

First report of *Meloidogyne incognita* infecting *Siegesbeckia orientalis* in China

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Short Report

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Abstract

In September 2024, stunted growth and root knot symptoms were observed on *Siegesbeckia orientalis* plants in tobacco fields of Weining County, Bijie City, Guizhou Province, China, suspected to be caused by root-knot nematode infection. Through sodium hypochlorite-acid fuchsin staining method, morphological characteristics, and SCAR molecular marker analysis, the pathogen was identified as *Meloidogyne incognita*. This study holds significant importance for expanding the known host range and preventing its spread to other crops. To our knowledge, this is the first report of *M. incognita* infecting *S. orientalis* in Guizhou, China.

Introduction

Siegesbeckia orientalis L., a traditional Chinese medicinal herb from the Asteraceae family, is recorded in the 2015 Pharmacopoeia of the People's Republic of China as derived from the dried aerial parts of three species: *S. orientalis*, *Siegesbeckia pubescens*, and *Siegesbeckia glabrescens*. It has a cold property, pungent-bitter flavor, and tropism to the liver and kidney channels (National Pharmacopoeia Commission 2015). Modern studies reveal its antiviral, anti-inflammatory, antitumor, and metabolic regulatory effects, showing therapeutic potential for rheumatoid arthritis, diabetes, and hypertension (Xu et al. 2021).

However, *Meloidogyne* spp., among the most destructive plant pathogens globally, threaten medicinal plant cultivation. These nematodes infect over 3,000 plant species, causing annual agricultural losses of \$157 billion (Pierre et al. 2008). In China, high-value medicinal plants like Panax ginseng, *Panax quinquefolius*, *Panax notoginseng*, *Scutellaria baicalensis*, and *Ophiopogon japonicus* are highly susceptible, compromising yield and quality (Gao et al. 2016). Root-knot nematodes have become a major biotic constraint for medicinal crop production, jeopardizing large-scale cultivation (Lin et al. 2004). Thus, monitoring nematodes in newly affected regions and host crops is critical to curbing transmission and developing targeted control strategies.

In September 2024, *S. orientalis* exhibiting stunted growth and mild chlorosis was identified in tobacco cultivation areas of Weining County, Bijie, Guizhou Province. Examination of uprooted plants revealed the presence of characteristic root galls. Microscopic observation of gall dissections showed the presence of juvenile nematodes, with morphological features matching *Meloidogyne* spp. infection. To determine the causative agent of root-knot nematode disease in *S. orientalis*, identification was performed using staining techniques, morphological analysis, and molecular approaches. This study aims to identify the *Meloidogyne* species causing disease in *S. orientalis* plants, which is significant for expanding the known host range of nematodes and developing precise control strategies against this pathogen.

Materials and methods

The study was conducted in a tobacco field located in Heishitou Town, Weining County, Bijie City, Guizhou Province (26°45'32"N, 103°59'24"E; altitude 2086.2 meters). Diseased *S. orientalis* plants (Fig. 1) were collected, placed in sampling bags, and transported to Guizhou Academy of Tobacco Science for

pathogen identification. For morphological identification, diseased *S. orientalis* samples were stained using the sodium hypochlorite-acid fuchsin method and observed. Thirty adult female nematodes and thirty second-stage juveniles (J2s) were then measured for morphological analysis. The J2 juveniles were obtained by extracting egg masses from root galls of infected samples, which were then surface-sterilized in a petri dish containing 5.25% NaClO, subsequently transferred to a sterile water petri dish, and incubated at 28°C for hatching and cultivation. Female nematodes were isolated by dissecting infected roots under an optical microscope, and perineal patterns were prepared following Duan's method (2011). Morphological identification is based on the original description of root-knot nematodes in the reference literature, with a focus on observing the perineal pattern of female adults and the following key measurements: body length (BL), stylet length (ST), and the distance from the dorsal esophageal gland opening to the stylet base (DGO). For J2 juveniles, body length (BL), body width (BW), stylet length (ST), DGO, and tail morphology were analyzed. All measurements were taken under a stereo microscope after slide preparation.

