Host response of Nicotiana benthamiana to the parasitism of five populations of root-lesion nematode, Pratylenchus coffeae, from China

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Abstract

In a recent survey of nematodes associated with tobacco in Shandong, China, the root-lesion nematode *Pratylenchus coffeae* was identified using a combination of morphology and molecular techniques. This nematode species is a serious parasite that damages a variety of plant species. The model plant benthi, *Nicotiana benthamiana*, is frequently used to study plant-disease interactions. However, it is not known whether this plant species is a host of *P. coffeae*. The objectives of this study were to evaluate the parasitism and pathogenicity of five populations of the root-lesion nematode *P. coffeae* on *N. benthamiana*. *N. benthamiana* seedlings with the same growth status were chosen and inoculated with 1,000 nematodes per pot. At 60 days after inoculation, the reproductive factors (Rf = final population densities (Pf)/initial population densities (Pi)) for *P. coffeae* in the rhizosphere of *N. benthamiana* were all more than 1, suggesting that *N. benthamiana* was a good host plant for *P. coffeae*. *Nicotiana. benthamiana* infected by *P. coffeae* showed weak growth, decreased tillering, high root reduction, and noticeable brown spots on the roots. Thus, we determined that the model plant *N. benthamiana* can be used to study plant-*P. coffeae* interactions.

Introduction

Benthi, *Nicotiana benthamiana* Domin, is an herbaceous plant in the Solanaceae family that was discovered in Australia before it spread to other countries in the 18th century. Benthi is frequently used to research the interaction between pathogen and host, plant innate immunity, and defense signal transduction because it is very sensitive to pathogens such as viruses, bacteria, oomycetes, and fungi (Goodin *et al.* 2008). After hundreds of years of development, *N. benthamiana* has evolved into an essential model plant in many pathogen and host interaction systems due to its simple cultivation, short growth cycle, quick reproduction, and high regeneration frequency (Goodin *et al.* 2008). Recently, *N. benthamiana* has been utilized as a model plant in comparative assessments of the developmental rate of the rice root-knot nematode, *Meloidogyne graminicola* Golden and Birchfield, 1965 on different hosts (Naalden *et al.* 2018).

Root-lesion nematodes (*Pratylenchus* spp.) are migratory endoparasitic nematodes that are widely distributed, with broad host ranges and damaging effects to their hosts. Their feeding activity and tunnelling inside the roots cause large cavities and necrosis of the cortex, resulting in debilitation of the root system and consequent arrest of plant growth, leaf chlorosis, and yield losses (Lamondia *et al.* 2003). Among the *Pratylenchus* species, the coffee root-lesion nematode, *P. coffeae* (Zimmerman, 1898) Filipjev and Schuurmans Stekhoven, 1941 is one of the most economically important species because of its wide distribution and severely damaging effects on a variety of ornamental plants, food, and cash crops including banana, citrus, coffee, maize, peanut, ramie, sesame, soybean, tobacco, wheat, and yam (Li *et al.* 2021). Morphological and molecular analyses have shown that *P. coffeae* is a species complex consisting of cryptic species (De Luca *et al.* 2012). Root-lesion nematode populations identified tentatively as *P. coffeae* occur in tobacco (*N. tabacum* L.) farms in Shandon Province, China. There is no information on the host status of *N. benthamiana* to *P. coffeae*.

Our study was conducted to: (i) morphologically define a root-lesion nematode population collected from the above mentioned tobacco fields and reared on carrot disks; (ii) provide molecular characterization and phylogenetic relationships of this population with other related species using ITS rRNA and 28S rRNA gene sequences; (iii) assess nematode reproduction rate and response of *N. benthamiana* to *P. coffeae* parasitism after exposing seedlings of this plant growing in pots containing 1800³ soil to an initial population density of 1,000 specimens of *P. coffeae* per pot for 60 days; and (iv) assess, in the same conditions of the above experiment,

nematode reproduction rate and the response of *N. benthamiana* to the parasitism of four putative *P. coffeae* populations from crops grown in different regions of China.

Materials and methods

Isolation and culture of nematodes

Samples of brown, rotting tobacco roots were collected from tobacco farms located in Weifang City, Shandong Province, China. Root-lesion nematodes were extracted from these root samples using a modified Baermann funnel method (Hooper *et al.* 2005) and assigned population number SD-YC-1. Under a stereomicroscope, a single female root-lesion nematode was hand-picked, cleaned with streptomycin sulfate, and placed on a carrot disk at 25°C in the dark for 90 days (Li *et al.* 2019). The cultured nematode population was then used for morphological and molecular analyses and the host study, together with four other putative populations of *P. coffeae* from crops grown in different regions of China (Table 1) and preserved in the Plant Nematode Laboratory of Henan Agricultural University.

Morphological identification of root-lesion nematode

For morphological identification, nematodes were killed by heat fixed in 4% FG solution (formalin:glycerin:water = 10:1:89), dehydrated, and then processed in pure glycerin and mounted in permanent glass slides using Xie's (2005) method. Nematode specimens were examined, measured using a Nikon Eclipse Ti-S (Nikon, Tokyo, Japan) ocular micrometer and photographed with the same microscope.

