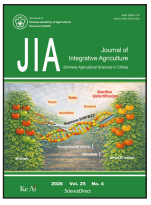
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RESEARCH ARTICLE

A novel effector of *Aphelenchoides besseyi*, AbPFN3, interacts with multiple host proteins to facilitate parasitic nematode and sustain infection in rice

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Highlights

- Identifies AbPFN3 as a novel *Aphelenchoides besseyi* effector upregulated in juveniles and secreted from esophageal glands.
- Reveals AbPFN3 promotes parasitism by targeting host proteins (OsAAC1/OsBAP31/OsSAUR50) across multiple cellular compartments.

Abstract

The rice white tip nematode (RWTN) *Aphelenchoides besseyi* secretes effectors that manipulate host plant cells to facilitate successful parasitism and sustain infection. Although the number of identified RWTN effectors remains limited, their mechanisms of interaction with host plants are largely unknown. Profilins (PFNs) function as molecular hubs that regulate complex interaction networks. To advance understanding of PFN3 in plant-parasitic nematodes, we identified an effector from *A. besseyi*, designated AbPFN3. *AbPFN3* is transcriptionally upregulated during the juvenile stage of the nematode, and *in situ* hybridization localized its expression to the esophageal glands. Three rice (*Oryza sativa*) proteins, ADP/ATP carrier protein 1 (OsAAC1), B-cell receptor-associated protein 31 (OsBAP31) and Small Auxin Up RNA 50 (OsSAUR50), were identified as interactors of AbPFN3, with interactions occurring in distinct cellular compartments, including the endoplasmic reticulum, cytoplasm, and plasma membrane. Transgenic analyses revealed that *AbPFN3* expression significantly increased plant height and upregulated *AAC1* and *BAP31*, while downregulating *RGA2* and *SAUR50*. This study characterizes AbPFN3 as a novel effector secreted by *A. besseyi* that interacts with multiple host proteins, highlighting its potential role in modulating host defense responses and cell development processes.

Keywords: *Aphelenchoides besseyi*, profilin 3, protein–protein interactions, effector

1. Introduction

Plant-parasitic nematodes (PPNs) pose a serious threat to global food production, causing up to USD 173 billion in annual economic losses. The rice white tip nematode (RWTN), *Aphelenchoides besseyi*, is a major pathogen of rice, named for the characteristic dry tip symptom it induces, and can reduce annual rice yields by up to 50%. This nematode exhibits a broad host range, parasitizing more than 200 plant species beyond rice. In recent years, RWTN has garnered increased attention due to reports of new hosts, including soybean and cotton (Meyer *et al.* 2017; Favoreto *et al.* 2018). Therefore, in-depth knowledge of the molecular mechanisms underlying RWTN pathogenicity is essential for understanding this nematode and developing safe, efficient control strategies.

Plant-parasitic nematodes secrete enzymes and other effector proteins into their hosts to establish successful infection and sustain nutrient uptake. These effectors

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facilitate the establishment and maintenance of parasitism. Numerous candidate effectors from PPNs have been identified through genomics, transcriptomics, and proteomics approaches. Secreted from esophageal gland cells, multiple enzymes, including cellulases, pectate lyases, serine carboxypeptidases, and cellulose-binding proteins, have been shown to support nematode parasitism (Rehman *et al.* 2009; Palomares-Rius *et al.* 2014; Huang *et al.* 2022; Yang *et al.* 2024). Other effectors, such as SPRYSEC-19, Mi-CRT, MjTTL5, and MgGPP, suppress plant defense responses through diverse mechanisms (Postma *et al.* 2012; Jaouannet *et al.* 2013; Lin *et al.* 2016; Chen *et al.* 2017). Additionally, effectors like HsIPT (Siddique *et al.* 2015) and chorismate mutase (CM) (Lambert *et al.* 1999) modulate host cell growth by directly or indirectly interfering with phytohormone signaling pathways. In recent years, plant peptide hormone mimics have emerged as key virulence factors. For instance, CEP (C-TERMINALLY ENCODED PEPTIDE) and CLAVATA3/EMBRYO SURROUND REGION-RELATED (CLE)-like (CLAVATA3/EMBRYO SURROUNDING REGION-like) peptides, when introduced into plants, alter root development (Mitchum *et al.* 2012). Similarly, RALF-like (rapid alkalinization factor and homologues) peptides exhibit canonical plant RALF activities and directly bind to the extracellular domain of FERONIA to modulate specific steps in nematode parasitism-related immune responses and cell expansion (Zhang *et al.* 2020).

