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附件 成果 材料

项目名称：小麦根腐线虫 Pc-ENG3 蛋白调控寄主免疫反应促进侵染的分子机制

培养对象姓名： 李 宇

培养单位： 河南农业大学

外文论文中文摘要

成果（论文）1：

1. Qin Ling, Lin Fankang, Lv Yunlong, Tai Zelin, Zhang Xu, Li Honglian, **Li Yu***(通讯作者), Wang Ke*. Identification of *Pratylenchus coffeae* as a causal agent of root rot disease in *Sorghum bicolor* in China. BMC Microbiology, 2025, 25, 41.

摘要：高粱（*Sorghum bicolor*）是一种重要的粮食作物和饲料作物。根腐线虫（*Pratylenchus* spp.）是一类致病性线虫，对多种粮食和经济作物造成严重经济损失。本研究在中国山西省的高粱田中发现了生长迟缓、根部呈褐色并腐烂的病株。通过改良的巴尔曼漏斗法分离出一种根腐线虫 GL-1 种群。结合形态学和分子生物学鉴定，确认其为咖啡短体线虫（*Pratylenchus coffeae*）。此外，我们通过温室盆栽接种测定了 GL-1 及其他四种 *P. coffeae* 种群对高粱的寄生性和致病性。接种 60 天后结果表明，这五种 *P. coffeae* 种群均能侵染并危害高粱。高粱是 *P. coffeae* 的理想宿主（ $Rf > 1$ ）。此外，与对照组相比，五种 *P. coffeae* 种群所导致的高粱地上部分鲜重和根部鲜重显著增加。接种组的病原性显著降低，根部出现褐色斑点甚至坏死腐烂。这五个种群对高粱均具有高度致病性，但各种群之间的致病性存在显著差异。本研究结果为高粱根腐线虫的识别和检测提供了科学依据。

RESEARCH

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Identification of *Pratylenchus coffeae* as a causal agent of root rot disease in *Sorghum bicolor* in China

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Abstract

Sorghum (*Sorghum bicolor*) is an important food and feed crop. Root-lesion nematodes (*Pratylenchus* spp.) are a group of pathogenic nematodes that cause severe economic losses in various food and cash crops. This study identified diseased sorghum plants with stunted growth and brown, rotting roots in sorghum fields in Shanxi Province, China. A species of root-lesion nematode was isolated by modified Baermann funnel method and named the GL-1 population. Afterward, the GL-1 population of root-lesion nematodes was identified as *P. coffeae* through a combination of morphological, rDNA-ITS and rDNA-28 S D2-D3 region techniques for molecular biological identification. We also conducted greenhouse experiments to assess the parasitism and pathogenicity of GL-1 and four other *P. coffeae* populations on sorghum through pot inoculation. At 60 days after inoculation, the results indicated that all five populations of *P. coffeae* were capable of infecting and causing damage to the sorghum plants. Sorghum is a suitable host for *P. coffeae* (with a reproduction factor > 1). Moreover, compared with those in the control group, the aboveground fresh weights and root fresh weights of sorghum in the five inoculation groups were significantly lower, and brown spots or even necrotic rot appeared on the roots. All five populations were highly pathogenic to sorghum, but there were significant differences in pathogenicity among the populations. This study provides a scientific basis for identifying and detecting root-lesion nematodes in sorghum.

Keywords *Sorghum bicolor*, Root-lesion nematode, Identification, Parasitism, Pathogenicity

Introduction

Sorghum (*Sorghum bicolor*), a member of the Gramineae family in the Sorghum genus, originated from Ethiopia, in Africa [1]. Sorghum is not only the world's fifth-most important food and feed crop, but it is also used in the energy and processing industries [2]. As of 2022, sorghum is grown on 7.29×10^5 hectare in China, with production reaching 3.32×10^8 t (<https://data.stats.gov.cn/easyquery.htm?cn=C01>). China's sorghum is primarily used in the production of liquor, including the world-famous 'China-Moutai', 'China-Wuliangye', etc., which are brewed with sorghum as the main raw material, supporting a brewing industry with distinct Chinese characteristics [3].

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Root-lesion nematodes (*Pratylenchus* spp.) are a very important group of migratory endoparasitic nematodes with a wide host range that, together with cyst nematodes and root-knot nematodes, are among the most damaging plant pathogenic nematodes worldwide [4]. The symptoms of root-lesion nematode damage are easily confused with those caused by a lack of water and fertilizer, soil-borne fungal diseases, and environmental stress [5]. Moreover, the mechanical damage caused by root-lesion nematodes when they infect plant roots provides more favorable conditions for infecting pathogens such as fungi and bacteria [6, 7]. Among the sorghum nematode diseases, three nematodes are more serious, namely, *Belonolaimus* spp., *Meloidogyne* spp. and *Pratylenchus* spp [8–10]. These nematode diseases can severely impair sorghum production, leading to substantial reductions in both yield and quality [11]. Consequently, it is imperative to implement effective monitoring and control strategies to manage sorghum nematode diseases efficiently.

In 2021, we detected weak growth and root rot symptoms in some sorghum plants from Shanxi Province, China. A large number of root-lesion nematodes were isolated using a modified Baermann funnel method [12], and the root-lesion nematodes collected in Shanxi Province were clearly identified as *P. coffeae* by morphological and molecular biology methods. Inoculation of greenhouse pots, based on the nematode host plant determination criteria proposed by Goo et al. [13] confirmed that sorghum is a suitable host for *P. coffeae* and that *P. coffeae* is highly pathogenic to sorghum. Koch's law experiments verified that the collected *P. coffeae* was the pathogen causing sorghum root rot in this study. Additionally, the parasitism and pathogenicity of five different populations of *P. coffeae* on sorghum and the differences in pathogenicity between populations were determined. In this study, sorghum root rot nematode disease caused by *P. coffeae*, was found for the first time in Shanxi Province, China. This is the first time that differences in the pathogenicity of different *P. coffeae* populations on sorghum have been clarified. This study provides a scientific basis for research related to the detection and integrated control of root rot in sorghum.

Materials and methods

Isolation and purification of nematodes

Diseased sorghum (Jinza 210) with dwarfed plants, reduced root systems and brown rot was found in a sorghum field in Yangfang village, Mengfeng township, Qingxu county, Taiyuan, Shanxi Province, China, and samples of the sorghum rhizosphere soil were subsequently collected. Root tissues from diseased plants were cut and mixed with inter-root soil, and root-lesion nematodes were isolated from the samples using a modified Baermann funnel method [12]. Single female root-lesion

nematodes were picked under a stereomicroscope, disinfected with 0.3% streptomycin sulfate solution about 6 h [14], washed with sterile water, inoculated onto carrot disks, and cultured in the dark at 25 °C for approximately 90 days to obtain a purified population of root-lesion nematodes [15], which were named the GL-1 population.

Morphological identification of root-lesion nematodes

Purely cultured root-lesion nematodes were picked onto concave slides containing sterile water under a stereomicroscope; subsequently, the nematodes were heat-killed, fixed in FG solution (formalin: Glycerin: water = 10 : 1 : 89) [16], and dehydrated by glycerol-ethanol dehydration. Lastly, a permanent microscopic mount was made by wax circle method [12]. The nematodes were measured and photographed with a Nikon Eclipse TI-S inverted microscope (Japan). The measurement and calculation of quantitative characteristics are based on deMan formula and Hooper et al. [12].

Molecular identification of root-lesion nematodes

The DNA of single nematodes was extracted by protease K method [17]. The internal transcribed spacer region (ITS) rDNA gene was amplified using primers TW81 (5'-GTTTCCGTAGGTGAACCTGC-3') and AB28 (5'-ATATGCTTAAGTTCAGCGGGT-3') [18]. The D2-D3 region of the 28 S rDNA gene was amplified using primers D2A (5'-ACAAGTACCGTGAGGGAAAGTTG-3') and D3B (5'-TCGGAAGGAACCAGCTACTA-3') [19]. The reaction system was formulated according to the instructions of 2×Magic Green Taq SuperMix Polymerase (TOLOBIO, Shanghai, China) for PCR. The reaction procedure was as follows: pre-denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 59.1 °C (ITS rDNA) or 51.9 °C (28 S rDNA) for 30 s, extension at 72 °C for 2 min, and a final extension at 72 °C for 5 min. The resulting PCR products were purified with a FastPure® Gel DNA Extraction Mini Kit (Vazyme Biotech Co., Ltd., Nanjing, China), connected to a one-step ZTOPO-Blunt/TA cloning vector (Zoman, Beijing, China), transferred to DH5α cells (TOLOBIO), and then sent to Sangon Biotech Co., Ltd. (Shanghai, China) for sequencing [15].

Phylogenetic relationship analysis of the root-lesion nematodes

The obtained sequences of the rDNA-ITS and rDNA 28 S D2-D3 regions of the GL-1 population were submitted to the GenBank database with the accession numbers PP531525 (rDNA-ITS) and PP531576 (28S rDNA), respectively, and then the corresponding gene sequences of *Pratylenchus* obtained from the GenBank database were aligned using the BLASTn algorithm (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Multiple sequence

comparison was performed in MEGA 7 using the ClustalW technique. The optimal nucleotide substitution model GTR+G+I was selected under the Akaike information criterion (AIC) using PAUP and MrModeltest 2.3, and a phylogenetic tree of 28 S and ITS sequences in *P. coffeae* was constructed using MrBayes 3.1 [20].

Parasitism and pathogenicity of five *P. coffeae* populations toward *Sorghum bicolor*

In this study, the parasitism and pathogenicity of four other populations of *P. coffeae* conserved in our laboratory and GL-1 population (Table 1) in sorghum were determined via pot inoculation experiments. Sorghum seeds were sterilized with 75% ethanol for 3–5 min, washed with sterile water and sown in sterile soil for 15 days of growth. The flowerpots used in the experiment were the same, with an outer diameter of 20 cm, an inner diameter of 17.5 cm, a bottom diameter of 13.5 cm and a height of 18 cm, which could hold a volume of about 3.5 L of nutrient soil. Subsequently, sorghum seedlings with uniform growth were selected and inoculated with a suspension of five *P. coffeae* populations at a concentration of 1000 nematodes/mL around the sorghum root system at a rate of 2000 nematodes/pot. The nematode suspension contains all the life stages of the nematodes, i.e., eggs, females, males, and juveniles. Five biological replicates were performed. The sorghum treatment without nematode inoculation was used as a control. All the pot experiments were replicated twice in the greenhouse (28 °C, 12 h light/12 h dark photoperiod). The potted seedlings were watered 1 day before inoculation with nematodes to moisten the soil, and the pots were not watered for 3 days after inoculation to ensure normal nematode infestation; then regular planting management was applied [21]. Sixty days after inoculation with nematodes, growth parameters such as the plant height, aboveground fresh weight and root fresh weight of sorghum were investigated, and symptoms of sorghum root infestation were observed and imaged [16]. The modified Baermann funnel method [12] was used to isolate and count the number of nematodes (Pf) in the sorghum inter-root region and to calculate the reproduction factor (Rf) of the nematodes. The host plant determination criteria proposed by Goo et al. [13] were used to determine whether sorghum was a host plant for *P. coffeae* during this test.

Histopathological observation

The *P. coffeae* in the root system of the differently treated sorghum plants were stained with acidic magenta and observed according to the methods by Bybd et al. [22]. Tissue sectioning of the sorghum root system was performed after dehydration, transparency, wax dipping, embedding, sectioning, staining and sealing according to the methods of Wang et al. [23] and Sasanelli et al. [24]. The sections were stained with Fanshi red-solid green dye and then observed and photographed under a microscope.

Data analysis

Statistical analysis was performed with SPSS 22.0 software (Chicago, USA) in this study. All the data were subjected to a one-way analysis of variance (ANOVA) and tested for differences among treatments at the 5% level by Duncan’s multiple range test (DMRT) [14].

Results

Field symptoms

As a result of root-lesion nematode infestation, the symptoms in the sorghum field were as follows: the aboveground plants were significantly shorter and weaker, the root systems were reduced, the roots showed obvious brown or black-brown spots, and parts of the root systems showed varying degrees of necrosis and decay (Fig. 1).

Morphological identification of *P. coffeae*

Morphological characteristics: The morphometric values for the female and male root-lesion nematodes harvested from the sorghum inter-roots in Shanxi Province, China were compared with the corresponding indices of other *P. coffeae* nematodes, as shown in Table 2; the microscopic observations of their morphometric features are shown in Fig. 2.

Females almost straight, when heat-killed (Fig. 2A). Head sclerotization heavy, extending backward. The labial region is low and slightly offset, containing 2 labial rings (Fig. 2D–G). The median bulb is ovoid (Fig. 2B) and the esophageal glands cover the anterior end of the intestine (Fig. 2C). Lateral fields with distinct four lines at mid-body (Fig. 2K). The oocytes in the ovary were arranged in a single row, and the spermatheca was distinct and round or oval (Fig. 2H–I). Tail subcylindrical,

Table 1 Nematode populations used in this study

Nematode population	Host	Sampling location	In vitro cultured	GenBank (ITS)	GenBank (28 S)
HN-K1	corn	Pingdingshan City, Henan Province	Carrot disks	OR417384	OR417283
SD-YC-1	tobacco	Weifang City, Shandong Province	Carrot disks	OQ449389	OQ449390
HN-A21	sesame	Xuchang City, Henan Province	Carrot disks	MN588280	MN588282
XC-344-1	soybean	Linyi City, Shandong Province	Carrot disks	MT879294	MT879295
GL-1	sorghum	Taiyuan City, Shanxi Province	Carrot disks	PP531525	PP531576



Fig. 1 Root rot field symptoms of sorghum caused by root-lesion nematodes in Shanxi Province, China

caudal end occasionally with distinctly irregular indentations (Fig. 2N-P). The morphology of the male was similar to that of the females after heat exposure (Fig. 2Q). The seminal vesicle is obvious (Fig. 2J). Male tail with slender spicules, narrowing to a pointed terminus (Fig. 2M).

The morphological characteristics of the root-lesion nematode population collected in this study were more consistent with those of *P. coffeae* in the article by Inserra et al. [25] and Xie [26], so the root-lesion nematode population collected from the inter-roots of sorghum from Shanxi Province, China, was tentatively identified as *P. coffeae*.

Molecular identification and phylogenetic analysis of *P. coffeae*

The rDNA-ITS and rDNA-28 S D2-D3 regions of the sorghum inter-root nematode from Shanxi Province, China were PCR-amplified and sequenced using the universal primers TW81/AB28 and D2A/D3B, and two sequences with lengths of 1086 bp and 811 bp, respectively, were obtained. A BLAST analysis of the NCBI database

Table 2 Morphometrics of a population of *Pratylenchus coffeae* collected in Taiyuan City, Shanxi Province, China

Character	GL-1 population		Pratylenchus coffeae population of Inserra et al. [25]	
	female	male	female	male
n	18	18	20	20
l	583.55 ± 30.47 (514.35–644.50)	536.58 ± 44.19 (482.90–641.80)	601.90 ± 51.40 (520.00–715.00)	589.10 ± 25.40 (558.50–647.50)
a	22.52 ± 2.62 (18.92–28.15)	28.85 ± 1.70 (26.29–32.00)	28.70 ± 3.10 (23.40–34.00)	30.80 ± 1.60 (28.70–33.90)
b	5.67 ± 0.20 (5.36–6.24)	5.86 ± 0.25 (5.25–6.20)	6.70 ± 0.40 (5.60–7.20)	6.70 ± 0.30 (6.00–7.30)
b'	4.81 ± 0.32 (4.02–5.26)	-	-	-
c	20.76 ± 2.38 (18.05–27.99)	21.18 ± 2.02 (18.12–26.70)	20.90 ± 2.80 (17.00–31.00)	21.80 ± 1.60 (19.40–25.40)
c'	1.91 ± 0.23 (1.52–2.38)	-	-	-
V	78.90 ± 1.70 (75.56–82.40)	-	80.50 ± 1.50 (76.00–82.50)	-
T	-	39.80 ± 5.51 (31.17–52.19)	-	-
DGO form stylet base	3.18 ± 0.42 (2.18–3.81)	3.37 ± 0.48 (2.35–3.94)	2.80 ± 0.50 (2.50–4.00)	3.60 ± 0.30 (3.00–4.00)
EP	87.40 ± 4.09 (79.21–94.07)	85.42 ± 4.37 (78.92–94.19)	-	-
Stylet length	16.23 ± 0.53 (15.36–17.29)	16.00 ± 0.72 (14.37–16.91)	16.90 ± 0.20 (16.50–17.00)	15.00 ± 0.40 (14.50–15.50)
Stylet shaft	10.59 ± 1.81 (7.04–13.21)	10.45 ± 1.65 (8.29–13.91)	-	-
Stylet knob height	2.55 ± 0.51 (1.68–3.41)	2.43 ± 0.47 (1.70–3.07)	-	-
Stylet knob width	3.86 ± 0.38 (3.19–4.54)	3.35 ± 0.54 (2.25–4.17)	-	-
Pharyngeal overlap	39.66 ± 8.05 (25.17–53.21)	29.33 ± 5.27 (24.31–46.90)	49.80 ± 10.30 (34.00–72.50)	-
Max body width	26.29 ± 3.78 (21.53–34.07)	18.70 ± 2.25 (15.09–23.98)	-	-
Tail length	28.46 ± 3.67 (22.05–35.70)	25.67 ± 4.21 (19.06–35.42)	29.00 ± 3.30 (21.50–36.00)	27.10 ± 1.90 (24.50–31.00)
Number of tail annuli	24.17 ± 1.58 (18–26)	-	-	-
Vulva to anus distance	86.86 ± 6.36 (76.10–99.24)	-	87.20 ± 12.80 (70.50–135.00)	-
Post-uterine sac length	27.70 ± 4.3 (18.21–35.20)	-	29.50 ± 6.50 (19.50–49.50)	-
Lateral field width	7.36 ± 0.57 (6.53–8.74)	5.90 ± 0.85 (4.15–8.53)	-	-
Vulval body diameter	19.13 ± 1.47 (16.72–22.07)	-	-	-
Anal body diameter	17.99 ± 2.94 (12.79–23.15)	-	-	-
Spicule length	-	17.10 ± 0.73 (15.41–18.29)	-	17.50 ± 0.60 (16.00–18.00)
Gubernaculum length	-	5.20 ± 0.3 (4.68–5.79)	-	5.30 ± 0.30 (5.00–5.50)

Notes: All measurements are in μm and in the form of mean $\pm$ 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 $\times$ 100/body length; T, distance from cloaca opening to anterior most part of testis/body length $\times$ 100%; DGO, distance between dorsal oesophageal gland opening and stylet knobs

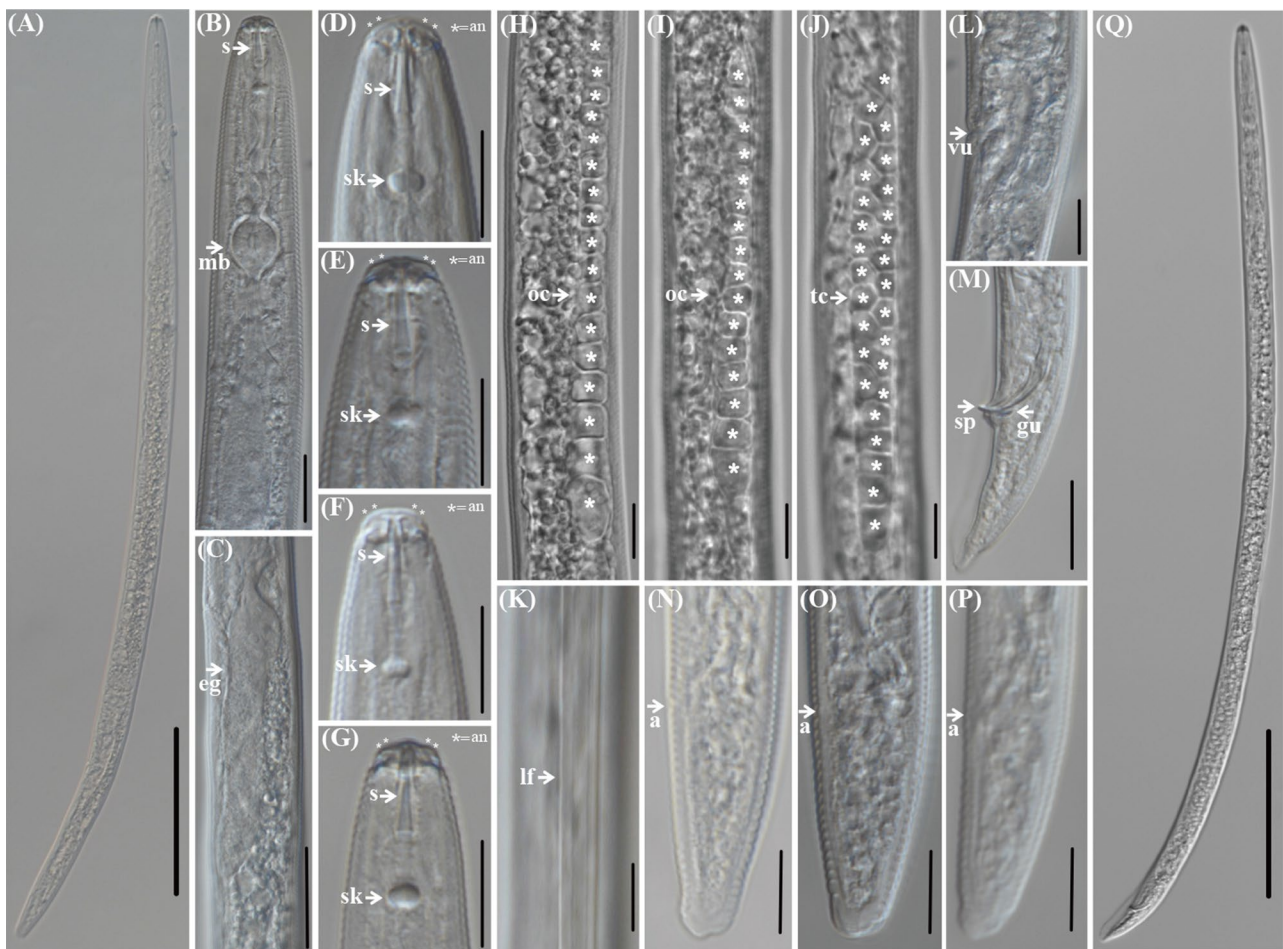


Fig. 2 Light micrographs of *Pratylenchus coffeae* from sorghum in Shanxi Province, China. (A) female entire body; (B) anterior region; (C) pharyngeal gland lobe overlapping the intestine; (D)–(G) lip region; (H)–(I) female anterior end of genital gland; (J) male anterior end of genital gland; (K) lateral field; (L) post-vulval region showing the post-uterine sac; (M) male tail region; (N)–(P) female tail region; (Q) male entire body. Scale bars: 100 µm ((A), (Q)) and 10 µm ((B)–(P)). S, stylet; mb, median bulb; e.g., esophageal glands; sk, stylet knob; an, annuli; oc, ovary cells; lf, lateral field; a, anal; tc, testis cells; vu, vulval; sp, spicules; gu, gubernaculum

showed that the sequence of the rDNA-ITS region from the root-lesion nematode obtained in this study (GenBank accession no. PP531525) was 100% similar to the rDNA-ITS region sequence of the root-lesion nematode *P. coffeae* (OQ592785, OQ592735). The sequence of the rDNA-28 S D2-D3 region (GenBank accession no. PP531576) showed 100% similarity to the sequence of the rDNA-28 S D2-D3 region of the root-lesion nematode *P. coffeae* (OQ592691, OQ592693).

The Bayesian phylogenetic tree constructed based on the sequences of the rDNA-ITS region contained one outgroup and 53 ingroups based on the *Nacobbus aberrans* population (Fig. 3), and the sequences of the root-lesion nematodes isolated from the sorghum root system in Shanxi Province clustered in the same highly supported branch as the reported *P. coffeae* rDNA-ITS region with a support rate of up to 100%. The Bayesian phylogenetic tree constructed based on the sequence of the rDNA-28 S D2-D3 region contained one outgroup

and 55 ingroups based on the *Trophurus sculptus* population (Fig. 4), and this root-lesion nematode sequence was clustered in the same highly supported branch as the reported *P. coffeae* rDNA-28 S D2-D3 region, with up to 100% similarity. The above molecular identification results based on the sequences of the rDNA-ITS and rDNA-28 S D2-D3 regions were consistent with the morphological findings, indicating that the root-lesion nematode GL-1 population isolated from sorghum in Shanxi Province, China was *P. coffeae*.

Parasitism and pathogenicity assays of five *P. coffeae* populations from *Sorghum bicolor*

The results of potting at 60 days after inoculation showed (Fig. 5A–F) that the root systems of the plants in the treatment groups inoculated with five different populations of *P. coffeae* showed different levels of damage. Compared with control plants not inoculated with the nematode, sorghum plants inoculated with *P. coffeae*

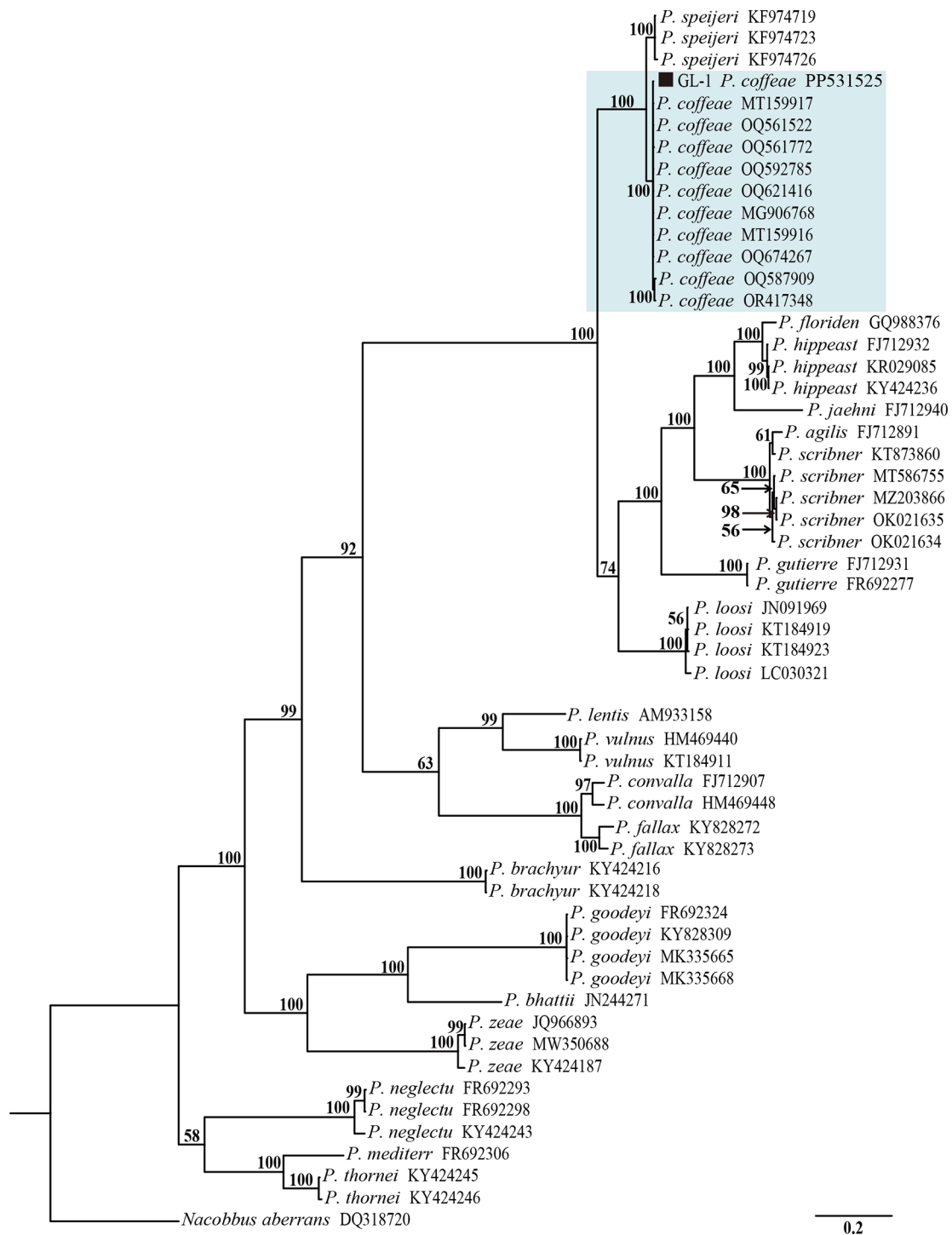


Fig. 3 Bayesian tree of *Pratylenchus* inferred from ITS rRNA gene sequences under the GTR+I+G model. Posterior probabilities of greater than 50% are given for appropriate clades. The newly obtained sequence is indicated with a black square

exhibited slow aboveground growth and significantly shorter and weaker plants compared to control plants not inoculated with the nematode. As a result of *P. coffeae* infestation, the root system of sorghum plants is significantly reduced, the roots tend to break off, the fibrous roots are reduced, and some of the roots develop brown

or dark brown spots. In the early stages of *P. coffeae* infestation, small, light brown or tan spots appeared in the sorghum root system (Fig. 5H), and as the disease continued to worsen and the nematodes continued to feed and reproduce in the root system, the spots in the sorghum root system continued to expand (Fig. 5I-K), ultimately

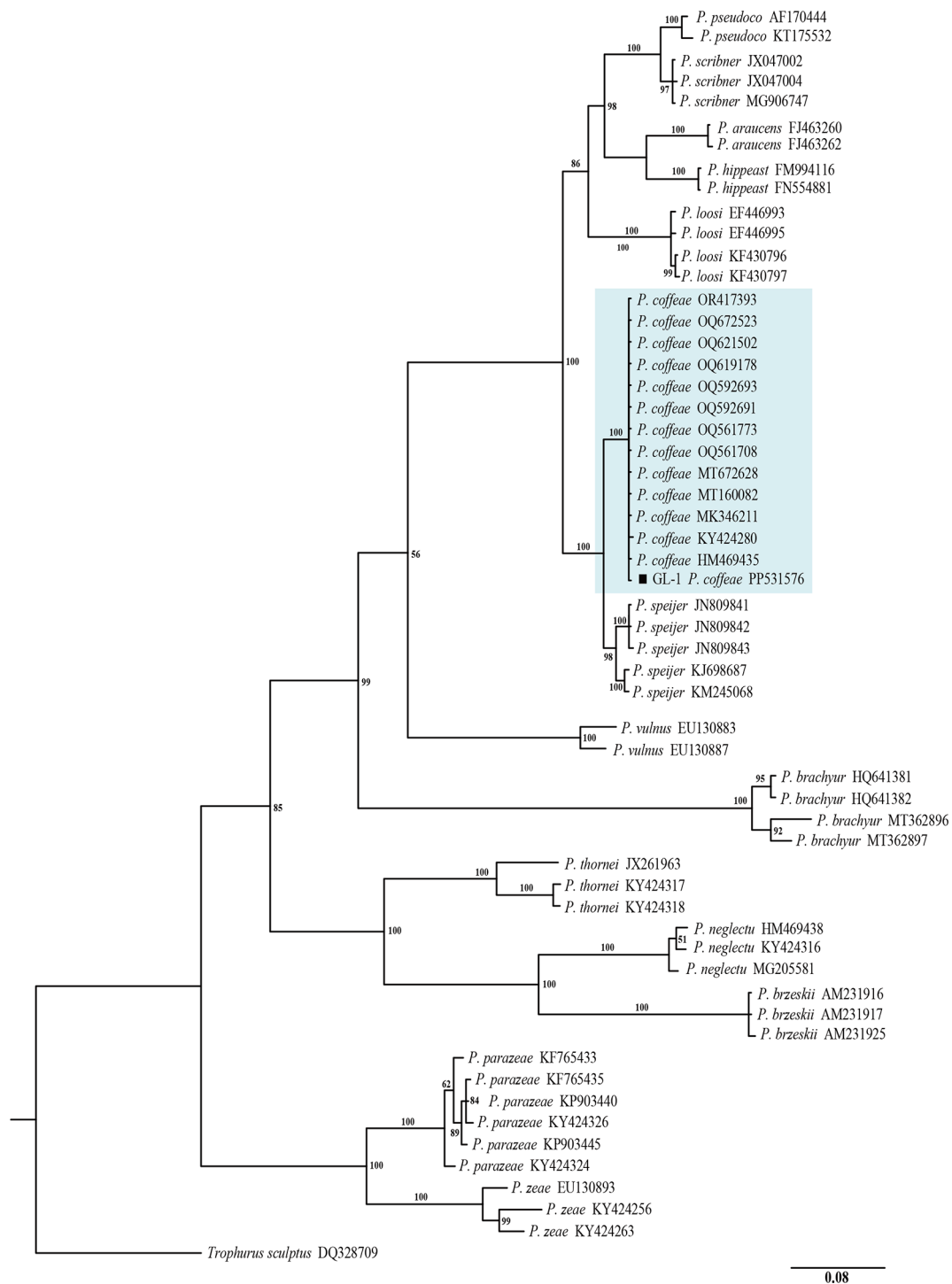


Fig. 4 Bayesian tree of *Pratylenchus* inferred from 28 S rRNA gene sequences under the GTR+I+G model. Posterior probabilities of greater than 50% are given for appropriate clades. The newly obtained sequence is indicated with a black square

leading to large, necrotic rot-like conditions throughout the root system (Fig. 5L). The roots of the control sorghum without nematode connections grew healthily and showed no symptoms of damage (Fig. 5G).

