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Research Note

Meloidogyne luci, a new infecting nematode species on common bean fields at Paraná State, Brazil

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Summary

Common bean diseased plants with symptoms of decline and root knots were collected in two growing areas in the municipality of Araucária, Paraná State (Brazil). Morphological (perineal patterns), biochemical (esterase phenotypes) and molecular (ITS1 sequences) studies allowed us to identify the infecting nematode as *Meloidogyne luci*. To our knowledge, this is the first formal record of *M. luci* parasitizing common bean in Paraná State, Brazil.
Keywords: Brazil; *Phaseolus vulgaris*; ITS1; *Meloidogyne luci*; taxonomy; etiology

Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the most important crops in Brazil and worldwide (FAO, 2015). Brazil is the second largest producer of the world and the main producer of beans in the Americas, with a total production of 3,435,370 t in approximately 3,152.917 ha (IBGE, 2013).

There are numerous limiting factors to common bean production, among them the phytonematodes occurrence. This plant species is usually infected by many nematode species, but *Meloidogyne* spp. (*i.e. M. incognita*, *M. javanica* and *M. arenaria*) are responsible for great damage worldwide (Sikora *et al*., 2005). These nematodes reduce the quality of vegetables and cause yield losses around 50 to 80 % per annum worldwide (Moens *et al*., 2009; Siddiqi, 2000).

Beyond these most common root-knot species, currently, other species have been cited damaging *P. vulgaris* around the world, as *M. enterolobii, M. chitwood, M. hapla*, and *M. brasiliensis* (Brito *et al*., 2003a,b; Hafez & Sundararaj, 1999; Charchar & Eisenback, 2002). *Meloidogyne luci* was detected on *P. vulgaris* in Distrito Federal, Brazil, but no damages were reported (Carneiro *et al*., 2008).

Common bean plants collected on the municipality of Araucária, Paraná State, Brazil, showed symptoms of decline and stunting. Roots showed clearly visible galls and egg masses. Therefore, we aimed to identify the infecting nematode species with the integration of morphological, biochemical and molecular approaches.

Materials and Methods

During a survey of nematode species on common bean fields in Paraná State, Brazil, galled root samples of cultivar Tuiuiú (Fig.1A) were sent, in June 2012, to the Nematology Laboratory from IAPAR, Instituto Agronômico do Paraná, collected in the municipality of Araucária (25°35'34"S, 49°24'36"W). Roots were washed with tap water and adult females were extracted from dissected roots; after, the extraction of nematodes was carried out according to Boneti and Ferraz (1981). Then, nematode population was estimated.

The specimens were identified through perineal patterns (Hartman & Sasser, 1985) and esterase phenotypes of 20 adult females extracted from dissected roots. Esterase phenotypes were determined using protein extract from one young egg-laying female for each reaction. For this purpose, females were placed in a hematocrit containing 5 µl of extraction solution (Carneiro *et al*., 2000), macerated and transferred to 7 % polyacrylamide gel slabs. Ho-

mogenates of the isolate IPR 81 of *M. javanica* (Mj) (J3; *Rm*: 1.0, 1.3 and 1.4) was our reference. Electrophoresis was performed according to Brito *et al*. (2004) using a Omniphor (Biosystems) equipment, at 4 °C, under constant voltage of 100 V for 15 min and 200 V for 30 min. Gel was stained for esterase activity using the α-naphtyl acetate substrate.

Scanning electron microscopy (SEM) analysis was also performed on females partially dissected from the roots. After dissection step, samples were transferred to glass vials containing 2 mL of Karnovsky fixative solution [2.5 % v v^{-1} glutaraldehyde and 2.5 % v v^{-1} paraformaldehyde in 0.05 M sodium cacodylate buffer + 0.001 M calcium chloride, pH 7.0] and stored at 4 °C. Samples were post-fixed in osmium tetroxide 1 % for 2h at 25 °C, and then dehydrated in a graded acetone series (30, 50, 70, 90 and 100 %) and dried to the critical point in CO_2 (Bal-tec CPD 030; Balzers, Germany). Root tissues were mounted on aluminium stubs, sputter coated with gold (Bal-tec SCD 050; Balzers, Germany) and examined in SEM (LEO-435 VP, Cambridge, England) operating at 20 kV with a working distance ranging from 10 to 30 mm.