Molecular identification and phylogenetic analysis of root-lesion nematodes

DNA from individual nematodes was extracted using protease K method (Wang *et al.* 2011). The rDNA ITS regions were amplified by polymerase chain reaction (PCR) using universal primers 18s (5′- TTGATTACGTCCCTGCCCTTT -3′) and 26S (5′- TTCACTCGCCGTTACTAGG -3′) (Vrain *et al.* 1992), and the 28S rDNA D2–D3 region was amplified by PCR with universal primers D2A (5′-ACAAGTACCGGGAAAGTTG-3′) and D3B (5′- TCGGAAGGAACCAGCTACTA-3′) (Subbotin *et al.* 2008). The specific primers TW81 (5′- GTTTCCGTAGGTGAACCTGC-3′) and coffeae group-specific (5′- CTTAAGCCATGTGCCAACTC-3′) (De Luca *et al.* 2012) were used for the specific detection of this nematode species. The PCR reaction system was prepared according to the instructions of KOD FX DNA polymerase (Toyobo, Japan). The PCR reaction conditions were as follows: pre-denatured at 94°C for 2 min, followed by 35 cycles (denatured at 98°C for 10 s, annealed at 58.2°C (ITS rDNA) or 51.7°C (28S rDNA)

for 30 s, extended at 68°C for 90 s), and final extension at 72°C for 10 min. PCR products were purified with a DNA gel recovery kit (Sangon Biotech (Shanghai) Co., Ltd., Shanghai, PR China), connected to a one-step ZTOPO-Blunt/TA cloning vector (Zoman, Beijing, PR China), transferred to DH5αcells, and then sent to Sangon Biotech (Shanghai, PR China) for sequencing. The newly obtained sequences were submitted to the GenBank database under the accession numbers: OQ449389 (rDNA-ITS) and OQ449390 (28s rDNA). These new consensus sequences for each gene of rDNA-ITS and 28S rRNA were aligned with corresponding published gene sequences of *Pratylenchus* obtained from the GenBank database using the nucleotide BLAST program in NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Multiple alignments of sequences were performed using the Clustal W technique in MEGA 7 (Tamura *et al.* 2011). The Akaike Information Criterion (AIC) was used to choose the best-fit model, using MrModeltest 2.3 (Nylander 2004). Sequence datasets were analysed with Bayesian inference (BI) Using MrBayes 3.2.7 (Huelsenbeck & Ronquist 2001). Posterior probabilities (PP) were given on appropriate clades. Outgroup taxa for ITS and D2-D3 datasets were selected according to Wang *et al.* (2015) and Subbotin *et al.* (2008).

Influence of initial densities of five *P. coffeae* populations on growth of *N. benthamiana* in pots

Seeds of *N. benthamiana* were washed three times with sterile water, treated with 75% ethanol for one min, disinfected with 12.5% sodium hypochlorite for 30 min, washed seven times with sterile water, and then sown in sterilized soil medium in 1800 cm^3 pots. After seed germination, seedlings were grown at 25°C with 16 h of sunshine and 8 h of darkness for 30 days in a greenhouse.

Five *P. coffeae* populations were cultured on carrot disks (Reise *et al.* 1987) for 50 days. The nematodes on carrot disks were then washed down with sterile water and suspended in water at a concentration of 1,000 nematodes/ml. Seedlings of *N. benthamiana* of the same growth status were chosen and inoculated with 1 ml water suspension containing 1,000 nematodes (Pi) per pot. Each nematode population inoculated on *N. benthamiana* was a treatment, and each treatment was set up with five replications. For the non-inoculated controls (ck), 1 ml of sterile water was inoculated on *N. benthamiana* in a pot. All pot experiments were repeated twice in a greenhouse (25 °C, 12 h light/12 h dark photoperiod) (Hahn *et al.* 1996).

Plants were harvested 60 days after inoculation. Plant height and fresh weight of shoot and roots were recorded. Meanwhile, the symptoms of the nematode infection were observed and photographed. The final population density (Pf) was obtained by extracting nematodes from soil and roots according to Li *et al.* (2021), and then the reproduction factor, which is a ratio of final (Pf) and initial (Pi) population densities, was determined. The host plant selection

Table 1. Nematode populations used in this study

Nematode population	Host	Sampling location	In vitro cultured
Pratylenchus coffeae (SD-YC-1)	tobacco	Weifang City, Shandong Province	Carrot disks
P. coffeae (XC-278-1)	tobacco	Nanyang City, Henan Province	Carrot disks
P. coffeae (HN-K1)	corn	Pingdingshan City, Henan Province	Carrot disks
P. coffeae (AH-015A2)	wheat	Suzhou City, Anhui Province	Carrot disks
P. coffeae (XC-344-1)	soybean	Linyi City, Shandong Province	Carrot disks

criteria considered Rf > 1 a good host, 1 ≥ Rf > 0 a poor host, and Rf = 0 a non-host, as proposed by Goo *et al.* (1997); this was used to evaluate whether *N. benthamiana* was a good host of *P. coffeae*. The data were analysed with SPSS 22.0 software(Chicago, USA), and Duncan's new multiple range test (DMRT) was used to make multiple comparisons at the 5% significance level to calculate the standard error (SE).