Sixty days after inoculation, large numbers of *P. coffeae* were isolated from sorghum roots and inter-root soil

(Table 3). Among them, sorghum inter-root soils inoculated with the HN-K1 and SD-YC-1 populations had the highest reproduction capacity, with averages of 4971 and 4632 nematodes and Rf values of 2.5 and 2.3, respectively. The Rf values of *P. coffeae* in the inter-root regions of sorghum plants inoculated with the HN-A21, XC-344-1

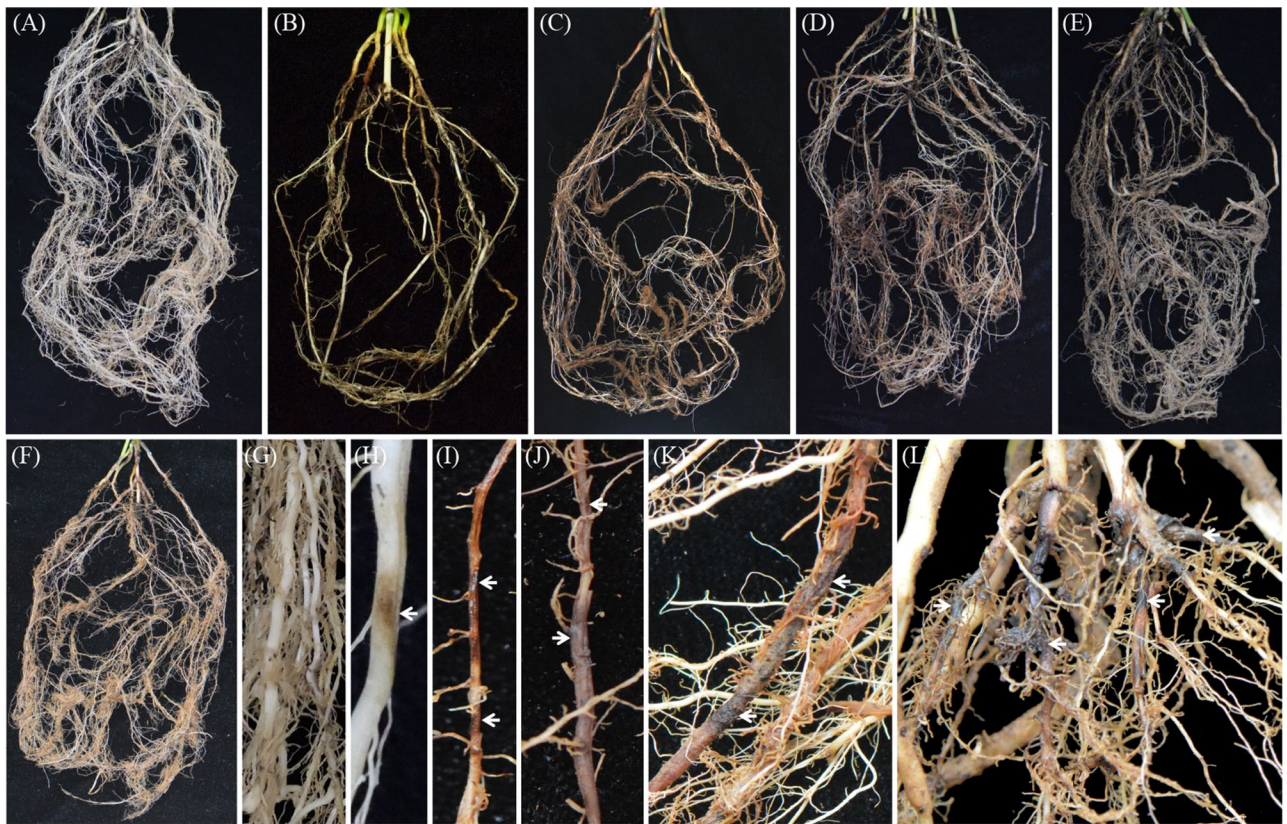


Fig. 5 Five populations of *Pratylenchus coffeae* suppressed the growth of *Sorghum bicolor* roots. **(A)** Root system of a non-inoculated plant; **(B)–(F)** roots of plants 60 days after inoculation with the HN-K1, SD-YC-1, HN-A21, XC-344-1 and GL-1 populations, respectively; **(G)** healthy roots; and **(H)–(L)** necrotic areas on the root systems from *Sorghum bicolor* inoculated with *P. coffeae*

Table 3 Final population densities (pf) and reproduction factor (rf) of five populations of *Pratylenchus coffeae* on *Sorghum bicolor* 60 days after the inoculation of 2000 nematodes/pot

Treat-ment popu-lation	Nematodes in the soil	Nematodes in the root	Total nematodes	Rf
CK	0	0	0	0
HN-K1	4038.0 ± 1226.1 ^a	595.0 ± 86.8 ^a	4633.0 ± 1252.9 ^a	2.3 ± 0.6 ^a
SD-YC-1	4358.0 ± 1460.0 ^a	613.0 ± 215.4 ^a	4971.0 ± 1670.1 ^a	2.5 ± 0.8 ^a
HN-A21	3936.8 ± 1891.8 ^a	460.8 ± 192.7 ^{ab}	4397.6 ± 2080.4 ^a	2.2 ± 1.0 ^a
XC-344-1	3792.6 ± 1137.9 ^{ab}	483.4 ± 85.3 ^a	4276.0 ± 1201.5 ^a	2.1 ± 0.6 ^a
GL-1	2907.6 ± 1019.2 ^b	306.8 ± 87.1 ^b	3214.4 ± 1080.4 ^b	1.6 ± 0.5 ^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. Reproductive factor (Rf) = final isolated nematodes/initial inoculation nematodes

and GL-1 populations were 2.2, 2.1 and 1.6, respectively. Based on Goo’s host determination criteria [13], all five populations of *P. coffeae* inoculates were capable of infesting and damaging sorghum and the sorghum was a suitable host for *P. coffeae* (Rf > 1), but there were differences in the fecundity of different sorghum populations.

Table 4 Effects of five different populations of *Pratylenchus coffeae* on the growth of *Sorghum bicolor* 60 days the inoculation 2000 nematodes/pot

Treatment population	Plant height (cm)	Fresh shoot weight (g)	Fresh root weight (g)
CK	158.8 ± 17.7 ^a	40.1 ± 11.3 ^a	36.5 ± 5.0 ^a
HN-K1	71.3 ± 4.3 ^c	21.2 ± 3.5 ^c	11.7 ± 1.2 ^c
SD-YC-1	69.9 ± 10.2 ^c	21.8 ± 9.8 ^{bc}	12.6 ± 5.2 ^c
HN-A21	78.2 ± 13.9 ^{bc}	21.7 ± 4.3 ^{bc}	14.9 ± 5.6 ^{bc}
XC-344-1	83.1 ± 8.0 ^{bc}	24.4 ± 11.5 ^{bc}	17.0 ± 6.6 ^{bc}
GL-1	102.4 ± 32.1 ^b	30.3 ± 9.9 ^b	20.3 ± 3.0 ^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

Sorghum plants from five different populations inoculated with *P. coffeae* as described above were surveyed to measure the plant height, aboveground fresh weight and root fresh weight to observe their pathogenicity (Table 4). The average aboveground plant height, aboveground fresh weight and root fresh weight of uninoculated healthy sorghum plants were 158.8 cm, 40.1 g and 36.5 g, respectively. Sorghum plants inoculated with the HN-K1 and SD-YC-1 populations had significantly shorter

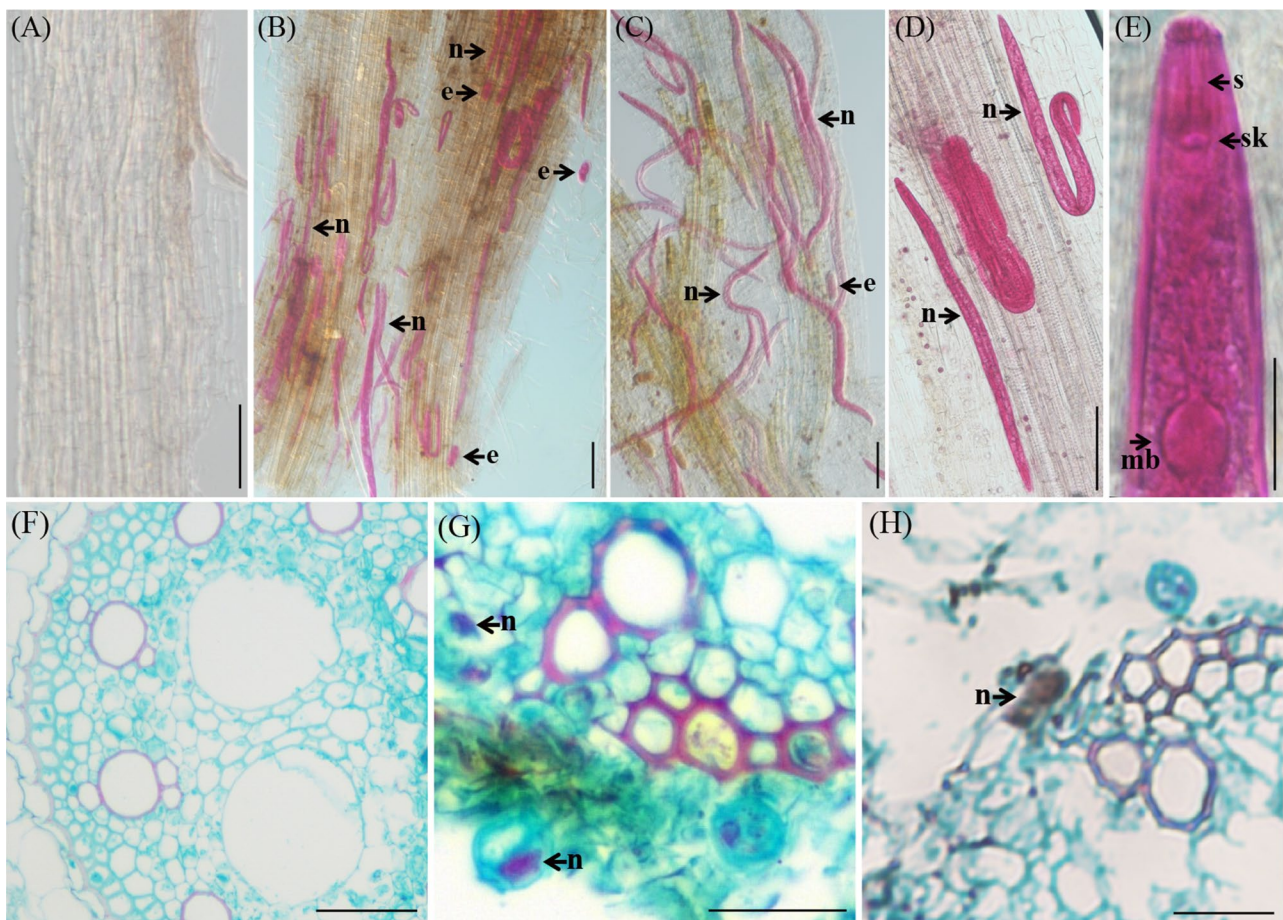


Fig. 6 *Sorghum bicolor* roots infected by *Pratylenchus coffeae*. (A) Healthy roots; (B)-(E) a large number of *P. coffeae* individuals and eggs in the cortex of the roots; (F) *P. coffeae* in *Sorghum* root cells; (G) cross section of undamaged roots; and (H)-(J) cross section of roots infected with *P. coffeae* showing vermiform nematodes in damaged epidermal cells. Scale bars: 100 µm (A)-(E); 20 µm (F); 50 µm (G)-(H). n, nematode; e, egg; s, stylet; sk, stylet knob; mb, median bulb

plants and more severely damaged root systems, with plant heights, aboveground fresh weights and root fresh weights of 71.3 cm, 21.2 g, and 11.7 g and 69.9 cm, 21.8 g, and 12.6 g, respectively, which were significantly lower ($P < 0.05$) than those of the uninoculated control. *Sorghum* plants inoculated with the GL-1 population exhibited relatively little plant growth and relatively severe root damage, with an average plant height, aboveground fresh weight, and root fresh weight of 102.4 cm, 30.3 g, and 20.3 g, respectively. In summary, the five different populations of *P. coffeae* tested here were all clearly pathogenic to sorghum, but there were significant differences in the pathogenicity of the different populations, with the strongest pathogenicity to sorghum being found in the HN-K1 and SD-YC-1 populations, followed by the HN-A21 and XC-344-1 populations, and the weakest pathogenicity to sorghum in the GL-1 population, which was isolated from Shanxi Province, China.

Histopathological observation

After acidic magenta staining of the morbid sorghum root system, a large number of *P. coffeae* individuals and eggs were observed within the roots under the microscope, indicating that the nematode can grow, develop and complete its life cycle within the sorghum root system of the test (Fig. 6B-E). The results of the pathologic tissue sections showed (Fig. 6G-H) that *P. coffeae* was present in the cells of sorghum roots, and due to the migration and feeding of this nematode, the cells of the root tissues were partially degraded, which in turn led to the decay and necrosis of the roots.

Discussion

In this study, we used a combination of morphological and molecular biological identification of root-lesion nematode species harvested from the inter-roots of sorghum in Shanxi Province, China. The results indicated that this nematode population was basically consistent with the morphological characteristics of *P. coffeae* as

previously reported. Phylogenetic trees also indicate that this nematode population is in the same branch as *P. coffeae* and is distinguished from other populations of the genus *Pratylenchus*. In this study, *P. coffeae* was confirmed as a pathogen causing root rot in sorghum by Koch's law tests. *P. coffeae* infests the root system of sorghum, causing cell necrosis, resulting in root rot and other symptoms. When the density of the nematode population is high, plant dwarfing can occur, and the sculpts leaves show signs of nutrient deficiency. In severe cases, the aboveground part of the plant fades to green, grows slowly, yellows, and even dies [5, 7].

The parasitism and pathogenicity of the *P. coffeae* GL-1 population and four other populations on sorghum were determined via greenhouse pot inoculation tests in our study. The host status and susceptibility of sorghum to four root-lesion nematodes were studied in greenhouse and field microplot experiments [10]. Sorghum was a good host for *P. zaeae*, *P. brachyurus* and *P. coffeae* which had reproduction indices (Pf/Pi) greater than 1, Sorghum was a nonhost for *P. crenatus*. Our findings are consistent with the findings of Motalaote's study, all five populations of *P. coffeae* from different host sources were able to cause root pathogenicity in sorghum, but there were significant differences in the pathogenicity of *P. coffeae* in sorghum from different regions or host sources. These differences may be due to environmental [27], anthropogenic [28], and intrinsic nematode factors [29], which are common to a wide range of root-lesion nematodes. Nematodes are adapted to and tamed over time in different environments, and humidity and temperature alter their reproductive and pathogenic capacities [30].

In agriculture, sorghum is often rotated or intercropped with crops such as soybeans, corn, or wheat to achieve greater yields [2]. Soybean and corn, among other crops, are suitable hosts of *P. coffeae* [31, 32]. This study demonstrated that *P. coffeae* isolated from the inter-root of soybeans and corn can infest sorghum roots. We hypothesize that *P. coffeae* is capable of infesting successive interspecific crops in the soil if all crops grown in a rotation or sets of crops are hosts of *P. coffeae*. In our study, this is the first time that *P. coffeae* can cause significant damage to sorghum in the field and it should continue to be a concern in sorghum cultivation in the future.

Conclusions

In this study, the pathogen causing sorghum root rot disease found in Shanxi Province, China, was identified as *P. coffeae*, and this nematode population and four other populations of *P. coffeae* conserved in our laboratory were subsequently subjected to a pot inoculation test to determine their parasitism and pathogenicity on sorghum plants. The indoor potting results demonstrated

that sorghum is a suitable host for *P. coffeae* ($R_f > 1$) and that all five different populations of *P. coffeae* can grow, develop, and complete their life cycles within the sorghum root system. All five different test *P. coffeae* populations were highly pathogenic to sorghum, but there were significant differences in pathogenicity between populations with different hosts or from different geographic origins. To our knowledge, this is the first report of root rot in *Sorghum bicolor*, caused by *P. coffeae* in China. This study provides a scientific basis for identifying and detecting root-lesion nematodes in sorghum.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12866-025-03759-1>.

Supplementary Material 1

Supplementary Material 2

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Author contributions

LY and WK conceived and designed experiments. QL, LFK and LYL identified *Pratylenchus coffeae*. QL and ZX sequenced and analyzed genetic data. QL and TZL conducted the phylogenetic analyses. QL and WK wrote the manuscript. WK and LHL revised the manuscript. All authors read and approved the final manuscript.

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Data availability

The datasets generated and analysed during the current study are available in the NCBI repository or in Annex 1 and 2 with the accession codes PP531525 (ITS) (<https://www.ncbi.nlm.nih.gov/nuccore/PP531525.1/>) and PP531576 (28 S) (<https://www.ncbi.nlm.nih.gov/nuccore/PP531576.1/>). The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper.

Declarations

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of authors. The diseased sorghum plants were sampled with the permission of the landowner. The authors confirm that all the experimental methods and plants complied with relevant institutional, national, and international guidelines and legislation.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

- Mekbib F. Genetic erosion of sorghum (*Sorghum bicolor* (L.) Moench) in the centre of diversity. Ethiopia Genetic Resour Crop Evol. 2008;55:351–64. <https://doi.org/10.1007/s10722-007-9240-7>.
- Baloch FS, Altaf MT, Liaqat W, Bedir M, Nadeem MA, Cömertpay G, et al. Recent advancements in the breeding of sorghum crop: current status and future strategies for marker-assisted breeding. Front Genet. 2023;14:1150616. <https://doi.org/10.3389/fgene.2023.1150616>.
- Li SG, Liu M, Liu F, Zou JQ, Lu XC, Diao XM. Current status and future prospective of sorghum production and seed industry in China. Scientia Agricultura Sinica. 2021;54:471–82. <https://doi.org/10.1016/j.rser.2021.111079>.
- Jones JT, Haegeman A, Danchin EG, Gaur HS, Helder J, Jones MG, et al. Top 10 plant-parasitic nematodes in molecular plant pathology. Mol Plant Pathol. 2013;14(9):946–61. <https://doi.org/10.1111/mpp.12057>.
- Bell CA, Lilley CJ, McCarthy J, Atkinson HJ, Urwin PE. Plant-parasitic nematodes respond to root exudate signals with host-specific gene expression patterns. PLoS Pathog. 2019;15:e1007503. <https://doi.org/10.1371/journal.ppat.1007503>.
- LaMondia JA. Interaction of *Pratylenchus penetrans* and *Rhizoctonia fragariae* in strawberry black root rot. J Nematology. 2003;35:17–22.
- Phani V, Khan MR, Dutta TK. Plant-parasitic nematodes as a potential threat to protected agriculture: current status and management options. Crop Prot. 2021;144:105573. <https://doi.org/10.1016/j.cropro.2021.105573>.
- Khanal C, Gc S, Harshman D. Host suitability of summer cover crops and peach rootstocks to the peach root-knot nematode, *Meloidogyne floridensis*. Plant Dis. 2024;108(3):582–6. <https://doi.org/10.1094/PDIS-07-23-1413-SC>.
- Koenning SR, Overstreet C, Noling JW, Donald PA, Becker JO, Fortnum BA. Survey of crop losses in response to phytoparasitic nematodes in the United States for 1994. J Nematology. 1999;31(4S):587–618.
- Motalaote B, Starr JL, Frederiksen RA, Miller FR. Host status and susceptibility of sorghum to *Pratylenchus* species. Revue De Nématologie. 1987;10(1):81–6. <https://api.semanticscholar.org/CorpusID:81542983>.
- Masudulla K, Azhar UK. Plant parasitic nematodes effectors and their crosstalk with defense response of host plants: a battle underground. Rhizosphere. 2021;17:100288. <https://doi.org/10.1016/j.rhisp.2020.100288>.
- Hooper DJ, Hallmann J, Subbotin SA. Methods for extraction, processing and detection of plant and soil nematodes. In: Luc M, Sikora RA, Bridge J, editors. Plant Parasitic nematodes in Subtropical and Tropical Agriculture. Wallingford: CABI Publishing; 2005. pp. 53–86.
- Goo MYC, Sipes BS. Host preference of *Radopholus citrophilus* from hawaiian anthurium among selected tropical ornamentals. Hort Sci. 1997;32:1237–8. <https://doi.org/10.21273/HORTSCI.32.7.1237>.
- Li Y, Wang K, Xie H, Xu CL, Wang DW, Li J, Huang X, Peng XF. Parasitism and pathogenicity of *Radopholus similis* to *Ipomoea aquatica*, *Basella rubra* and *Cucurbita moschata* and genetic diversity of different populations. J Integr Agric. 2016;15:120–34. [https://doi.org/10.1016/S2095-3119\(14\)61003-0](https://doi.org/10.1016/S2095-3119(14)61003-0).
- Wang K, Xie H, Li Y, Wu WJ, Xu CL. Morphology and molecular analysis of *Paratylenchus nanjingensis* n. sp. (nematoda: Paratylenchinae) from the rhizosphere soil of *Pinus massoniana* in China. J Helminthol. 2016;90:166–73. <https://doi.org/10.1017/S0022149X14000819>.
- Sun M, Niu W, Shi L, Lv Y, Fu B, Xia Y, Li H, Wang K, Li Y. Host response of *Nicotiana benthamiana* to the parasitism of five populations of root-lesion nematode, *Pratylenchus coffeae*, from China. J Helminthol. 2023;97:e73. <https://doi.org/10.1017/S0022149X2300055X>.
- Tanaka R, Kikuchi T, Aikawa T, Kanzaki N. Simple and quick methods for nematode DNA preparation. Appl Entomol Zool. 2012;47:291–4. <https://doi.org/10.1007/s13355-012-0115-9>.
- Nadler SA, Félix M, Frisse LM, Sternberg PW, Ley PD, Thomas WK. Molecular and morphological characterisation of two reproductively isolated species with mirror-image anatomy (Nematoda: Cephalobidae). Nematology. 1999;1:591–612. <https://doi.org/10.1163/156854199508559>.
- Subbotin SA, Sturhan D, Chizhov VN, Vovlas N, Baldwin JG. Phylogenetic analysis of *Tylenchida* Thorne, 1949 as inferred from D2 and D3 expansion fragments of the 28S rRNA gene sequences. Nematology. 2006;8:455–74. <https://doi.org/10.1163/156854106778493420>.
- Kumar S, Tamura K, Nei M. MEGA: molecular evolutionary genetics analysis software for microcomputers. Comput Appl Biosciences: CABIOS. 1994;10:189–91. <https://doi.org/10.1093/bioinformatics/10.2.189>.
- Hahn ML, Sarah JL, Boisseau M, Vines NJ, Wright DJ, Burrows PR. Reproductive fitness and pathogenicity of selected *Radopholus* populations on two banana cultivars. Plant Pathol. 1996;45:1–9. <https://doi.org/10.1046/j.1365-3059.1996.d01-128.x>.
- Bybd DW, Kirkpatrick T, Barker KR. An improved technique for clearing and staining plant tissues for detection of nematodes. J Nematology. 1983;15:142–3.
- Wang K, Li Y, Xie H, Wu WJ, Xu CL. Pin nematode slow decline of *Anthurium Andeanum*, a new disease caused by the pin nematode *Paratylenchus shenzhenensis*. Plant Dis. 2016;100:940–5. <https://doi.org/10.1094/PDIS-07-15-0777-RE>.
- Sasaneli N, Vovlas N, Trisciuzzi N, Cantalapiedra-Navarrete C, Palomares-Rius JE, Castillo P. Pathogenicity and host-parasite relationships of *Heterodera cruciferae* in cabbage. Plant Dis. 2013;97:333–8. <https://doi.org/10.1094/PDI-07-12-0699-RE>.
- Inserra RN, Duncan LW, Troccoli A, Dunn D, Handoo ZA, Troccoli A, Vovlas N. *Pratylenchus jaehni* sp. n. from citrus in Brazil and its relationship with *P. coffeae* and *P. loosi* (Nematoda: Pratylenchidae). Nematology. 2001;3:653–65. <https://doi.org/10.1163/156854101753536028>.
- Xie H. Taxonomy of plant nematodes. 2nd Edn. Beijing, China: Higher Education Press; 2005.
- Fatemi E, Jung C. Pathogenicity of the root-lesion nematode *Pratylenchus neglectus* depends on pre-culture conditions. Sci Rep. 2023;13:19642. <https://doi.org/10.1038/s41598-023-46551-9>.
- Reddy PP. Nematode diseases and their management. Sustainable Crop Protection under protected cultivation. Singapore: Springer; 2016. pp. 177–85. <https://doi.org/10.1007/978-981-287-952-3>.
- Golden AM, Et AL. Terminology and identity of intraspecific forms of the soybean cyst nematode (*Heterodera glycines*). Plant Disease Report. 1970;54:544–6.
- van den Berg E, Marais M, Swart A. Nematode morphology and classification. In: Fourie H, Spaull V, Jones R, Daneel M, De Waele D, editors. Nematology in South Africa: a View from the 21st Century. Switzerland: Springer; 2017. pp. 33–71. <https://doi.org/10.1007/978-3-319-44210-5>.
- Li Y, Li J, Hu YJ, Wang K, Yuan HX, Sun BJ, Li HL, Lei B. Occurrence of root-lesion nematode *Pratylenchus coffeae* on corn in Xinjiang Uygur autonomous region, China. Plant Dis. 2022. <https://doi.org/10.1094/PDIS-09-21-1988-PDN>.
- Wang K, Hao PH, Liu YK, Xia YH, Sun BJ, Li HL, Li Y. Occurrence of *Pratylenchus coffeae* causing root rot of soybean in Shandong Province of China. Plant Dis. 2021;105:127. <https://doi.org/10.1094/PDIS-08-20-1740-PDN>.

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外文论文中文摘要

成果（论文）2:

2. Wang Shuo, Wang Ke, Niu Wenlong, Lin Fankang, Dababat. Abdelfattah A, Shi Yan, Yuan Hongxia, Xia Yanhui, Li Honglian, **Li Yu***(通讯作者). *Pratylenchus coffeae* effector Pc-ENG3 targets the maize TCTP protein to facilitate parasitism. *The Crop Journal*, 2026, 14(2), 388–399.

摘要：咖啡短体线虫（*Pratylenchus coffeae*）是一种广泛分布的迁徙性内寄生线虫，对多种作物造成显著产量损失，其成功寄生依赖于分泌调节宿主细胞过程的效应蛋白。本研究中，我们鉴定出一种新型的 β -1,4-内切葡聚糖酶基因 *Pc-ENG3*，其在咖啡短体线虫的食道腺细胞和生殖腺特异性表达，并在寄生阶段显著上调。酵母信号肽检测证实，*Pc-ENG3* 的 N 端信号肽具有功能，表明其可能参与分泌。在咖啡线虫中沉默 *Pc-ENG3* 后，其对玉米的侵染能力明显下降。将 *Pc-ENG3* 转入拟南芥（*Arabidopsis thaliana*）后，可抑制由 *flg22* 触发的活性氧生成。*Pc-ENG3* 的积累导致对线虫感染的易感性增强。酵母两杂交和荧光素酶补体成像分析显示，*Pc-ENG3* 与玉米 *ZmTCTP2a* 之间存在直接相互作用，而 *ZmTCTP2a* 是一种在细胞增殖和免疫调节中起调控作用的翻译后调控型肿瘤蛋白。在玉米中抑制 *ZmTCTP2a* 的表达会增加对咖啡短体线虫的易感性，并削弱对多种病原的免疫应答。综上所述，我们的研究揭示了一种此前未被阐明的效应子-宿主相互作用：*PcENG3* 靶向 *ZmTCTP2a*，从而抑制免疫反应以及根尖细胞的增殖与分化，进而促进线虫寄生。本研究解析了效应蛋白 *Pc-ENG3* 反向调控玉米对线虫的抗病能力，通过“抗病抑制+组织重塑”双重策略促进线虫寄生的分子机制。



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Pratylenchus coffeae effector PcENG3 targets the maize TCTP protein to facilitate parasitism

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ABSTRACT

Pratylenchus coffeae is a widespread migratory endoparasitic nematode that causes substantial yield losses across a broad range of crops. Successful parasitism depended on the secretion of effector proteins that modulate host cellular processes. In this study, we identified a novel β -1,4-endoglucanase effector, PcENG3, which was specifically expressed in the esophageal gland cell and gonadal tissues of *P. coffeae* and was significantly upregulated during parasitic stages. A yeast signal sequence trap assay confirmed the N-terminal signal peptide of PcENG3 was functional, suggesting its potential for secretion. Silencing PcENG3 in *P. coffeae* markedly impaired its infectivity on maize (*Zea mays* L.). Transgenic expression of PcENG3 in *Arabidopsis thaliana* suppressed flg22-triggered reactive oxygen species (ROS) accumulation, leading to enhanced susceptibility to nematode infection. Yeast two-hybrid and luciferase complementation imaging assays revealed a direct interaction between PcENG3 and maize ZmTCTP2a, a translationally controlled tumor protein involved in cell proliferation and immune regulation. Notably, suppression of ZmTCTP2a expression in maize increased susceptibility to *P. coffeae* and compromised basal immunity against multiple pathogens. Collectively, our findings uncovered a previously uncharacterized effector-host interaction in which PcENG3 targeted ZmTCTP2a to suppress immunity and alter root cap cell proliferation and differentiation, thereby promoting nematode parasitism. This work provided new insights into the molecular mechanisms underlying *P. coffeae* pathogenicity and host susceptibility.

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1. Introduction

Root lesion nematodes (RLNs; *Pratylenchus* spp.) represent a major threat to global agriculture, causing significant yield losses across a broad range of economically important crops [1]. Unlike sedentary endoparasitic nematodes such as root-knot nematodes (RKNs; *Meloidogyne* spp.) and cyst nematodes (CNs; *Heterodera* and *Globodera* spp.), which establish permanent feeding sites, RLNs maintain a migratory lifestyle throughout their life cycle [2–4]. The motile second-stage juveniles (J2) of RLNs penetrate the host root, where they feed and migrate intracellularly within the cortex [5,6]. This activity disrupts cellular organization, impairs root development, and increases the susceptibility of parasitized tissues to secondary infections by fungi and bacteria, thereby exacerbating

damage to the host plant [7,8]. However, due to the non-specificity of symptoms, the agricultural impact of RLNs is often underestimated.

To establish successful infections, plant-parasitic nematodes (PPNs) secrete an array of effector proteins that reprogram host cell physiology, development, and defense [9,10]. These effectors enable the nematode to modulate host metabolic and signaling pathways, thereby facilitating nutrient acquisition and evading host immunity [6,11]. For example, the *M. incognita* effector Mi2G02 hijacked the plant trihelix transcription factor GT-3a, thereby modulating developmental pathways in *Arabidopsis thaliana* [12]. Similarly, the *M. enterolobii* effector MeMSP1 targeted glutathione S-transferase (GSTF) proteins in *A. thaliana*, reconfiguring host metabolism to enhance nematode fitness [13]. Additionally, the isochorismatase Mi-ISC-1 of *M. incognita* suppressed salicylic acid (SA)-mediated defense by interfering with the SA biosynthetic pathway, thereby compromising host immunity [14].

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Like other pathogens, plant parasitic nematodes (PPNs) secrete diverse effectors to suppress pattern-triggered immunity (PTI) and facilitate parasitism. For example, RsVAP, a venom allergen-like protein from *Radopholus similis*, was shown to suppress host defense responses, thereby facilitating parasitism [6]. Similarly, the secreted effector BxSCD1 from *Bursaphelenchus xylophilus* was found to inhibit reactive oxygen species (ROS) accumulation and callose deposition, contributing significantly to virulence during host colonization [15]. The Mg16820 effector from *M. graminicola* suppressed R2/Avr2-triggered effector-triggered immunity (ETI) in *Nicotiana benthamiana* and inhibited flg22-induced ROS bursts [16]. The *H. avenae* effector Ha18764, a G16B09-like protein, was upregulated during infection and suppressed host hypersensitive response (HR) and callose deposition when expressed in tobacco [17]. MiCTL1a, a C-type lectin effector from *M. incognita*, reduced *Arabidopsis* catalase activity, modulates redox homeostasis, and altered susceptibility to RKN by regulating stress-responsive gene expression [10].