Among these identified effectors, several interact with multiple host proteins. For example, the nematode effector 25A01 interacts with various plant proteins, including an *Arabidopsis* F-box-containing protein, chalcone synthase, and the translation initiation factor eIF-2β, to enhance host susceptibility (Pogorelko *et al.* 2016). HsSNARE1 (soluble NSF attachment protein receptor) promotes cyst nematode disease by interacting with both AtSNAP2 and AtPR1 (Zhao *et al.* 2022). Likewise, MgMO237 facilitates parasitism by *Meloidogyne graminicola* through interactions with three endogenous rice proteins, thereby suppressing basal plant immunity during later parasitic stages (Chen *et al.* 2018).

Profilins (PFNs) are small actin-binding proteins that are essential in all examined organisms. Their primary role is to regulate actin polymerization dynamics during cellular motility. Beyond actin binding, profilins serve as hubs that orchestrate a complex network of molecular interactions, including nucleotide-exchange activity and promotion of filament elongation at free barbed ends. The interplay between PFNs and actin has been investigated in nematodes; notably, in the root-knot nematode, MiPFN3 suppresses the aberrant plant growth phenotype caused by misexpression of reproductive actin (*AtACT1*), disrupts actin polymerization, and diminishes the filamentous actin network (Leelarasamee *et al.* 2018).

The primary objectives of this study were to identify a profilin domain-containing effector protein in RWTN and to functionally characterize its molecular mechanisms of action. Here, we identified the profilin domain-containing protein AbPFN3 and elucidated its indirect modulation of the host plant's immune response through interaction with B-cell receptor-associated protein 31 (BAP31), as well as its influence on plant physiology and development *via* interactions with Small Auxin Up RNA 50 (SAUR50) and ADP/ATP carrier protein 1 (AAC1). These findings collectively reveal the functional diversity of the effector AbPFN3 in nematode parasitic infection.

2. Materials and methods

2.1. Biological materials and culture conditions

The *A. besseyi* used in this study were isolated from rice leaves and cultured on carrot disks according to previously described methods (Tulek *et al.* 2009). To assess nematode infestation, pot experiments were conducted using the rice cultivar (*Oryza sativa* L. *japonica* cv. Nipponbare). Briefly, rice seedlings were grown in 0.5 L plastic containers filled with approximately 400 g of sterilized soil and maintained at 28°C under a 16 h light/8 h dark photoperiod with 60–80% relative humidity in a controlled growth chamber. Seeds of the Col-0 ecotype of *Arabidopsis thaliana* were maintained in our laboratory. Surface-sterilized seeds of *A. thaliana* (including wild-type and transgenic lines) were sown and cultured on Murashige and Skoog (MS) medium (Sigma-Aldrich, St. Louis, MO, USA). Rice protoplasts (*O. sativa*) were prepared as previously described (He *et al.* 2016).

2.2. Gene amplification and sequence analysis

The full-length sequence of *AbPFN3* was amplified using specific primers PFN3f/PFN3r (Appendix A). PCR was performed according to the manufacturer's instructions for KOD Plus Neo (TOYOBO, Osaka, Japan) at an annealing temperature of 60°C, using *A. besseyi* cDNA as template. The cDNA sequence of *AbPFN3* was confirmed by sequencing. SignalP 5.0 (https://services.healthtech.dtu.dk/service.php?SignalP-5.0) and TMHMM server v. 2.0 (https://services.healthtech.dtu.dk/service.php?TMHMM-2.0) were used to predict the signal peptide and transmembrane domains in AbPFN3. Conserved domains of AbPFN3 were predicted using Pfam (https://www.ebi.ac.uk/interpro/) and SMART (https://smart.embl.de/). Subcellular localization of the protein was predicted using PSORT (https://psort.hgc.jp/).

To construct the phylogenetic tree, the amino acid sequence of AbPFN3 was used to query the NCBI nonredundant protein database (nr), and 15 amino acid sequences from 12 species were selected for comparative analysis. Phylogenetic analyses were performed using MEGA 7 software (Kumar *et al.* 2016).

2.3. Quantitative reverse-transcriptase PCR (qRT-PCR)

After 50 days of culturing, nematodes on carrot disks were isolated according to Huang *et al.* (2022). Mixed-stage nematodes were suspended in sterile water, and 1,000 females, males, juveniles, and eggs were individually collected under a microscope. Total RNA was extracted from eggs, juveniles, males, and females of *A. besseyi* using the miRNeasy Micro Kit (Qiagen, Düsseldorf, Germany). cDNA templates for qPCR were synthesized with HiScript II Q RT SuperMix (Vazyme, Nanjing, China). qPCR was performed on a CFX96 qPCR instrument using primers PFNqf/PFNqr, following the protocol of the AceQ qPCR SYBR Green Master Mix Kit (Vazyme). Additionally, primers

act-f/act-r were designed to amplify fragments of the internal reference gene (*Actin*). For qRT-PCR analysis of transgene expression in stable transgenic lines, RNA was extracted from seeds or whole plants of *A. thaliana*, and qRT-PCR was performed as described (Huang *et al.* 2019). Relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method. Each experiment included three biological replicates and was repeated three times. Significant differences in *AbPFN3* expression among RWTN developmental stages were analyzed by Duncan's multiple range test ($P<0.05$), while differences in transgenic *Arabidopsis* relative to wild-type Col-0 were assessed using one-way ANOVA in SPSS 25 (IBM, Armonk, NY, USA). Primers for qRT-PCR are listed in Appendix A.