Histopathological observation

At the end of the experiment, roots of *N. benthamina* seedlings inoculated with different populations of *P. coffeae* were washed with tap water to remove soil particles and processed for histological examination. Roots were stained according to Bybd *et al.* (1983) and then examined under the microscope. Paraffin root sections were made according to Wang *et al.* (2016) and Sasanelli *et al.* (2013). Infected roots were cleansed with water, divided into 1 cm segments and fixed in FAA fixative for 48 h, dehydrated, embedded in paraffin, and then sectioned. Sections were stained with safranin and fast green solution and photographed using a Nikon Eclipse Ti-S optical microscope.

Results

Morphological characterization of SD-YC-1 population

The SD-YC-1 population collected from tobacco in Weifang City, Shandong Province was identified as *P. coffeae* based on the morphological characterization. The morphometric data are listed in Table 2.

Female: Body straight or slightly bent into the shape of the letter c after heat relaxation (Figure 1a). Labial framework heavily sclerotised; labial region with two annuli; stylet 15.3 ± 0.4 (14.6–16.1) μm long; orifice of dorsal pharyngeal gland approximately 3.0 ± 0.4 (2.2–3.8) μm posterior to stylet base (Figure 1d–f). Pharyngeal gland lobe overlapping intestine ventrally for about 0.7 to 2 times

Table 2. Morphometrics of a population of *Pratylenchus coffeae* collected in Weifang City, Shandong Province

Character	SD-YC-1 population		Inserra *et al.* (2001)	
	female	male	female	male
n	16	17	20	20
L	629.5 ± 29.52 (575.1–673.4)	542.3 ± 21.35 (494.1–587.7)	601.9 ± 51.4 (520.0–715.0)	589.1 ± 25.4 (558.5–647.5)
a	29.2 ± 2.25 (24.5–35.1)	31.2 ± 1.0 (29.5–32.5	28.7 ± 3.1 (23.4–34.0)	30.8 ± 1.6 (28.7–33.9)
b	6.0 ± 0.3 (5.4–6.5)	5.6 ± 0.2 (5.4–6.2)	6.7 ± 0.4 (5.6–7.2)	6.7 ± 0.3 (6.0–7.3)
b'	4.7 ± 0.3 (4.2–5.0)	–	–	–
c	21.1 ± 1.3 (19.1–24.7)	21.5 ± 1.3 (18.9–23.6)	20.9 ± 2.8 (17–31.0)	21.8 ± 1.6 (19.4–25.4)
c'	2.1 ± 0.24 (1.5–2.6)	–	–	–
V	81.1 ± 1.0 (79.1–82.3)	–	80.5 ± 1.5 (76.0–82.5)	–
T	–	39.1 ± 4.2 (30.8–45.0)	–	–
DGO form stylet base	3.0 ± 0.4 (2.2–3.8)	2.8 ± 0.5 (2.1–4.3)	2.8 ± 0.5 (2.5–4.0)	3.6 ± 0.3 (3.0–4.0)
EP	87.3 ± 4.0 (79.1–95.4)	79.3 ± 2.7 (75.1–85.5)	–	–
Stylet length	15.3 ± 0.4 (14.6–16.1)	15.2 ± 0.3 (14.7–15.6)	16.9 ± 0.2 (16.5–17.0)	15.0 ± 0.4 (14.5–15.5)
Stylet shaft	8.9 ± 0.4 (8.1–9.8)	7.3 ± 0.3 (6.2–8.1)	–	–
Stylet knob height	2.4 ± 0.4 (1.7–3.3)	2.0 ± 0.3 (1.6–2.6)	–	–
Stylet knob width	3.9 ± 0.4 (3.2–4.6)	3.0 ± 0.3 (2.4–3.6)	–	–
Pharyngeal overlap	28.8 ± 5.1 (17.4–36.4)	24.6 ± 4.0 (16.4–35.2)	49.8 ± 10.3 (34.0–72.5)	–
Max body width	21.7 ± 1.6 (18.0–23.8)	17.4 ± 0.6 (16.3–18.2)	–	–
Tail length	30.0 ± 2.6 (24.4–34.9)	25.3 ± 1.5 (23.2–28.2)	29.0 ± 3.3 (21.5–36)	27.1 ± 1.9 (24.5–31.0)
Number of tail annuli	23.0 ± 2.0 (18.0–28.0)	–	–	–
Vulva to anus distance	89.3 ± 7.3 (75.2–101.9)	–	87.2 ± 12.8 (70.5–135)	–
Post-uterine sac length	26.0 ± 3.8 (18.6–33.4)	–	29.5 ± 6.5 (19.5–49.5)	–
Lateral field width	8.0 ± 0.7 (6.2–8.9)	5.9 ± 0.5 (5.1–6.9)	–	–
Vulval body diameter	19.0 ± 1.6 (15.5–21.4)	–	–	–
Anal body diameter	14.5 ± 2.3 (11.9–21.9)	–	–	–
Spicule length	–	18.5 ± 0.7 (17.4–19.7)	–	17.5 ± 0.6 (16.0–18.0)
Gubernaculum length	–	5.1 ± 0.3 (4.6–5.6)	–	5.3 ± 0.3 (5.0–5.5)