Among these effectors, β -1,4-endoglucanases (ENGs) played particularly important roles in nematode invasion and migration [18]. These enzymes were capable of degrading cellulose in plant cell walls, thereby facilitating mechanical penetration and movement through host tissues [19,20]. ENGs were detected in the secretomes of several PPNs and were often localized in esophageal gland cells [21,22]. Functional studies in *G. rostochiensis* demonstrated that cellulase genes significantly affected nematode infectivity [23]. In addition to their cell wall-degrading activity, ENGs might have contributed to nutrient release by breaking down complex polysaccharides into soluble sugars that supported nematode development [24]. Although ENGs were considered conserved virulence factors, their specific molecular targets in migratory endoparasites such as *Pratylenchus* spp. remained largely unexplored.

Translationally controlled tumor protein (TCTP) is an evolutionarily conserved protein that has been implicated in diverse biological processes, including cell proliferation, organogenesis, and stress tolerance across eukaryotes [25,26]. In *A. thaliana*, TCTP promoted mitotic growth by modulating the cell cycle, influencing root and shoot architecture [27]. Similar roles were observed in rice (*Oryza sativa*), where OsTCTP played an important role in mercury tolerance in rice [28]. Moreover, TCTP was linked to enhanced abiotic stress tolerance through its involvement in ROS-scavenging pathways [29], suggesting a key role in maintaining plant homeostasis under environmental constraints.

In this study, we identified and functionally characterized a novel effector, PcENG3, from *P. coffeae*. PcENG3 was expressed in the nematode's esophageal gland and gonad. Our data showed that PcENG3 directly interacted with the maize ZmTCTP2a, and that this interaction perturbed the expression of ZmTCTP2a and associated glucose metabolism genes involved in cell proliferation. Functional assays revealed that silencing ZmTCTP2a impaired maize root development and increased host susceptibility to both *P. coffeae* and the fungal pathogen *Ustilago maydis*. Together, these findings uncovered a previously uncharacterized mechanism by which migratory nematodes subverted host cell development and immunity, shedding new light on the molecular underpinnings of *P. coffeae* parasitism.

2. Materials and methods

2.1. Nematodes and plant materials

Carrot callus tissues were prepared according to the method previously described [30]. *P. coffeae* populations (HNK1, Henan Province, China) were extracted using a modified Baermann funnel

technique [31]. Maize plants (susceptible cultivar Zhengdan 958) were grown under a 16 h light/8 h dark photoperiod at 28 °C. Seeds of *A. thaliana* (ecotype Columbia, Col-0) were surface-sterilized and sown on MS medium plates containing 4.7 g L⁻¹ MS basal salts with vitamins, 20 g L⁻¹ sucrose, 3.5 g L⁻¹ Gelam Gum, and adjusted to pH 5.7. *N. benthamiana* and *A. thaliana* plants were cultivated under controlled environmental conditions at 22 °C with a 16 h light/8 h dark cycle.

2.2. Sequence analysis

The sequence of PcENG3 in root-lesion nematodes (RLNs) was obtained from *P. coffeae* RNA-seq datasets and the National Center for Biotechnology Information (NCBI; <https://www.ncbi.nlm.nih.gov>). Protein domain architecture was analyzed and validated using the Simple Modular Architecture Research Tool (SMART) in normal mode (<http://smart.embl-heidelberg.de/smart/>) [32]. Multiple sequence alignments were performed using DNAMAN software.

2.3. RNA extraction and reverse transcription quantitative realtime PCR

Total RNA was extracted from *P. coffeae* and plant samples using the HiPure Total RNA Plus Mini Kit (Magen, Guangzhou, Guangdong, China) according to the manufacturer's protocol. Complementary DNA (cDNA) synthesis and reverse transcription quantitative real-time PCR (RT-qPCR) were performed using the PrimeScript RT Reagent Kit with gDNA Eraser (RR047A; TaKaRa, Kyoto, Japan). Relative gene expression levels were calculated using the 2^{-ΔΔCT} method [33]. For normalization, *P. coffeae* Pc18s (MG906739), maize ZmActin1 (J01238), and *Arabidopsis* AtActin2 (ES659082) were used as internal reference genes. Each experiment was performed with at least three independent biological replicates, and each RT-qPCR reaction included three technical replicates.

2.4. In situ hybridization

A fragment of the PcENG3 was amplified using gene-specific primers (Table S2) and used as a template for RNA probe synthesis. Digoxigenin (DIG)-labeled sense and antisense RNA probes were generated using the DIG RNA Labeling Kit (Roche, Mannheim, Germany) according to the manufacturer's instructions. The *in situ* hybridization protocol was adapted from previously described with minor modifications [22]. *P. coffeae* individuals were collected from carrot callus cultures and fixed overnight in 4% paraformaldehyde. Fixed nematodes were then finely chopped on glass slides using sterile blades to expose internal tissues. The resulting fragments were hybridized with DIG-labeled RNA probes at 55 °C overnight. Hybridization signals were detected using anti-DIG antibodies conjugated to alkaline phosphatase. Samples were imaged using a confocal laser scanning microscope (Nikon A1HD25, Tokyo, Japan).

2.5. PcENG3 transgenic Arabidopsis plants

The coding sequence of PcENG3, excluding the N-terminal signal peptide, was amplified and cloned into the modified pEarleyGate 202 binary vector to generate the expression construct pEarleyGate 202-PcENG3. The resulting plasmid was introduced into *A. tumefaciens* strain GV3101 via electroporation. Transformation of wild-type *A. thaliana* ecotype Columbia-0 (Col-0) was performed using the floral dip method, as previously described [34]. Homozygous T₃ lines were identified by reverse transcription PCR (RT-PCR) analysis. To assess the impact of PcENG3 expression on nematode

infection, transgenic lines were inoculated with 200 *P. coffeae* individuals per plant. Root samples were harvested and thoroughly washed at 35 d post-inoculation (dpi), and nematode populations were quantified under a stereomicroscope.

2.6. PTI assays

To detect ROS, four-week-old homozygous T3 *Arabidopsis* lines were treated with 1 $\mu\text{mol L}^{-1}$ flg22, and ROS production was measured using a luminol-based chemiluminescence assay, as previously described [35]. For gene expression analysis, seedlings were treated with flg22 following the same protocol, and total RNA was extracted for transcript analysis. The expression levels of *AtWRKY33* (AT2G38470), *AtWRKY30* (AT5G24110), and *AtWRKY29* (AT4G23550) were quantified by reverse transcription quantitative PCR (RT-qPCR).

2.7. Yeast secretion assays

The yeast secretion assay was conducted following the method described [36,37]. The predicted signal peptide (SP) sequence of *PcENG3* was synthesized and then introduced into the pSUC2 vector, which contains a truncated invertase gene (*SUC2*) lacking the start codon (ATG). The resulting construct was subsequently transformed into the yeast strain YTK12. Invertase enzymatic activity was assessed by monitoring the reduction of TTC dye to insoluble, red-colored triphenylformazan.

2.8. Synthesis of double-stranded RNA (dsRNA) and in vitro RNA interference (RNAi)

The *in vitro* RNA interference (RNAi) assay was performed as previously described [38]. A 409-bp PCR product carrying the T7 promoter was amplified from the *PcENG3* of *P. coffeae*. Single-stranded RNA (ssRNA) was synthesized using the ScriptMAX™ Thermo T7 Transcription Kit (TOYOBO, Osaka, Japan), and complementary ssRNAs were mixed and annealed to form double-stranded RNA (dsRNA). Female *P. coffeae* were isolated from carrot callus cultures and incubated with dsRNA targeting *PcENG3* at a concentration of 2 ng μL^{-1} . After treatment, 30 female *P. coffeae* were inoculated onto fresh carrot callus and incubated in the dark at 25 °C for 60 d. Following the incubation period, the callus was homogenized, and the numbers of nematodes and eggs were quantified.

2.9. Virus-induced gene silencing

The SGN-VIGS tool (<https://vigs.solgenomics.net>) was used to design specific silencing fragments targeting the *PcENG3*, *ZmPDS*, and *ZmTCTP2a* cDNA sequences. These fragments were individually cloned into the tobacco rattle virus RNA 2 (TRV2) vector, as previously described [39,40], and the resulting recombinant constructs were transformed into *Agrobacterium tumefaciens* strain C58C1. Virus-induced gene silencing (VIGS) in maize was performed as follows: maize seeds were surface-sterilized, and the seed coats were removed. *Agrobacterium* cultures containing TRV1 and TRV2 were mixed in equal volumes and applied to the seeds. The seeds were then rinsed three times with sterile water before being sown in sterilized nutrient soil. TRV-infected and wild-type maize plants were inoculated with 1000 *P. coffeae*. Nematodes were collected at 15 d post-inoculation (dpi) for RT-qPCR analysis, as described above, to assess gene silencing efficiency. Additionally, the *P. coffeae* population and related growth parameters of maize were evaluated at 60 dpi.

2.10. Subcellular localization assay

The coding sequences of *PcENG3* and *ZmTCTP2a* were amplified using gene-specific primers and cloned into the pGWC entry vector. Subsequently, Gateway LR recombination was performed to transfer the inserts into the destination vector pEarleyGate104, resulting in C-terminal GFP fusion constructs (*PcENG3*-GFP and *ZmTCTP2a*-GFP). These constructs were introduced into *A. tumefaciens* strain GV3101 and used for transient expression in the leaves of four-week-old transgenic *N. benthamiana* plants expressing the H2B nuclear marker. Leaf infiltration was conducted via *Agrobacterium*-mediated transformation, and fluorescent signals were visualized 48 h post-infiltration using a laser scanning confocal microscope (Nikon A1HD25, Tokyo, Japan).

2.11. Yeast two-hybrid assay

The *PcENG3* (excluding the signal peptide sequence) was amplified and cloned into the pGBKT7 vector to serve as the bait construct. This construct was introduced into *Saccharomyces cerevisiae* strain Y2H Gold using the Matchmaker Gold Yeast Two-Hybrid (Y2H) System (Clontech, Palo Alto, CA, USA). Yeast co-transformation and interaction assays were performed as previously described [10]. To identify potential interacting partners of *PcENG3*, a maize cDNA library was generated from mRNA isolated from maize roots infected with *P. coffeae* at 2, 5, 8, and 10 d post-inoculation (dpi). The resulting cDNA fragments were cloned into the pGADT7 vector and transformed into *S. cerevisiae* strain Y187 using the Make Your Own “Mate & Plate” Library System (Clontech, Mountain View, CA, USA). This library was subsequently screened against the *PcENG3* bait construct using the Y2H assay.

2.12. Firefly luciferase complementation imaging (LCI) assay

The coding sequences (CDS) of *PcENG3* and *ZmTCTP2a* were amplified and cloned into the pCambia1300-cLUC and pCambia1300-nLUC vectors to generate the *PcENG3*-nLUC and cLUC-*ZmTCTP2a* constructs, respectively. Positive control constructs (SAR-cLUC and SGF-nLUC) were generated using the same cloning strategy. All constructs were individually introduced into *A. tumefaciens* strain GV3101 and co-infiltrated into *N. benthamiana* leaves. At two d post-infiltration, leaf tissues were treated with 0.2 mmol L^{-1} luciferin solution (Thermo Fisher Scientific, Waltham, MA, USA), and luminescence was detected using a low-light cooled CCD imaging system (NightOWL II LB983, Berthold, Bad Wildbad, Germany).

2.13. Pull-down assay

The coding sequences of *PcENG3* and *ZmTCTP2a* were amplified and cloned into the pET-32a and pGEX-4 T-1 vectors to generate the *PcENG3*-His and *ZmTCTP2a*-GST fusion constructs, respectively. Each construct was individually transformed into *E. coli* strain Transetta (DE3) for protein expression. The purified fusion proteins were incubated together in 1× TBS buffer containing 1 mmol L^{-1} PMSF at 4 °C for 1 h. Subsequently, 20 μL of glutathione magnetic beads (Yeasen Biotech, Shanghai, China) were added to the mixture, followed by an additional 1-hour incubation at 4 °C. After incubation, the samples were boiled for 10 min in SDS sample loading buffer. The eluted proteins were separated by SDS-PAGE and analyzed by Western blotting using anti-His and anti-GST antibodies (ABclonal Technology, Wuhan, Hubei, China).

2.14. BiFC assay

The full-length coding sequence of *PcENG3* (excluding the signal peptide) was amplified and cloned into the pGWC entry vector, and subsequently recombined into the pEarlyGate201-nYFP destination vector via Gateway cloning to generate the *PcENG3*-nYFP construct. The *ZmTCTP2a* coding sequence was cloned into the pEarlyGate201-cYFP vector to produce the cYFP-*ZmTCTP2a* construct. Both constructs were introduced into *A. tumefaciens* strain GV3101 and transiently expressed in *N. benthamiana* leaves through agroinfiltration. At 48 h post-infiltration, YFP fluorescence was observed using a laser scanning confocal microscope (Nikon A1HD25, Tokyo, Japan), indicating potential protein–protein interactions.

2.15. *Ustilago maydis* infection assay in maize

The susceptibility of maize to *U. maydis* infection was assessed using 3-week-old maize plants. The *U. maydis* strains U02 and B48 were cultured at 25 °C until the optical density at 600 nm (OD₆₀₀) reached 2.0. Equal volumes of the two cultures were mixed at a 1:1 ratio to prepare the inoculum. A total of 2 mL of the resulting suspension was drawn into a syringe and injected into the whorl of each maize plant. Prior to inoculation, the suspension was thoroughly mixed to ensure uniformity. Tumor formation was evaluated at 30 dpi by counting the number of tumors per plant.

2.16. Protein extraction, sodium dodecyl sulphate polyacrylamide gel electrophoresis and Western blot assay

Fresh *N. benthamiana* and *A. thaliana* leaves were collected and ground into a fine powder using liquid nitrogen. A lysis buffer containing 60% SDS, 100 mmol L⁻¹ Tris-HCl, and 2% β-mercaptoethanol was added to prepare the lysate. Each protein sample was individually homogenized and then analyzed by SDS-PAGE. Detection was carried out using specific primary antibodies, followed by incubation with secondary antibodies. The detection signal was visualized using an imaging apparatus (GE Amersham Imager 680RGB, Boston, MA, USA).

3. Results

3.1. *PcENG3* was a candidate effector involved in *P. coffeae* parasitism

PcENG3 was identified as a candidate effector based on RNA-seq analysis. The *PcENG3* protein shared over 95% sequence identity with known endoglucanases (ENGs) and comprised 357 amino acids, including a 21-amino-acid N-terminal signal peptide and a predicted cellulase functional domain. To determine the spatial expression pattern of *PcENG3* in *P. coffeae*, *in situ* hybridization was performed. Strong hybridization signals were detected in the esophageal gland cell (EG) and gonads when the reverse RNA probe was used (Fig. 1A), whereas no signal was observed with the sense control probe (Fig. 1A). RT-qPCR analysis revealed that *PcENG3* was expressed throughout all developmental stages of *P. coffeae*, with significantly higher transcript levels in adult females and males (Fig. 1B; $P < 0.05$). During the initial phase of infection, *PcENG3* expression reached its maximum level at 2 dpi and subsequently remained elevated, suggesting a pivotal role during the early stages of parasitism (Fig. 1C).

To investigate the functional role of *PcENG3*, double-stranded RNAs (dsRNAs) targeting *PcENG3* and *GFP* (control) were applied to mixed-stage nematodes. qRT-PCR analysis showed that *PcENG3* dsRNA induced a maximum silencing efficiency of 62% after 36 h of incubation (Fig. S1). Compared to controls, *PcENG3*-silenced

P. coffeae caused significantly less tissue maceration in carrot callus (Fig. 1D). Additionally, both nematode numbers and egg production were significantly reduced by 26% and 27.5%, respectively (Fig. 1E). These results suggested that *PcENG3* was critical not only for parasitism but also for the reproductive success of *P. coffeae*.

3.2. *PcENG3* encoded a secreted effector

To evaluate the functionality of the *PcENG3* signal peptide (SP), a yeast secretion assay was performed using invertase-mediated conversion of sucrose as a readout. Yeast transformants expressing the *PcENG3*-SP, similar to those expressing the known effector Avr1b-SP (positive control), were able to grow on synthetic tryptophan (Trp) dropout medium supplemented with sucrose. This growth indicated successful secretion of invertase, thereby restoring the yeast's ability to metabolize sucrose (Fig. 2). In contrast, the untransformed YTK12 strain failed to grow under the same conditions, and its culture remained colorless after TTC treatment. These results demonstrated that the signal peptide of *PcENG3* was functional in directing protein secretion and supported the conclusion that *PcENG3* encoded a secreted effector protein.

3.3. Ectopic expression of *PcENG3* promoted the infection of *P. coffeae* and suppressed plant defense responses

To investigate the role of *PcENG3* in plant-nematode interactions, two independent transgenic *Arabidopsis* lines (*PcENG3*-1 and *PcENG3*-2) expressing *PcENG3* were generated. These lines exhibited shorter primary roots and a lower number of lateral roots than WT plants (Fig. S2). This suggested that *PcENG3* might have impacted the promotion of *P. coffeae* infection. The expression of *PcENG3* was confirmed at both the transcript and protein levels by RT-qPCR (Fig. 3B) and Western blotting (Fig. S3), respectively. To assess whether *PcENG3* influenced plant immunity, PTI suppression assays were performed. Following flg22 treatment, transgenic *Arabidopsis* lines expressing *PcENG3* displayed significantly reduced ROS accumulation compared to WT (Fig. 3A). Additionally, transcript levels of key defense-related genes, including *AtWRKY33*, *AtWRKY30*, and *AtWRKY29*, were markedly downregulated in *PcENG3*-expressing plants following flg22 exposure (Fig. 3C). To determine whether *PcENG3* enhanced nematode parasitism, *P. coffeae* infection levels were assessed in the transgenic lines. At 30 dpi, the number of nematodes in *PcENG3*-expressing plants was significantly increased by 47% compared to WT (Fig. 3D). Collectively, these findings indicated that *PcENG3* functioned as an effector that interfered with host immunity and promoted *P. coffeae* parasitism by modulating plant defense responses and root development.

3.4. *PcENG3* silencing reduced *P. coffeae* parasitism

To elucidate the role of *PcENG3* in the pathogenicity of *P. coffeae* during host infestation, a Tobacco Rattle Virus (TRV)-mediated RNA interference (RNAi) strategy was employed to suppress *PcENG3* expression in feeding nematodes within maize roots (Figs. 4A, S4A). RT-qPCR analysis confirmed effective gene silencing, with a 58% reduction in *PcENG3* transcript levels in nematodes extracted from RNAi-treated plants compared to controls (Fig. 4D). As indicated by Fig. 4B, the nematode population was reduced by 37% in maize plants with silenced *PcENG3* at 60 dpi. Furthermore, the average number of nematodes per gram of maize roots was reduced by 35% (Fig. S4C). Nematode staining further revealed fewer *P. coffeae* individuals in the RNAi-treated maize roots, indicating enhanced plant resistance (Fig. 4C). Consistently, the fresh shoot and root weights of RNAi-treated maize increased by 18%

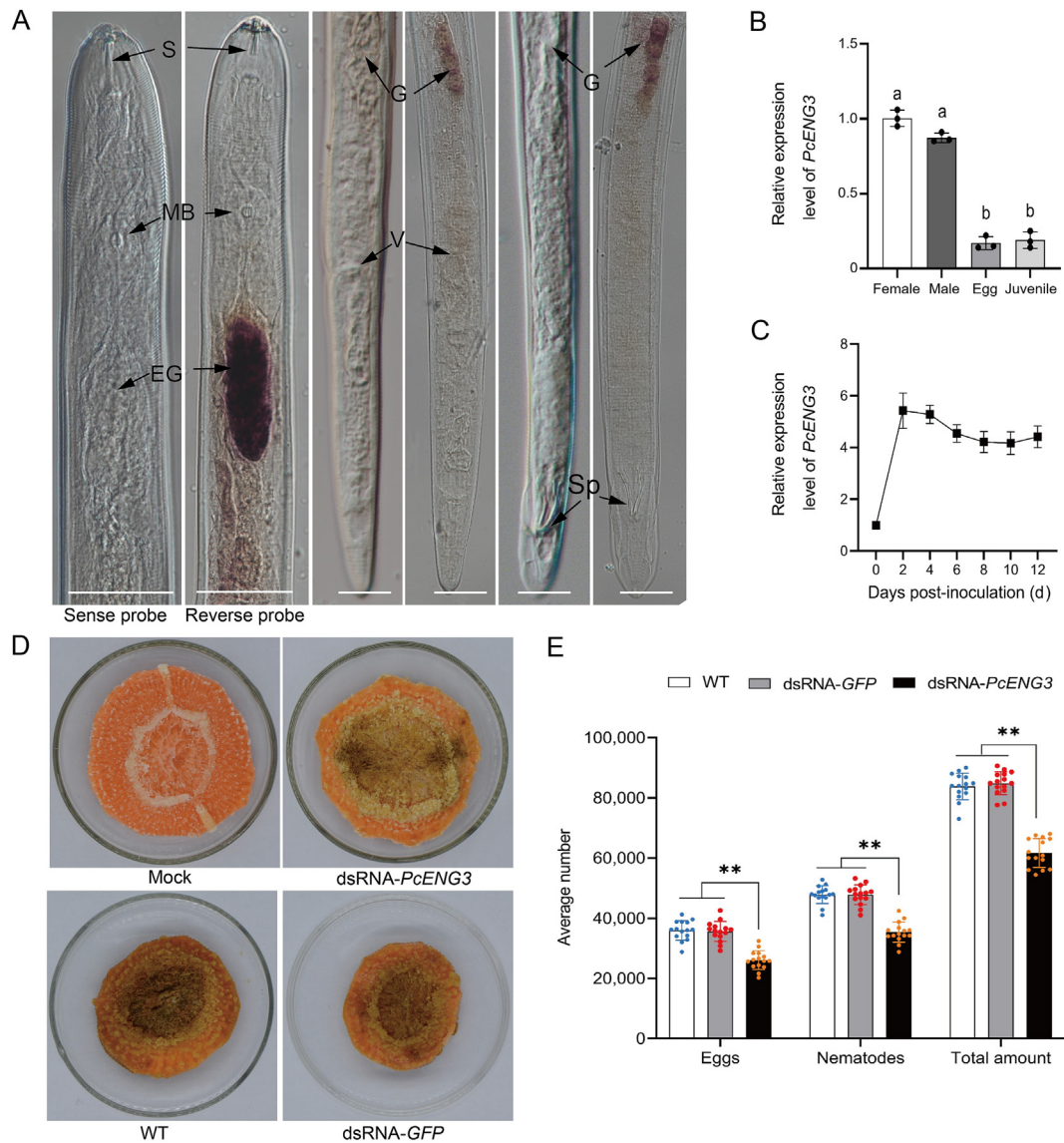


Fig. 1. Localization and expression profile of *PcENG3* of *P. coffeae*. (A) *In situ* hybridization showed *PcENG3* transcripts expressed in the esophageal gland (EG) and gonad of the *P. coffeae*, which were hybridized with sense and antisense cDNA probes from *PcENG3*. S, stylet; MB, medium bulb; EG, esophageal gland; G, gonad; V, vulva; Sp, spicule. Scale bars, 20 μ m. (B) The developmental expression pattern of *PcENG3* was quantified by RT-qPCR. The results of RT-qPCR were calculated via the $2^{-\Delta\Delta CT}$ method. Values are means $\pm$ SE. (C) Expression profile of *PcENG3* in planta. The results of RT-qPCR were calculated via the $2^{-\Delta\Delta CT}$ method. Values are means $\pm$ SE. (D) dsRNA-*PcENG3* mediated RNA interference reduced the reproductive capacity of *P. coffeae*. Mock, blank control; WT, wild type; GFP, green fluorescent protein. (E) Suppressed the expression of the *PcENG3* significantly reduced the pathogenicity of *P. coffeae*. Values are means $\pm$ SD ($n = 15$). **, $P < 0.01$ by Student's *t*-test.

and 25%, respectively, compared to the WT (Figs. 4D, S4B). These findings suggested that *PcENG3* contributed to *P. coffeae* parasitism.

3.5. *PcENG3* interacted with maize translationally controlled tumor protein (*ZmTCTP2a*)

To identify host targets of *PcENG3*, a yeast two-hybrid (Y2H) screen was performed using a maize cDNA library, with *PcENG3* (lacking the signal peptide) as bait. Several candidate interacting proteins were identified, among which the maize translationally controlled tumor protein *ZmTCTP2a* (AF548024) was captured most frequently-eight independent times-exhibiting strong interaction signals. To map the interaction region, two truncated versions of *PcENG3*, including BD-*PcENG3* (amino acids 65–315), were constructed. Four candidate proteins-*ZmTCTP2a*, a senescence-associated protein (NM001111815), a ubiquitin-conjugating enzyme (XM035961568), and *ZmHSP21* (a maize heat

shock protein; EU958034)-were subjected to yeast cotransformation assays with *PcENG3* (Table S1). Among these, only yeast co-expressing *PcENG3* and *ZmTCTP2a* grew robustly on SD/-Leu/-Trp/-Ade/-His medium supplemented with X- α -Gal, indicating a specific protein-protein interaction (Fig. 5A). To further confirm this interaction, a GST pull-down assay was performed using recombinant GST-tagged *ZmTCTP2a* and His-tagged *PcENG3* expressed in *E. coli*. The results showed that *PcENG3* was specifically pulled down by GST-*ZmTCTP2a* (Fig. 5B). Consistent with this, split luciferase complementation imaging (LCI) in *N. benthamiana* leaves confirmed the *in vivo* interaction between *PcENG3* and *ZmTCTP2a* (Fig. 5C), with SAR-cLUC and SGF-nLUC serving as a positive control.

To validate this interaction at the subcellular level, bimolecular fluorescence complementation (BiFC) vectors were constructed by fusing *PcENG3* to the N-terminal half of YFP (nYFP) and *ZmTCTP2a* to the C-terminal half (cYFP). Following *Agrobacterium*-mediated

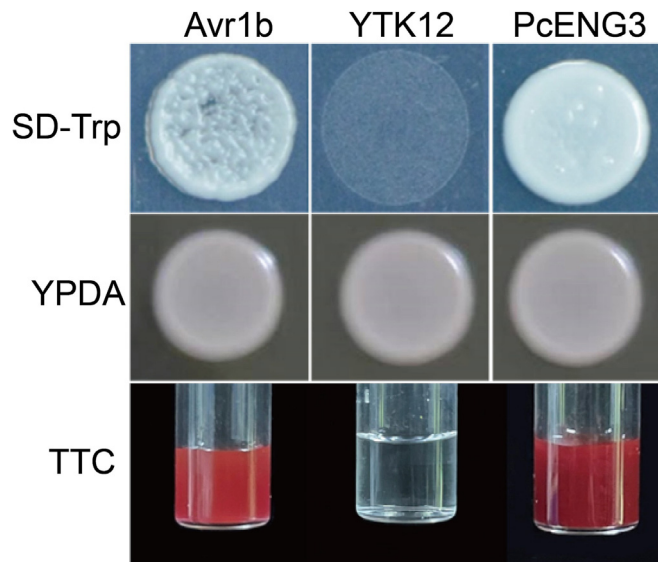


Fig. 2. Validation of PcENG3 SP with a yeast system. Yeast strain YTK12 was transformed with either the empty vector pSUC2 (negative control), pSUC2-PcENG3^{SP}, or pSUC2-Avr1b^{SP} (positive control). The transformed yeast strains were then evaluated for growth on SD/-Trp medium and invertase activity using a colorimetric TTC assay.

transient expression in *N. benthamiana* leaf cells, strong yellow fluorescence was observed in the nYFP-PcENG3 + cYFP-ZmTCTP2a combination, but not in control samples (nYFP-PcENG3 + cYFP),

confirming the interaction *in planta* (Fig. 5D). Furthermore, a sub-cellular localization analysis was conducted by expressing PcENG3-GFP and ZmTCTP2a-GFP in a transgenic *N. benthamiana* line expressing the H2B nuclear localization protein marker through transient expression. The results demonstrated that PcENG3 and ZmTCTP2a predominantly localize to the cytoplasm and nucleus (Fig. S5), consistent with the BiFC results. Collectively, these findings demonstrated that PcENG3 specifically interacted with ZmTCTP2a, both *in vitro* and *in vivo*, suggesting a potential role in modulating host cellular processes during *P. coffeae* parasitism.

3.6. Downregulation of ZmTCTP2a expression in maize increased susceptibility to pathogen infection

To investigate the role of ZmTCTP2a in maize defense, TRV-mediated virus-induced gene silencing (VIGS) was employed. Silencing of *ZmPDS*, used as a visual marker control, resulted in characteristic photobleaching symptoms in maize leaves (Figs. 6A, S6). Silencing efficiency of *ZmTCTP2a* was confirmed by RT-qPCR, revealing a significant 33% reduction in transcript levels compared with the control (Fig. 6C). *RNAi-ZmTCTP2a* maize plants exhibited a significant decrease in both shoot and root fresh weight relative to wild-type plants at 60 dpi (Fig. 6C). Furthermore, the quantification of nematodes revealed an approximate 19% increase in the population of *P. coffeae* in the silenced lines (Fig. 6B, C). This finding suggests that the silenced lines exhibit an enhanced susceptibility. Similarly, in the *U. maydis* infection assay, *RNAi-ZmTCTP2a* plants developed significantly more and larger tumors compared with wild-type plants at 30 dpi. Furthermore, several

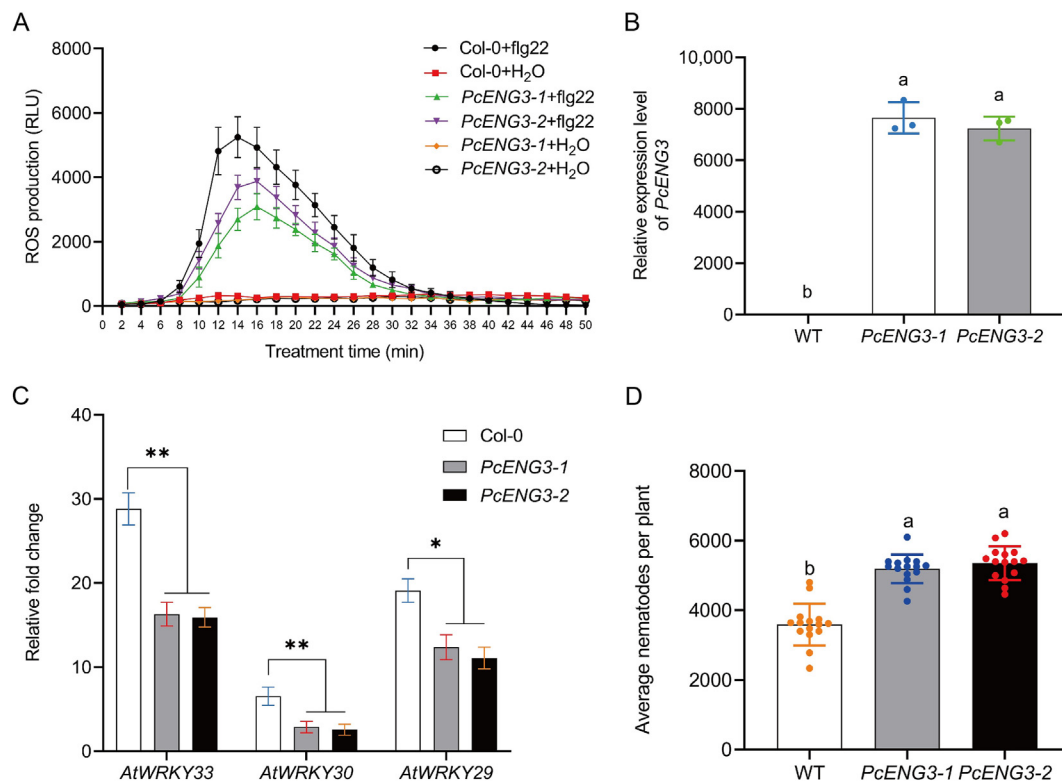


Fig. 3. PcENG3 is involved in *P. coffeae* parasitism and suppresses plant defense responses triggered by flg22. (A) PcENG3 inhibits flg22-induced ROS production in PcENG3-transgenic *Arabidopsis* lines. (B) Relative expression level of PcENG3 in two independent ectopic-expressing *Arabidopsis* lines. (C) RT-qPCR analysis revealed that the expression levels of *Arabidopsis* defense genes *AtWRKY30*, *AtWRKY33*, and *AtWRKY29* in response to flg22 were significantly reduced in transgenic *Arabidopsis* lines compared to WT (Col-0). Values are means $\pm$ SD. *, $P < 0.05$; **, $P < 0.01$ by Student's *t*-test. (D) *P. coffeae* infection was increased in transgenic *Arabidopsis* expressing PcENG3 as compared to wild-type (Col-0) lines. The numbers of *P. coffeae* were counted at 30 dpi. Values are means $\pm$ SD ($n = 15$). Different letters represent significant differences ($P < 0.05$ by one-way ANOVA).