2.4. *In situ* hybridization

Using the purified PCR product of *AbPFN3* as template, two fragments containing the T7 promoter sequence at the 5′ or 3′ ends were amplified separately. DIG-labeled sense and antisense probes were synthesized from these fragments using DIG RNA Labeling Mix (Roche, Basel, Switzerland) according to the manufacturer's protocol. *In situ* hybridization was performed as previously described (de Boer *et al.* 1998). After hybridization at 47°C for 14 h, nematodes were examined using a Leica DM1000 LED microscope (Leica, Wetzlar, Germany).

2.5. Subcellular localization analysis

To observe the subcellular localization of *AbPFN3* in the host plant, *AbPFN3* was cloned into pAN580 fused with GFP at the C terminus. The GFP-fusion plasmid was introduced into rice protoplasts using the method described previously (Mravec *et al.* 2009). Pro35S:SPER-mKATE was used as an endoplasmic reticulum marker. GFP fluorescence was excited at 488 nm, and emission was detected between 509/526 nm. Chlorophyll autofluorescence was excited at 640 nm, and emission was detected between 640/700 nm. Fluorescence signals were captured using a Nikon C2-ER microscope (Nikon, Tokyo, Japan) in the dark at room temperature. Empty vectors expressing eGFP served as controls.

2.6. Co-immunoprecipitation mass spectrometry (Co-IP/MS) assay

To identify proteins interacting with AbPFN3, AbPFN3 and its associated proteins were immunoprecipitated and subjected to mass spectrometry. Two hundred mixed-stage RWTNs were inoculated onto rice plants at the two-leaf stage. Ten days post-inoculation, infected rice leaves were collected, frozen in liquid nitrogen, and stored at −80°C. Proteins were extracted from leaf samples as detailed previously (Wei *et al.* 2021). Constructs pBWA(V)HS:*AbPFN3*-GFP and pBWA(V)HS:GFP were generated to express *AbPFN3* or GFP, respectively. Bait protein precipitation and Co-IP/MS analysis were performed as described (Sukarta *et al.* 2022). Protein identification was carried out by matching spectra from each run to a database comprising translated ESTs and UniProt entries for *O. sativa* using MaxQuant software.

To verify interactions between AbPFN3 and candidate proteins, a pull-down assay was performed using a rice protoplast transient expression system as described (An *et al.* 2022).

2.7. Bimolecular fluorescence complementation (BiFC) assay

For the BiFC assay, the coding sequences of *AbPFN3* and candidate interacting genes (*OsSAUR50*, *OsBAP31*, and *OsAAC1*) were cloned into pXY104 or pXY106 to generate pXY104-*AbPFN3* and pXY106-(*OsSAUR50*, *OsBAP31*, and *OsAAC1*). All BiFC constructs were transformed into *Agrobacterium tumefaciens* GV3101, and combinations were co-transformed into rice protoplasts *via* PEG-mediated transfection as described (Song *et al.* 2021). Reconstituted YFP signals were observed 48 h post-transfection under the GFP channel of a Nikon C2-ER microscope.

2.8. Generation of transgenic plants

For *AbPFN3* overexpression, the *AbPFN3* coding sequence was cloned into the pBWA(V)HS vector to generate pBWA(V)HS:*AbPFN3*.

The overexpression construct was introduced into *Agrobacterium tumefaciens* strain EHA105. *Arabidopsis* transformation was carried out using the floral dip method as previously described (Zhao *et al.* 2022). Transformed seedlings were grown under standard growth conditions. Harvested seeds were screened on 1/2 MS medium supplemented with hygromycin to select positive transformants. Putative transgenic lines were further confirmed by RT-PCR using primers listed in Appendix A, and seeds were collected from verified positive plants. T2 plants were genotyped by PCR prior to phenotypic and gene expression analyses.

2.9. Statistical analysis

All data were derived from three independent biological replicates and presented as mean±standard error (SE). One-way ANOVA was performed using GraphPad Prism 7, followed by Tukey's multiple comparison test at a significance level of $P<0.05$. Student's *t*-test was used to compare means between experimental and control groups to determine statistical significance.