Molecular identification was conducted using SCAR markers, with genomic DNA extracted from female nematodes following Yang's method (2020). PCR amplification was performed in a 50 µL reaction system containing 25 µL of 2×Taq PCR Master Mix, 1 µL DNA template, 1 µL each of four species-specific primer pairs (Mi-F/Mi-R, Far/Rar, Fjav/Rjav, Fha/Rha), and 22 µL ddH₂O. The PCR conditions included initial denaturation at 94°C for 5 min; 35 cycles of denaturation (94°C, 30 s), primer-specific annealing (58–64°C, 30 s), and extension (30 s–1 min); followed by final extension at 72°C for 7 min (Meng et al. 2004; Zijlstra et al. 2000; Dong et al. 2001). Products were analyzed by 1% agarose gel electrophoresis, sequenced, and BLAST-aligned before GenBank submission. For pathogenicity testing under Koch's postulates, seven 3-week-old *S. orientalis* seedlings were inoculated with ~ 1,000 J2s in sterilized soil (26–28°C), with three uninoculated controls.

Results

The staining results clearly showed purple-red pear-shaped adult females, slender J2 larvae, and elongated oval eggs in the roots (Fig. 2A-C). Morphometric measurements (mean ± range) showed: (1) Females (n = 30): BL = 501.2–720.1 µm, ST = 14.1–16.9 µm, DGO = 2.1–3.8 µm; perineal patterns (n = 10) had circular-oval shapes with distinct dorsal arches and dense striae (Fig. 2D); (2) J2 juveniles (n = 30): BL = 336.2–413.9 µm, BW = 13.2–16.7 µm, ST = 9.5–12.8 µm, DGO = 2.1–3.5 µm, tail length = 38.3–54.5 µm. These morphological characteristics matched the original description of *M. incognita* (Eisenback & Hirschmann 1981).

Molecular analysis confirmed *M. incognita* by SCAR-PCR amplification of a 955-bp species-specific fragment (Fig. 2I), showing 100% sequence identity with reference strains MK410954.1 and MN728679.1 (GenBank accession PQ885372.1). Pathogenicity tests revealed that inoculated plants showed stunted growth and root galls at 40 days post-inoculation, while controls remained healthy (Fig. 2E-H). Re-isolated nematodes from infected roots were confirmed as *M. incognita* through morphological and molecular identification, fulfilling Koch's postulates.

Discussion

Meloidogyne spp. are highly destructive pathogenic nematodes that not only directly reduce crop yield and quality but also weaken host resistance, creating opportunities for secondary pathogen infections. This ultimately leads to complex root diseases characterized by tissue necrosis and rot (Gao, 2016). Although traditionally serving as a medicinal plant, *S. orientalis* frequently grows as a weed in tobacco fields and functions as an alternative host for various pests and pathogens, thereby increasing the difficulty of disease management. While *Meloidogyne hapla* infections of *S. orientalis* have been reported in Shaanxi, China (Yang et al., 2024), to our knowledge, this represents the first confirmed case of *M. incognita* infection in *S. orientalis* in China. This study expands the known host range of root-knot nematodes, providing a basis for developing efficient and environmentally friendly control strategies. The findings may help reduce crop losses, promote sustainable development of the tobacco industry, and offer references for managing nematode diseases in other crops.

Declarations

Conflict of interest

These authors declare that they have no conflict of interest.