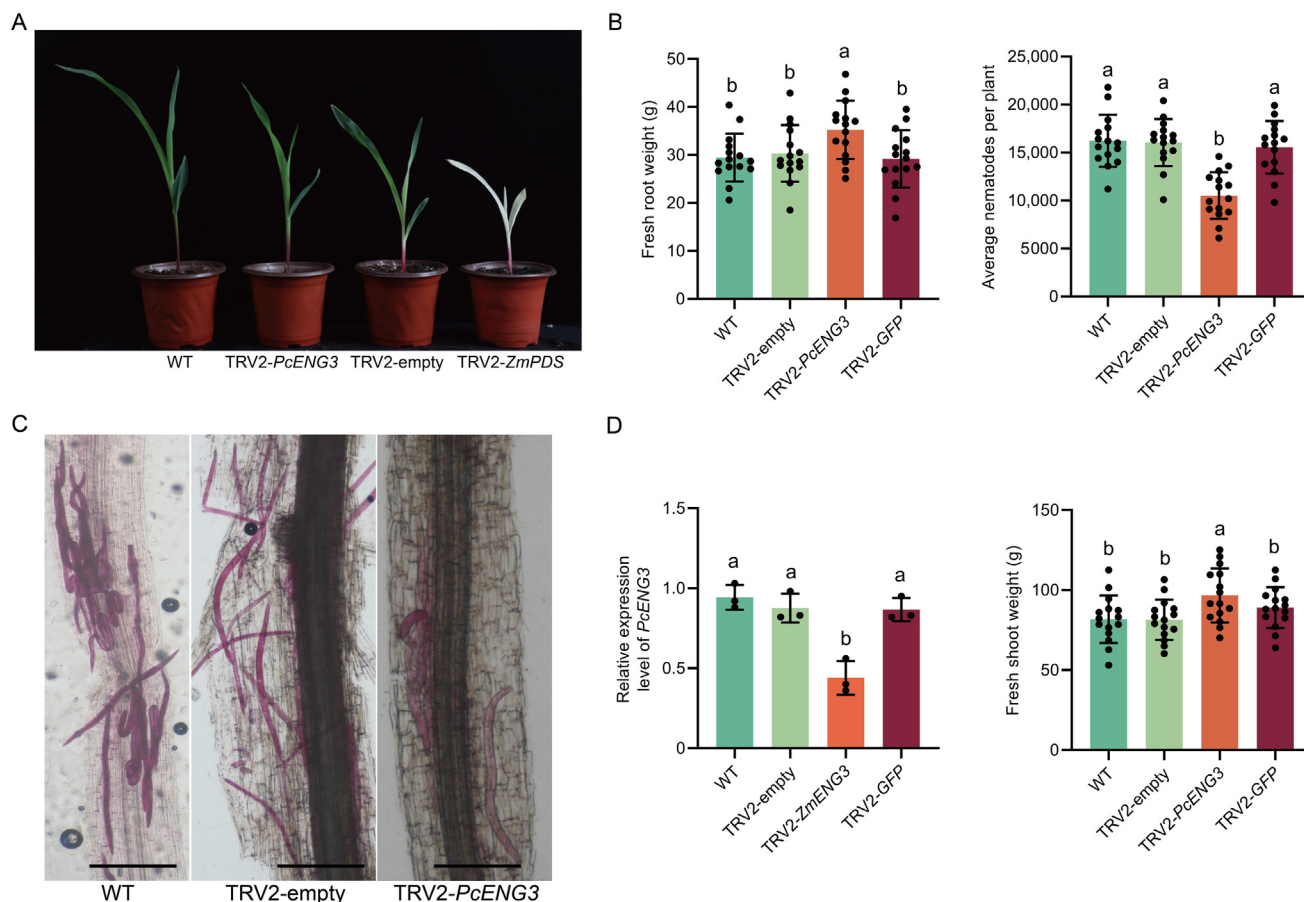


Fig. 4. Host-derived TRV-mediated silencing of *P. coffeae* *PcENG3* ectopic expression in the maize. (A) Maize phenotypes, treated with *A. tumefaciens* carrying TRV2-*ZmPDS* exhibiting a photobleaching phenotype. No significant change was observed in the TRV2-empty and wild-type (WT) plants. (B) TRV-mediated RNAi of *PcENG3* in maize reduced the ability of *P. coffeae* infection. Values are means $\pm$ SD ($n = 15$), with distinct letters representing statistically significant differences ($P < 0.05$ by one-way ANOVA). (C) The population of *P. coffeae* in the TRV2-*PcENG3* roots of maize lines was decreased significantly compared with WT. Scale bars, 100 μ m. (D) TRV-mediated RNAi of *PcENG3* significantly increased the resistance of maize to *P. coffeae* infection. *Pc18S* was used as the internal control. GFP, green fluorescent protein. Values are means $\pm$ SE ($n = 15$), with different letters representing significant differences ($P < 0.05$ by one-way ANOVA).

maize varieties with the *ZmTCTP2a* silenced expression exhibited significant differences in susceptibility to *U. maydis*. The sensitive maize varieties (ZD958) displayed a significantly higher incidence of larger and more numerous tumors, whereas the resistant varieties (YD959 and YD8348) showed increased susceptibility to *U. maydis* (Fig. 6D, E). Moreover, an analysis of defense-related genes (*ZmPAL*, *ZmLOX*, and *ZmSOD*) in TRV2-*ZmTCTP2a* maize indicated that the expression levels of these defense-related genes were significantly reduced by 38%, 24%, and 38%, respectively (Fig. S7). Together, these results demonstrated that *ZmTCTP2a* played a critical role in maize defense against both *P. coffeae* and fungal pathogens.

3.7. Maize *ZmTCTP2a* influenced root development in maize through the regulation of energy metabolism-related genes

Silencing of *ZmTCTP2a* significantly impaired primary root elongation and reduced lateral root formation during the seedling stage in maize (Fig. 7A, B). At later developmental stages, pronounced reproductive defects were observed, male inflorescences failed to produce pollen, while female ear development was abnormal (Fig. 7C). These findings suggested that *ZmTCTP2a* was crucial for both root development and reproductive growth. To further explore its role in root development, the root tip structure was examined using Safranin-fast green staining. Comparative analysis between the TRV2-*ZmTCTP2a* and TRV2-*GFP* lines revealed distinct

morphological differences (Fig. 7A). Root length was reduced 35% in TRV2-*ZmTCTP2a* plants compared to the TRV2-*GFP* (Fig. S9C). Additionally, root cap cell numbers were markedly decreased 45% (Fig. S9B), and both the apical meristem and root cap cells exhibited signs of incomplete development (Fig. S9A). These results indicated that *ZmTCTP2a* regulated root cell proliferation, differentiation, and lateral root formation.

To investigate the role of *ZmTCTP2a* in maize energy metabolism, we analyzed the expression of genes involved in key metabolic pathways. Specifically, we focused on genes associated with glycolysis (*ZmH XK1*, *ZmH XK2*, *ZmPK1*, *ZmPK2*, *ZmPFK1*, *ZmPFK2*, *ZmPFK3*), the pentose phosphate pathway (PPP) (*ZmG6PD1*, *ZmG6PD2*), and the tricarboxylic acid (TCA) cycle (*ZmCS1*). The results revealed significantly downregulated expression of *ZmH XK1*, *ZmH XK2*, *ZmPK1*, *ZmPK2*, *ZmPFK1*, *ZmPFK3*, *ZmG6PD2*, and *ZmCS1* (Fig. 7D, E). These findings demonstrated that *ZmTCTP2a* played a key role in regulating genes involved in energy metabolism.

4. Discussion

Effectors are critical molecular tools employed by sedentary and migratory plant-parasitic nematodes. Such as RKNs and CNs, to induce and maintain specialized feeding structures through the manipulation of host developmental pathways [41,42]. A well-characterized example is the *H. glycines* effector HgSYV46, which

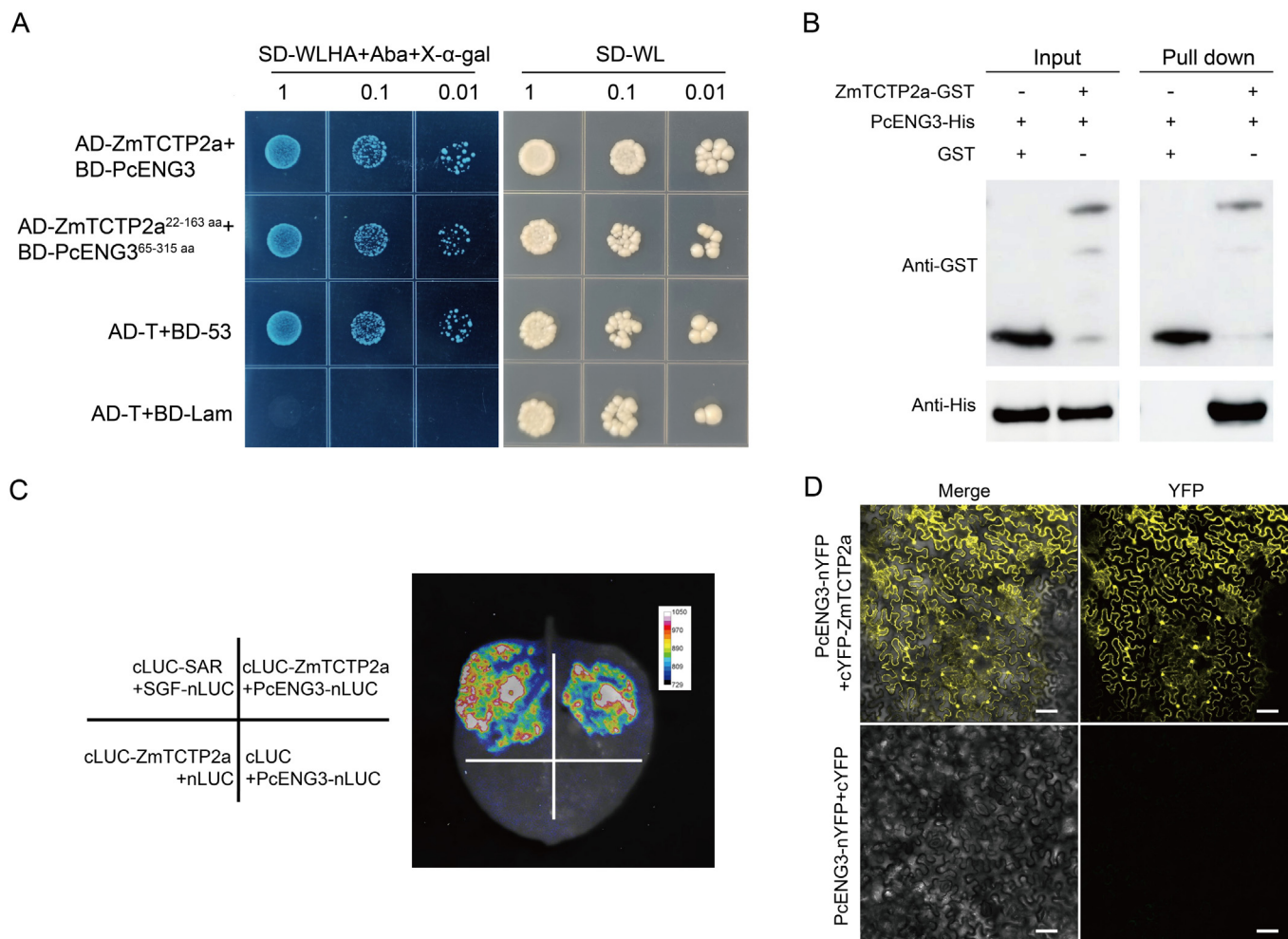


Fig. 5. PcENG3 interacts with maize ZmTCTP2a. (A) Yeast two-hybrid analysis of the interaction between PcENG3 and ZmTCTP2a. Y2H gold yeast cells harboring bait and prey vectors were grown normally on SD-WL (SD/-Trp/-Leu) media. After the addition of 1 mg mL⁻¹ aureobasidin A and 20 mg mL⁻¹ X-α-Gal, the yeast gold cells cultured on the SD-WLHA selective media (SD/-Trp/-Leu/-His/-Ade) appeared bluish. Yeast cells containing pGBKT7-53 and pGADT7-T served as the positive controls, while yeast cells containing pGBKT7-Lam and pGADT7-T served as the negative controls. (B) ZmTCTP2a-GST associated with PcENG3-His in a GST pull-down assay. GST was utilized as a control. (C) The interaction between PcENG3 and ZmTCTP2a was assessed via a luciferase complementation assay (LCL). A series of plasmids were introduced into *A. tumefaciens*, which were subsequently infiltrated into four discrete regions of *N. benthamiana* leaves. Luciferase activities were subsequently measured 2 dpi. The measurement of luciferase activity was performed using a CCD imaging apparatus. A combination expressing SGF-nLUC + cLUC-SAR was used as a positive control. (D) Bimolecular fluorescent complementation (BiFC) confirmed the interaction between PcENG3 and ZmTCTP2a *in planta*. PcENG3 and ZmTCTP2a were individually fused with the N-terminal (nYFP) and C-terminal (cYFP) halves of the yellow fluorescent protein (YFP). The YFP signal was detected by confocal microscopy at 48 h post-infiltration.

mimics the activity of the CLAVATA3/ESR (CLE) family in *A. thaliana*, thereby hijacking host signaling cascades to promote cell division and feeding site formation [43]. In contrast, migratory nematodes such as *P. coffeae* do not establish permanent feeding sites, and whether they utilize similar effector-mediated strategies to modulate host development has remained largely unexplored. Our study provided evidence that *P. coffeae* employs a novel β-1,4-endoglucanase effector PcENG3 and demonstrated that PcENG3 was expressed in esophageal glands and played a pivotal role during *P. coffeae* parasitism. It exerted a physical interaction with maize translationally controlled tumor protein (ZmTCTP2a). This interaction led to the suppression of normal root development, contributing to enhanced parasitism. Members of the endoglucanase (ENG) family are widely conserved across nematode species and have been implicated in cell wall degradation and host invasion [21,22,44], but our findings highlight an additional role for PcENG3 in modulating host regulatory networks.

To facilitate their intracellular migration, plant-parasitic nematodes (PPNs) secrete an array of cell wall degrading enzymes (CWDEs), such as β-1,4-endoglucanase (ENGs), which facilitate cell

wall loosening and tissue penetration. Comparative genomic analyses have revealed that genes encoding CWDEs are consistently present in PPNs but absent in free-living and non-parasitic plant-associated nematodes, highlighting their evolutionary linkage to parasitism [45]. The cellulase *Pv-eng-1* from *P. vulnus* and the pectate lyases *Bx-pel-1* and *Bx-pel-2* from *Bursaphelenchus xylophilus* have been shown to degrade host plant cell walls, a process that plays a critical role in facilitating nematode invasion and intercellular migration [22,46]. Among PPNs, *Meloidogyne* spp. possess the most extensive CWDE repertoire, with 81 predicted genes, a feature potentially underpinning their remarkably broad host range [47]. These results provide compelling evidence that ENGs play a pivotal role in facilitating nematode parasitism.

TCTP is a highly conserved protein found in all eukaryotes, initially identified in *Mus musculus* fibroblasts [48]. It functions in diverse cellular processes, including cell proliferation, growth, apoptosis, and responses to abiotic and biotic stresses [49]. In plants, TCTP has emerged as a central regulator of growth and development. For example, *A. thaliana* TCTP loss-of-function mutants exhibit severe developmental defects and premature

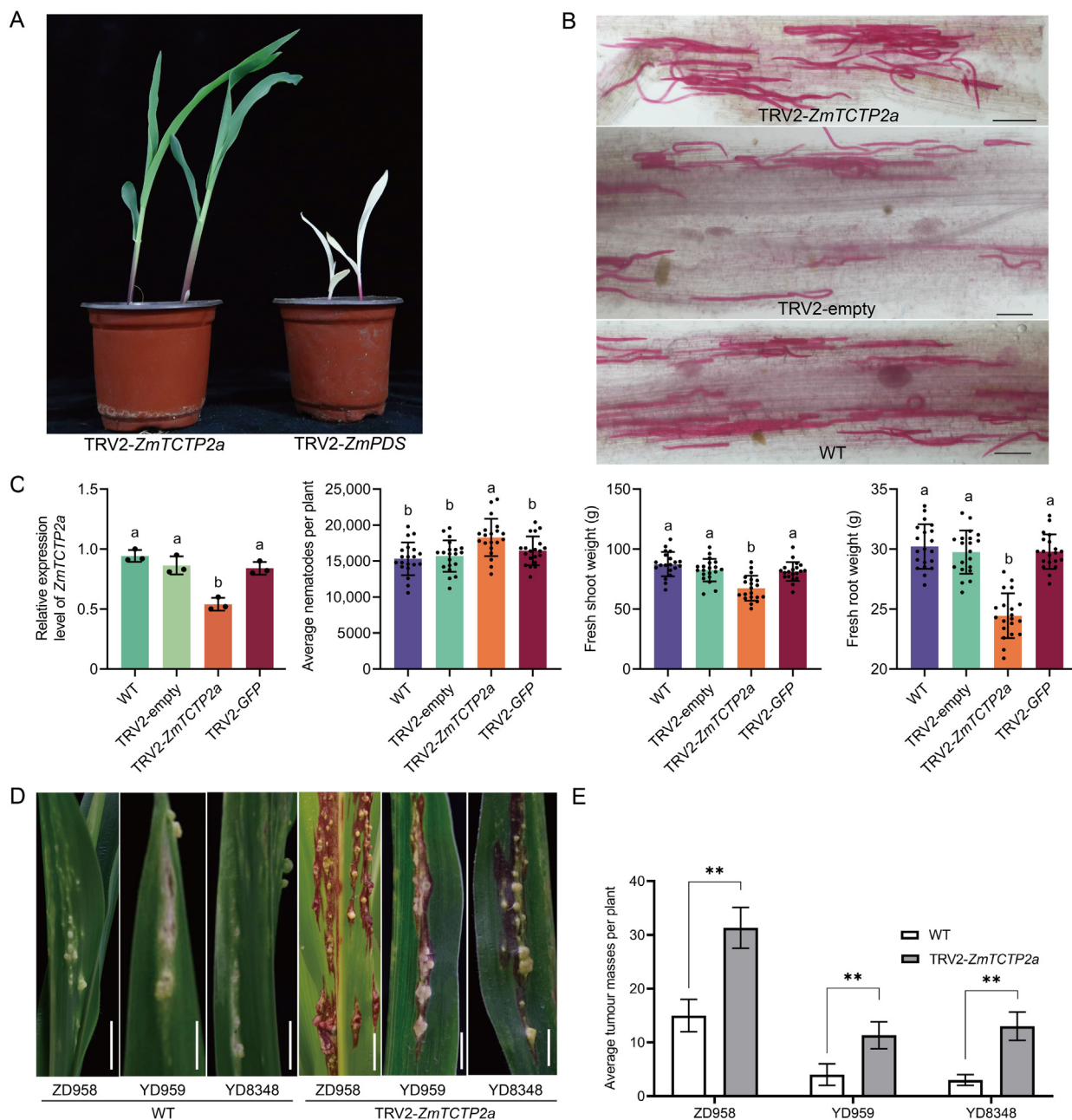


Fig. 6. Reduced expression of *ZmTCTP2a* in maize increased vulnerability to pathogen infection. (A) Maize phenotypes, treated with *A. tumefaciens* carrying TRV2-ZmPDS exhibiting a photobleaching phenotype. No significant phenotypic characteristics change was observed in the TRV2-ZmTCTP2a plants. (B) The population of *P. coffeae* in the roots of TRV2-ZmTCTP2a maize lines was increased significantly compared with WT. Scale bars, 200 μ m. (C) TRV-mediated RNAi of *PcENG3* in maize reduced the ability of *P. coffeae* infection. *ZmActin* was used as an internal control. GFP, green fluorescent protein. Values are means $\pm$ SD, with different letters indicating significant differences at $P < 0.05$ by one-way ANOVA. (D) Resistance to *U. maydis* was impeded in TRV2-ZmTCTP2a maize lines. ZD958, *U. maydis*-resistant maize varieties; YD959 and YD8348 are maize varieties that exhibit susceptibility to *U. maydis*. Scale bars, 2 cm. (E) Downregulation of *ZmTCTP2a* expression in maize increased susceptibility to *U. maydis*.

senescence [27]. In maize, we showed that silencing of *ZmTCTP2a* significantly impaired primary and lateral root development and led to reproductive abnormalities during late developmental stages, underscoring its importance in tissue-specific cell division and differentiation.

Beyond its developmental functions, TCTP has been increasingly recognized as a component of immune signaling [50]. Recent evidence suggests that TCTP can be secreted and may participate in defense responses. Notably, TCTP homologs from parasitic species such as *Plasmodium falciparum* and *Schistosoma mansoni* have been detected in the bloodstream of infected hosts, where they are associated with immune modulation and inflammatory responses [51].

In plants, *M. enterolobii* secretes a TCTP-like effector, MeTCTP, which is highly expressed in the dorsal esophageal gland and functions to suppress host programmed cell death, thereby facilitating parasitism [52]. This convergent strategy suggests that TCTP represents a valuable molecular target for parasitic organisms seeking to suppress host immunity. Our findings indicated that transgenic *Arabidopsis* plants expressing *PcENG3* showed lower ROS production and lower expression of PTI marker genes compared with WT plants after being treated with flg22. These changes correlated with increased susceptibility to *P. coffeae*, strongly suggesting that *PcENG3* interfered with immune responses. Likewise, *ZmTCTP2a* silencing in maize enhances susceptibility not only to *P. coffeae*

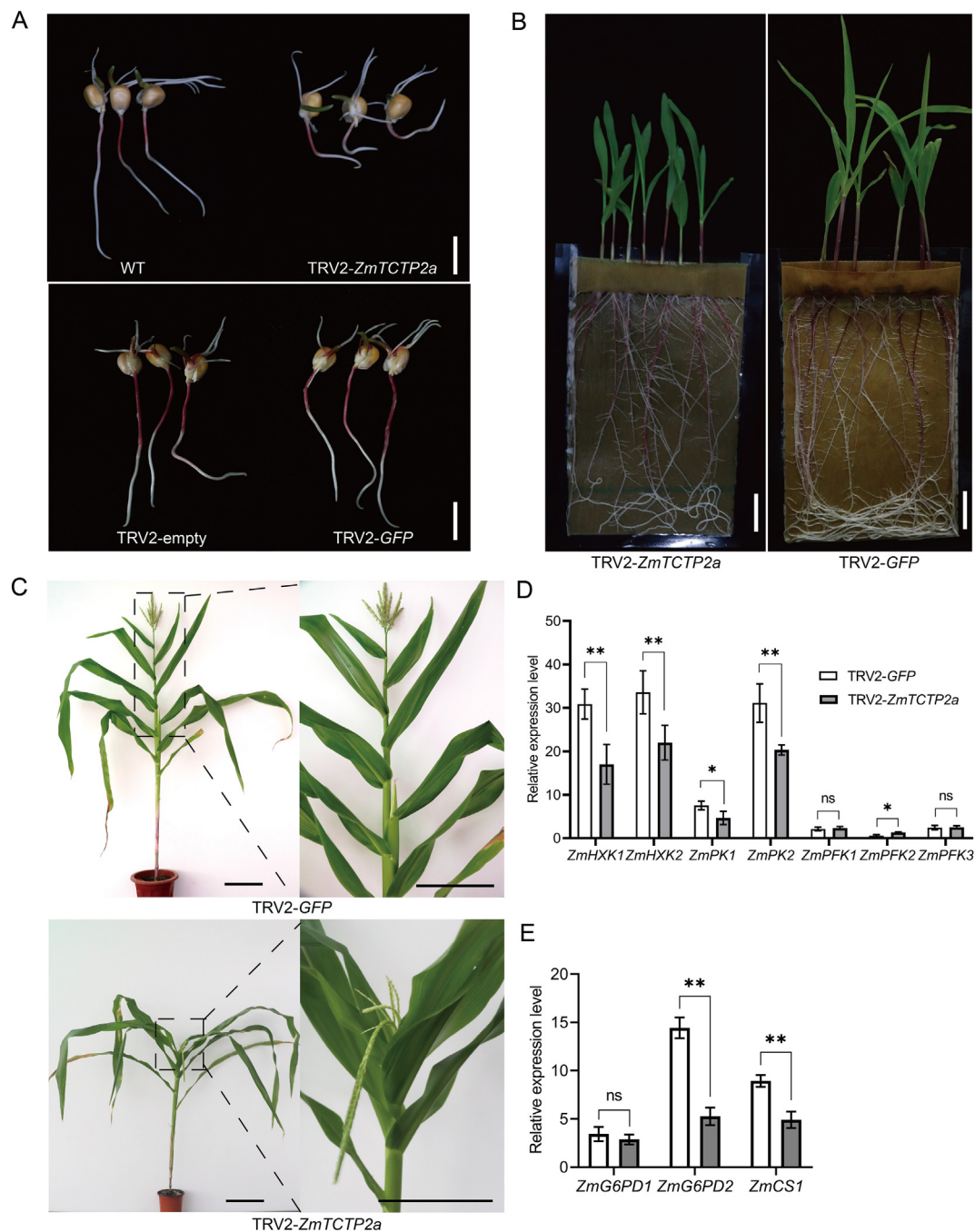


Fig. 7. *ZmTCTP2a* expression in maize regulates the development of root and modulates energy metabolism-related genes. (A) maize phenotypes, relative root length, and relative lateral root numbers of silenced expression of *ZmTCTP2a* maize lines compared with the controls. Scale bars, 1 cm. (B) The silencing of *ZmTCTP2a* expression in maize significantly reduced root length. Scale bars, 4 cm. (C) Morphology of TRV2-GFP and TRV2-ZmTCTP2a maize plants. Scale bars, 20 cm. (D) The expression level of glycolysis related genes in silenced expression of *ZmTCTP2a* maize lines. Expression levels were measured by reverse transcription real-time quantitative (RT-qPCR), and values are means $\pm$ SD. ns, not significant; *, $P < 0.05$; **, $P < 0.01$ by Student's *t*-test. (E) The expression level of the pentose phosphate pathway and the tricarboxylic acid related genes in silenced expression of *ZmTCTP2a* lines. Values are means $\pm$ SD. **, $P < 0.01$; ns, not significant by Student's *t*-test.

but also to *U. maydis*. These results revealed a previously uncharacterized role for TCTP in defense against diverse pathogens and suggested that nematodes may coopt host TCTP to create a permissive environment for infection. Similarly, the MjTTL5 effector from *M. javanica* has been shown to interact with the thioredoxin reductase catalytic subunit (AtFTRc) in *A. thaliana*, enhancing the scavenging of ROS to suppress PTI [53]. This modulation of the host redox state facilitates nematode infection and compromises plant resistance. *M. incognita* effectors calreticulin (MiCRT) have been shown to downregulate the expression of defense-related genes, thereby attenuating plant immune responses [54,55]. *M. incognita* effector

MiCE108 specifically targets the *A. thaliana* cysteine protease RD21A and promotes its degradation through the endosomal trafficking pathway or the 26S proteasome system. This targeted degradation leads to a reduction in RD21A protease activity, thereby attenuating plant immunity mediated by papain-like cysteine proteases (PLCPs) [56].

Furthermore, we showed that suppressed *ZmTCTP2a* expression affected key genes involved in energy metabolism, aligning with the regulatory roles of *PcENG3* and *ZmTCTP2a*. Recent studies suggest that pathogens frequently target energy metabolism to influence host development and susceptibility. For instance, *Botrytis*

cinerea alters glycolytic and PPP pathways in *A. thaliana* to promote tissue necrosis [57]. Similarly, *M. incognita* modulates root energy metabolism to establish feeding sites in hosts [58], and *Fusarium graminearum* in maize disrupts carbohydrate and amino acid metabolism to weaken resistance [59]. Furthermore, *Ralstonia solanacearum* alters metabolic gene expression in tomato, compromising vascular integrity [60]. Together with our results, these studies underscore the central role of energy metabolism in regulating host development and immunity. *ZmTCTP2a* may act as a molecular hub that integrates metabolic flux with defense, where its silencing disrupted this balance, promoting pathogen colonization and impairing growth.

In conclusion, this study identified PcENG3 as a novel effector from *P. coffeae* that modulated host cell function by suppressing *ZmTCTP2a*, thereby facilitating nematode parasitism. These findings contribute to our understanding of effector diversity in migratory nematodes and highlight a mechanism by which RLNs promote infection by targeting conserved regulators of development and immunity. Unraveling such interactions will not only improve our understanding of host-pathogen co-evolution but may also inform the development of new strategies for durable nematode resistance.

5. Conclusions

This study identified PcENG3, a novel β -1,4-endoglucanase effector from *P. coffeae*, as a critical virulence factor that facilitated nematode parasitism by targeting the maize protein *ZmTCTP2a*. *PcENG3* was specifically expressed in the nematode's esophageal glands and gonad, and was secreted during parasitic stages. Functional analyses demonstrated that *PcENG3* suppressed host immunity and enhanced susceptibility through direct interaction with *ZmTCTP2a*, a key regulator of cell proliferation and immune responses. Silencing *PcENG3* significantly impaired nematode infectivity, while transgenic expression of *PcENG3* in *A. thaliana* inhibited flg22-induced ROS production. Moreover, suppression of *ZmTCTP2a* in maize increased plant vulnerability to *P. coffeae* and other pathogens. These findings reveal an uncharacterized effector-host interaction mechanism and underscore how *P. coffeae* manipulates host cellular processes to establish infection. This study deepens our understanding of nematode pathogenesis and offers a promising target for developing nematode-resistant crops.

Accession numbers

The gene sequences referenced in this study are available from The *Arabidopsis* Information Resource (<https://www.arabidopsis.org/>) and the GenBank/EMBL database (<https://www.ncbi.nlm.nih.gov/genbank/>) under the following accession numbers: *AtActin2* (ES659082), *AtWRKY33* (AT2G38470), *AtWRKY30* (AT5G24110), *AtRBOHF* (AT1G64060), *Pc18s* (MG906739), *PcENG3* (PV575806), *ZmActin1* (J01238), *ZmPDS* (LOC542329), and *ZmTCTP2a* (AF548024).

CRedit authorship contribution statement

Shuo Wang: Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ke Wang:** Writing – original draft, Methodology, Formal analysis, Data curation. **Fankang Lin:** Methodology, Formal analysis, Data curation. **Wenlong Niu:** Software, Methodology, Data curation. **Abdelfattah A. Dababat:** Writing – review & editing, Software, Methodology, Data curation. **Yan Shi:** Methodology, Data curation. **Hongxia Yuan:** Supervision, Methodology, Formal analysis. **Yanhui Xia:** Software, Methodology, Formal analysis, Data

curation. **Honglian Li:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Yu Li:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data for this article can be found online at <https://doi.org/10.1016/j.cj.2025.10.013>.

References

- [1] J.T. Jones, A. Haegeman, E.G. Danchin, H.S. Gaur, J. Helder, M.G. Jones, T. Kikuchi, R. Manzanilla-López, J.E. Palomares-Rius, W.M. Wesemael, R.N. Perry, Top 10 plant-parasitic nematodes in molecular plant pathology, *Mol. Plant Pathol.* 14 (2013) 946–961.
- [2] D.G. Bartlem, M.G.K. Jones, U.Z. Hammes, Vascularization and nutrient delivery at root-knot nematode feeding sites in host roots, *J. Exp. Bot.* 65 (2014) 1789–1798.
- [3] B. Favery, M. Quentin, S. Jaubert-Possamai, P. Abad, Gall-forming root-knot nematodes hijack key plant cellular functions to induce multinucleate and hypertrophied feeding cells, *J. Insect Physiol.* 84 (2016) 60–69.
- [4] T. Hewezi, Cellular signaling pathways and posttranslational modifications mediated by nematode effector proteins, *Plant Physiol.* 182 (2020) 31–40.
- [5] E. Fatemi, C. Jung, Pathogenicity of the root lesion nematode *Pratylenchus neglectus* depends on pre-culture conditions, *Sci. Rep.* 13 (2023) 19642.
- [6] Y. Lu, S. Yang, W. Chen, H. Xie, C. Xu, Advances in migratory plant endoparasitic nematode effectors, *Int. J. Mol. Sci.* 25 (2024) 6435.
- [7] P. Castillo, N. Vovlas, *Pratylenchus* (Nematoda: Pratylenchidae): Diagnosis, Biology, Pathogenicity and Management, *Pratylenchus Diagnosis Biology Pathogenicity & Management*, Koninklijke Brill, NV, Leiden, the Netherlands, 2007.
- [8] Y. Li, J. Li, Y. Hu, K. Wang, H. Yuan, B. Sun, H.L. Li, B. Lei, Occurrence of root-lesion nematode *Pratylenchus coffeae* on corn in Xinjiang Uygur Autonomous Region, China, *Plant Dis.* 106 (2022) 3219.
- [9] P. Vieira, C. Gleason, Plant-parasitic nematode effectors-insights into their diversity and new tools for their identification, *Curr. Opin. Plant Biol.* 50 (2019) 37–43.
- [10] J. Zhao, Q. Sun, M. Quentin, J. Ling, P. Abad, X. Zhang, Y. Li, Y. Yang, B. Favery, Z. Mao, B. Xie, A *Meloidogyne incognita* C-type lectin effector targets plant catalases to promote parasitism, *New Phytol.* 232 (2021) 2124–2137.
- [11] J. Mejias, N.M. Truong, P. Abad, B. Favery, M. Quentin, Plant proteins and processes targeted by parasitic nematode effectors, *Front. Plant Sci.* 10 (2019) 970.
- [12] J. Zhao, K. Huang, R. Liu, Y. Lai, P. Abad, B. Favery, H. Jian, J. Ling, Y. Li, Y. Yang, B. Xie, M. Quentin, Z. Mao, The root-knot nematode effector Mi2G02 hijacks a host plant trihelix transcription factor to promote nematode parasitism, *Plant Commun.* 5 (2024) 100723.
- [13] Y. Chen, Q. Liu, X. Sun, L. Liu, J. Zhao, H. Jian, *Meloidogyne enterolobii* MeMSP1 effector targets the glutathione-S-transferase phi GSTF family in *Arabidopsis* to manipulate host metabolism and promote nematode parasitism, *New Phytol.* 240 (2023) 2468–2483.
- [14] L. Qin, J. Zhao, Q. Liu, X. Sun, H. Jian, An unconventionally secreted effector from the root-knot nematode *Meloidogyne incognita* suppresses plant immunity by disrupting the isochlorismate pathway for salicylic acid biosynthesis, *Mol. Plant Pathol.* 22 (2021) 1526–1539.
- [15] T. Wen, X. Wu, L. Hu, Y. Qiu, L. Rui, Y. Zhang, X. Ding, J. Ye, A novel pine wood nematode effector, BxSCD1, suppresses plant immunity and interacts with an ethylene-forming enzyme in pine, *Mol. Plant Pathol.* 22 (2021) 1399–1412.
- [16] S. Bali, C. Gleason, Unveiling the diversity: plant parasitic nematode effectors and their plant interaction partners, *Mol. Plant-Microbe Interact.* 37 (2024) 179–189.
- [17] S. Yang, Y. Dai, Y. Chen, J. Yang, D. Yang, Q. Liu, H. Jian, A novel G16B09-like effector from *Heterodera avenae* suppresses plant defenses and promotes parasitism, *Front. Plant Sci.* 10 (2019) 66.