Genomic DNA was obtained according to NaOH method (Stanton *et al*., 1998). Amplification of the 18S-ITS1-28S region of ribosomal DNA was performed using the Kit Taq PCR Master Mix (Promega) and the nematode universal primers rDNA2 (5'-TTGATTACGTCCCTGCCCTTT-3') and rDNA1.58S (5'-ACGAGCCGAGTGATCCACCG-3'). In a microcentrifuge tube were added 25 µl of the Kit Taq PCR Master Mix, 1.5 µl (0.3 microM) from each primer, 18 µl water mili-Q and 4µl total DNA. The DNA was subjected to a PCR with the following specifications: 94 °C (2 min); followed by 40 cycles at 94 °C (1 min), 57 °C (1 min) and 72 °C (2 min) (Cherry *et al*., 1997).

DNA sequences were analyzed using BLASTn megablast (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) and deposited in GenBank

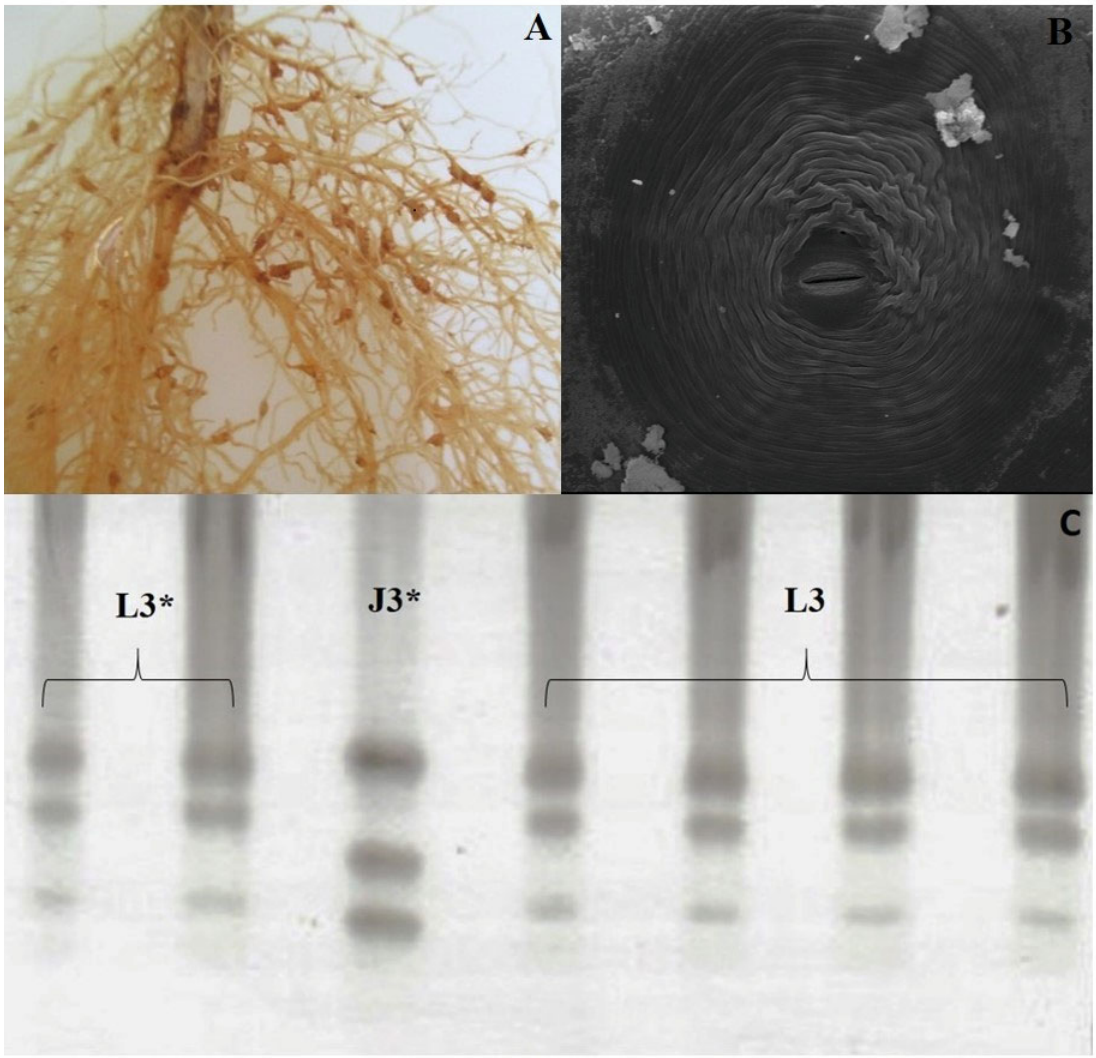

Fig.1. Common bean roots showing natural galls caused by *Meloidogyne luci* (A), perineal pattern of *M. luci* (B), and esterase phenotype of *M. luci* detected in common bean (L3: *M. luci* from Araucária, PR; L3*: *M. luci* reference isolate; J3*: *M. javanica* reference isolate) (C)