Notes: All measurements are in μm and in the form of mean ± standard deviation (range). n, number of specimens measured; L, body length; a, body length/greatest body width; b, body length/length from the lips to the junction of oesophageal gland and intestine; b', body length/length from the lips to oesophageal gland end; c, body length/tail length; c', tail length/tail diameter at anus; V, distance of vulva from the lips × 100/body length; T, distance form cloaca opening to anterior most part of testis/body length × 100%; DGO, distance between dorsal oesophageal gland opening and stylet knobs.
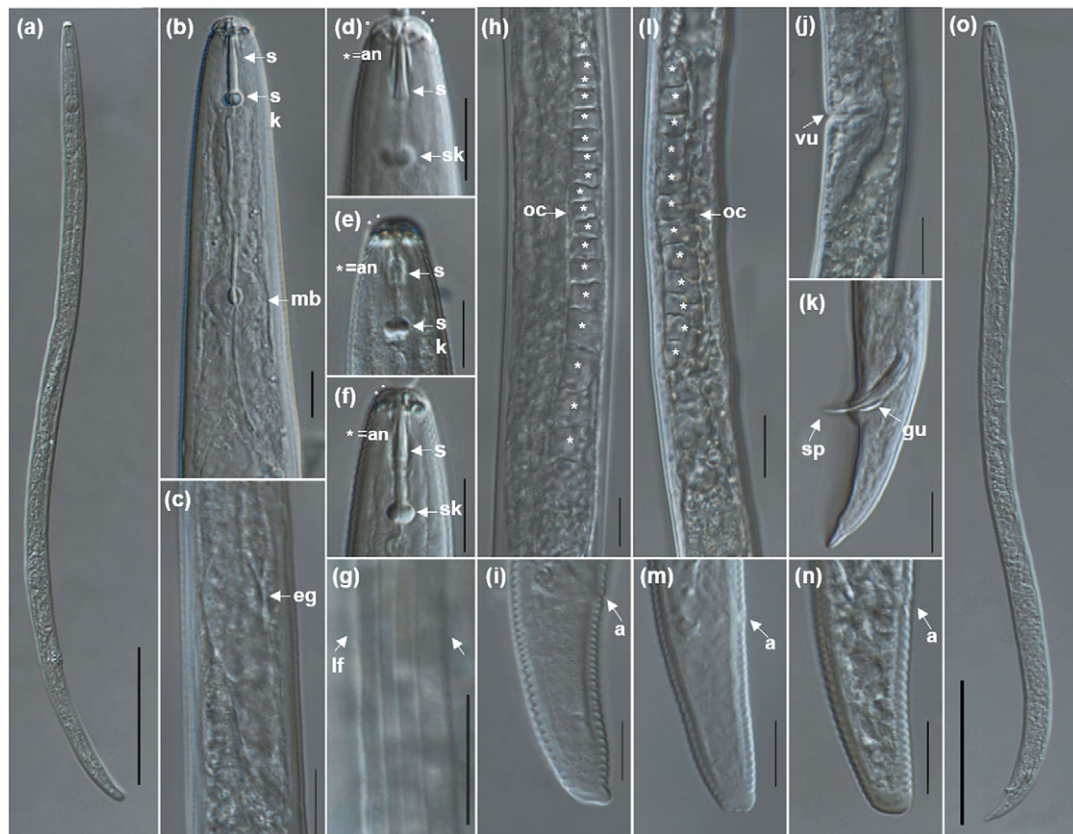
Figure 1. Light micrographs of *Pratylenchus coffeae* from tobacco in Shandong Province, China. (a) female entire body; (b) anterior region; (c) pharyngeal gland lobe overlapping the intestine; (d)–(f) lip region; (g) lateral field; (h)–(i) ovaries showing oocytes(oc); (j) post-vulval region showing the post-uterine sac; (k) spicules; (l)–(n) female tail region; (o) male entire body. Scale bars: 100 μm ((a), (o)) and 10 μm ((b)–(n)). An, annuli; s, stylet; sk, stylet knob; oc, ovary cells; lf, lateral feld; mb, median bulb; eg, pharyngeal glands; vu, vulva; a, anus; sp, spicules; gu, gubernaculum.

the maximum body width (Figure 1c). Lateral fields with four longitudinal lines (Figure 1g). Excretory pore immediately posterior to hemizonid. Ovary with oocytes in one row (Figure 1h). Spermatheca rounded to oval filled with sperm (Figure 1i). The length of the post-uterine sac 0.9–1.6 times the width of the vulval body diameter (Figure 1j). Tail tapering slightly, terminus mostly broadly rounded, varying from somewhat narrower to almost truncate, usually with 18–28 annuli (Figure 1l–n).