- [18] Y. Yan, G. Smant, J. Stokkermans, L. Qin, J. Helder, T. Baum, A. Schots, E. Davis, Genomic organization of four beta-1,4-endoglucanase genes in plant-parasitic cyst nematodes and its evolutionary implications, *Gene* 220 (1998) 61–70.
- [19] A. Haegeman, B. Vanholme, J.T. Jones, *Meloidogyne* species effectors target plant cell walls, *Trends Plant Sci.* 17 (2012) 221–227.
- [20] M.G. Mitchum, R.S. Hussey, T.J. Baum, Plant parasitic nematode effectors, *Curr. Opin. Plant Biol.* 16 (2013) 440–448.
- [21] X. Wang, D. Meyers, Y. Yan, T. Baum, G. Smant, R. Hussey, E. Davis, *In planta* localization of a beta-1,4-endoglucanase secreted by *Heterodera glycines*, *Mol. Plant-Microbe Interact.* 12 (1999) 64–67.
- [22] E. Fanelli, A. Troccoli, E. Picardi, C. Pousis, F. De Luca, Molecular characterization and functional analysis of four β -1,4-endoglucanases from the root-lesion nematode *Pratylenchus vulnus*, *Plant Pathol.* 63 (2014) 1436–1445.
- [23] Q. Chen, S. Rehman, G. Smant, J.T. Jones, RNA interference in *Globodera rostochiensis*: silencing of cellulase genes impairs infectivity, *Mol. Plant-Microbe Interact.* 18 (2005) 621–625.
- [24] A. Haegeman, S. Mantelin, J.T. Jones, G. Gheysen, Functional roles of effectors of plant-parasitic nematodes, *Gene* 492 (2012) 19–31.
- [25] M. Tuynder, G. Fiucci, S. Prieur, A. Lespagnol, A. Géant, S. Beaucourt, D. Dufaut, S. Besse, L. Susini, J. Cavarelli, D. Moras, R. Amson, A. Telerman, Translationally controlled tumor protein is a target of tumor reversion, *Proc. Natl. Acad. Sci. U. S. A.* 101 (2004) 15364–15369.
- [26] U.A. Bommer, B.J. Thiele, The translationally controlled tumour protein (TCTP), *Int. J. Biochem. Cell Biol.* 36 (2004) 379–385.
- [27] F. Brioude, A.M. Thierry, P. Chambrier, B. Mollereau, M. Bendahmane, Translationally controlled tumor protein is a conserved mitotic growth integrator in animals and plants, *Proc. Natl. Acad. Sci. U. S. A.* 107 (2010) 16384–16389.
- [28] Z.Q. Wang, G.Z. Li, Q.Q. Gong, G.X. Li, S.J. Zheng, OsTCTP, encoding a translationally controlled tumor protein, plays an important role in mercury tolerance in rice, *BMC Plant Biol.* 15 (2015) 123.
- [29] M. Guo, Q. Zhang, Z. Sun, The role of TCTP in reactive oxygen species-scavenging pathways and stress tolerance in plants, *Environ. Exp. Bot.* 189 (2022) 104550.
- [30] R. Reise, G. Jacob, Preparation of carrot callus tissues for plant regeneration, *Plant Cell Rep.* 6 (1987) 497–500.
- [31] D.T. Kaplan, C.A. Beasley, Extraction of nematodes from soil using a modified Baermann funnel technique, *J. Nematol.* 29 (1997) 100–103.
- [32] J. Schultz, F. Milpetz, P. Bork, C.P. Ponting, SMART: a simple modular architecture research tool, *Bioinformatics* 16 (2000) 849–850.
- [33] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method, *Methods* 25 (2001) 402–408.
- [34] X. Zhang, R. Henriques, S.S. Lin, Q.W. Niu, N.H. Chua, *Agrobacterium*-mediated transformation of *Arabidopsis thaliana* using the floral dip method, *Nat. Protoc.* 1 (2006) 641–646.
- [35] Y. Kadota, K. Shirasu, C. Zipfel, Regulation of the NADPH oxidase RBOHD during plant immunity, *Plant Cell Physiol.* 56 (2015) 1472–1780.
- [36] M. Gu, X. Li, Z. Zhang, Yeast secretion assay for studying protein secretion in *Saccharomyces cerevisiae*, *Yeast* 28 (2011) 423–433.
- [37] W. Zhang, H. Li, L. Wang, S. Xie, Y. Zhang, R. Kang, M. Zhang, P. Zhang, Y. Li, Y. Hu, M. Wang, L. Chen, H. Yuan, S. Ding, H. Li, A novel effector, CsSp1, from *Bipolaris sorokiniana*, is essential for colonization in wheat and is also involved in triggering host immunity, *Mol. Plant Pathol.* 23 (2022) 218–236.
- [38] S. Devanapally, S. Ravikumar, A.M. Jose, Double-stranded RNA made in *C. elegans* neurons can enter the germline and cause transgenerational gene silencing, *Proc. Natl. Acad. Sci. U. S. A.* 112 (2015) 2133–2138.
- [39] N. Fernandez-Pozo, N. Menda, J.D. Edwards, S. Saha, I.Y. Tecle, S. Strickler, L.A. Mueller, The Sol Genomics Network (SGN)-from genotype to phenotype to breeding, *Nucleic Acids Res.* 43 (2015) D1036–D1041.
- [40] J. Zhang, D. Yu, Y. Zhang, K. Liu, K. Xu, F. Zhang, J. Wang, G. Tan, X. Xie, Q. Ji, L. Zhao, C. Li, Vacuum and Co-cultivation agroinfiltration of (germinated) seeds results in Tobacco Rattle Virus (TRV) mediated whole-plant virus-induced gene silencing (VIGS) in wheat and maize, *Front. Plant Sci.* 8 (2017) 393.
- [41] T. Hewezi, T.J. Baum, Manipulation of plant cells by cyst and root-knot nematode effectors, *Mol. Plant-Microbe Interact.* 26 (2013) 9–16.
- [42] M. Holterman, A. Karegar, P. Mooijman, H. van Megen, S. van den Elsen, J. Helder, Disparate gain and loss of parasitic abilities among nematode lineages, *PLoS ONE* 12 (2017) e0185445.
- [43] X. Wang, M.G. Mitchum, B. Gao, C. Li, H. Diab, T.J. Baum, R.S. Hussey, E.L. Davis, A parasitism gene from a plant-parasitic nematode with function similar to CLAVATA3/ESR (CLE) of *Arabidopsis thaliana*, *Mol. Plant Pathol.* 6 (2005) 187–191.
- [44] G. Smant, J.P.W.G. Stokkermans, Y. Yan, J.M. de Boer, T.J. Baum, X. Wang, J. Helder, Endogenous cellulases in animals: Isolation of β -1,4-endoglucanase genes from two species of plant-parasitic cyst nematodes, *Proc. Natl. Acad. Sci. U. S. A.* 95 (1998) 4906–4911.
- [45] A. Haegeman, J.T. Jones, E.G. Danchin, Horizontal gene transfer in nematodes: a catalyst for plant parasitism?, *Mol. Plant-Microbe Interact.* 24 (2011) 879–887.
- [46] T. Kikuchi, J.T. Jones, T. Aikawa, H. Kosaka, N. Ogura, A family of glycosyl hydrolase family 45 cellulases from the pine wood nematode *Bursaphelenchus xylophilus*, *FEBS Lett.* 572 (2004) 201–205.
- [47] P. Abad, J. Gouzy, J. Aury, M. Castagnone-Sereno, P. Danchin, E.G. Deleury, P. Wincker, Genome sequence of the metazoan plant-parasitic nematode *Meloidogyne incognita*, *Nat. Biotechnol.* 26 (2008) 909–915.
- [48] M.G. Thomas, G.J. Johannes, C.D. Stiles, Identification of a growth-related, translationally controlled protein in mouse fibroblasts, *Proc. Natl. Acad. Sci. U. S. A.* 78 (1981) 5712–5716.
- [49] Y. Yang, F. Yang, Y. Xiong, Cell growth and apoptosis regulation by TCTP in plants and animals, *Cell Res.* 15 (2005) 274–280.
- [50] N. Amzallag, B.J. Passer, D. Allan, E. Segura, C. Thery, B. Goud, A. Telerman, TSAP6 facilitates the secretion of translationally controlled tumor protein via exosomes, *Nat. Cell Biol.* 6 (2004) 603–609.
- [51] S.M. MacDonald, T. Rafnar, J. Langdon, L.M. Lichtenstein, Molecular identification of an IgE-dependent histamine-releasing factor, *Science* 269 (2010) 688–690.
- [52] K. Zhuo, J. Chen, B. Lin, J. Wang, F. Sun, J. Liao, A *Meloidogyne enterolobii* effector MeTCTP suppresses programmed cell death to promote parasitism, *Front. Microbiol.* 8 (2017) 1711.
- [53] B. Lin, K. Zhuo, S. Chen, L. Hu, L. Sun, X. Wang, L.H. Zhang, J. Liao, A novel nematode effector suppresses plant immunity by activating host reactive oxygen species-scavenging system, *New Phytol.* 209 (2016) 1159–1173.
- [54] S. Jaubert, J.B. Laffaire, P. Abad, M.N. Rosso, A polygalacturonase of animal origin isolated from a plant parasitic nematode, *FEBS Lett.* 579 (2005) 4101–4106.
- [55] M. Jaouannet, M. Magliano, M.J. Arguel, M. Gourgues, E. Evangelisti, P. Abad, M. N. Rosso, The root-knot nematode calreticulin Mi-CRT is a key effector in plant defense suppression, *Mol. Plant-Microbe Interact.* 26 (2013) 97–105.
- [56] J. Yu, Q. Yuan, C. Chen, T. Xu, Y. Jiang, W. Hu, A. Liao, J. Zhang, X. Le, H. Li, X. Wang, A root-knot nematode effector targets the *Arabidopsis* cysteine protease RD21A for degradation to suppress plant defense and promote parasitism, *Plant J.* 118 (2024) 1500–1515.
- [57] W. Zhang, F. Zhao, L. Jiang, C. Chen, L. Wu, Z. Liu, S. Xu, *Botrytis cinerea* induces metabolic shifts in *Arabidopsis thaliana* that promote disease susceptibility, *Plant Cell Environ.* 42 (2019) 3262–3276.
- [58] H. Zhang, Y. Zhao, Q. Sun, Y. Yang, H. Peng, Root-knot nematode *Meloidogyne incognita* rewires host root metabolism to support feeding site development, *Mol. Plant Pathol.* 25 (2024) 145–158.
- [59] Y. Sun, C. Wang, Y. Wang, H. Liu, B. Li, *Fusarium graminearum* reprograms primary metabolism in maize to impair host resistance, *Plant Physiol. Biochem.* 167 (2021) 702–710.
- [60] M. Shi, T. Zhou, X. Zhang, Y. Wang, Q. Li, *Ralstonia solanacearum* interferes with tomato vascular metabolism to promote wilt disease, *New Phytol.* 237 (2023) 1346–1358.

外文论文中文摘要

成果（论文）3:

3. Gao Lingling, Lv Yunlong, Zhang Can, Niu Wenlong, Li Honglian, **Li Yu**, Wang Ke*. Discovery of *Pratylenchus zae* causing root rot of winter wheat in Anhui Province of China. Plant disease, 2025, 109(8), 1794.

摘要: 根腐线虫-玉米短体线虫 (*Pratylenchus zae*) 是一种严重危害多种粮食作物和经济作物中的植物病原线虫。小麦是中国安徽省最重要的粮食作物之一。本课题组在中国安徽省亳州市瑶阳县冬小麦田开展了根腐线虫调查。样本采集于小麦收获前约 20 天。调查发现小麦植株生长不良，根部有明显褐色病斑。使用改良的曼漏斗分离根腐线虫。在 8 个采集样本中检测到根腐线虫，每克根部平均含 51 条线虫，每 100 立方厘米土壤中含有 128 条线虫。依据形态学和分子生物学特征确认该种群的形态特征和分子序列与玉米短体线虫相一致。形态学和分子数据均证实了 *P. zae* 的存在。致病力结果表明该线虫种群能有效感染并繁殖于该小麦品种上。此前仅在中国山西省报告过 *P. zae* 侵染冬小麦的情况。这是中国安徽省首次报道玉米短体线虫侵染冬小麦。由于 *P. zae* 可能严重危害冬小麦，应采取战略性措施防止该线虫扩散至其他地区。

Diseases Caused by Nematodes

Discovery of *Pratylenchus zae* Causing Root Rot of Winter Wheat in Anhui Province of China

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Root-lesion nematode (RLN), *Pratylenchus zae* Graham, 1951, is a serious nematode pest in a number of agronomic and industrial crops (Liu et al. 2017). Wheat (*Triticum aestivum*) is one of the most important food crops in Anhui Province of China. In May 2023, a survey was conducted for RLNs in winter wheat fields in Woyang County of Bozhou City, Anhui Province, China. The samples were collected approximately 20 days before wheat (cv. Gushenmai 19) harvest. The plants were growing poorly, with distinct brown lesions on the wheat roots. The RLNs were extracted with the modified Baermann funnel apparatus (Hooper et al. 2005). The RLNs were found in eight collected samples, an average of 51 nematodes per gram of root and 128 nematodes per 100 cm³ of soil. The extracted RLNs were disinfected with 0.3% streptomycin sulfate, and one individual female nematode was cultured on carrot disks at 25°C for propagation (Wang et al. 2021). The RLN species identification was based on morphological and molecular criteria. The main morphological measurements of adult females ($n = 15$) included body length = 490.5 μm (mean) $\pm$ 13.1 (SD) (range = 471.3 to 523.1 μm), stylet length = 16.1 μm $\pm$ 0.7 (14.0 to 16.9 μm), tail length = 27.3 μm $\pm$ 5.3 (18.9 to 34.1 μm), $a = 24.8 \pm 1.8$ (21.9 to 27.8), $b = 5.2 \pm 0.3$ (4.5 to 5.9), $c = 17.8 \pm 1.9$ (14.9 to 20.3), and two annules on the lip region. No males were found in the specimens. The morphological characters of this population are consistent with *P. zae* as described previously

(Castillo and Vovlas 2007). Furthermore, the individual female was used for the molecular identification, and the DNA was extracted as described previously (Wang et al. 2011). The primers D2A/D3B (Subbotin et al. 2006) and TW81/AB28 (Vovlas et al. 2011) were used to amplify the rDNA 28S D2-D3 region and the rDNA-internal transcribed spacer (ITS) region, respectively. The PCR products were sequenced, and the newly obtained sequences of the rDNA 28S D2-D3 region (782 bp) and the rDNA-ITS region (669 bp) were submitted to NCBI. The obtained 28S D2-D3 region sequence (GenBank accession nos. PQ859281 and PV083139) had 100% identity with *P. zae* sequences available from GenBank (KY424256 and KY424263). The obtained ITS sequences in this study (PQ857687 and PV089700) had more than 98% identity with *P. zae* sequences available from GenBank (OP456372 and OP466367). Both morphological and molecular data confirmed the presence of *P. zae*. To validate the reproductive capacity of the RLNs on winter wheat, a greenhouse experiment was conducted at 25°C, following the modified Koch's postulate protocol. Wheat plants were grown in eight 1.5-liter pots, and each with three plants (cv. Gushenmai 19) was inoculated with 1,000 *P. zae*. Two months after inoculation, the wheat roots were washed, and brown lesions were observed. RLNs in the soil and roots were extracted as previously described, and the population was found to have increased substantially. The average of final number of nematodes per pot was approximately 1,859 in soil and 793 in roots. The reproduction factors (final population/initial population) were greater than 1 (mean 2.65). Those results confirmed that this nematode population infects and reproduces well on this wheat cultivar. *P. zae* has only been reported on winter wheat in Shanxi Province of China (Liu and Liu 2007). To our knowledge, this is the first report of *P. zae* infecting winter wheat in Anhui Province of China. Since *P. zae* can cause considerable damage to winter wheat, strategic measures should be taken to prevent the spread of *P. zae* to other regions.

References:

- Castillo, P., and Vovlas, N. 2007. *Pratylenchus* (Nematoda: Pratylenchidae): Diagnosis, Biology, Pathogenicity and Management. Koninklijke Brill NV, Leiden, the Netherlands.
- Hooper, D. J., et al. 2005. Page 53 in: Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. 2nd ed. CABI, Wallingford, U.K.
- Liu, X., et al. 2017. Eur. J. Plant Pathol. 148:435.
- Liu, X. Y., and Liu, W. Z. 2007. J. Qingdao Agric. Univ. 24:175.
- Subbotin, S. A., et al. 2006. Nematology 8:455.
- Vovlas, N., et al. 2011. Plant Pathol. 60:762.
- Wang, J. L., et al. 2011. Plant Quar. 25:32.
- Wang, K., et al. 2021. Plant Dis. 105:1227.

The author(s) declare no conflict of interest.

Keywords: cereals and grains, nematodes, pathogen detection

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外文论文中文摘要

成果（论文）4:

4. Wang Shuo, Niu Wenlong, Wang Ke, Xu Feifei, Dababat. Abdelfattah A, Shi Yan, Yuan Hongxia, Wang Hua, Xia Yanhui, Li Honglian, **Li Yu***(通讯作者). *Pratylenchus coffeae* effector Pc-ENG2 targets the maize Chitinase ZmCHI5 to promote parasitism. Journal of Agricultural and Food Chemistry, 2025, 73(43), 27435–27449.

摘要：咖啡短体线虫（*Pratylenchus coffeae*）是一种具有重要致病性的根部病原线虫，其分泌效应蛋白以调控宿主生理过程并促进寄生。本研究对 PcENG2 进行了研究，该蛋白为一种 β -1,4-内切葡聚糖酶，定位在线虫食道腺细胞中，并在感染过程中被分泌到植物组织。PcENG2 的表达在 *P. coffeae* 早期寄生阶段显著上调。通过免疫定位和免疫电子显微镜分析表明，PcENG2 可促进细胞壁和糊精的降解。此外，在拟南芥中异位表达时，PcENG2 还抑制了宿主活性氧（ROS）的产生，并增强了宿主对 *P. coffeae* 的易感性。PcENG2 能直接与玉米（*Zea mays*）中的几丁质酶相互作用，而几丁质酶是关键防御相关蛋白。这种相互作用可抑制几丁质酶的酶活性，从而保护线虫卵免受降解。病毒诱导的基因沉默（VIGS）实验表明，ZmCHI5 在玉米中对线虫具有广谱抗性。综上所述，这些发现表明 PcENG2 是一种关键效应蛋白，能够调节宿主免疫应答，从而促进 *P. coffeae* 的侵染。

Pratylenchus coffeae Effector PcENG2 Targets the Maize Chitinase ZmCHI5 to Promote Parasitism

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ABSTRACT: *Pratylenchus coffeae*, a damaging root-lesion nematode, secretes effectors to manipulate host processes and promote parasitism. Here, we characterize PcENG2, a β -1,4-endoglucanase localized in the nematode esophageal gland cells and secreted during infection. PcENG2 expression is upregulated during the early parasitic stage of *P. coffeae*. PcENG2 promotes cell wall and callose degradation, as demonstrated by immunolocalization and immunoelectron microscopy. It also suppresses host reactive oxygen species (ROS) production and enhances susceptibility to *P. coffeae* when ectopically expressed in *Arabidopsis*. PcENG2 directly interacts with maize (*Zea mays* L.) chitinase, a key defense-related protein. This interaction suppresses chitinase enzymatic activity and protects nematode eggs from degradation. Virus-induced gene silencing (VIGS) demonstrated that ZmCHI5 is essential for broad-spectrum nematode resistance in maize. Collectively, these findings identify PcENG2 as a key effector that modulates host immunity to promote *P. coffeae* infection.

KEYWORDS: *Pratylenchus coffeae*, effector, maize chitinase, interaction, plant immunity

1. INTRODUCTION

Plant–parasitic nematodes (PPNs) are major threats to global agriculture, causing estimated annual losses exceeding US\$150 billion.¹ Recent changes in agricultural practices and environmental conditions, including global warming, water scarcity, and continuous straw return, have exacerbated their impact on crop production.² Among them, root-lesion nematodes (RLNs) are highly prevalent and cause substantial economic damage, which has likely been underestimated.³

During migration and feeding, RLNs secrete a suite of effector proteins into host cells via their stylets. These effectors facilitate nematode invasion, migration, and suppression of host immune responses.^{4,5} Among these effectors, β -1,4-endoglucanases (ENGs) are key cell wall-degrading enzymes that enable nematode entry and migration by breaking down plant cell walls.^{6,7} The plant cell wall serves the first physical barrier against pathogen invasion, and its enzymatic degradation is prerequisite for successful parasitism.^{3,8}

Molecular cloning and characterization of ENGs in several migratory and sedentary nematodes have revealed structural and functional diversity. For instance, *Pratylenchus penetrans* expresses two ENGs (*Pp-eng-1* and *Pp-eng-2*), differing in the presence of a cellulose-binding domain (CBD), reflecting potential functional specialization.⁹ Similarly, two ENGs from *Ditylenchus destructor* (*Dd-eng-1a* and *Dd-eng-2*) were shown to be secreted from subventral pharyngeal glands and display strong cellulase activity. Notably, silencing these genes significantly reduced nematode infectivity, underscoring their crucial roles in parasitism.¹⁰ These findings highlight the conserved yet diversified nature of ENGs in plant–parasitic nematodes and provide the rationale for investigating specific ENG functions in *P. coffeae*.

ENGs are widely conserved and found in archaea, bacteria, fungi, insects, and animals.^{11,12} In RLNs, ENGs randomly cleave β -1,4-glycosidic bonds in cellulose, generating oligosaccharides that not only aid in physical penetration but also activate plant basal immunity, including pattern-triggered immunity (PTI) and reactive oxygen species (ROS) production.^{13,14} Although plant ROS signaling plays a central role in restricting nematode invasion, the mechanisms by which plant–parasitic nematodes suppress ROS-mediated defenses remain poorly defined.^{15,16} Notably, a recent study reported that an effector from *Bursaphelenchus xylophilus* inhibits host ROS accumulation by directly interacting with a class III peroxidase, highlighting a novel strategy for nematode immune suppression.¹⁷ Most effectors produced by plant–parasitic nematodes aid parasitism by countering plant defense mechanisms.¹⁸ For example, MiCTL1, a *Meloidogyne incognita* C-type lectin-like effector, has been shown to interact with plant catalases, thereby modulating the redox balance of host defense responses, thus promoting the establishment of parasitism.¹⁹

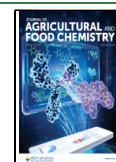
Plant chitinases, particularly class I and II glycoside hydrolase family 19 (GH19) enzymes, play a pivotal role in plant immunity by hydrolyzing chitin, a key structural component of fungal cell walls and certain nematode eggshells.²⁰ Several studies have shown that plant chitinases

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are upregulated upon nematode infection and contribute to resistance by degrading the chitin-rich structures of nematodes, such as the eggshell and cuticle.^{21–23} In *Arabidopsis*, overexpression of chitinases conferred enhanced resistance to cyst and root-knot nematodes.²² Conversely, some pathogens and parasitic nematodes have evolved effectors that interfere with chitinase-mediated defense. For instance, the fungal pathogen *Verticillium dahliae* secretes effectors that bind and inhibit plant chitinases to facilitate colonization.^{24,25} However, direct suppression of host chitinase activity by nematode effectors remains poorly understood.

PPNs have evolved highly sophisticated strategies to manipulate host cellular processes and suppress immune responses, thereby ensuring successful parasitism. A key aspect of this interaction is the secretion of effector proteins into host tissues via the nematode's stylet. These effectors operate through various mechanisms, including modulating host transcription, suppressing immune signaling, altering hormone balance, and remodeling cell walls and plasmodesmata.^{26,27} In response, host plants activate a range of defense mechanisms upon nematode invasion, such as PTI, effector-triggered immunity (ETI), cell wall reinforcement, and the production of ROS and antimicrobial proteins.^{28,29} However, nematodes can evade or suppress these defense responses through their effector proteins. For instance, some cyst and root-knot nematode effectors have been shown to directly interfere with MAP kinase signaling and salicylic acid biosynthesis, thus inhibiting the host's immune responses.^{27,30,31} While significant progress has been made in understanding the functions of effectors in sedentary nematodes, the molecular mechanisms by which migratory nematodes, such as *Pratylenchus* spp., manipulate host immunity remain largely unexplored.

While extensive research has focused on sedentary nematodes such as *Meloidogyne* and *Heterodera*, less is known about the effector-mediated strategies employed by migratory species like *P. coffeae*. In this study, we investigate the interaction between the ENG effector PcENG2 from *P. coffeae* and maize chitinase ZmCHI5, a key defense-related enzyme that targets nematode eggs and cuticles. We demonstrate that PcENG2 inhibits ZmCHI5 activity, thereby attenuating host defense responses and promoting nematode parasitism. These findings reveal a novel mechanism by which *P. coffeae* suppresses maize immunity, providing new insights into host-nematode molecular interactions.

2. MATERIALS AND METHODS

2.1. Nematodes and Plant Materials. The carrot callus tissue was prepared following previously described.³² Various populations of *P. coffeae* were subjected to surface disinfection with a 0.3% streptomycin sulfate solution for a period of 8 h, after which the samples were rinsed with double-distilled water. The nematode populations were then introduced onto the carrot callus tissue. Surface-sterilized maize (Zhengdan 958) seeds were planted into pots containing a mixture of soil and substrate (1:1) and cultivated under a 16 h light cycle at 22 °C. Seeds of *Arabidopsis thaliana* (ecotype Columbia, Col-0) were planted on MS medium plates (4.7 g/L Murashige & Skoog containing vitamins, 20 g/L sucrose, pH 5.7, 3.5 g/L Gelam Gum). *Nicotiana benthamiana* plants and *A. thaliana* cultivated under a 16 h light cycle at 22 °C.

2.2. Sequence Analysis. The PcENG2 sequence was retrieved from *P. coffeae* RNA-seq data. The identification of structural domains of the protein was achieved by the National Center for Biotechnology Information (NCBI) database (<https://www.ncbi.nlm.nih.gov>). Sequence alignment analysis was conducted using DNAMAN software.

2.3. RNA Extraction, cDNA Synthesis, and RT-qPCR. Total RNA was extracted from *P. coffeae* and maize was performed via the RNAiso Plus (Takara D9108A, Kyoto, Japan). The synthesis of complementary DNA (cDNA) using Prime Script Reverse Transcriptase (Takara, Tokyo, Japan), and real-time quantitative PCR (RT-qPCR) were conducted with the SuperPrep II Cell Lysis & RT Kit (TOYOBO SCQ-501, Osaka, Japan). The subsequent analysis of the data was conducted utilizing the 2^{−ΔΔC_t} method.³³ *P. coffeae* Pc18s (MG906739), maize ZmActin (J01238) or *Arabidopsis* AtActin2 (ES659082) served as internal reference genes. Each reaction comprised at least three biological replicates, with each including four technical repeats. A comprehensive list of primers utilized in this study is provided in Table S3.

2.4. In Situ Hybridization and Immunolocalization of *P. coffeae* on Maize. The PcENG2 fragment was generated through PCR amplification using specific primers (Table S3). The *in situ* hybridization experiments was conducted on *P. coffeae* following previously described.^{19,34} A polyclonal antibody targeting PcENG2 was produced by ABclonal Biotechnology (Wuhan, China) using 75–259 aa of the complete protein sequence, excluding the signal peptide. The specificity of the anti-PcENG2 antibody was evaluated through the implementation of western blot analysis. After a 2 day infection of maize roots with *P. coffeae*, the roots were subjected to paraffin embedding, sectioning, dewaxing, and hydration procedures.¹⁹ The tissue sections were subsequently treated with 3% BSA (Solarbio, Beijing, China) for 30 min. The maize root sections were subsequently incubated 2 h at 15 °C with an anti-PcENG2 antibody (diluted 1:300). The sections were incubated with CY3-conjugated goat anti-rabbit IgG (H + L) (diluted 1:200) (ABclonal Biotechnology, Wuhan, China) at 25 °C for 1 h. The sections were subsequently rinsed 1 min three times with sterile water and incubated with DAPI (Solarbio, Beijing, China) in the dark. Imaging was performed via confocal microscopy (Nikon A1HD2S, Tokyo, Japan) and scanner (Pannoramic MIDI, Budapest, Hungary).

2.5. PcENG2 Transgenic *Arabidopsis* Plants. The PcENG2 coding sequence lacking signal peptide was cloned into the modified pEarleyGate 202 binary vector to construct pEarleyGate 202-PcENG2. The construct was introduced into *Agrobacterium tumefaciens* strain GV3101 and used to transform wild-type (WT) *A. thaliana* ecotype Columbia-0 (Col-0) following the floral dip method.³⁵ The selection of transgenic plants was conducted on MS plates containing BASTA.³¹ Homozygous T3 seeds of transgenic *Arabidopsis* lines were verified by PCR. To assess the effect of PcENG2 expression on nematode infection, *Arabidopsis* lines were inoculated with 200 *P. coffeae*. The roots were gently washed, collected, and nematode populations were quantified at 35 days post-inoculation (dpi). Each infection assay included over 20 replicate plants, with three independent replicates conducted.

2.6. PTI Assays. For the reactive oxygen species detection assay, four-week-old *A. thaliana* seedlings were treated with 1 μM flg22 and carried out in a manner consistent with the luminol-based method that had previously been described.³⁶ For RNA extraction, *A. thaliana* seedlings treated with flg22 were prepared as described above. The transcript abundances of AtWRKY33 (AT2G38470), AtWRKY30 (AT5G24110), and AtRBOHF (AT1G64060) were assessed by RT-qPCR.

2.7. Virus-Induced Gene Silencing (VIGS) of PcENG2. The tobacco rattle virus RNA 2 (TRV2) vector was used to clone cDNA fragments specific for silencing PcENG2 and ZmPDS, as described previously.³⁷ The coding fragments of PcENG2 were amplified and inserted into TRV2 to generate the TRV2-PcENG2 construct and then transformed into the *A. tumefaciens* strain C58C1. Maize seeds were surface sterilized and removed seed coats gently. Subsequently, an equivalent volume of *A. tumefaciens* containing a mixture of TRV1 and TRV2 with corresponding fragments was infiltrated into maize seeds. The mixture was then incubated at 100 rpm in the dark for 18 h. Individual maize plants were then inoculated with 1000 *P. coffeae*. After 60 days, the abundance of *P. coffeae* in the maize roots and maize growth were assessed. The efficiency of the gene silencing was detected by performing RNA extraction from the maize roots in

different treatment groups via the TRIzol liquid nitrogen grinding method. Each treatment consisted of at least 20 maize plants with three biological replicates.

2.8. Subcellular Localization Assay. Target-specific primers (Table S3) were used to amplify the coding sequences of PcENG2 and ZmCHIS via PCR. The amplified sequences were then cloned into the pGWC initial vector and subsequently subjected to gateway recombination with pEarleyGate104-GFP. This process generated the constructs PcENG2-GFP and ZmCHIS-GFP, which were cloned into the *A. tumefaciens* strain GV3101. These constructs were transiently expressed in 4-week-old *N. benthamiana* leaves through agro-infiltration. After 48 h, the infiltrated leaves were imaged using a laser scanning confocal microscope (Nikon A1HD25, Tokyo, Japan).