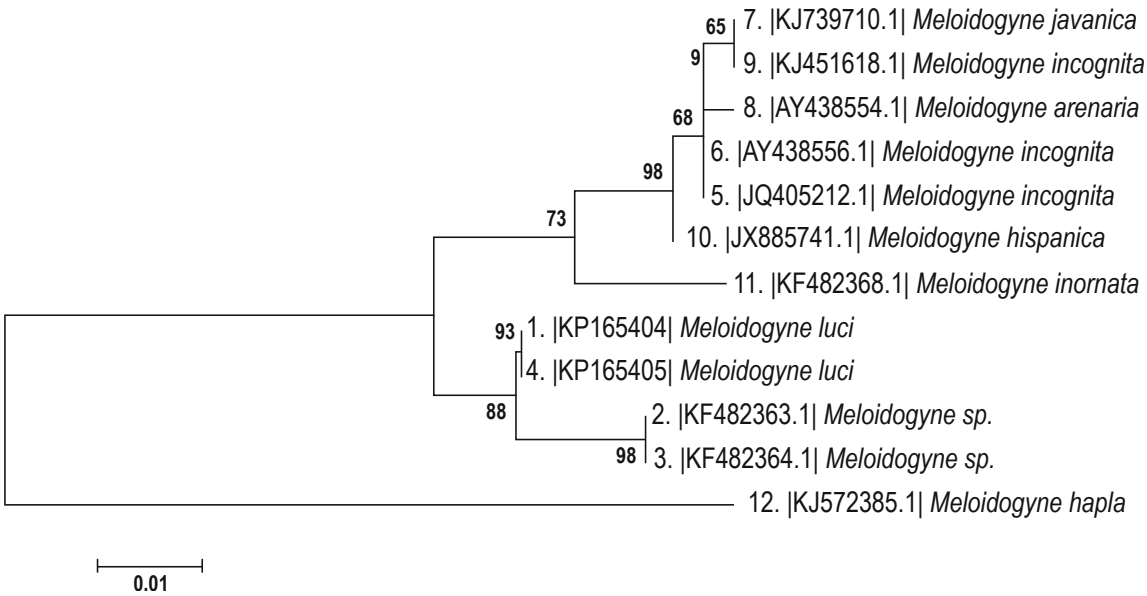

Fig.2. Phylogenetic tree (Maximum Likelihood) resulting from alignment of the partial sequences of the 18S-ITS1-5.8S of populations of *Meloidogyne* spp. Bootstrap values were obtained from 1,000 replicates. Populations isolated from common bean plants are indicated by KP165404 and KP165405

(KP165404 and KP165405). The ITS1 sequences were aligned using CLUSTAL W (Tompson *et al*., 1997). Dendrogram was obtained by MEGA 6.06 (Tamura *et al*., 2013). Model with lowest BIC (Bayesian Information Criterion) scores was considered to describe the substitution pattern. Then, a phylogenetic tree was constructed using Maximum Likelihood with Kimura 2-parameter model (Gamma distribution) and complete deletion (1,000 replicates). *Meloidogyne hapla* (KJ572385.1) was used as outgroup taxa.

Results and Discussion

The population density in the samples was 82 nematodes per gram of roots. Characters observed on both perineal patterns and biochemical analysis were consistent with those described for *M. luci.* Females showed an oval to squarish perineal pattern with a low to moderately high dorsal arc and without shoulders (Fig.1B) (Carneiro *et al*., 2014). Biochemically, we obtained L3 (*Rm*: 1.05, 1.10, 1.25) esterase phenotypes, unique trait for *M. luci* (Fig.1C) (Carneiro *et al*., 2014).

In relation to ITS1 sequences, amplicons of 376 and 417 pb in length obtained showed 97 % and 99 % identity with known sequences of *M. luci* (accession number KF482363.1 and KF482364.1). Phylogenetic analysis with maximum likelihood of those sequences placed our *Meloidogyne* isolate in a clade (90 % bootstrap support) which included only *M. luci* sequences available in the GenBank database, thus confirming its identity (Fig.2).

To our knowledge, this is the first report of *M. luci* parasitizing common bean in Paraná State; previously, it was associated with bean in Braslândia, Distrito Federal, Brazil (Carneiro *et al*., 2013). In general way, common bean is a good host for the major species of *Meloidogyne*; now, this species is included as a new concern for growers and technicians. Additional studies should be conducted in order to determine its distribution and estimates of damage.

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