Males: Body shorter and more slender than females (Figure 1o). Lip region, stylet, and median pharyngeal bulb slightly weaker than females. Spicules slender, slightly ventrally curved; gubernaculum 4.6–5.6 μm long; bursa enveloping tail tip; tail tip pointed (Figure 1k).

Remarks: The morphological characters of the nematode population collected in Weifang City, Shandong Province, were consistent with the description of a topotype population of *P. coffeae* reported by Inserra *et al.* (2001), except that the overlap at the pharyngeal gland was slightly shorter (17.4–36.4 μm vs. 34.0–72.5 μm).

Molecular characterization and phylogenetic analysis of *P. coffeae*

One 1247 bp rDNA-ITS sequence and one 781 bp D2-D3 region of 28S rDNA sequence were obtained from this population, and the specific primer TW81/coffeae group-specific amplified only one sequence length of 421bp. This is consistent with the literature on *P. coffeae*. The newly obtained rDNA-ITS sequence (OQ449389) showed the highest similarity (99.68%) with a *P. coffeae* sequence (KR106219) from Ruichang City, Jiangxi Province. The newly obtained 28S rDNA sequence (OQ449390) shared 99.74% similarity with the *P. coffeae* sequence from two other populations (MT586754, MN750755). The coffeae group-specific sequence (OR363694) showed 99.53% similarity with the *P. coffeae* sequence from two other populations (OQ674268, MW513459).

The Bayesian phylogenetic tree (Figure 2) constructed based on rDNA-ITS contained 50 sequences. In the *P. coffeae* species complex, *P. speijeri* was isolated into a single branch, which was highly supported (100%). The newly obtained sequence and four additional populations—XC-278-1, HN-K1, AH-015A2, and XC-344-1— clustered together with other *P. coffeae* rDNA-ITS sequences in a highly supported branch (100%). The Bayesian phylogenetic tree (Figure 3) constructed based on the rDNA-28S D2-D3 region contained 46 sequences. Two species of the *P. coffeae* species complex, *P. speijeri* and *P. coffeae*, formed a highly supportive branch (100%). In the monophylline formed by *P. coffeae*, *P. speijeri* formed a single sprig and obtained a high level of support (100%). The newly obtained sequence and the four additional populations—XC-278-1, HN-K1, AH-015A2, and XC-344-1—of *P. coffeae* clustered in a 100% supported clade.

Parasitism of *P. coffeae* on *N. benthamiana*

After 60 days of inoculation, many *P. coffeae* were isolated from the *N. benthamiana* root and rhizosphere soil, and the reproduction factors (Rf) were all greater than 1 in each treatment (Table 3). The
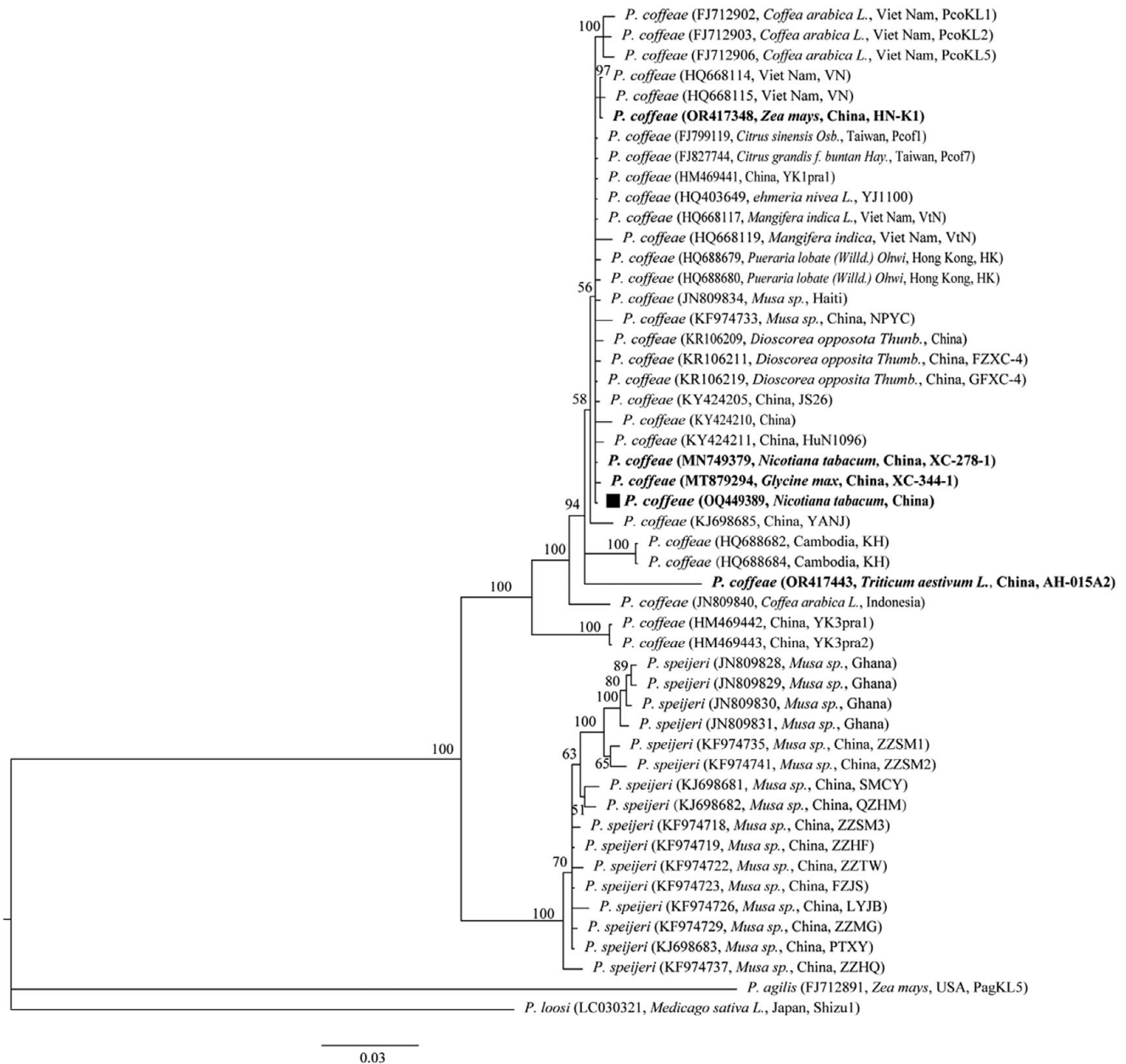
Figure 2. Bayesian tree of *Pratylenchus* as inferred from ITS rRNA gene sequences under GTR+I+G model. Posterior probabilities more than 50% are given for appropriate clades. Newly obtained sequence is indicated in bold font.

