Meloidogyne incognita parasitizing *Ophiopogon japonicus* in Rio Grande do Sul state, Brazil

Izabelle Scheffer Romagna¹ · Emanuele Junges¹ · Rodrigo Ferraz Ramos² · Zaida Ines Antonioli² · Cristiano Bellé²

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Abstract

Ophiopogon japonicus plants showing symptoms of root-knot nematode infestation were observed in the municipality of São Vicente do Sul, Rio Grande do Sul state, Brazil. Based on the morphological identification, esterase phenotypes and molecular analyses of COII and 16S region, the causal agents of the observed symptoms were identified as *Meloidogyne incognita*, with pathogenicity confirmed by fulfilling Koch's postulates. This is the first report of *M. incognita* in *O. japonicus* in Brazil.

Keywords Root-knot nematodes · Mondo grass · Occurrence · Identification

Root-knot nematodes (*Meloidogyne* spp.) are the most damaging group of plant-parasitic nematodes to ornamental plants (Brito et al. 2010). As serious pathogens of many woody ornamental species, root-knot nematodes limit productivity by damaging numerous nursery crops directly and by forming disease complexes with certain soil-borne fungal pathogens (Singh et al. 2013). Furthermore, root-knot nematode populations often thrive and cause damage on perennial hosts for many months and years.

Ophiopogon japonicus (mondo grass) consists of evergreen, grass-like perennials used in garden landscapes as casual groundcovers in small areas. Plants grow in filtered sun to full shade, and are regarded as fairly drought tolerant for short periods. It has been widely used by landscapers, as a decorative effect in the composition of gardens, in replacement of grass, or as borders and boundaries.

Mondo grass plants were collected within the Municipality of São Vicente do Sul, Rio Grande do Sul State, Brazil. Symptoms of galls due to infection by *Meloidogyne* spp. were clearly visible on roots of infected plants. In order to identify the plant-parasitic nematode species infecting roots of these

mondo grass, a combination of morphological, biochemical, and molecular analyses were employed.

In February 2019, samples of mondo grass (Fig. 1a–b) exhibiting many galls on the roots were detected and sampled in areas from São Vicente do Sul (29°42'14"S; 54°41'49"W), Rio Grande do Sul State, Brazil. Roots were washed with tap water and adult female nematodes extracted from the roots, where after the nematode population density was determined (Coolen and D'Herde 1972).

The root-knot nematode species was first identified using perineal-pattern ($n = 20$) morphology (Hartman and Sasser 1985). The morphometric measures of second-stage juvenile nematodes (J2) ($n = 20$) and adult female ($n = 20$) were also obtained after they had been heat killed and mounted on slides (formalin 2%) (Cobb 1918), using the following characteristics for J2: body length (L), stylet length, greatest body diameter, dorsal esophageal gland orifice (DGO), tail length, and hyaline tail terminus. The de Man's ratios (a and c) were also obtained (Siddiqi 2000). For adult females the following characteristics were measured: body length, stylet length and DGO. Microscope slides of perineal patterns were deposited (LB0013) in the Universidade Federal de Santa Maria (UFSM) Collection, Rio Grande do Sul state, Brazil.

Furthermore, the esterase phenotypes of 20 adult females that were isolated from infected roots were determined. This was done using protein extracts from one young, egg-laying female for each reaction. For this purpose, each of the 20 females were placed in a hematocrit containing 2 μ l of the extraction solution (20% sucrose, 1% Triton X-100) (Carneiro et al. 2000), macerated, and transferred to 7.5% polyacrylamide gel slabs. Females of a single population of *M. javanica* (J3; Rm: 1.00, 1.25 and

✉ Cristiano Bellé
crbelle@gmail.com

¹ Instituto Federal de Educação, Ciência e Tecnologia Farroupilha, Campus São Vicente do Sul, São Vicente do Sul, Rio Grande do Sul 97420-000, Brazil

² Universidade Federal de Santa Maria, Centro de Ciências Rurais, Departamento de Solos, Santa Maria, Rio Grande do Sul 97105-900, Brazil

Fig. 1 **a** Mondo grass (*Ophiopogon japonicus*) roots showing galls caused by *Meloidogyne incognita* infection, **b** close up of galls on mondo grass roots

1.40) were used as a reference (Carneiro and Almeida 2001). Electrophoresis was performed according to Carneiro and Almeida (2001) at 4 °C, under a constant voltage of 90 V for 130 min. The gel was stained for esterase activity using the α -naphthyl acetate substrate.