3. Results

3.1. Identification and sequence analysis of the *AbPFN3* from *A. besseyi*

Based on previously published transcriptome data from two RWTN populations exhibiting differing levels of pathogenicity and reproduction (Wang *et al.* 2018), a gene homologous to *Meloidogyne incognita MiPFN3* was selected for functional investigation in RWTN. The complete cDNA sequence of this gene was obtained and contains a 381 bp open reading frame (ORF) encoding a 126-amino acid profilin domain-containing protein. This protein shares 59.52% identity with *Globodera pallida* profilin 3 (GpPFN3) and 40.60% identity with *A. thaliana* profilin 5 (Fig. 1-A); thus, the gene was designated *AbPFN3*. Analysis using SignalP predicted no N-terminal

signal peptide for secretion, and TMHMM detected no putative transmembrane domains (Appendix B). Phylogenetic analysis revealed that the five profilin proteins from RWTN cluster into two distinct groups, with AbPFN3 grouping closely with profilins from other plant-parasitic nematodes, suggesting potential functional conservation (Fig. 1-B).

3.2. *AbPFN3* upregulated in *A. besseyi* esophageal gland during parasitic stages

In situ hybridization using a DIG-labeled antisense probe revealed strong signal localization in the esophageal glands of *A. besseyi* (Fig. 2-A). No hybridization signals were detected in nematodes or eggs hybridized with the control sense probe.

Quantitative PCR (qPCR) analysis of *AbPFN3* transcription across four developmental stages of *A. besseyi* showed the highest relative expression in juveniles, followed by females and then males, with minimal expression in eggs (Fig. 2-C). These results suggest that *AbPFN3* encodes a protein secreted from esophageal gland cells and may play a role during the parasitic stages of *A. besseyi*.

3.3. *AbPFN3* is localized in multiple subcellular compartments

The fluorescence emitted by the GFP fusion of AbPFN3 overlapped with chlorophyll autofluorescence, confirming the WoLF PSORT prediction of chloroplast localization (Fig. 3-A). In addition, strong merged fluorescence signals were observed on the endoplasmic reticulum upon co-expression of AbPFN3:GFP with the ER marker protein mKATE:SPPER in rice protoplast cells, further confirming its localization to the endoplasmic reticulum (Fig. 3-B).

3.4. Screening of AbPFN3 interacting proteins using Co-IP/MS

In rice protoplasts, we performed co-immunoprecipitation coupled with mass spectrometry (Co-IP/MS) to identify putative interacting proteins of AbPFN3. A total of 92 proteins were identified, of which 21 were quantified (Appendix C). Among these, 11 proteins were considered potential interactors of AbPFN3. To extract biologically meaningful insights, we manually annotated these proteins based on published literature that convincingly reported their subcellular localization and/or specific function, including a leucine-rich repeat (LRR) protein (OsRGA2, Os01g0937300), ADP/ATP carrier protein 1 (OsAAC1, Os05g0302700), B-cell receptor-associated protein 31 (OsBAP31, Os06g0367500), and auxin response protein SAUR50 (OsSAUR50, Os04g0517900) (Table 1).

3.5. Validation of protein–protein interactions

To further confirm the accuracy of the IP-MS method and gain insight into the function of *AbPFN3*, we performed pull-down assays and BiFC imaging. Although both Co-IP/MS and pull-down experiments suggest a potential interaction between OsRGA2 and AbPFN3, no fluorescence signal was observed