2.9. Yeast Two-Hybrid (Y2H) Assay. The coding sequence of PcENG2 (lacking the signal peptide) was amplified and cloned into the pGBKT7 vector to serve as bait. This construct was introduced into the *Saccharomyces cerevisiae* Y2H Gold strain using the Matchmaker Gold Yeast Two-Hybrid (Y2H) System (Clontech, Palo Alto, CA, U.S.A.). Yeast cotransformation and interaction tests were performed as previously described.¹⁹ To identify potential interacting partners of PcENG2, a maize cDNA library was constructed from mRNA isolated from maize roots infected with *P. coffeae* at 2, 5, 8, and 10 dpi. The cDNA fragments were cloned into the pGADT7 vector and transformed into the *S. cerevisiae* Y187 strain using the Make Your Own "Mate & Plate" Library System (Clontech, Mountain View, CA, U.S.A.). This library was then used to screen for interactions with the PcENG2 bait construct in a Y2H assay.

2.10. Firefly Luciferase Complementation Imaging Assay. The coding sequences (CDS) of PcENG2 and ZmCHIS were amplified and inserted into pCambia1300-cLuc or pCambia1300-nLuc to generate PcENG2-nLuc and cLuc-ZmCHIS construct. The positive controls (SAR-cLuc and SGF-nLuc) were generated via the same method. These constructs were separately introduced into *A. tumefaciens* strain GV3101 and subsequently infiltrated into *N. benthamiana* leaves. The leaves were examined 2 days after being re-infiltrated with a 0.2 mM luciferin solution (Thermo Scientific, U.S.A.). Luciferase activity was assessed via a low-light cooled CCD imaging system (Berthold NightOWL II LB983, Bad Wildbad, Germany).

2.11. BiFC Assay. The complete coding sequence of PcENG2 lacking the signal peptide was subjected to amplification and subsequent cloning into the pGWC initial vector, followed by gateway recombination with pEarleyGate201-nYFP to create the PcENG2-nYFP construct. ZmCHIS was cloned into pEarleyGate201-cYFP to produce the cYFP-ZmCHIS construct. The GV3101 strain was used for the transformation of constructs and was transiently expressed in *N. benthamiana* leaves by agroinfiltration. After 48 h, YFP fluorescence was assessed using a laser scanning confocal microscope (Nikon A1HD25, Tokyo, Japan). YFP fluorescence was collected at wavelengths ranging from 525–570 nm.

2.12. Pull-Down Assay. The coding sequences of PcENG2 and ZmCHIS were amplified and inserted into pET-32a or pGEX-4T-1 to generate PcENG2-His and ZmCHIS-GST construct. All the constructs were transformed individually into the *Escherichia coli* strain Rosetta2 (DE3) for protein expression. Combined and incubated purified ZmCHIS-GST and PcENG2-His in 1× TBS buffer supplemented with 1 mM PMSF at 4 °C for 1 h. Next, added 20 μ L of glutathione magnetic beads (Yeasen Biotech, Shanghai, China) to each sample and further incubated the samples at 4 °C for 1 h. The samples were boiled individually for 10 min in sodium dodecyl sulfate (SDS) sample loading buffer. The eluted proteins were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and subsequently immunoblotting with anti-His and anti-GST antibodies (ABclonal Technology, Wuhan, China) in western blot analysis.

2.13. Generation of the ZmCHIS Transgenic Maize Plants and the Broad-Spectrum Resistance to *Pratylenchus* spp. Infection Assay. To assess the silencing efficiency of *ZmCHIS* in the maize lines, RT-qPCR was employed, following a similar methodology as previously described.³⁷ The maize lines were inoculated with

1000 *P. coffeae*. After 60 days, the nematode and various maize growth parameters, were collected and quantified. Each experimental treatment group consisted of more than 15 maize plants, and the experiment was independently replicated three times. The TRV-mediated *ZmCHIS* RNAi maize was inoculated with four species of nematodes: *P. coffeae*, *P. zeae*, *P. scribneri*, and *P. thornei*, to evaluate its resistance to infection. After 60 days, the nematodes were counted via the modified Baermann funnel method.

2.14. Immunogold Labeling for Transmission Electron Microscopy. Roots from maize plants were collected at 2 dpi. They were fixed in a solution containing 1.5% glutaraldehyde precooled 100 mM PBS (pH 7.4) for 3 h at 25 °C. After three 2 min washes in PBS, the samples were embedded in LR White resin (Sigma, U.S.A.). Ultrathin sections (70 nm) were sliced using an ultramicrotome (Leica EM UC-7, Wetzlar, Germany) and mounted on nickel grids. The grids were blocked with TBST containing 1% BSA for 1 h at 20 °C. Primary anti-PcENG2 antibodies (ABclonal Biotechnology, China; 1:500) were applied overnight incubation. Secondary goat antirabbit antibodies conjugated to 10 nm gold particles (Sigma G7402; 1:20) were subsequently incubated for 30 min, followed by TBST and double-distilled water washes. The samples were captured using a transmission electron microscope (Hitachi HT7800, Tokyo, Japan).

2.15. Scanning Electron Microscopy Analysis on the Ovicidal Activity of Chitinase. The *P. coffeae* eggs were collected from the carrot culture medium, rinsed with sterile water, and the impact of *ZmCHIS* on the eggs was assessed via a suspension containing 1000 eggs. The suspension was incubated with *ZmCHIS* expressed *in vitro* at 25 °C for 36 h.³⁸ To confirm the nematocidal activity of *ZmCHIS*, *ZmCHIS* was heated at 100 °C for 10 min followed by immersion in a 10 min ice bath, after which the nematode eggs were treated for 24 h and 48 h, and the hatching rate was observed under a dissecting microscope. To evaluate the impact of *ZmCHIS* on the development of the *P. coffeae* eggs, scanning electron microscopy (SEM) analysis was conducted following the steps of a previous method.³⁹ The eggs were fixed in glutaraldehyde at 4 °C, washed three 15 min times with 0.1 M phosphate buffer (pH 7.4). Next, the samples were fixed in 1% osmium tetroxide (Ted Pella, Inc.) in a 0.1 M phosphate buffer (PBS, pH 7.4) at room temperature for 2 h, followed by three 15 min washes with a 0.1 M PBS (pH 7.4). Dehydration was carried out using a gradient of ethanol samples (30, 50, 70, 90, 95, and 100%) for 15 min. The samples were dried in a critical point dryer (Quorum K850, East Sussex, England) and then sputter-coated with gold for 30 s on both sides of the conductive carbon film via an ion sputtering apparatus.³⁹ The dried samples were mounted on metallic stubs using carbon adhesive tabs and sputter-coated with gold for 30 s. Subsequently, photomicrographs were captured using a scanning electron microscope (Hitachi SU8100, Tokyo, Japan).

2.16. Protease Activity Assays. Protease activity assays were performed as described.⁴⁰ Protein concentrations were determined via a BCA protein quantification kit (Sangon Biotech, Shanghai, China). His-PcENG2 and boiled that were combined with GST-ZmCHIS, thoroughly mixed, respectively. To determine chitinase activity, 100 μ L of enzyme solution (0.1 mg/mL), citrate–disodium hydrogen phosphate buffer (PBS), and 2% colloidal chitin (pH 7.0) were separately reacted at 50 °C for 10 min. The reaction was terminated by boiling in a water bath and then rapidly cooled by ice–water mixture. Subsequently, dinitrosalicylic acid (DNS) reagent induced color development. The samples were then transferred to an enzyme plate, and absorbance was measured at 540 nm. For the blank control, PBS buffer was mixed with 2% colloidal chitin (pH 7.0) as the blank control. Chitinase activity was subsequently determined using a standard curve. Each experimental condition was performed in triplicate to ensure reproducibility and accuracy.

2.17. Protein Extraction, SDS–PAGE, and Western Blot Analysis. The freshly collected *N. benthamiana* and *A. thaliana* leaves were ground into a fine powder via liquid nitrogen. The lysate was prepared by adding a lysis buffer consisting of 60% SDS, 100 mM Tris–HCl, and 2% β -mercaptoethanol.⁴¹ Each protein sample was

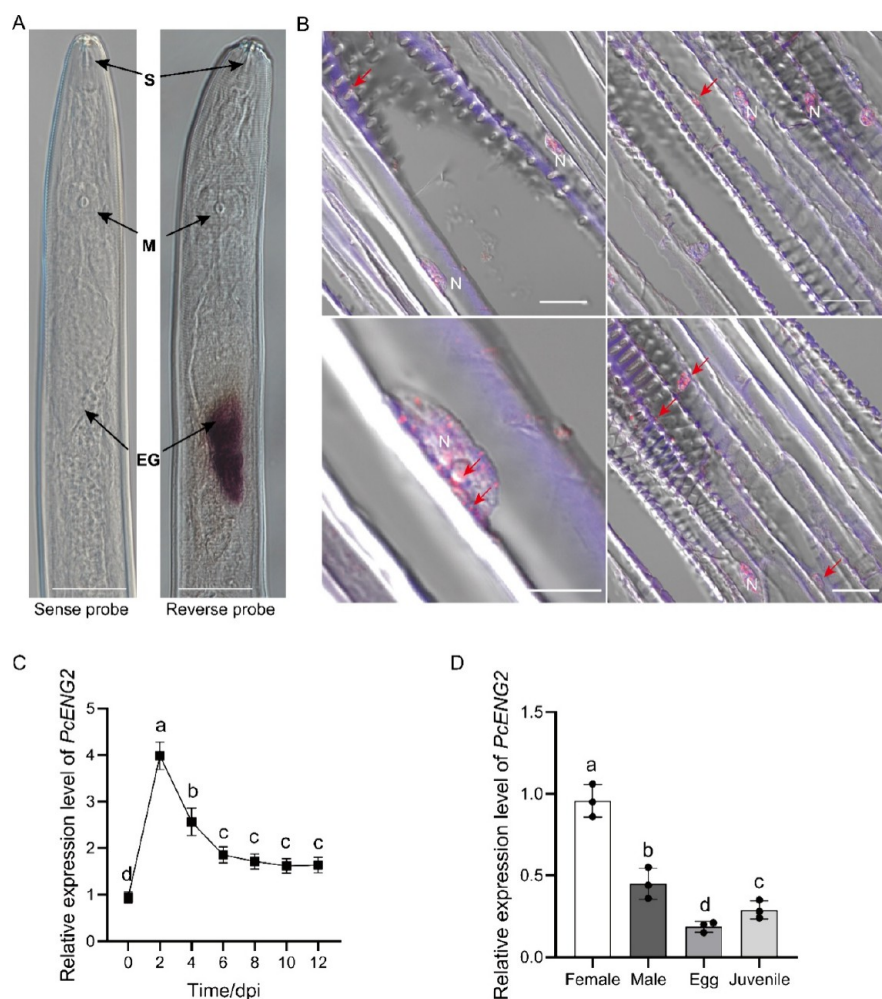


Figure 1. Localization and expression profile of the *P. coffeae* effector PcENG2. (A) *In situ* hybridization (ISH) showed PcENG2 transcripts expressed in the esophageal gland (EG) of *P. coffeae*. S, stylet; M, median bulb; EG, esophageal gland. Bars, 20 μ m. (B) PcENG2 localization was examined in sectioned maize roots and *P. coffeae* using immunolocalization with anti-PcENG2 antibody (1:200). Fluorescence was detected using a CY3-labeled goat anti-mouse IgG secondary antibody. The PcENG2 signal, indicated by red arrows, was observed in the nematode. N, nematode; Bars, 200 μ m. (C) Expression profile of PcENG2 in planta. The results of RT-qPCR were calculated via the $2^{-\Delta\Delta C_t}$ method. The standard deviation of three technical replicates and biological replicates was calculated, and the experiment was repeated three times. The bars represent the mean $\pm$ SE. Distinct letters represent statistically significant differences ($p < 0.05$, one-way ANOVA). (D) Expression profile of PcENG2 in the *P. coffeae*. The relative expression level of PcENG2 was quantified using RT-qPCR, and the fold-change values were calculated using the $2^{-\Delta\Delta C_t}$ method. The bars represent the means $\pm$ SE. Distinct letters represent statistically significant differences ($p < 0.05$, one-way ANOVA).

homogenized individually and then subjected to SDS–PAGE analysis. Detection was performed using specific primary antibodies, followed by incubation with secondary antibodies. The detection signal was visualized via an imaging apparatus (GE Amersham Imager 680RGB, Boston, U.S.A.).

2.18. Statistical Analysis. All data was performed via GraphPad Prism software version 8.0.2. To compare multiple groups against a control group, one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test was employed. Alternatively, pairwise comparisons were conducted via two-tailed *t* tests.

3. RESULTS

3.1. PcENG2 Is a Candidate Effector and Involved in *P. coffeae* Parasitism. PcENG2 (XC�70975) was identified as a putative secreted protein from *P. coffeae* RNA-seq data. Although initially uncharacterized as an effector, it shares more than 95% sequence identity with known ENGs from *P. penetrans* and *P. vulnus*. PcENG2 comprises 333 amino acids, including a 22-aa signal peptide and a conserved cellulase domain. To determine the expression location of PcENG2

mRNA in *P. coffeae*, *in situ* hybridization assays were performed to investigate the expression location of PcENG2 mRNA in *P. coffeae*. The results revealed a distinct hybridization signal was observed in the esophageal glands when the PcENG2 antisense strand RNA probe from PcENG2 was used, while no hybridization signal was observed with the sense strand RNA probe (Figure 1A).

To confirm PcENG2 secretion by *P. coffeae* into maize roots, immunolocalization was conducted on maize root sections using rabbit anti-PcENG2 polyclonal antibody (Figure S2D and Table S1). Western blot analysis showed the expected 37 kDa bands in infected roots (Figure S1). Immunohistochemical analysis on maize sections revealed PcENG2 fluorescence signals in the migrating *P. coffeae*. Furthermore, PcENG2 accumulation was observed along nematode migration channels and adjacent plant tissues (Figure 1B and Figure S2A and C). As anticipated, no signal was detected in planta when using preimmune serum (Figure S2B). These results

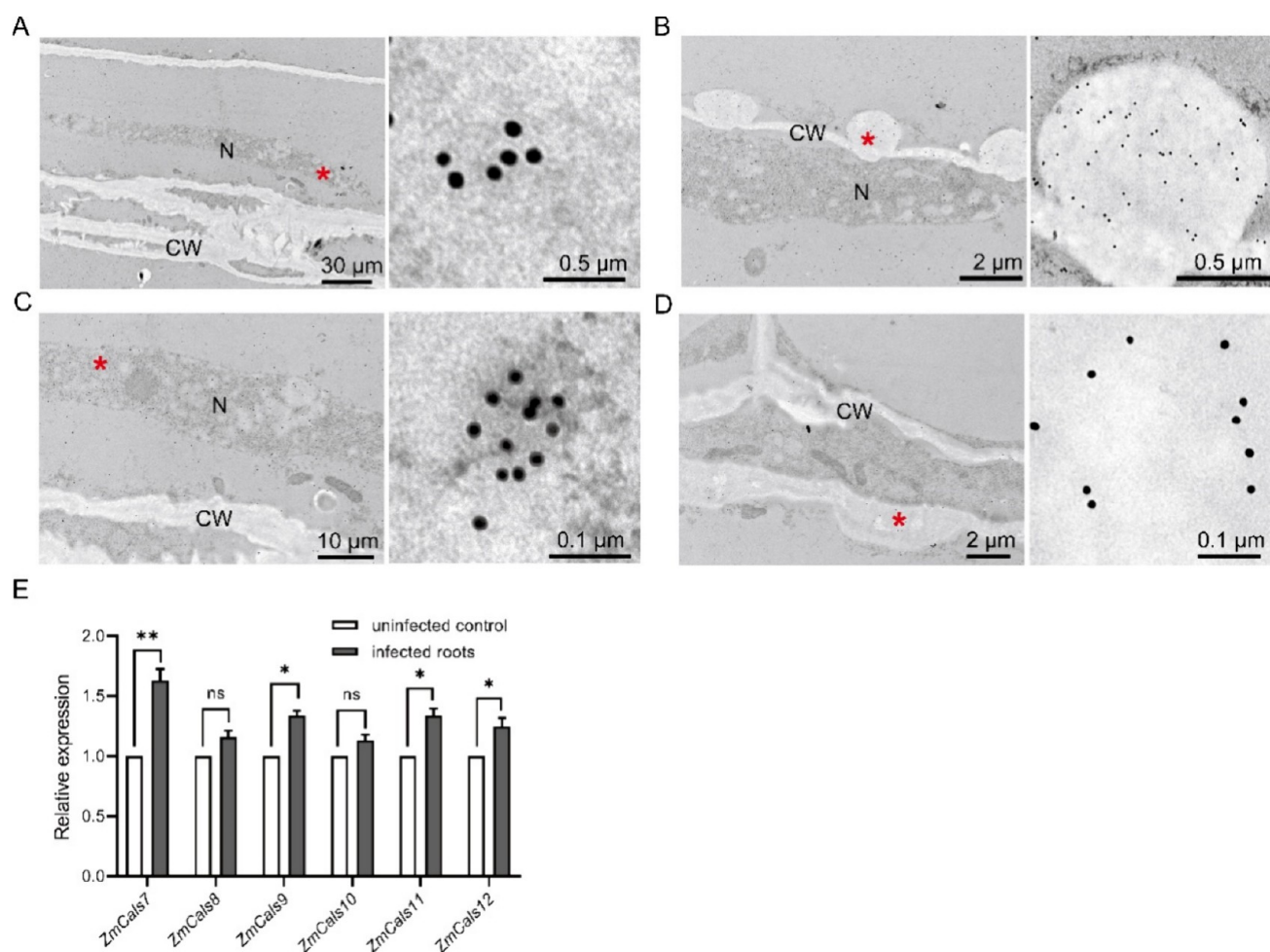


Figure 2. PcENG2 induces the degradation of cell wall and callose. (A) PcENG2 is detected throughout the entire body and notably accumulated in the esophageal gland (red asterisk indicated). (B) IEM showed PcENG2 is localized in callose (red asterisk indicated), which is distributed in the cell wall in a mastoid shape. Bar, 2 μ m, bars in the insets, 0.5 μ m. (C) PcENG2 is observed localized and is secreted through the cloaca (red asterisk indicated). CW, cell wall; N, nematode. Bar, 10 μ m, bars in the insets, 0.1 μ m. (D) PcENG2 distributed in thickened cell walls (red asterisk indicated); CW, cell wall. Bar, 2 μ m, bars in the insets, 0.2 μ m. (E) RT-qPCR analyzed the expression levels of maize *ZmCals* in both uninfected maize roots and roots infected with the RLNs, including *ZmCals7*–*ZmCals12* at 7 dpi. Bars represent the mean $\pm$ SD. Asterisks indicate significant differences compared to WT plants, as determined by Student's *t* test (*, $p < 0.05$; **, $p < 0.01$; ns, not significant).

indicate the secretion of the PcENG2 effector *in planta* during *P. coffeae* parasitism.

RT-qPCR analysis revealed that the transcriptional expression of *PcENG2* peaked at 2 dpi, followed by a gradual decline to a stable level (Figure 1C). In addition, *PcENG2* was expressed throughout all developmental stages of *P. coffeae*, with low expression observed in eggs and juvenile stages, and significantly higher expression detected in females (Figure 1D; $p < 0.05$). These results suggest that *PcENG2* is strongly induced during the early stages of host invasion and may play a critical role in nematode parasitism.

3.2. PcENG2 Is a *P. coffeae* Effector That Promotes Maize Cell Wall and Callose Degradation. Callose formation in the plant cell wall is induced by various biotic and abiotic stresses and is believed to contribute to the host plant's defense and immunity.^{42–44} Previous studies have reported the thickened cell wall and mastoid structures composed of callose act as a physical barrier to block the pathogen's penetration and spread.⁴⁵ This prevents the pathogen from accessing the plant's internal tissues and nutrients, thereby limiting its growth and reproduction. However, research on the functions of callose following *P.*

coffeae infection remains limited. Maize roots at 2 dpi were sectioned and subjected to immunoelectron microscopy (IEM) using an anti-PcENG2 antibody. The results showed that PcENG2 was predominantly localized in the esophageal glands of *P. coffeae* and was secreted through the cloaca and hypodermis along the nematode's body, excluding the stylet (Figure 2C and Figure S3A and B). Additionally, callose was found to be irregularly and asymmetrically distributed in plant cell walls. However, in the cell wall channels invaded by the nematode, callose was largely degraded (Figure 2B and Figure S3C and D). Consistent with the classic substrate specificity of β -1,4-endoglucanases, our *in vitro* assay using purified PcENG2 and laminarin (a β -1,3-glucan) showed no detectable hydrolytic activity (Figure S4). Consistently, PcENG2 signals were also detected extensively in thickened host cell walls (Figure 2D), suggesting its role in host cell wall modification. To further explore host responses, we examined the expression levels of maize callose synthase genes (*ZmCals*) in both uninfected and *P. coffeae*-infected roots. RT-qPCR analysis revealed a significant upregulation of *ZmCals* transcripts in infected roots compared to uninfected controls (Figure 2E),

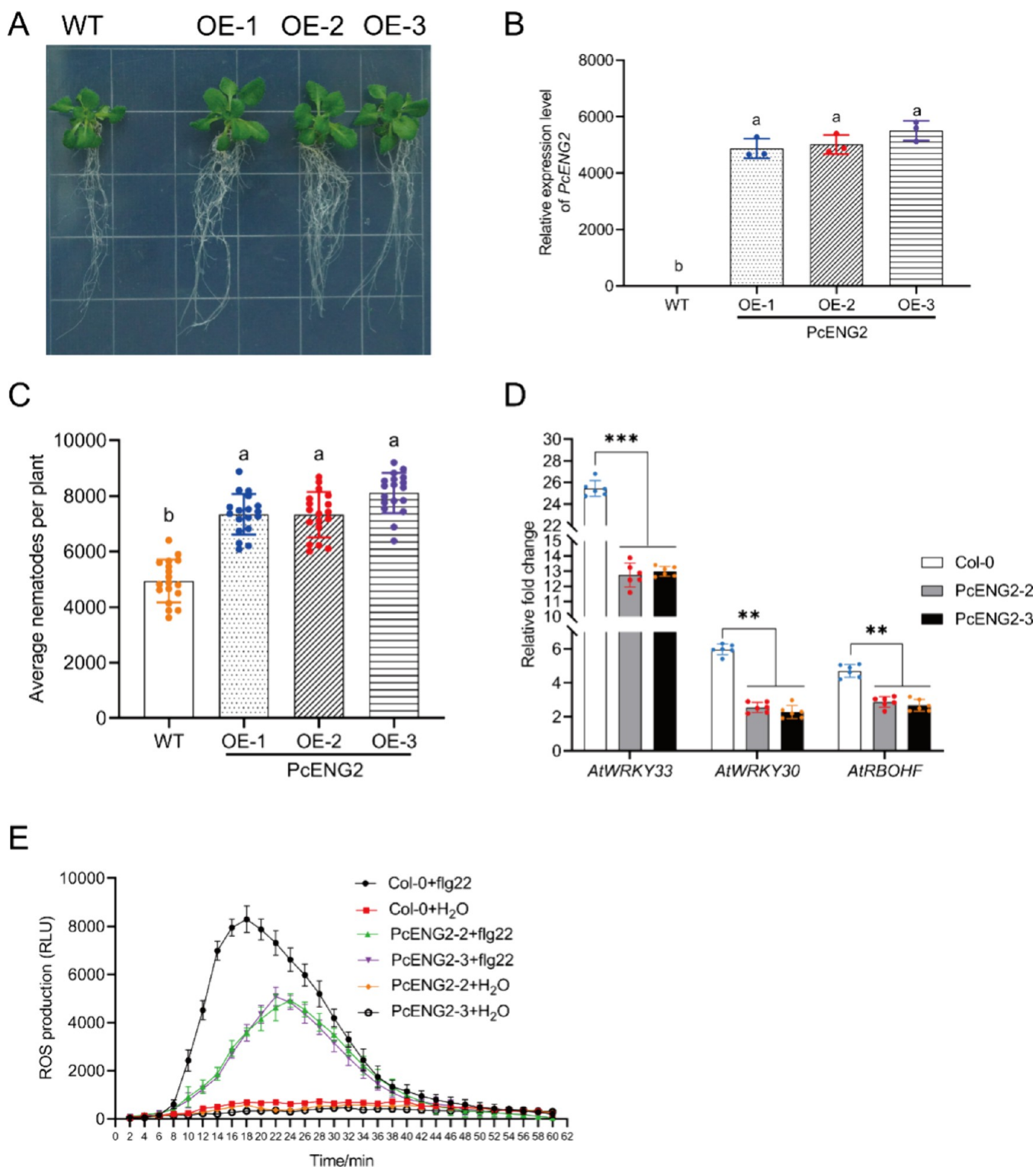


Figure 3. *PcENG2* is involved in *P. coffeae* parasitism and suppresses plant defense responses triggered by flg22. (A) Comparison of lateral root numbers between *A. thaliana* lines. (B) Relative expression level of *PcENG2* in three independent ectopic-expressing *Arabidopsis* lines. OE-1, 2, 3, *PcENG2* ectopic-expressing *Arabidopsis* lines. (C) *P. coffeae* infection was increased in transgenic *Arabidopsis* expressing *PcENG2* as compared to WT (Col-0) lines. The numbers of *P. coffeae* masses were counted at 30 dpi. Data are presented as means $\pm$ SD ($n = 18$). Distinct letters represent statistically significant differences ($p < 0.05$, one-way ANOVA). (D) RT-qPCR analysis revealed that the expression levels of *Arabidopsis* defense genes *AtWRKY30*, *AtWRKY33*, and *AtRBOHF* in response to flg22 were significantly reduced in transgenic *Arabidopsis* lines compared to WT (Col-0). Bars represent the mean $\pm$ SD, with asterisks indicating statistically significant differences (**, $p < 0.01$; ***, $p < 0.001$, Student's *t* test). All tests were conducted three times and yielded the similar findings each time. (E) *PcENG2* inhibit flg22-induced ROS production in two *PcENG2*-transgenic *Arabidopsis* lines.

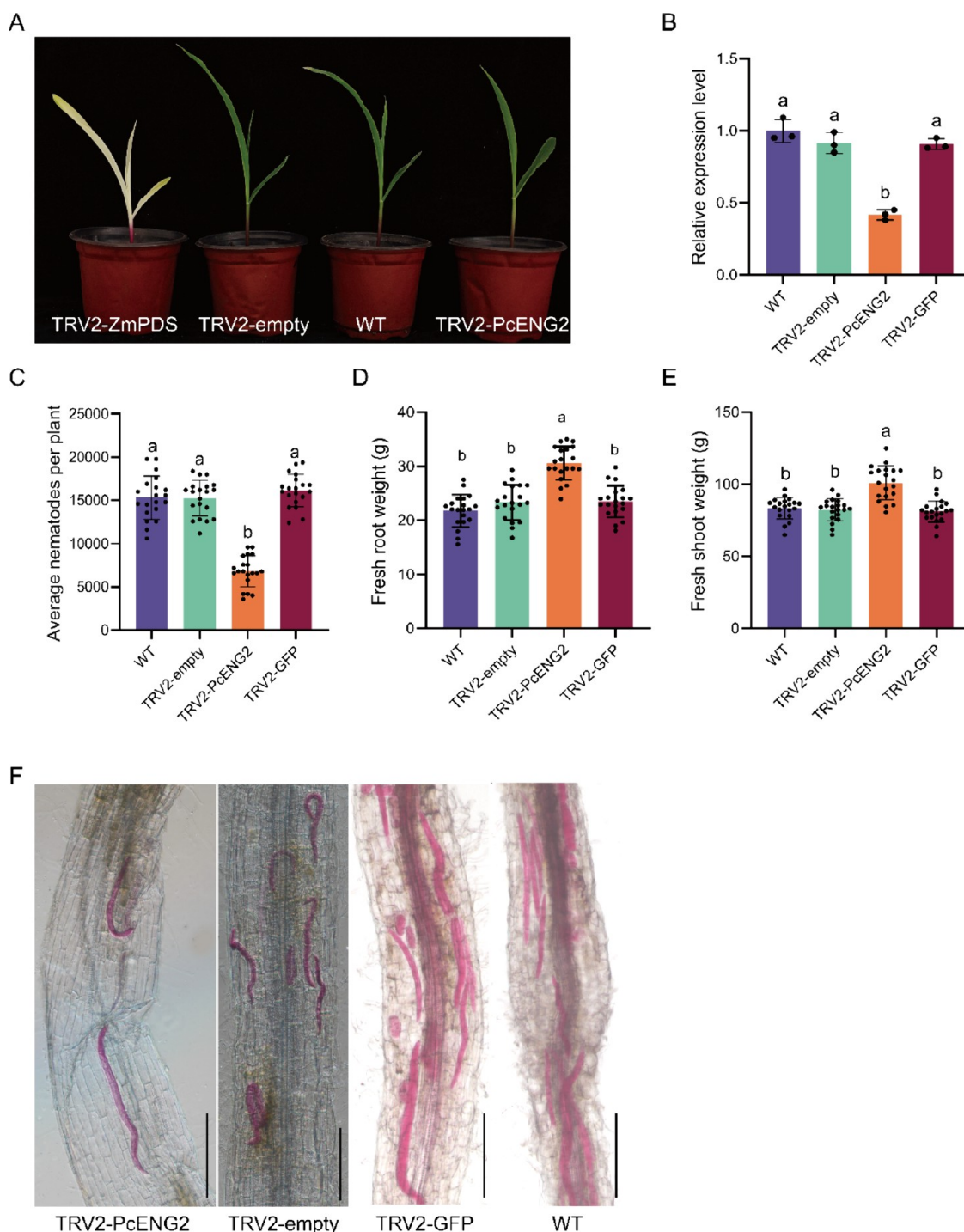


Figure 4. Host-derived tobacco rattle virus (TRV)-mediated silencing of *P. coffeae* *PcENG2* ectopic expression in the maize. (A) Maize phenotypes, treated with *A. tumefaciens* carrying TRV2-ZmPDS exhibiting a photobleaching phenotype. No significant change was observed in the TRV2-empty and WT plants. (B) Expression levels of *PcENG2* changes in different maize lines with *P. coffeae*. *Pc18S* was used as the internal control. GFP, green fluorescent protein. (C) *P. coffeae* infection was decreased in TRV2-PcENG2 as compared to WT. (D) Weight of fresh maize root per plant at 60 dpi. (E) Weight of fresh shoot weight per plant at 60 dpi. Data are presented as means $\pm$ SE ($n = 20$), with distinct letters represent statistically significant differences ($p < 0.05$, one-way ANOVA). (F) Population of *P. coffeae* in the TRV2-PcENG2 transgenic maize roots were decreased significantly compared with WT. Scale bars, 200 μ m.

indicating an active host attempt to reinforce cell walls during nematode invasion.