SD-YC-1 *P. coffeae* population had the highest reproduction, reaching 4.2. According to the host plant selection criteria (Goo *et al.* 1997), *N. benthamiana* is a good host of *P. coffeae*. The staining study revealed that a significant number of nematodes and eggs were found in the roots (Figure 4a–d). Histological sections (Figure 4e–f) revealed that *P. coffee* was primarily present in the cortex of the roots of *N. benthamiana*, with no evidence of presence of nematodes in the stele.

Pathogenicity of different populations of P. coffeae

Sixty (60) days after inoculation, *N. benthamiana* plants showed weak growth, decreased tillering, high root reduction, and noticeable brown spots on the roots in comparison to uninoculated plants (Figure 5, Figure 6a). The disease spots were initially small, enlarged

gradually, and then the entire roots became necrotic and decayed (Figure 6c–f).

The fresh shoot weight and fresh root weight of inoculated *N. benthamiana* were significantly lower than those of the non-inoculated plants ($P < 0.05$), but there was no clear trend in plant height between the inoculated and uninoculated plants (Table 4). The fresh shoot and root weights of *N. benthamiana* inoculated with SD-YC-1 *P. coffeae* population were 13.3 g and 1.8 g, respectively, which were the lowest of all treatments. The fresh shoot and root weights of *N. benthamiana* inoculated with *P. coffeae* AH-015A2 population (18.2 g and 4.0 g, respectively) were less affected by the colonization of this population. As a result, the tested populations were clearly pathogenic to *N. benthamiana* in this study; however, there were differences in pathogenicity among different populations. The *P. coffeae* SD-YC-1 population from

Figure 3. Bayesian tree of *Pratylenchus* as inferred from 28S rRNA gene sequences under GTR+I+G model. Posterior probabilities more than 50% are given for appropriate clades. Newly obtained sequence is indicated in bold font.

Table 3. Final population densities (Pf) and reproduction factor (Rf) of five populations of *Pratylenchus coffeae* on *Nicotiana benthamiana* 60 days after the inoculation of 1000 nematodes/pot

Code	Nematodes in soil	Nematodes in root	Total nematodes	Rf
ck(non-inoculated)	0	0	0	0
SD-YC-1	3724 ± 783[a]	452 ± 179[a]	4176 ± 691[a]	4.2 ± 0.7[a]
XC-278-1	2772 ± 309[b]	330 ± 150[b]	3102 ± 4457[b]	3.1 ± 0.4[b]
HN-K1	3265 ± 222[a,b]	338 ± 138[a,b]	3603 ± 279[a,b]	3.6 ± 0.3[a,b]
AH-015A2	1714 ± 127[c]	80 ± 33[c]	1794 ± 127[c]	1.8 ± 0.1[c]
XC-344-1	1980 ± 233[c]	171 ± 84[b,c]	2151 ± 185[c]	2.2 ± 0.2[c]