Genomic deoxyribonucleic acid (DNA) was ultimately obtained from females using the NaOH method (Stanton et al. 1998). Amplification of the mitochondrial region between the cytochrome oxidase subunit II (COII) and 16S rRNA genes (mtDNA) was performed using nematode universal primers C2F3 (5'-GGTCAATGTTTCAGAAATTTGTGG-3') and 1108 (5'-TACCTTTGACCAATCACGCT-3') (Powers and Harris 1993).

DNA sequence was compared using Basic Local Alignment Search Tool (BLAST) and deposited in GenBank as accession number MK861920. The COII and 16S sequence were aligned using CLUSTAL W (Tompson et al. 1994). A dendrogram was generated using MEGA 7 (Tamura et al. 2013), and then a phylogenetic tree was constructed using a Maximum Likelihood algorithm based on the Jukes-Cantor model and complete deletion (1000 replicates).

The nematode population density observed in the sample was 1350 J2 per root. The J2 s had the following morphometric characters: length (L) = 355.19 ± 25.75 (325–487) μm , a = 24.05 ± 5.00 (20.5–27.5), c = 9.00 ± 1.00 (5.2–11.5), stylet length = 10.50 ± 0.55 (9.5–13.0) μm , dorsal esophageal gland orifice (DGO) = 2.3 ± 0.35 (1.7–3.0) μm , tail length = 41.5 ± 1.27 (40.0–49.5) μm and hyaline tail terminus = 10.8 ± 0.90 (10.3–11.0) μm . Morphometric features of females included

L = 630.5 ± 35.0 (540.5–690.5) μm , stylet length = 14.1 ± 0.5 (12.5–15.5) μm , and DGO = 3.5 ± 0.3 (3.0–4.2) μm . The female's perineal patterns included a high and square dorsal arch, with wavy striae bending toward the lateral lines and the absence of distinct lateral line incisures (Fig. 2a–d). The overall morphology and morphometrics of the population of *M. incognita* correspond with the original description (Hunt and Handoo 2009).

The esterase activity showed an esterase pattern with two bands (Rm = 1.05 and 1.10) (Fig. 2e) which corresponds with the I2 phenotype of *M. incognita*. This is an important characteristic since it differentiates *M. incognita* from other *Meloidogyne* spp. (Carneiro et al. 1996). The COII and 16S sequence correspond with known sequences of *M. incognita* (KP001569, KP001567, KP001571 and KP001570) that are available in Genbank. Phylogenetic analyses of those sequences using maximum likelihood, placed the *Meloidogyne* population isolated from mondo grass during this study in a clade with the *M. incognita* sequences from GenBank, thereby confirming it as *M. incognita* (Fig. 3).

In greenhouse tests, mondo grass plantlets were maintained in pots with 2000 dm³ sterilised soil; eight replicates were inoculated with 5000 eggs and J2 s from the original population of *M. incognita*, in addition to a non-inoculated control. Plants were maintained under greenhouse conditions at 22–27 °C, with watering as needed. After 60 days, the inoculated plants exhibited galled root systems similar to plants observed in the field; the nematode reproduction factor (final population/initial population) was 17.50. The non-inoculated

Fig. 2 a–d *Meloidogyne incognita* perineal patterns (Scale for light microscopy photos = 10 μ m). e Esterase phenotypes of *M. incognita* detected in mondo grass (Est. I2: *M. incognita* from São Vicente do Sul, RS, Brazil); J3*: *Meloidogyne javanica* reference isolate

plants did not exhibit any galls. The morphological and molecular characteristics of the re-isolated root-knot nematode were identical to those of *M. incognita*.

This is the first report of *M. incognita* parasitizing mondo grass (*Ophiopogon japonicus*) plants in Brazil. This finding has great importance for the garden industry, and nursery

Fig. 3 A phylogenetic tree (Maximum Likelihood) resulting from alignment of the mitochondrial DNA region between the cytochrome oxidase subunit II (*COII*) and 16S rRNA gene (mtDNA) populations of *Meloidogyne* spp. (Bootstrap values were obtained from 1000 replicates and populations isolated from mondo grass plants)

industry in Brazil, considering this nematode may damage mondo grass plants and become an additional problem for this crop.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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