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Figures

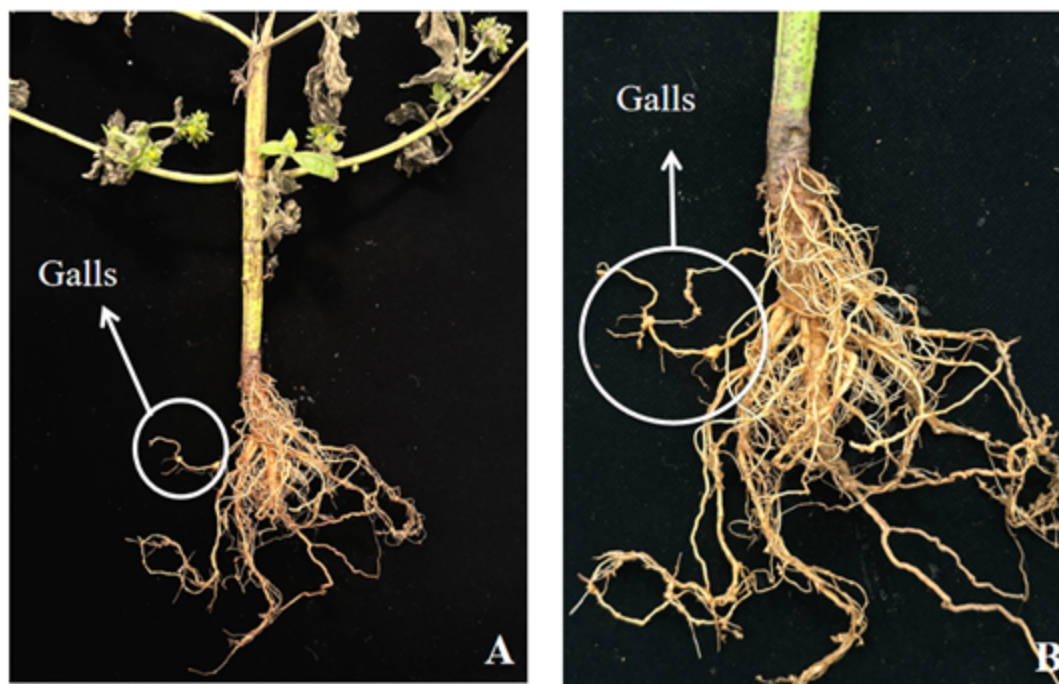


Figure 1

Field observations and root-knot nematode symptoms on *S. orientalis*. A: Naturally infected *S. orientalis* plants collected from the field; B: Root galls formed on susceptible roots, a typical symptom of

nematode infection.

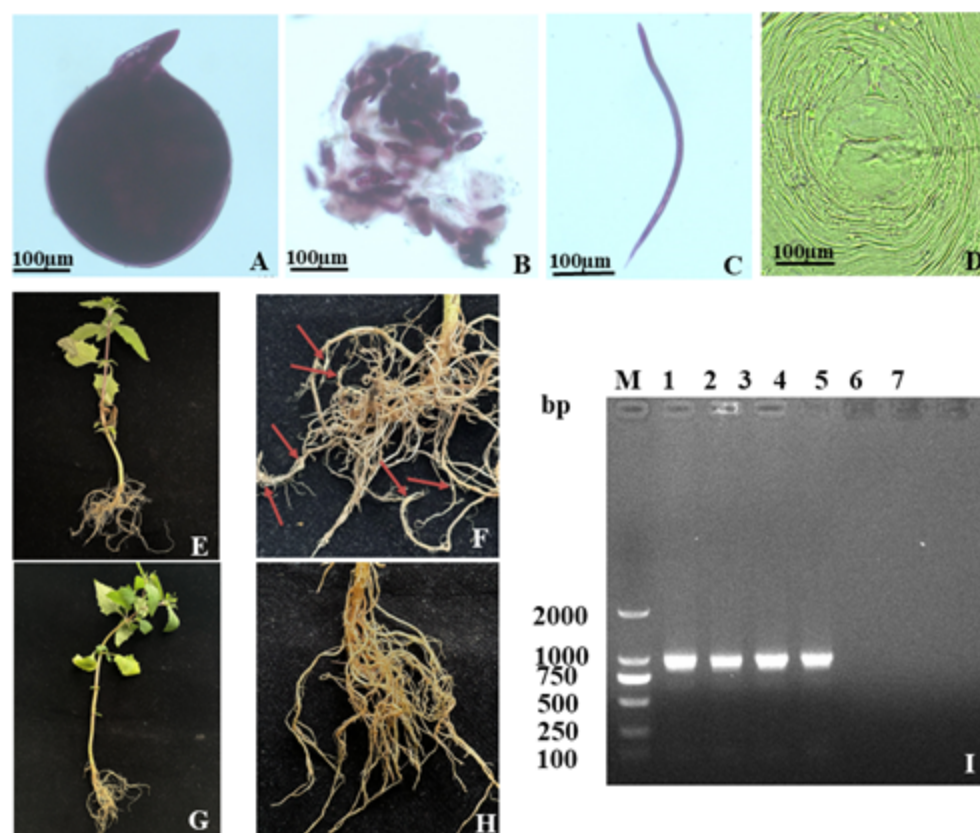


Figure 2

Staining, molecular identification, and pathogenicity verification of root-knot nematodes in *S. orientalis* based on Koch's postulates. A: Female; B: Egg; C: Second-stage juvenile (J2); D: Female perineal pattern; E-H: Pathogenicity assay of *Meloidogyne incognita* (E: Inoculated-whole plant; F: Inoculated-root system; G: Control-whole plant; H: Control-root system); I: SCAR marker analysis (Lanes 1-4 show 955-bp fragments amplified with species-specific primers Mi-F/Mi-R; Lane 5: Far/Rar amplification; Lane 6: Fjav/Rjav amplification; Lane 7: Fha/Rha amplification)