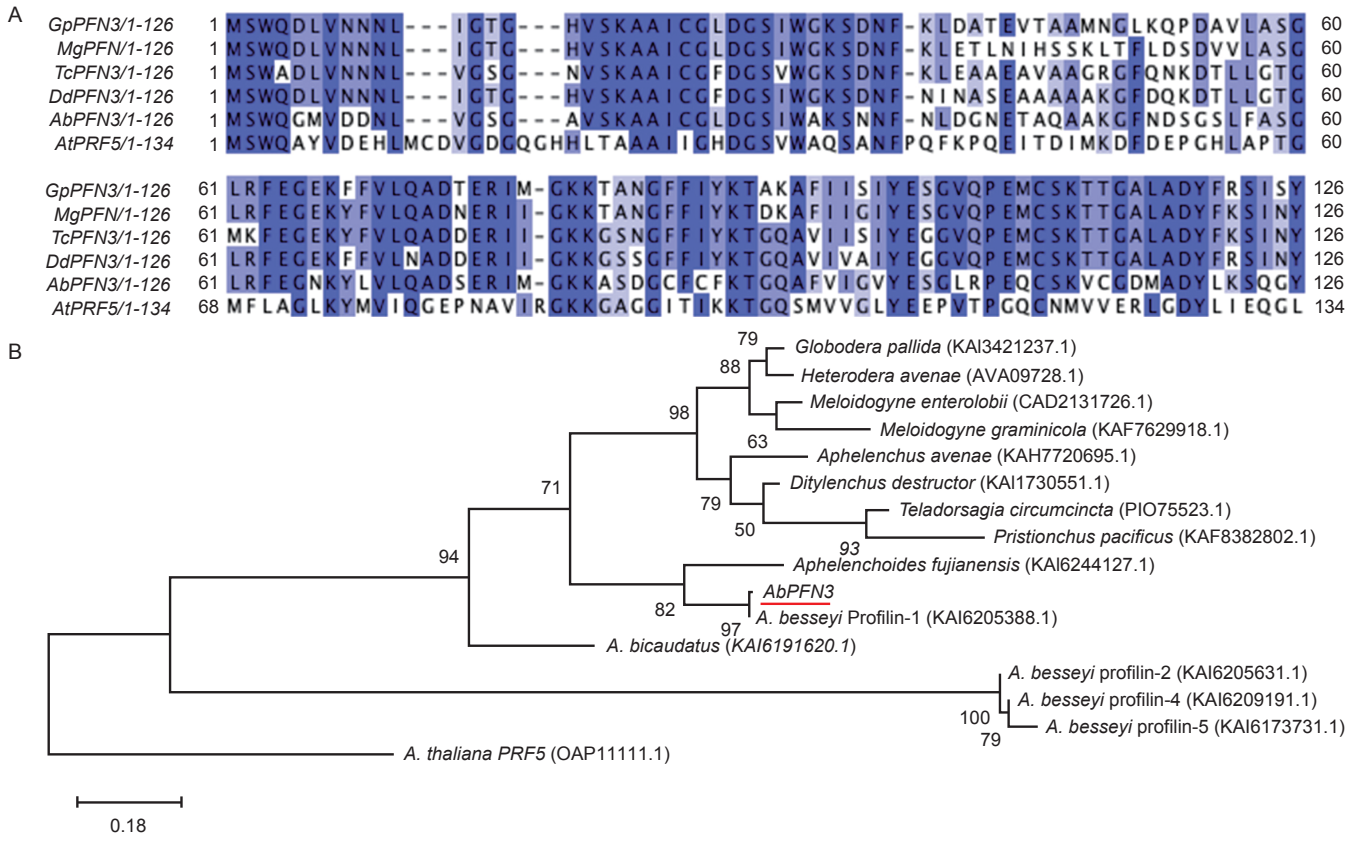


Fig. 1 Sequence analysis of *AbPFN3*. A, multiple sequence alignment of AbPFN3 with homologs from other nematodes and *Arabidopsis thaliana*. B, maximum-likelihood phylogenetic tree inferred from profilin (PFN) sequences.

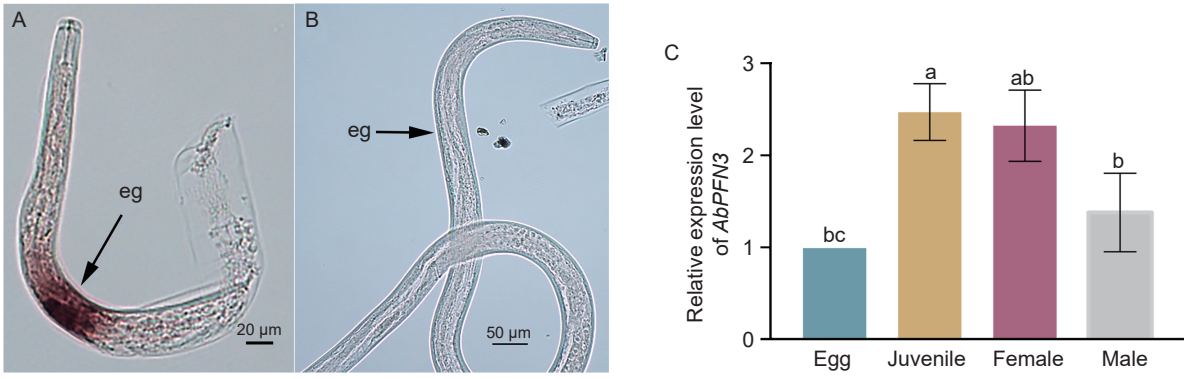


Fig. 2 Expression of *AbPFN3* in *Aphelenchoides besseyi*. A, *AbPFN3* was expressed in the esophageal glands. B, no hybridization signals were observed in the control nematodes. eg, esophageal glands. C, relative expression of *AbPFN3* in *A. besseyi* at different life stages. Bars indicate the standard error of the mean (n=3), and different letters indicate significant differences (P<0.05) between treatments.