Note: Data are mean ± standard error of five replicates. Different lowercase letters within the same columns indicate significant difference at 0.05 level. The same below. Reproductive factor (Rf) = final isolated nematodes/initial inoculation nematodes.
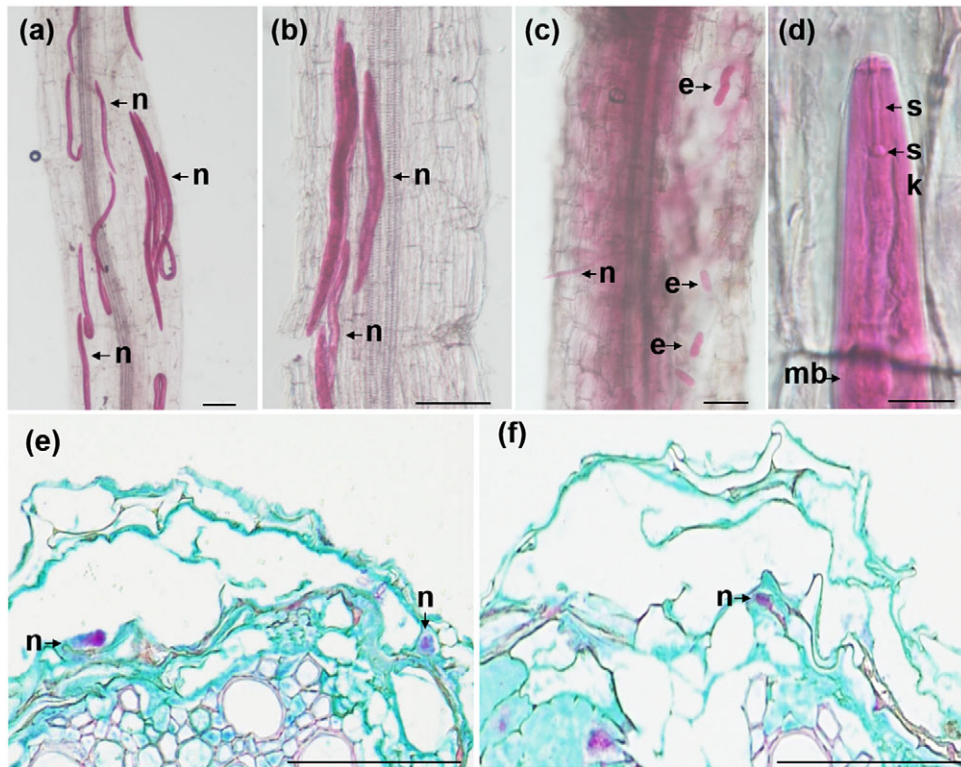
Figure 4. *Nicotiana benthamiana* roots infected by *Pratylenchus coffeae*. (a)–(d), a large number of *P. coffeae* in the cortex of the roots; (e)–(f) root cross sections showing large cavity in the cortical parenchyma. Note sectioned nematode bodies (n). Scale bars: 100 μm ((a)–(c) and (e)–(f)); 10 μm (d).

Figure 5. Above-ground growth of *Nicotiana benthamiana* 60 days after inoculation with five populations of *Pratylenchus coffeae*. ck, non-inoculated plant; 1–5, infected plants with poor growth 60 days after inoculation with HN-K1, SD-YC-1, XC-278-1, AH-015A2, and XC-344-1 populations of *P. coffeae*.

Weifang, Shandong, had the strongest pathogenicity, and the AH-015A2 population from Suzhou, Anhui Province, had the weakest pathogenicity.