3.3. Ectopic Expression of *PcENG2* Promotes the Infection of *P. coffeae* and Suppresses Plant Defense Responses. To investigate the role of *PcENG2* in plant-nematode interactions, three independent transgenic *A.*

thaliana lines (*PcENG2*-OE1, *PcENG2*-OE2, and *PcENG2*-OE3) constitutively expressing *PcENG2* were generated, and their susceptibility to *P. coffeae* was evaluated. Notably, these transgenic lines developed significantly more lateral roots compared to WT plants (Figure 3A). The presence of *PcENG2* transcripts and proteins in the transgenic lines was confirmed

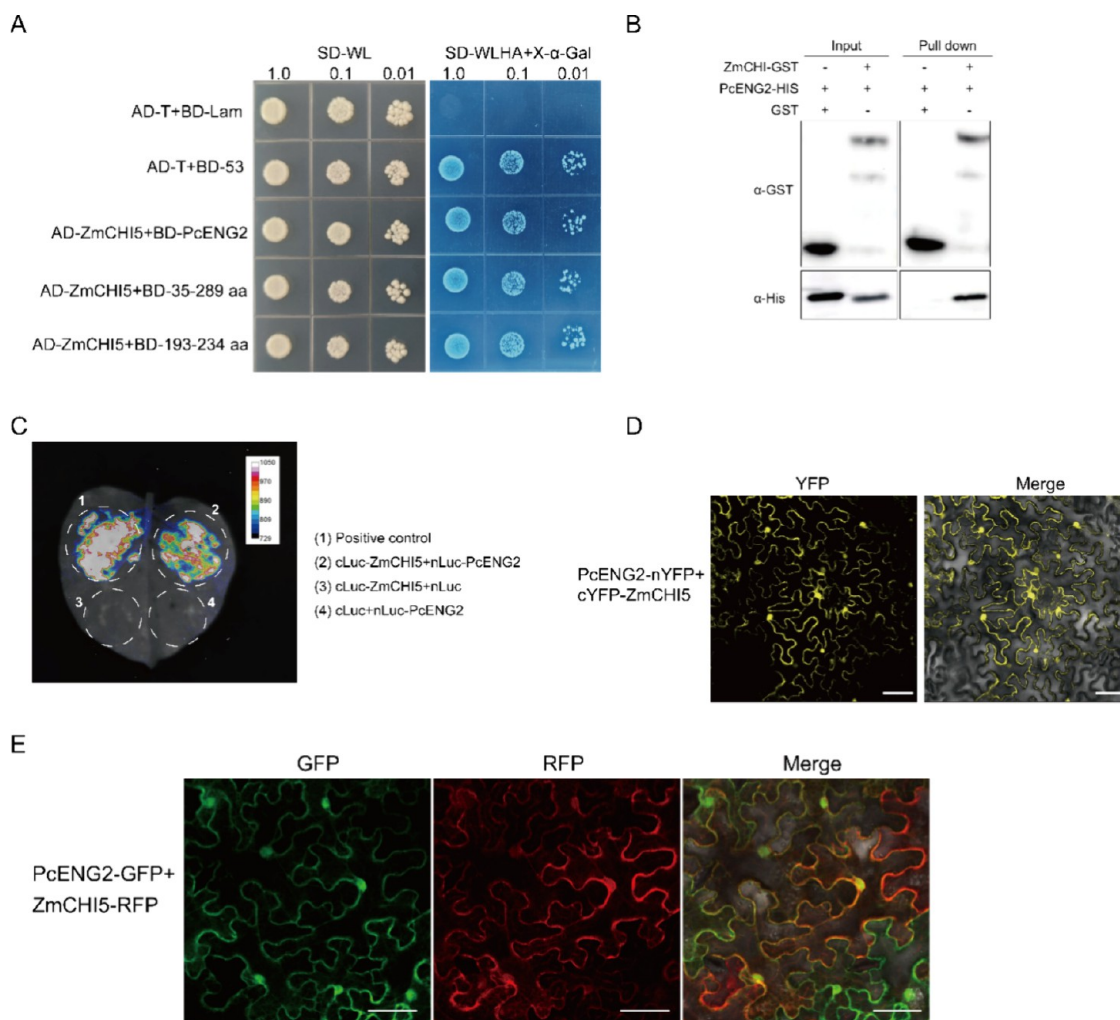


Figure 5. *P. coffeae* PcENG2 interacts with maize chitinase ZmCHI5. (A) Yeast two-hybrid analysis of the interaction between PcENG2 and ZmCHI5. Y2H gold yeast cells harboring bait and prey vectors were grown normally on SD-WL (SD/−Trp/−Leu) media. After the addition of 1 mg/mL aureobasidin A and 20 mg/mL X-α-Gal, the yeast gold cells cultured on the SD-WLHA selective media (SD/−Trp/−Leu/−His/−Ade) appeared bluish. Yeast cells containing pGBKT7-53 and pGADT7-T served as the positive controls, while yeast cells containing pGBKT7-Lam and pGADT7-T served as the negative controls. (B) ZmCHI5-GST associated with PcENG2-His in a GST pull-down assay. GST was utilized as a control. (C) Interaction between PcENG2 and ZmCHI5 was assessed via a luciferase complementation assay (LCI). A series of plasmids were introduced into *A. tumefaciens*, which were subsequently infiltrated into four discrete regions of *N. benthamiana* leaves. Luciferase activities were subsequently measured 2 dpi. The measurement of luciferase activity was performed using a CCD imaging apparatus. A combination expressing SGF-nLuc + cLuc-SAR were used as a positive control. (D) Bimolecular fluorescent complementation (BiFC) confirmed the interaction between PcENG2 and ZmCHI5 in planta. PcENG2 and ZmCHI5 were individually fused with the N-terminal (nYFP) and C-terminal (cYFP) halves of the yellow fluorescent protein (YFP). The YFP signal was detected by confocal microscopy at 48 h post-infiltration. Scale bars, 10 μm. (E) Subcellular localization of PcENG2 and ZmCHI5 in *N. benthamiana* leaf cells. Confocal microscopy was used to detect the colocalization of PcENG2 and ZmCHI5 in *N. benthamiana* leaves at 2 dpi. Scale bars, 10 μm.

by RT-qPCR and western blot analysis, respectively (Figure 3B and Figure S5A and B), indicating that *PcENG2* expression may interfere with normal root development. Furthermore, the transgenic *PcENG2*-expressing lines exhibited significantly higher susceptibility to *P. coffeae* compared to WT, with a 48% increase in nematode mass formation observed at 30 dpi (Figure 3C). Pattern-triggered immunity (PTI) suppression assays revealed that these transgenic lines produced significantly lower levels of reactive ROS in response to flg22, a conserved 22-amino acid peptide derived from bacterial flagellin that activates PTI in plants, compared to WT plants (Figure 3E). To further investigate the impact of *PcENG2* on plant immune responses, the expression levels of key defense-related genes, including *AtWRKY33*, *AtWRKY30*, and *AtRBOH*, were examined following flg22 elicitation. These

genes were significantly downregulated in *PcENG2*-expressing lines compared to WT (Figure 3D), suggesting that *PcENG2* suppresses defense signaling pathways. Collectively, these findings demonstrate that the effector *PcENG2* facilitates *P. coffeae* infection by altering host root development and suppressing basal immune responses in *Arabidopsis*.

3.4. *PcENG2* Silencing Reduces *P. coffeae* Parasitism.

To elucidate the role of *PcENG2* during *P. coffeae* infestation, tobacco rattle virus (TRV)-mediated RNA interference (RNAi) was employed to suppress *PcENG2* expression in maize roots colonized by feeding *P. coffeae* (Figure 4A and Figure S6A). The silencing efficiency was confirmed by RT-qPCR, which showed that *PcENG2* transcript levels in parasitic nematodes were significantly reduced by 58% compared to the control group (Figure 4B). At 60 dpi, *P. coffeae* population

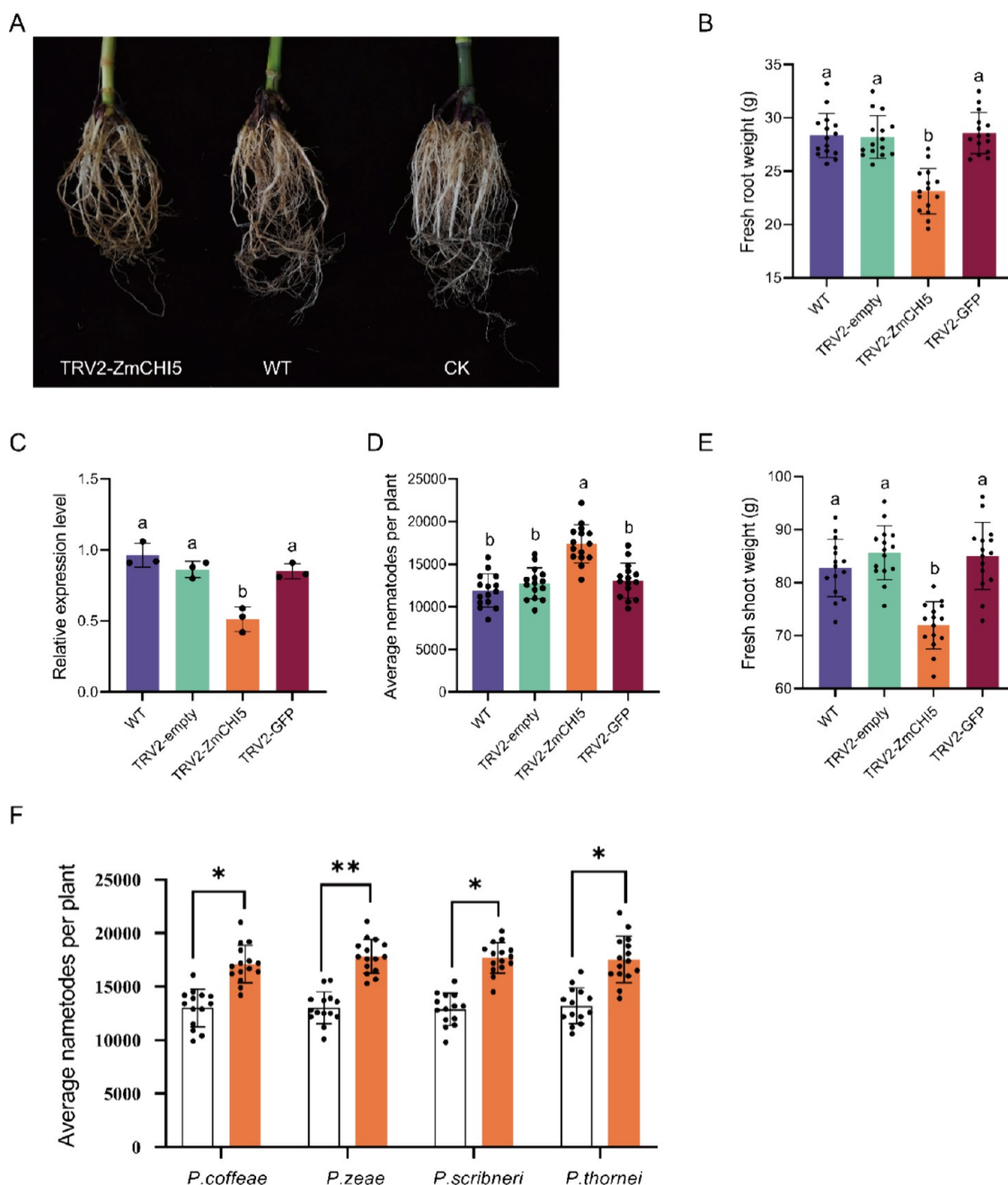


Figure 6. Determination of maize resistance to *P. coffeae* after TRV-mediated RNAi silencing of *ZmCHIS*. (A) Root lesion areas of *P. coffeae*-induced were significantly increased in the TRV2-*ZmCHIS* maize lines. (B) Fresh root weight per plant of maize lines at 60 dpi. The values presented are the means $\pm$ SE ($n = 15$), with different letters indicate significant differences ($p < 0.05$, one-way ANOVA). (C) Expression levels of *ZmCHIS* changes in different maize lines at 14 dpi with *Agrobacterium*. *ZmActin* was used as an internal control. GFP, green fluorescent protein. (D) Number of nematodes masses produced on different maize lines at 60 dpi. (E) Fresh shoot weight per plant of maize lines at 60 dpi. (F) Total numbers of *P. coffeae*, *P. zeae*, *P. scribneri*, and *P. thornei* masses were counted in the TRV-mediated maize RNAi-*ZmCHIS* at 60 dpi with 1000 nematodes, and maize *ZmCHIS* presented broad-spectrum resistance to RLN infestation. The values presented are the means $\pm$ SE ($n = 15$), and asterisks denote significant differences (*, $p < 0.05$; **, $p < 0.01$; Student's *t* test).

density in maize roots was quantified. A significant 55.4% reduction in nematode numbers was observed in *PcENG2*-silenced plants compared to the control (Figure 4C). In nematode staining assays, wild-type maize exhibited greater susceptibility to *P. coffeae* than *PcENG2*-silenced plants, as evidenced by fewer and less severe infection sites (Figure 4F and Figure S6B). In addition, *PcENG2* silencing improved plant growth, as demonstrated by a 17.4% increase in fresh shoot weight and a 28.7% increase in root weight relative to

controls (Figure 4D and E). These results suggest that *PcENG2* contributes significantly to nematode parasitism and host damage during *P. coffeae*.

3.5. *PcENG2* Interacts with Maize Chitinase. To identify the function of *PcENG2*, we screened a maize Y2H cDNA library using *PcENG2* (without signal peptide) as bait. Several maize proteins potentially interacting with *PcENG2* were identified, among which *ZmCHIS* was captured the most 12 times with a strong signal (Table S2). We constructed two

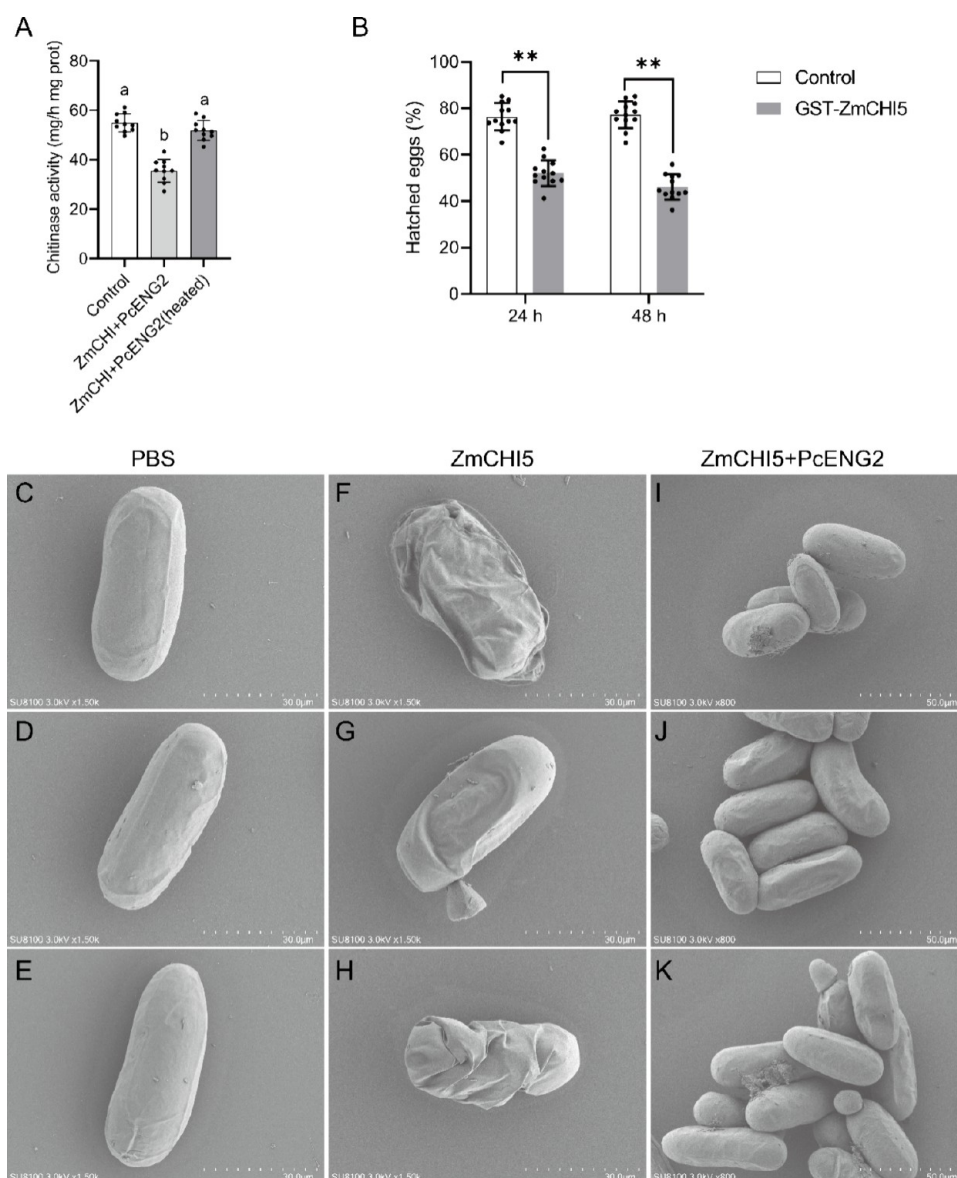


Figure 7. Morphological changes in the *P. coffeae* eggs of incubation with the maize ZmCHI5. (A) Effects of PcENG2 on ZmCHI5 chitinase enzyme activity. The values presented are the means $\pm$ SE ($n = 10$), with different letters indicate significant differences ($p < 0.05$, one-way ANOVA). (B) Effects of GST-ZmCHI5 on the hatching rate after 24 and 48 h of incubation with the eggs. The significance of the results was determined by Student's test. The asterisks indicate significant differences (**, $p < 0.01$). (C–E) No observable change on the surface of the eggs incubated with PBS. Bars, 30 μ m. (F–H) Eggs surface exposed to ZmCHI5 presented crumpling and breakage. Bars, 30 μ m. (I–K) No significantly surface damage was observed in the eggs incubated with the mixture of ZmCHI5 and PcENG2. Bars, 50 μ m.

truncated versions of PcENG2, BD-PcENG2 (35–289 aa) and BD-PcENG2 (193–234 aa) to identify its interaction (Figure S7). Then, two candidate proteins, ZmCHI5 (maize chitinase 5; ALP46634) and ZmCP1 (maize cysteine protease 1; AQK46675) were further examined for interactions with PcENG2 using yeast two-hybrid cotransformation assays. The results showed that yeast coexpression PcENG2 and ZmCHI5 grew on SD/–Leu/–Trp/–Ade/–His/medium with the supplementation of X- α -Gal (Figure 5A). To identify the interaction between PcENG2 and ZmCHI5, a GST pull-down assay was conducted using GST-tagged ZmCHI5 and His-tagged PcENG2 expressed in *E. coli*, revealing that PcENG2 was specifically pulled down by GST-ZmCHI5 (Figure 5B). Furthermore, the split luciferase complementation imaging (LCI) experiment was performed to further confirm the interaction, and the results demonstrated that PcENG2 and

ZmCHI5 also physically interact in *N. benthamiana* cells (Figure 5C). SAR-cLuc and SGF-nLuc were coexpressed as a positive control.

The BiFC verification vector was constructed by fusing the PcENG2 protein to the N terminus of nYFP and the ZmCHI5 protein to the N terminus of cYFP. The interaction was verified via *Agrobacterium*-mediated transient transformation in *N. benthamiana* leaf cells. While no fluorescence was detected in the nYFP-PcENG2 + cYFP group, the verification group (nYFP-PcENG2 + cYFP-ZmCHI5) exhibited yellow fluorescence signals in the nucleus and plasma membrane, indicating that PcENG2 interacts with ZmCHI5 at the cell membrane (Figure 5D). We conducted colocalization studies of the two proteins, PcENG2 and ZmCHI5. The results confirmed that both PcENG2 and ZmCHI5 colocalize in the nucleus and plasma membrane (Figure 5E). Furthermore, subcellular

localization analysis of ZmCHIS-GFP in transgenic *N. benthamiana* cells revealed that ZmCHIS predominantly localizes to the cytoplasm and nucleus (Figure S8), consistent with the BiFC results. These findings provide evidence for a specific interaction between PcENG2 and the maize chitinase ZmCHIS, both *in vitro* and *in vivo*.

3.6. Chitinase Affects *P. coffeae* Parasitism. To clarify the role of ZmCHIS in *P. coffeae* infestation, RNAi was employed to silence ZmCHIS expression in maize (Figure S9). Infestation assays revealed that ZmCHIS-silenced maize exhibited reduced resistance to *P. coffeae*. Notably, ZmCHIS-silenced maize roots displayed more extensive tissue damage and a greater number of nematode-induced lesions at 60 dpi, resulting in a marked reduction in root fresh weight compared to WT plants (Figure 6A and B). RT-qPCR analysis confirmed a significant 49% reduction in ZmCHIS transcript levels compared to the control group (Figure 6C). Specifically, the number of invading nematodes increased significantly by 46% (Figure 6D), and fresh root were significantly decreased by 19% compared to WT (Figure 6E).

To assess whether ZmCHIS confers broad-spectrum resistance to root-lesion nematodes (RLNs), TRV2-ZmCHIS maize roots were inoculated with four RLN species: *P. coffeae*, *P. zeae*, *P. thornei*, and *P. scribneri*. At 60 dpi, nematodes were extracted using a modified Baermann funnel method and quantified. ZmCHIS-silenced maize showed significantly higher susceptibility to all four RLN species compared to controls (Figure 6F), indicating that ZmCHIS-mediated chitinase activity plays a critical and broad-spectrum role in host resistance against RLN infections.

3.7. Biological Activities of ZmCHIS Suppress the *P. coffeae* Egg Development. To investigate the molecular interaction between PcENG2 and ZmCHIS, recombinant His-tagged PcENG2 and GST-tagged ZmCHIS proteins were expressed *in vitro*. Enzymatic activity assays revealed that His-PcENG2 significantly inhibited the chitinase activity of GST-ZmCHIS, resulting in a 22.2% reduction compared to the control (Figure 7A). In contrast, co-incubation with heat-denatured His-PcENG2 did not affect GST-ZmCHIS activity, indicating that the inhibition is dependent on the structural integrity of PcENG2 and suggesting that PcENG2 specifically suppresses ZmCHIS enzymatic function.

To assess the biological relevance of ZmCHIS activity, egg hatching assays were performed using *P. coffeae* eggs. *In vitro* incubation with GST-ZmCHIS significantly inhibited nematode egg hatching. After 24 h, the hatching rate was reduced by 31.7%, and after 48 h, a 40.3% reduction was observed compared to the control (Figure 7B). Scanning electron microscopy (SEM) further revealed that GST-ZmCHIS treatment altered egg morphology and disrupted development. Normal eggs displayed smooth, intact surfaces without visible defects (Figure 7C–E). However, following 48 h of GST-ZmCHIS exposure, eggs exhibited clear surface damage, including invaginations and cracking (Figure 7F–H). Notably, these structural damages were absent when eggs were co-incubated with both GST-ZmCHIS and His-PcENG2, suggesting that PcENG2 effectively suppresses the deleterious effects of ZmCHIS on nematode eggs (Figure 7I–K).

4. DISCUSSION

RLNs are migratory endoparasitic nematodes that feed freely on plant roots and are often underestimated due to the non-specific symptoms they induce.^{3,46} In this study, we identified

and characterized a novel effector, PcENG2, expressed in the esophageal gland cells of *P. coffeae*. PcENG2 is secreted into host root tissues via the stylet, cloaca, and hypodermis during parasitism. It functions to subvert callose and ZmCHIS mediated plant immunity, thereby promoting nematode parasitism (Figure 8).

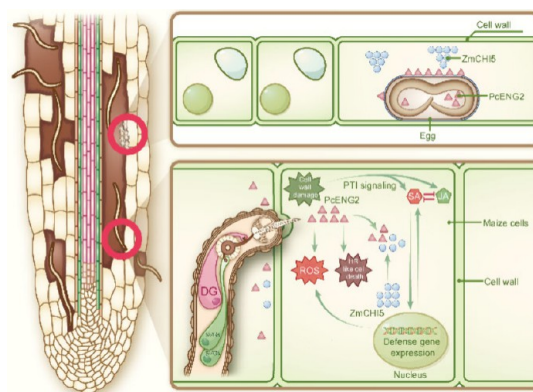


Figure 8. Model for the involvement of maize ZmCHIS in the immune response during its interaction with *P. coffeae* PcENG2. The PcENG2 effector protein is released into plant cells during *P. coffeae* infection, suppressed the immune response induced by cell wall damage. PcENG2 targets maize chitinase ZmCHIS, preventing it from degrading chitin in the nematode body wall and eggshell, thereby weakening plant immunity stimulated by chitin oligosaccharides and promoting nematode parasitism.

PcENG2 belongs to a group of cell wall-degrading enzymes (CWDEs) with a conserved cellulase domain that hydrolyzes cellulose. Previous studies have suggested that ENGs in PPNs may have originated from horizontal gene transfer events from bacteria or fungi.⁴⁷ For instance, ENGs in *Bursaphelenchus xylophilus* are thought to have been acquired from bacteria.^{48,49} CWDEs are crucial for the successful parasitism of PPNs, as evidenced by their strong expression in esophageal glands and secretion into host tissues during infection.^{8,50,51} For example, ENGs such as HgENG2 and GrENG1 are secreted through the stylet and can be detected along the nematode migration path.^{52,53} Other studies have shown that *H. schachtii* secretes a cellulose-binding protein (CSP-1) that interacts with *Arabidopsis* pectin methylesterase (PME33) to modify the cell wall, thereby facilitating invasion.⁵⁴ In the present study, we demonstrated that PcENG2 is upregulated during early infection stages. Immunohistochemical and immuno-electron microscopy analyses confirmed that PcENG2 is secreted into the apoplast and functions to degrade host cell walls and callose. The localization of PcENG2 in esophageal glands and along the body, particularly near the stylet, cloaca, and hypodermis, supports its active role in breaching physical barriers during root penetration. These findings are consistent with previous reports on ENGs localization in other nematodes.^{47,51,52}

The plant cell wall is primarily composed of cellulose, hemicellulose, lignin, and pectin, and acts as a critical defense barrier against pathogen invasion.⁵⁵ Callose, a β -1,3-glucan component of the cell wall, reinforces plant defenses upon pathogen attack.^{56,57} Plants increase callose deposition to restrict pathogen movement.^{58,59} However, nematodes have evolved strategies to overcome these barriers by secreting effectors that degrade structural components. For example,

RKNs release cellulases and pectinases to weaken the cell wall and facilitate invasion.⁶⁰ In barley, silencing of *HvGSL6* led to reduced callose accumulation and increased susceptibility to powdery mildew.⁶¹ The failure of PcENG2 to degrade laminarin is in agreement with substrate specificity reported for canonical β -1,4-endoglucanases, which are unable to act on β -1,3 linkages unless part of mixed-linkage polymers (e.g., β -1,3:1,4 glucan that adjacent β -1,3 and β -1,4 bonds).⁶² While some enzymes in other GH families (e.g., GH1 β -glucosidases) can act on both β -1,3 and β -1,4 bonds, these are structurally and mechanistically distinct from PcENG2-like endoglucanases.⁶³ Therefore, our data strongly support that PcENG2 does not directly degrade callose *in vitro*. Although PcENG2 does not directly hydrolyze laminarin *in vitro*, similar indirect mechanisms have been documented in other pathosystems: for example, cell wall-degrading enzymes such as endo-1,4- β -glucanases can modulate the host apoplast and enable or enhance the activity of host-derived β -1,3-glucanases.^{64,65} Plants themselves can upregulate β -1,3-glucanases in response to cell wall loosening.⁶⁶ In nematode-induced feeding sites, host cellulases and expansins are reported to facilitate cell wall remodeling and potentially synergize with nematode-secreted enzymes.⁶⁷ Thus, PcENG2 may act indirectly to reduce callose deposition by altering cell wall permeability or triggering host glucanase responses, consistent with our *in planta* observations of reduced callose at infection sites.

Chitinases play a central role in plant defense by directly degrading chitin in pathogens and by regulating immune responses, including ROS production.⁶⁸ ROS accumulation in the apoplast is a key component of early defense signaling.^{13,69} Nematodes counteract this through effector proteins; for example, *M. javanica* secretes MjTTL5 to modulate the plant ROS scavenging system.³¹ Overexpression of chitinase genes can enhance resistance to fungal and bacterial pathogens, as demonstrated in rice, tobacco, and other crops.^{70,71} Interestingly, chitinases are also essential in some pathogens; e.g., *Plasmodium* and *Toxoplasma gondii* require chitinases for life cycle progression.^{72,73} In this study, we identified ZmCHI5 as a key chitinase targeted by PcENG2. ZmCHI5 contributes to maize defense against biotic and abiotic stress.⁷⁴ PcENG2 suppresses ZmCHI5 activity, as shown *in vitro*, thereby diminishing maize immune responses and promoting *P. coffeae* parasitism. Virus-induced gene silencing (VIGS) of ZmCHI5 enhanced plant susceptibility, supporting its role in defense.

Chitinases also restrict nematode development by degrading the chitin in eggshells. We demonstrated that ZmCHI5 inhibited egg hatching, whereas PcENG2 protected nematode eggs from chitinase degradation. Plant chitinases can also degrade chitin in insect eggshells and exoskeletons, disrupting molting and reproduction.^{20,75} Thus, PcENG2 helps *P. coffeae* evade both plant immune signaling and direct structural attack. Chitinases are linked to hormonal signaling pathways as well. Ethylene (ET) promotes chitinase expression during pathogen challenge,⁷⁶ and AtCTL1 regulates root development by modulating ET biosynthesis.⁷⁷ Salicylic acid (SA) also boosts ROS production and pathogenesis-related gene expression, amplifying immune responses.⁷⁸ Hence, suppression of ZmCHI5 by PcENG2 likely impairs both direct chitin hydrolysis and downstream signaling cascades.

Over the past decades, significant progress has been made in elucidating the molecular interactions between sedentary PPNs, such as root-knot (*Meloidogyne* spp.) and cyst nematodes (*Heterodera* spp.), and their host plants. These

nematodes secrete effector proteins that are essential for establishing and maintaining specialized feeding structures—giant cells or syncytia—by modulating host transcription, hormone pathways, and immunity.^{26,27} In contrast, migratory PPNs such as *Pratylenchus* spp. do not form permanent feeding sites but instead move destructively through root tissues, requiring a distinct set of effectors that facilitate repeated penetration, suppression of immune responses, and nutrient acquisition at multiple sites.²⁹ Our study on PcENG2 offers new insight into this migratory parasitism strategy. Unlike sedentary nematodes that rely heavily on effectors targeting transcriptional reprogramming, PcENG2 acts both as a cell wall-degrading enzyme and an immune suppressor. By degrading cell wall components and subverting chitinase-mediated defenses, PcENG2 enables *P. coffeae* to overcome mechanical and biochemical barriers encountered during migration. These findings underscore the adaptability and versatility of migratory PPNs effectors and point to PcENG2 as a model for understanding effector-driven pathogenesis in migratory nematodes.

These insights deepen our understanding of how migratory nematodes suppress host immunity through effector-mediated mechanisms, and provide potential targets for the development of nematode-resistant crops. In conclusion, our study demonstrates that PcENG2 targets and suppresses the activity of maize chitinase ZmCHI5, thereby attenuating host basal immunity and facilitating the successful parasitism of *P. coffeae*.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jafc.5c06425>.

Nine supplemental figures and three supplemental tables: antigen protein detection, immunolocalization and immunogold labeling (Figures S1–S3), enzyme activity analyses of PcENG2 (Figure S4), verification of transgenic and RNAi plants (Figures S5, S6, and S9), schematic illustration of PcENG2 structures (Figure S7), subcellular localization studies (Figure S8), lists of antiserum ELISA results (Table S1), yeast two-hybrid candidate targets (Table S2), and primers used in this study (Table S3) (PDF)

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Author Contributions

S.W., H.L., and Y.L. conceived the project, interpreted the results, and wrote the manuscript. S.W., W.N., F.X., and K.W. performed the experiments. Y.S., A.D., H.Y., H.W., and Y.X. collected and analyzed the data. All authors read and approved the final manuscript.