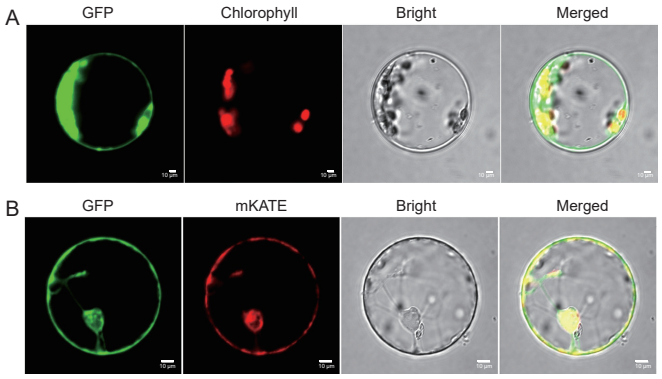


Fig. 3 Subcellular localization of AbPFN3 in endoplasmic reticulum and chloroplast. A, AbPFN3 localized in endoplasmic reticulum. B, AbPFN3 localized in chloroplast. Scale bar=10 μm.

following co-expression of AbPFN3-N-YFP and OsRGA2-C-YFP in rice protoplasts (Fig. 4-A). In contrast, the interactions between AbPFN3 and three other proteins, OsAAC1, OsBAP31, and SAUR50, were successfully validated by BiFC. The AbPFN3–OsAAC1 interaction localized to the endoplasmic reticulum (Fig. 4-D); the AbPFN3–OsBAP31 interaction occurred in the cytoplasm (Fig. 4-E); and the AbPFN3–SAUR50 interaction was detected at the plasma membrane and in the cytoplasm (Fig. 4-F).

3.6. AbPFN3 manipulates multiple processes in host cells

To investigate the role of AbPFN3 in regulating host growth, development, and disease resistance, we generated transgenic *Arabidopsis* plants overexpressing AbPFN3. Through sequence alignment, we identified *Arabidopsis* genes with the highest similarity to *OsRGA2*, *OsBAP31*, *OsSAUR50*, and *OsAAC1*. The expression levels of these homologous genes were then assessed by qPCR. Expression of *RGA2* (NC_003070.9) and *SAUR50* was suppressed, whereas that of *BAP31* (At5g42570) and *AAC1* (NM_111692.3) was enhanced (Fig. 5-A).

Transgenic *Arabidopsis* plants were significantly taller than wild-type plants (Fig. 5-C and D). Given that SAUR50 is an interacting partner of AbPFN3, we hypothesized that

Table 1 AbPFN3 specific interactors with known subcellular localization and function

Gene	Description	Subcellular location	Reference	Function	Verification
atpA	ATP synthase subunit alpha	Chloroplast	Tang *et al.* (2004)		–
RPL2	60S ribosomal protein L2	Mitochondrial	Diedrich *et al.* (2000)	Association of the ribosomal subunits, tRNA binding and peptidyl transfer	–
Os01g0886600	CLP protease regulatory subunit CLPX1	Mitochondria	Halperin *et al.* (2001)	Proteolytic	–
Os03g63410	Elongation factor Tu	Chloroplast	Fu *et al.* (2012)	Abiotic stresses	–
Os01g0937300	Disease resistance protein RGA2	Nucleus	Silverstone *et al.* (2001)	Plant immunity	Pull down
Os02g0183500	Uncharacterized LOC4328523	–	–		–
Os06g0367500	Similar to B-cell receptor-associated protein 31-like containing protein	Endoplasmic reticulum	Quistgaard (2021)	Protein sorting and transport; proapoptotic signaling	Pull down BiFC
Os05g0302700	ADP,ATP carrier protein 1	Mitochondria	Fiore *et al.* (1998)	ADP/ATP transport	BiFC
Os01g0299500	Uncharacterized LOC4325774	–	–		–
Os05g0352700	TATA box-binding protein-associated factor RNA polymerase I subunit B	Ribosomal	Mishal and Luna-Arias (2022)	Initiation of transcription	–
Os04g0517900	Auxin-responsive protein SAUR50	Chloroplast/ Nucleus	Zhang *et al.* (2021)	Cell elongation	Pull down BiFC