Discussion

In plant pathology, the term "pathogenicity" is typically used to describe an organism's capacity to cause illness or the extent of physiological harm that pathogens inflict on their host plants (Shaner *et al.* 1992). The pathogenicity of one population of root-lesion nematodes may be different for different hosts, and one species from different hosts or geographical regions may also differ in pathogenicity of the same host. In this study, five populations of *P. coffeae* showed differences in pathogenicity of *N. benthamiana*. These pathogenicity differences may be due to long-term adaptation of nematodes to the environment (Tian *et al.* 2019). We found bodies and eggs of *P. coffeae* in the roots of *N. benthamiana*,
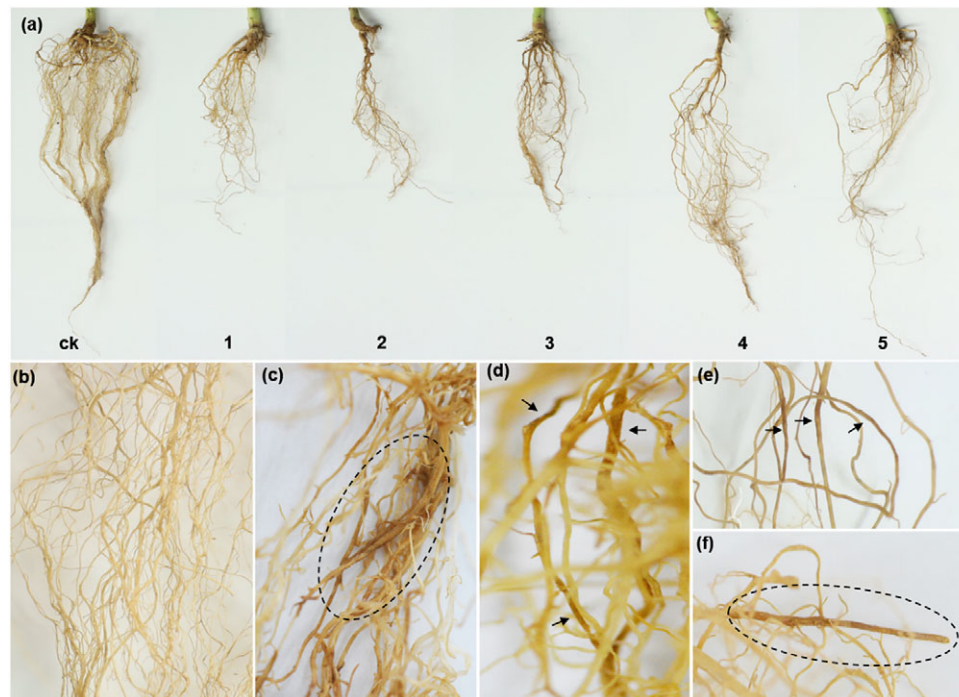
Figure 6. Growth suppression of *Nicotiana benthamiana* roots induced by five populations of *Pratylenchus coffeae*. ck, root system of non-inoculated plant; 1–5, roots of plants 60 days after inoculation with populations HN-K1, SD-YC-1, XC-278-1, AH-015A2, and XC-344-1, respectively; (b) uninoculated plant root system; (c)–(f) necrotic areas on root systems of *N. benthamiana* inoculated with *P. coffeae*.

Table 4. Effects of five different populations of *Pratylenchus coffeae* on the growth of *Nicotiana benthamiana* 60 days the inoculation 1000 nematodes/pot

Code	Plant height (cm)	Fresh shoot weight (g)	Fresh root weight (g)
ck(non-inoculated)	$43.0 \pm 0.5^{b,c}$	28.0 ± 3.4^{a}	8.4 ± 1.0^{a}
HN-K1	45.3 ± 2.6^{a}	$14.2 \pm 1.1^{c,d}$	2.2 ± 0.7^{c}
SD-YC-1	$44.5 \pm 6.2^{a,b}$	13.3 ± 1.7^{d}	1.8 ± 0.6^{c}
XC-278-1	38.4 ± 1.5^{d}	$16.5 \pm 2.7^{b,c,d}$	3.2 ± 0.4^{b}
AH-015A2	$40.4 \pm 0.8^{c,d}$	18.2 ± 1.0^{b}	4.0 ± 0.3^{b}
XC-344-1	$39.5 \pm 1.8^{c,d}$	$17.4 \pm 0.8^{b,c}$	3.6 ± 0.2^{b}

Note: Data are mean ± standard error of five replicates. Different lowercase letters within the same columns indicate significant difference at 0.05 level. The same below.

indicating that *P. coffeae* can successfully parasitise and complete its life history in the roots of *N. benthamiana*, which is the same as that of peanut (Liao *et al.* 2015), sesame (Li *et al.* 2020), and soybean (Wang *et al.* 2021).

　　N. benthamiana is an important model plant for studying plant-pathogen interaction, subcellular localization, and plant genetic engineering (Goodin *et al.* 2008). Therefore, functional studies of host-pathogen interactions using *N. benthamiana* are likely to reveal the molecular mechanism of pathogenesis of Solanaceae crops, and they may be of great significance for the prevention and control of plant diseases (Dong *et al.* 2007). In this study, we identified that *N. benthamiana* was a good host of *P. coffeae* and can be used to study the interaction with *P. coffeae*. The genome of *P. coffeae* is has been sequenced, and analysis of the genome sequence helps to learn better about the genes that may control the pathogenic effect, and *N. benthamiana* can be used as a model plant to mine response genes involved in plant-pathogen interaction. Using the efficient and stable genetic transformation of *N. benthamiana*, we can obtain overexpression or silencing of the interacting genes in transgenic plants; observe the phenotype of the transgenic plants;

study the impact of the transgenic plants to nematodes; and analyse the interaction mechanism between nematode effect proteins and *N. benthamiana*. These may provide new ideas and targets for the prevention and control of *P. coffeae*.

Conclusions

In this study, a root-lesion nematode was isolated from the rhizosphere of tobacco in Weifang City, Shandong Province, China. Morphological and molecular identification showed that the nematode species was a representative of *P. coffeae*. Parasitism and pathogenicity tests suggested that *N. benthamiana* was a good host plant to *P. coffeae*. The results of this study indicate *N. benthamiana* may be suitable for studying interactions between this root-lesion nematode species and its hosts.

Competing interest. The authors declare that there are no conflicts of interest.

Ethical standard. Written informed consent was obtained from all participants prior to the publication of this study.

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