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Notes

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ABBREVIATIONS USED

PPN, plant–parasitic nematode; RLN, root-lesion nematode; ENG, β -1,4-endoglucanase; PTI, pattern-triggered immunity; ROS, reactive oxygen species; GH19, glycoside hydrolase family 19; ETI, effector-triggered immunity; NCBI, National Center for Biotechnology Information; cDNA, complementary DNA; RT-qPCR, real-time quantitative polymerase chain reaction; WT, wild type; dpi, days post-inoculation; TRV, rattle virus RNA; Y2H, yeast two-hybrid; CDS, coding sequence; SDS, sodium dodecyl sulfate; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; SEM, scanning electron microscopy; DNS, dinitrosalicylic acid; RNAi, RNA interference; YFP, yellow fluorescent protein; CWDE, cell wall-degrading enzyme; VIGS, virus-induced gene silencing; SA, salicylic acid; ET, ethylene

REFERENCES

- (1) Abad, P.; Gouzy, J.; Aury, J. M.; Castagnone-Sereno, P.; Danchin, E. G. J.; Deleury, E.; Perfus-Barbeoch, L.; Anthouard, V.; Artiguenave, F.; Blok, V. C.; et al. Genome sequence of the metazoan plant–parasitic nematode *Meloidogyne incognita*. *Nat. Biotechnol.* **2008**, *26* (8), 909–915.
- (2) Wang, X.; Cheng, R.; Xu, D.; Huang, R.; Li, H.; Jin, L.; Wu, Y.; Tang, J.; Sun, C.; Peng, D.; et al. MG1 interacts with a protease inhibitor and confers resistance to rice root-knot nematode. *Nature Communications*. **2023**, *14* (1), 3354.
- (3) Lu, Y.; Yang, S.; Chen, W.; Xie, H.; Xu, C. Advances in migratory plant endoparasitic nematode effectors. *International Journal of Molecular Sciences*. **2024**, *25* (12), 6435.
- (4) Vanholme, B.; Van Thuyne, W.; Vanhouteghem, K.; De Meutter, J.; Cannoot, B.; Gheysen, G. Molecular characterization and functional importance of pectate lyase secreted by the cyst nematode *Heterodera schachtii*. *Molecular Plant Pathology*. **2007**, *8* (3), 267–278.
- (5) Molloy, B.; Shin, D. S.; Long, J.; Pellegrin, C.; Senatori, B.; Vieira, P.; Thorpe, P. J.; Damm, A.; Ahmad, M.; Vermeulen, K.; et al. The origin, deployment, and evolution of a plant–parasitic nematode effectorome. *Plos Pathogens*. **2024**, *20* (7), No. e1012395.
- (6) Popeijus, H.; Overmars, H.; Jones, J.; Blok, V.; Goverse, A.; Helder, J.; Schots, A.; Bakker, J.; Smant, G. Degradation of plant cell walls by a nematode. *Nature*. **2000**, *406* (6791), 36–37.
- (7) Vieira, P.; Maier, T. R.; Eves-van den Akker, S.; Howe, D. K.; Zasada, I.; Baum, T. J.; Eisenback, J. D.; Kamo, K. Identification of candidate effector genes of *Pratylenchus penetrans*. *Molecular Plant Pathology*. **2018**, *19* (8), 1887–1907.
- (8) Smant, G.; Stokkermans, J. P.; Yan, Y.; de Boer, J. M.; Baum, T. J.; Wang, X.; Hussey, R. S.; Gommers, F. J.; Henrissat, B.; Davis, E. L.; et al. Endogenous cellulases in animals: isolation of beta-1, 4-endoglucanase genes from two species of plant–parasitic cyst nematodes. *Proceedings of the National Academy of Sciences of the United States of America*. **1998**, *95* (9), 4906–4911.
- (9) Uehara, T.; Kushida, A.; Momota, Y. PCR-based cloning of two beta-1,4-endoglucanases from the root-lesion nematode *Pratylenchus penetrans*. *Nematology*. **2001**, *3* (4), 335–341.
- (10) Peng, H.; Peng, D.; Long, H.; He, W.; Qiao, F.; Wang, G.; Huang, W. Characterisation and functional importance of β -1,4-endoglucanases from the potato rot nematode, *Ditylenchus destructor*. *Nematology*. **2014**, *16*, 505–517.
- (11) Patel, A.; Shah, A. Purification and characterization of novel, thermostable and non-processive GH5 family endoglucanase from *Fomitopsis meliae* CFA 2. *International Journal of Biological Macromolecules*. **2021**, *182*, 1161–1169.
- (12) Shirai, T.; Ishida, H.; Noda, J.; Yamane, T.; Ozaki, K.; Hakamada, Y.; Ito, S. Crystal structure of alkaline cellulase K: insight into the alkaline adaptation of an industrial enzyme. *Journal of molecular biology*. **2001**, *310* (5), 1079–1087.
- (13) Jwa, N.-S.; Hwang, B. K. Convergent evolution of pathogen effectors toward reactive oxygen species signaling networks in plants. *Frontiers in Plant Science* **2017**, *8*, 1687.
- (14) Mittler, R.; Zandalinas, S. I.; Fichman, Y.; Van Breusegem, F. Reactive oxygen species signalling in plant stress responses. *Nature Reviews Molecular Cell Biology*. **2022**, *23* (10), 663–679.
- (15) Gillet, F.-X.; Bournaud, C.; de Souza Júnior, J. D. A.; Fatima Grossi-de-Sa, M. Plant–parasitic nematodes: towards understanding molecular players in stress responses. *Annals of Botany*. **2017**, *119* (5), 775–789.
- (16) Bleau, J. R.; Spoel, S. H. Selective redox signaling shapes plant–pathogen interactions. *Plant Physiology*. **2021**, *186* (1), 53–65.
- (17) Rui, L.; Wen, T.; Qiu, Y.; Yang, D.; Ye, J.; Wu, X. A pioneer nematode effector suppresses plant reactive oxygen species burst by interacting with the class III peroxidase. *Plant Cell and Environment*. **2024**, *47* (10), 3813–3827.
- (18) Rosso, M. N.; Jones, J. T.; Abad, P. RNAi and functional genomics in plant parasitic nematodes. *Annual Review of Phytopathology*. **2009**, *47*, 207–232.

- (19) Zhao, J.; Sun, Q.; Quentin, M.; Ling, J.; Abad, P.; Zhang, X.; Li, Y.; Yang, Y.; Favery, B.; Mao, Z.; et al. A *Meloidogyne incognita* C-type lectin effector targets plant catalases to promote parasitism. *New Phytologist*. **2021**, 232 (5), 2124–2137.
- (20) Grover, A. Plant chitinases: genetic diversity and physiological roles. *Critical Reviews in Plant Sciences*. **2012**, 31 (1), 57–73.
- (21) Qin, L.; Kudla, U.; Roze, E. H. A.; Goverse, A.; Popeijus, H.; Nieuwland, J.; Overmars, H.; Jones, J. T.; Schots, A.; Smant, G.; et al. Plant degradation: a nematode expansin acting on plants. *Nature*. **2004**, 427 (6969), 30.
- (22) Hamamouch, N.; Li, C.; Seo, P. J.; Park, C. M.; Davis, E. L. Expression of *Arabidopsis* pathogenesis-related genes during nematode infection. *Molecular Plant Pathology*. **2011**, 12 (4), 355–364.
- (23) Gao, B.; Allen, R.; Maier, T.; Davis, E. L.; Baum, T. J.; Hussey, R. S. Identification of putative parasitism genes expressed in the esophageal gland cells of the soybean cyst nematode *Heterodera glycines*. *Molecular plant-microbe interactions*. **2001**, 14 (10), 1247–1254.
- (24) Han, L.; Li, Y.; Wang, F.; Wang, W.; Liu, J.; Wu, J.; Zhong, N.; Wu, S.; Jiao, G.; Wang, H.; et al. The cotton apoplastic protein CRR1 stabilizes chitinase 28 to facilitate defense against the fungal pathogen *Verticillium dahliae*. *Plant Cell*. **2019**, 31 (2), 520–536.
- (25) Kombrink, A.; Rovenich, H.; Shi Kunne, X.; Rojas Padilla, E.; van den Berg, G. C. M.; Domazakis, E.; de Jonge, R.; Valkenburg, D. J.; Sanchez Vallet, A.; Seidl, M. F.; et al. *Verticillium dahliae* LysM effectors differentially contribute to virulence on plant hosts. *Molecular Plant Pathology*. **2017**, 18 (4), 596–608.
- (26) Haegeman, A.; Mantelin, S.; Jones, J. T.; Gheysen, G. Functional roles of effectors of plant–parasitic nematodes. *Gene*. **2012**, 492 (1), 19–31.
- (27) Hewezi, T.; Baum, T. J. Manipulation of plant cells by cyst and root-knot nematode effectors. *Molecular Plant-Microbe Interact.* **2013**, 26 (1), 9–16.
- (28) Goverse, A.; Smant, G. The activation and suppression of plant innate immunity by parasitic nematodes. *Annual Review of Phytopathology*. **2014**, 52, 243–265.
- (29) Kaloshian, I.; Teixeira, M. Advances in plant-nematode interactions with emphasis on the notorious nematode genus *Meloidogyne*. *Phytopathology*. **2019**, 109 (12), 1988–1996.
- (30) Li, Q.; Yang, Y.; Bai, X.; Xie, L.; Niu, S.; Xiong, B. Systematic analysis and functional characterization of the chitinase gene family in *Fagopyrum tataricum* under salt stress. *BMC Plant Biology* **2024**, 24 (1), 1222.
- (31) Lin, B.; Zhuo, K.; Chen, S.; Hu, L.; Sun, L.; Wang, X.; Zhang, L.; Liao, J. A novel nematode effector suppresses plant immunity by activating host reactive oxygen species-scavenging system. *New Phytologist*. **2016**, 209 (3), 1159–1173.
- (32) Reise, R. W.; Huettel, R. N.; Sayre, R. M. Carrot callus tissue for culture of endoparasitic nematodes. *Journal of Nematology* **1987**, 19 (3), 387–389.
- (33) Livak, K. J.; Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) Method. *Methods*. **2001**, 25 (4), 402–408.
- (34) De Boer, J. M.; McDermott, J. P.; Wang, X. H.; Maier, T.; Qui, F.; Hussey, R. S.; Davis, E. L.; Baum, T. J. The use of DNA microarrays for the developmental expression analysis of cDNAs from the esophageal gland cell region of *Heterodera glycines*. *Molecular Plant Pathology* **2002**, 3 (4), 261–270.
- (35) Zhang, X.; Henriques, R.; Lin, S.; Niu, Q.; Chua, N. *Agrobacterium*-mediated transformation of *Arabidopsis thaliana* using the floral dip method. *Nature Protocols*. **2006**, 1 (2), 641–646.
- (36) Mendy, B.; Wang'ombe, M. W.; Radakovic, Z. S.; Holbein, J.; Ilyas, M.; Chopra, D.; Holton, N.; Zipfel, C.; Grundler, F. M. W.; Siddique, S. *Arabidopsis* leucine-rich repeat receptor-like kinase NILR1 is required for induction of innate immunity to parasitic nematodes. *Plos Pathogens*. **2017**, 13 (4), No. e1006284.
- (37) Fernandez-Pozo, N.; Rosli, H. G.; Martin, G. B.; Mueller, L. A. The SGN VIGS tool: user-friendly software to design virus-induced gene silencing (VIGS) constructs for functional genomics. *Molecular Plant*. **2015**, 8 (3), 486–488.
- (38) Kraus, G. A.; Vander Louw, S. J.; Tylka, G. L.; Soh, D. H. Synthesis and testing of compounds that inhibit soybean cyst nematode egg hatch. *Journal of Agricultural and Food Chemistry*. **1996**, 44 (6), 1548–1550.
- (39) Sousa, A. J. S.; Souza, P. F. N.; Gifoni, J. M.; Dias, L. P.; Freitas, C. D. T.; Oliveira, J. T. A.; Sousa, D. O. B.; Vasconcelos, I. M. Scanning electron microscopy reveals deleterious effects of *Moringa oleifera* seed exuded proteins on root-knot nematode *Meloidogyne incognita* eggs. *International Journal of Biological Macromolecules*. **2020**, 154, 1237–1244.
- (40) Gao, F.; Zhang, B.; Zhao, J.; Huang, J.; Jia, P.; Wang, S.; Zhang, J.; Zhou, J.; Guo, H. Deacetylation of chitin oligomers increases virulence in soil-borne fungal pathogens. *Nature Plants*. **2019**, 5 (11), 1167–1176.
- (41) Andrade, L. B. d. S.; Oliveira, A. S.; Ribeiro, J. K. C.; Kiyota, S.; Vasconcelos, I. M.; de Oliveira, J. T. A.; de Sales, M. P. Effects of a novel pathogenesis-related class 10 (PR-10) protein from *Crotalaria pallida* roots with papain inhibitory activity against root-knot nematode *Meloidogyne incognita*. *Journal of Agricultural and Food Chemistry*. **2010**, 58 (7), 4145–4152.
- (42) Tenhaken, R. Cell wall remodeling under abiotic stress. *Frontiers in Plant Science*. **2015**, 5, 771.
- (43) Engelsdorf, T.; Gigli-Bisceglia, N.; Veerabagu, M.; McKenna, J. F.; Vaahtera, L.; Augstein, F.; Van der Does, D.; Zipfel, C.; Hamann, T. The plant cell wall integrity maintenance and immune signaling systems cooperate to control stress responses in *Arabidopsis thaliana*. *Science Signaling*. **2018**, 11 (536), No. ea03070.
- (44) Diana, S. G.; Kamila, G. J.; Ewa, K.; Malgorzata, K.; Monika, T.; Emilia, G.; Kaja, S.; Magdalena, R.; Karolina, U.; Monika, K.; et al. The effect of silicon supplementation and drought stress on the deposition of callose and chemical components in the cell walls of the *Brassica napus* roots. *BMC Plant Biology*. **2024**, 24 (1), 1249.
- (45) German, L.; Yeshvekar, R.; Benitez-Alfonso, Y. Callose metabolism and the regulation of cell walls and plasmodesmata during plant mutualistic and pathogenic interactions. *Plant Cell and Environment*. **2023**, 46 (2), 391–404.
- (46) Jones, J. T.; Haegeman, A.; Danchin, E. G. J.; Gaur, H. S.; Helder, J.; Jones, M. G. K.; Kikuchi, T.; Manzanilla-Lopez, R.; Palomares-Rius, J. E.; Wesemael, W. M. L.; et al. Top 10 plant–parasitic nematodes in molecular plant pathology. *Molecular Plant Pathology*. **2013**, 14 (9), 946–961.
- (47) Fanelli, E.; Troccoli, A.; Picardi, E.; Pousis, C.; De Luca, F. Molecular characterization and functional analysis of four β -1,4-endoglucanases from the root-lesion nematode *Pratylenchus vulnus*. *Plant Pathology*. **2014**, 63 (6), 1436–1445.
- (48) Danchin, E. G. J.; Rosso, M.; Vieira, P.; de Almeida-Engler, J.; Coutinho, P. M.; Henrissat, B.; Abad, P. Multiple lateral gene transfers and duplications have promoted plant parasitism ability in nematodes. *Proceedings of the National Academy of Sciences of the United States of America*. **2010**, 107 (41), 17651–17656.
- (49) Kikuchi, T.; Cotton, J. A.; Dalzell, J. J.; Hasegawa, K.; Kanzaki, N.; McVeigh, P.; Takanashi, T.; Tsai, I. J.; Assefa, S. A.; Cock, P. J. A.; et al. Genomic insights into the origin of parasitism in the emerging plant pathogen *Bursaphelenchus xylophilus*. *Plos Pathogens*. **2011**, 7 (9), No. e1002219.
- (50) Rosso, M. N.; Favery, B.; Piote, C.; Arthaud, L.; De Boer, J. M.; Hussey, R. S.; Bakker, J.; Baum, T. J.; Abad, P. Isolation of a cDNA encoding a beta-1,4-endoglucanase in the root-knot nematode *Meloidogyne incognita* and expression analysis during plant parasitism. *Molecular plant-microbe interactions*. **1999**, 12 (7), 585–591.
- (51) Gao, B. L.; Allen, R.; Davis, E. L.; Baum, T. J.; Hussey, R. S. Developmental expression and biochemical properties of a β -1,4-endoglucanase family in the soybean cyst nematode, *Heterodera glycines*. *Molecular Plant Pathology*. **2004**, 5 (2), 93–104.
- (52) Wang, X.; Meyers, D.; Yan, Y.; Baum, T.; Smant, G.; Hussey, R.; Davis, E. In planta localization of a beta-1,4-endoglucanase

secreted by *Heterodera glycines*. *Molecular plant-microbe interactions*. **1999**, 12 (1), 64–67.

(53) Goellner, M.; Smant, G.; De Boer, J. M.; Baum, T. J.; Davis, E. L. Isolation of Beta-1,4-endoglucanase genes from *Globodera tabacum* and their expression during parasitism. *Journal of Nematology*. **2000**, 32 (2), 154–165.

(54) Hewezi, T.; Howe, P.; Maier, T. R.; Hussey, R. S.; Mitchum, M. G.; Davis, E. L.; Baum, T. J. Cellulose binding protein from the parasitic nematode *Heterodera schachtii* interacts with *Arabidopsis* pectin methylesterase: cooperative cell wall modification during parasitism. *Plant Cell*. **2008**, 20 (11), 3080–3093.

(55) Somerville, C.; Bauer, S.; Brininstool, G.; Facette, M.; Hamann, T.; Milne, J.; Osborne, E.; Paredez, A.; Persson, S.; Raab, T.; et al. Toward a systems approach to understanding plant-cell walls. *Science*. **2004**, 306 (5705), 2206–2211.

(56) Abou-Saleh, R. H.; Hernandez-Gomez, M. C.; Amsbury, S.; Paniagua, C.; Bourdon, M.; Miyashima, S.; Helariutta, Y.; Fuller, M.; Budtova, T.; Connell, S. D.; et al. Interactions between callose and cellulose revealed through the analysis of biopolymer mixtures. *Nature Communications*. **2018**, 9, 4538.

(57) Niu, Q.; Zhang, P.; Su, S.; Jiang, B.; Liu, X.; Li, C.; Yu, T.; Yi, H.; Tang, J.; Cao, M. Characterization and expression analyses of callose synthase enzyme (*Cals*) family genes in maize (*Zea mays* L.). *Biochemical Genetics*. **2022**, 60 (1), 351–369.

(58) Boller, T.; He, S. Y. Innate immunity in plants: an arms race between pattern recognition receptors in plants and effectors in microbial pathogens. *Science*. **2009**, 324 (5928), 742–744.

(59) Wang, Y.; Li, X.; Fan, B.; Zhu, C.; Chen, Z. Regulation and function of defense-related callose deposition in plants. *International Journal of Molecular Sciences*. **2021**, 22 (5), 2393.

(60) Rai, K. M.; Balasubramanian, V. K.; Welker, C. M.; Pang, M.; Hii, M. M.; Mendu, V. Genome wide comprehensive analysis and web resource development on cell wall degrading enzymes from phytoparasitic nematodes. *BMC Plant Biology*. **2015**, 15, 187.

(61) Chowdhury, J.; Schober, M. S.; Shirley, N. J.; Singh, R. R.; Jacobs, A. K.; Douchkov, D.; Schweizer, P.; Fincher, G. B.; Burton, R. A.; Little, A. Down-regulation of the *glucan synthase-like 6* gene (*HvGsl6*) in barley leads to decreased callose accumulation and increased cell wall penetration by *Blumeria graminis* f. sp. *hordei*. *New Phytologist*. **2016**, 212 (2), 434–443.

(62) Henrissat, B.; Davies, G. Structural and sequence-based classification of glycoside hydrolases. *Current Opinion in Structural Biology*. **1997**, 7 (5), 637–644.

(63) Béguin, P.; Aubert, J. P. The biological degradation of cellulose. *FEMS Microbiol Reviews*. **1994**, 13 (1), 25–58.

(64) Paccanaro, M. C.; Sella, L.; Castiglioni, C.; Giacomello, F.; Martínez-Rocha, A. L.; D'Ovidio, R.; Schäfer, W.; Favaron, F. Synergistic effect of different plant cell wall-degrading enzymes is important for virulence of *Fusarium graminearum*. *Molecular Plant-Microbe Interact*. **2017**, 30 (11), 886–895.

(65) Goellner, M.; Wang, X.; Davis, E. L. Endo- β -1,4-glucanase expression in compatible plant–nematode interactions. *Plant Cell*. **2001**, 13 (10), 2241–2255.

(66) Perrot, T.; Pauly, M.; Ramírez, V. Emerging Roles of β -Glucanases in plant development and adaptive responses. *Plants*. **2022**, 11 (9), 1119.

(67) Bohlmann, H.; Sobczak, M. The plant cell wall in the feeding sites of cyst nematodes. *Frontiers in Plant Science*. **2014**, 5, 89.

(68) Chen, J.; Piao, Y.; Liu, Y.; Li, X.; Piao, Z. Genome-wide identification and expression analysis of chitinase gene family in *Brassica rapa* reveals its role in clubroot resistance. *Plant Science*. **2018**, 270, 257–267.

(69) Allan, A. C.; Fluhr, R. Two distinct sources of elicited reactive oxygen species in tobacco epidermal cells. *Plant Cell*. **1997**, 9 (9), 1559–1572.

(70) Richa, K.; Tiwari, I. M.; Devanna, B. N.; Botella, J. R.; Sharma, V.; Sharma, T. R. Novel chitinase gene *LOC_Os11g47510* from indica rice tetep provides enhanced resistance against sheath blight pathogen *Rhizoctonia solani* in rice. *Frontiers in Plant Science*. **2017**, 8, 596.

(71) Dong, X.; Zhao, Y.; Ran, X.; Guo, L.; Zhao, D. Overexpression of a new chitinase gene *EuCHIT2* enhances resistance to *Erysiphe cichoracearum* DC. in tobacco plants. *International Journal of Molecular Sciences*. **2017**, 18 (11), 2361.

(72) Barletta, A. B. F.; Smith, J. C.; Burkart, E.; Bondarenko, S.; Sharakhov, I. V.; Criscione, F.; O'Brochta, D.; Barillas-Mury, C. Mosquito midgut stem cell cellular defense response limits *Plasmodium* parasite infection. *Nature Communications*. **2024**, 15 (1), 1422.

(73) Fu, Y.; Tomita, T.; Weiss, L. M.; West, C. M.; Sibley, L. D. *Toxoplasma* chitinase-like protein orchestrates cyst wall glycosylation to facilitate effector export and cyst turnover. *Proceedings of the National Academy of Sciences of the United States of America*. **2025**, 122 (5), No. e2416870122.

(74) Khan, R. S.; Iqbal, A.; Bibi, A.; Khalil, I.; Ul Islam, Z.; Jan, F.; Khalid, A.; Abdalla, A. N.; Wadood, A. Plant chitinases: Types, structural classification, antifungal potential and transgenic expression in plants for enhanced disease resistance. *Plant Cell Tissue and Organ Culture*. **2024**, 156 (3), 75.

(75) Arakane, Y.; Muthukrishnan, S. Insect chitinase and chitinase-like proteins. *Cellular and Molecular Life Sciences*. **2010**, 67 (2), 201–216.

(76) Broglie, K. E.; Gaynor, J. J.; Broglie, R. M. Ethylene-regulated gene expression: molecular cloning of the genes encoding an endochitinase from *Phaseolus vulgaris*. *Proceedings of the National Academy of Sciences of the United States of America*. **1986**, 83 (18), 6820–6824.

(77) Gu, S. Y.; Wang, L. C.; Cheuh, C. M.; Lo, W. S. *CHITINASE LIKE1* regulates root development of dark-grown seedlings by modulating ethylene biosynthesis in *Arabidopsis thaliana*. *Frontiers in Plant Science*. **2019**, 10, 600.

(78) Durner, J.; Klessig, D. F. Inhibition of ascorbate peroxidase by salicylic acid and 2,6-dichloroisonicotinic acid, two inducers of plant defense responses. *Proceedings of the National Academy of Sciences of the United States of America*. **1995**, 92 (24), 11312–11316.



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肖甜甜(18510102238)

发文日:

2025 年 03 月 25 日



申请号: 202510355930.7

发文序号: 2025032501647830

专利申请受理通知书

根据专利法第 28 条及其实施细则第 43 条、第 44 条的规定, 申请人提出的专利申请已由国家知识产权局受理。现将确定的申请号、申请日等信息通知如下:

申请号: 2025103559307

申请日: 2025 年 03 月 25 日

申请人: 河南农业大学

发明人: 李宇, 王珂, 李洪连, 王硕, 施艳, 袁虹霞

发明创造名称: 一种玉米翻译控制肿瘤蛋白 ZmTCTP 及其应用

经核实, 国家知识产权局确认收到文件如下:

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2026年度河南省高等学校优秀科技成果评定结果公示（科技进步类）

项目名称	主要完成单位	主要完成人	等次
乡村振兴进程中相对贫困治理与共同富裕的耦合模式创新及路径实现	河南大学, 安徽财经大学	刘俊英, 耿明斋, 冯海龙, 李晓敏, 田丰韶, 朱磊, 胡志强	壹等
大跨度空间结构智能设计与建造关键技术及工程应用	河南大学, 北京市建筑设计研究院股份有限公司, 浙江大学, 浙江东南网架股份有限公司, 上海非解构数字科技有限公司, 中国建筑第七工程局有限公司, 中国建筑第八工程局有限公司	杜文凤, 薛红京, 周观根, 王洁, 高博青, 李洋, 蔡青, 赫中营, 陈耀东, 王友明, 朱黎明, 朱文兵, 蔡晓博, 贾恒, 王超	壹等
功能蛋白肽精准创制关键技术及应用	河南大学, 河南工业大学, 三全食品股份有限公司, 广东省农业科学院水稻研究所, 许昌学院, 河南牧业经济学院	黄继红, 侯银臣, 廖爱美, 潘龙, 周靖波, 王磊, 冯志强, 王道杰, 高雪丽, 田海龙, 张芯蕊, 杨金初, 李浩, 汪桢	壹等
高效安全的高维异构数据挖掘关键技术及应用	河南大学, 中核勘察设计研究有限公司, 郑州数智技术研究院有限公司	赵雅靓, 袁科, 韩宏宇, 王金科, 苏文杰, 范明虎, 苗长伟, 党兰学, 程杨, 孔令鹏	壹等
纤维/纤维聚合物增强混凝土结构性能提升关键技术与应用	河南工程学院, 中建海峡建设发展有限公司, 河南城建学院, 河海大学, 中国建筑第七工程局有限公司	陈刚, 张峰, 尤培波, 朱海堂, 袁健松, 王伟强, 郜玉芬, 王耀, 赵亮平, 宋帅奇	壹等
面向精准医疗的多模态大数据智能挖掘关键技术创新及示范应用	河南工学院, 上海交通大学, 河南师范大学, 河南医药大学, 华兰安康生物股份有限公司, 郑州大学	王鲜芳, 魏冬青, 王伟, 杜昊泽, 崔俊伟, 张华菲, 刘依锋, 王晓雷, 侯淑梅, 孙斌, 杜志勇, 张东方, 庞笑笑, 郭赛迪, 吕秋杰	壹等

项目名称	主要完成单位	主要完成人	等次
河南省地方羊种质资源创新应用	河南牧业经济学院, 中国农业科学院北京畜牧兽医研究所, 河南省畜牧技术推广总站, 河南省农业科学院动物疫病防控研究所(河南省农业科学院动物免疫学重点实验室), 中亨农牧(河南)有限公司, 新乡市雨轩清真食品股份有限公司, 浚县鑫林牧业有限公司	权凯, 魏彩虹, 王献伟, 李君, 韩浩园, 王慧华, 邢广旭, 金美林, 刘昆, 施会彬, 李春龙, 王拥庆, 王峰, 张道江, 余世锋	壹等
花生高产抗病育种关键技术创新与新品种培育及应用	河南农业大学	殷冬梅, 唐月异, 李拴柱, 滕开琼, 赵凯, 赵昆昆, 李培培, 马金娜, 周彦忠, 任锐, 巩方平, 李宪挺, 李振华	壹等
苹果品种结构优化与轻简栽培关键技术创新及应用	河南农业大学	郑先波, 白团辉, 焦健, 王苗苗, 张坤玺, 刘昱, 宋春晖, 史江莉, 万然, 郝鹏博, 徐宛玉, 赵玉洁, 申亚文, 丛柳, 李明	壹等
黄淮夏玉米主要病虫害成灾规律与绿色防控技术创新及应用	河南农业大学, 河南省植物保护检疫站, 中国农业大学, 先正达(上海)作物保护科技有限公司, 河南云飞科技发展有限公司, 河南省济源白云实业有限公司, 漯河市农业科学院	施艳, 李洪连, 张国彦, 杨雪, 赵曼, 王芹芹, 李宇, 范在丰, 徐永伟, 范志业, 张利娟, 周国涛, 袁虹霞, 贾少锋, 方分分	壹等
农林生物质复合材料低碳加工与高质利用关键技术	河南农业大学, 千年舟新材科技集团股份有限公司, 南京林业大学, 西南林业大学, 南京工程学院, 华南理工大学, 浙江树人学院	彭万喜, 陆胤, 陆铜华, 贾翀, 宋国辉, 徐开蒙, 武书彬, 郑海涛, 王世伟, 田得君, 石洋, 李城, 岳肖晨, 王璐瑶, 韩丹丹	壹等

成果关联性自评与专家认定表

本项目的研究思路是：1 明确黄淮地区根腐线虫的发生分布情况、病原种类、优势种及其致病力；2 以优势种（咖啡短体线虫）为后续的研究对象，获得强致病力和弱致病力种群的转录组数据，筛选侵染致病相关的基因；3 在咖啡短体线虫侵染阶段显著上调表达的基因中筛选致病相关基因，对其进行后续的功能验证，明确致病基因编码蛋白与寄主互作蛋白相互作用促进线虫侵染的分子机制。

成果（论文）1：

1. Qin Ling, Lin Fankang, Lv Yunlong, Tai Zelin, Zhang Xu, Li Honglian, **Li Yu***(通讯作者), Wang Ke*. Identification of *Pratylenchus coffeae* as a causal agent of root rot disease in *Sorghum bicolor* in China. BMC Microbiology, 2025, 25, 41. （第一标注）

本项成果属于本资助项目研究内容的第 1 部分内容（明确黄淮地区根腐线虫的发生分布情况、病原种类等）。

本研究在中国山西省的高粱田中发现了生长迟缓、根部呈褐色并腐烂的病株，通过形态学和分子生物学鉴定为咖啡短体线虫(*Pratylenchus coffeae*)，通过柯赫氏法则验证 *P. coffeae* 是引起该病害的病原。本研究首次报道了引起中国高粱根腐病的病原为 *P. coffeae*，该线虫对高粱均具有高度致病性，但各种群之间的致病性存在显著差异。本研究结果为高粱根腐线虫病的识别和检测提供了科学依据。

成果（论文）2：

2. Wang Shuo, Wang Ke, Niu Wenlong, Lin Fankang, Dababat. Abdelfattah A, Shi Yan, Yuan Hongxia, Xia Yanhui, Li Honglian, **Li Yu***(通讯作者). *Pratylenchus coffeae* effector Pc-ENG3 targets the maize TCTP protein to facilitate parasitism. The Crop Journal, 2026, 14(2), 388–399. （第二标注）

本项成果属于本资助项目研究内容的第 3 部分内容（明确咖啡短体线虫致病相关蛋白 Pc-ENG3 与寄主玉米 ZmTCTP2a 蛋白互作促进线虫侵染的分子机制）。

本研究中，我们鉴定出一种新型的 β -1,4-内切葡聚糖酶基因 *Pc-ENG3*，其在咖啡短体线虫的食道腺细胞和生殖腺特异性表达，并在寄生阶段显著上调。证实 *Pc-ENG3* 的 N 端信号肽具有功能，表明其可能参与分泌。*Pc-ENG3* 在线虫侵染过程中发挥重要作用。*Pc-ENG3* 蛋白与玉米 *ZmTCTP2a* 互作，通过抑制免疫反应以及根尖细胞的增殖与分化，进而促进线虫寄生。本研究解析了效应蛋白 *Pc-ENG3* 反向调控玉米对线虫的抗病能力，通过“抗病抑制+组织重塑”双重策略促进线虫寄生的分子机制。

成果（论文）3：

3. Gao Lingling, Lv Yunlong, Zhang Can, Niu Wenlong, Li Honglian, **Li Yu**, Wang Ke*. Discovery of *Pratylenchus zae* causing root rot of winter wheat in Anhui Province of China. Plant disease, 2025, 109(8), 1794. （第三标注）

本项成果属于本资助项目研究内容的第 1 部分（明确黄淮地区根腐线虫的发生分布情况、病原种类等）。本研究首次在中国安徽发现由玉米短体线虫 *Pratylenchus zae* 侵染引起的小麦根腐病，该成果完善了黄淮地区小麦根腐线虫的种类及其对寄主的致病力。

成果（论文）4：

4. Wang Shuo, Niu Wenlong, Wang Ke, Xu Feifei, Dababat. Abdelfattah A, Shi Yan, Yuan Hongxia, Wang Hua, Xia Yanhui, Li Honglian, **Li Yu***(通讯作者). *Pratylenchus coffeae* effector *Pc-ENG2* targets the maize Chitinase *ZmCHI5* to promote parasitism. Journal of Agricultural and Food Chemistry, 2025, 73(43), 27435–27449. （第四标注）

本项成果属于本资助项目研究内容第 3 部分（明确咖啡短体线虫致病相关蛋白 *Pc-ENG3* 与寄主玉米 *ZmTCTP2a* 蛋白互作促进线虫侵染的分子机制）的延伸内容。

本项目主要研究的基因是 *Pc-ENG3*，但在该基因的研究过程中我们克隆得到了其家族基因 *Pc-ENG2*，两者的相似度非常高，我们猜测 *Pc-ENG2* 可能与 *Pc-ENG3* 同时起作用或者具有 *Pc-ENG3* 没有的功能，以促进线虫的侵染寄生，因此我们在研究 *Pc-ENG3* 的过程中结合其他项目顺带一起研究了 *Pc-ENG2* 的功能。*PcENG2* 与玉米几丁质酶 *ZmCHI5* 互作，通过抑制宿主活性氧（ROS）的产生，并增强了宿主对 *P. coffeae* 的易感性，促进线虫的侵染

寄生。以上结果表明 Pc-ENG2 和 Pc-ENG3 分别与寄主不同的抗性蛋白互作，通过调控寄主的免疫反应或抗性进而促进线虫的侵染和寄生，研究结果完善了咖啡短体线虫 ENG 蛋白家族的功能解析，具有重要的意义。

成果（专利）5：

5. 中国发明专利，一种玉米翻译控制肿瘤蛋白 ZmTCTP 及其应用，申请号：2025103559307，申请日期：2025.3.25，实审。

本项成果属于本资助项目研究内容第 3 部分内容（咖啡短体线虫致病相关蛋白 Pc-ENG3 与玉米 ZmTCTP2a 蛋白互作促进线虫侵染的分子机制）。

玉米翻译控制肿瘤蛋白 ZmTCTP2a 在玉米抗病中的功能尚未见报道，本研究首次明确了玉米 *ZmTCTP2a* 是重要的抗根腐线虫病基因，其编码蛋白 ZmTCTP2a 与咖啡短体线虫 Pc-ENG3 蛋白互作。玉米 *ZmTCTP2a* 是一个重要的抗病基因，在后期的玉米抗病育种中具有重要的研究价值。因此，本团队以玉米 ZmTCTP2a 为主体在 2025.3.25 日申请了国家发明专利，目前处于实审阶段。

河南农业大学植物保护学院

2026.8.13