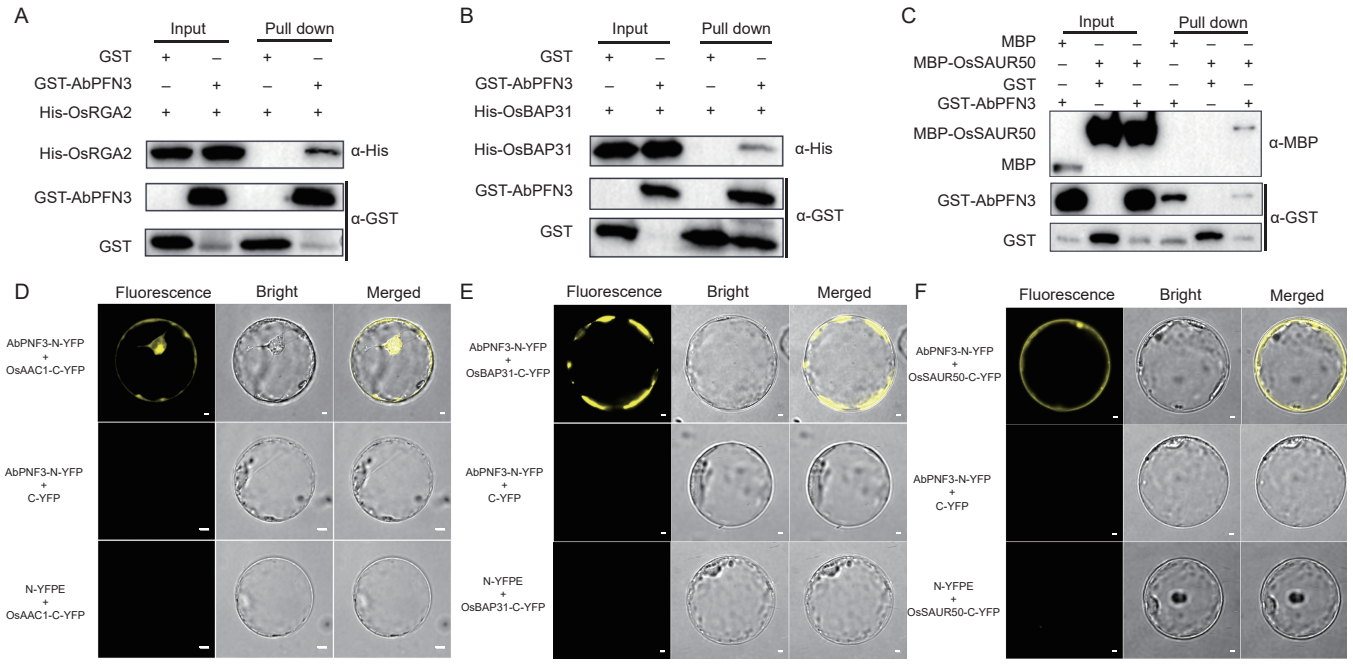


Fig. 4 Identification of interacting proteins of AbPFN3 in rice. A–C, pull-down assays for AbPFN3. Recombinant proteins, as indicated, were expressed in *Escherichia coli* and mixed together, followed by GST or MBP pull-down and immunoblot analysis. Interactions between AbPFN3 and OsRGA2 (A), AbPFN3 and OsBAP31 (B), AbPFN3 and OsSAUR50 (C), along with GST and MBP alone, served as negative controls. D–F, BiFC assays for AbPFN3. The N-terminal half of yellow fluorescent protein (YFP) fused to AbPFN3 and the C-terminal half of YFP fused to OsAAC1 (D), OsBAP31 (E), and OsSAUR50 (F) were co-expressed in rice protoplasts. Controls included AbPFN3-N-YFP with empty vector (C-YFP), OsAAC1 (OsBAP31, OsSAUR50)-C-YFP with empty vector (N-YFP), and empty vectors alone. Scale bar=10 μm.

AbPFN3 might influence plant growth. To test this, we examined the expression of 13 *Arabidopsis SAUR* genes from five classesexhibiting distinct expression patterns (Sun *et al.* 2016) using quantitative real-time PCR. *SAUR16*, *SAUR17*, *SAUR44*, and *SAUR45* were up-regulated in transgenic lines relative to wild-type, with *SAUR45* showing a 320-fold increase. In contrast, several other *SAUR* family members, including *SAUR6*, *SAUR14*, *SAUR15*, *SAUR50*, *SAUR51*, and *SAUR54*, were down-regulated. Transcripts of *SAUR12*, *SAUR46*, and *SAUR47* were undetectable in both transgenic and wild-type plants (Fig. 5-B).

4. Discussion

We have identified a profilin gene, *AbPFN3*, as a novel nematode effector that is upregulated during the parasitic life stages of RWTN. It potentially regulates host cell metabolism through interactions with SAUR50, AAC1, and BAP31, thereby facilitating nematode parasitism. The *A. besseyi* genome contains five profilin genes, more than any other known nematode species. In contrast, *C. elegans* possesses three profilin genes, all of which exhibit actin-binding activity. To date, profilin in parasitic nematodes has been characterized only in *M. incognita*, where it promotes nematode invasion by interacting with actin. Our study confirms that profilins secreted by plant-parasitic nematodes can also interact with multiple host proteins to enhance parasitism.

