prot* 44:13–17. <https://doi.org/10.1016/j.cropro.2012.10.015>
- Pinheiro JB, Amaro GB (2010) Ocorrência e controle de nematoides nas principais espécies cultivadas de cucurbitáceas. *Embrapa Hortaliças Circular técnica* 88:1–7
- Sikora RA, Fernandez E (2005) Nematode parasites of vegetables. In Luc M, Sikora RA, Bridge J (eds.) Plant parasitic nematodes in subtropical and tropical agriculture Wallingford, UK, CABI Publishing, CAB International, pp. 319–393
- Stanton JM, McNicol CD, Steele V (1998) Non-manual lysis of second-stage *Meloidogyne* juveniles for identification of pure and mixed samples based on the polymerase chain reaction. *Australas Plant Pathol* 27:112–115. <https://doi.org/10.1071/AP98014>
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 30:2725–2729. <https://doi.org/10.1093/molbev/mst197>
- Tompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22:4673–4680
- Zijlstra C, Donkers-Venne DTHM, Fargette M (2000) Identification of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* using sequence characterized amplified region (SCAR) based PCR assays. *Nematology* 2:847–853