Plant-parasitic nematodes possess remarkable abilities to alter plant development, physiology, and immunity by secreting proteins or other molecules into both the plant cytoplasm (Lin *et al.* 2013; Lilley *et al.* 2018) and apoplast (Vieira *et al.* 2011; Eves-van den Akker *et al.* 2014). Since the discovery of a non-coding motif termed the 'DOG-box' in the promoter regions of nematode effectors (Eves-van den Akker *et al.* 2016), the conventional criteria for effector identification, such as the presence of a signal peptide and absence of transmembrane domains, are no longer strictly required. Similar DOG-box motifs have since been identified in *Bursaphelenchus xylophilus* (Espada *et al.* 2018) and *Heterodera glycines* (Masonbrink *et al.* 2019), based on deep genomic data mining. Although the *A. besseyi* genome has been sequenced (Ji *et al.* 2023), DOG-box motifs have not yet been detected. AbPFN3 represents an atypical plant-parasitic nematode effector lacking a canonical secretion signal sequence, raising the possibility that a DOG-box motif exists in its promoter region. Several alternative secretion mechanisms may facilitate the release of such proteins: direct translocation across the plasma membrane (Type I), ABC transporters (Type II), membrane vesicle transport (Type III), and transmembrane protein-mediated export (Type IV). The occurrence of effectors without classical signal peptides is not unprecedented in plant-parasitic nematodes; for instance, MiPFN3 has been directly detected in nematode secretions (Leelarasamee *et al.* 2018). Additional examples include Mi-14-3-3 and Mi-GSTS-1 (Jaubert *et al.* 2004; Dubreuil *et al.* 2007). Notably, *AbPFN3* is expressed in the nematode's esophageal glands, indicating that it is secreted *via* the stylet directly into the host plant.

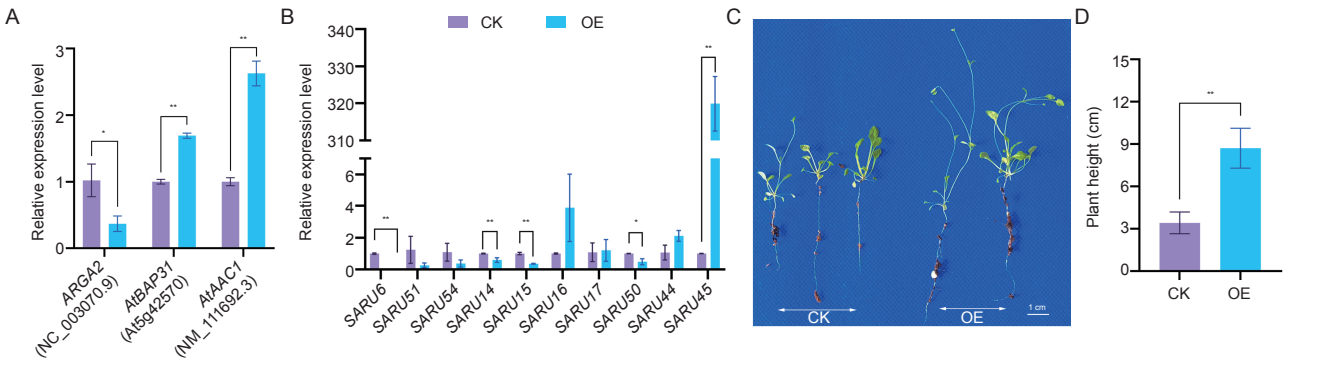


Fig. 5 Phenotypic and gene expression differences in transgenic *Arabidopsis thaliana*. A, relative expression of *AbPFN3*-interacting genes in *A. thaliana*. B, relative expression of 10 SAUR family genes in AbPFN3-overexpressing *A. thaliana*. C, phenotypic differences between AbPFN3-overexpressing and wild-type *A. thaliana*. D, plant height of transgenic *Arabidopsis*. OE, AbPFN3-overexpressing *A. thaliana*; CK, wild-type *A. thaliana*. *AAC1*, NM_111692.3; *BAP31*, *At5g42570*; *RGA2*, NC_003070.9. Bars indicate the standard error of the mean (n=3). Symbols * and ** indicate P<0.05 and P<0.01, respectively.

Previous studies have shown that expression of *MiPFN3* in protoplasts specifically disrupts actin filament organization and structure (Leelarasamee *et al.* 2018). Our findings suggest that plant-parasitic nematode PFN proteins localize not only to the actin cytoskeleton but also to the endoplasmic reticulum, plasma membrane, and cytoplasm, implying broader functional roles. We observed that AbPFN3 interacts with OsAAC1 on the endoplasmic reticulum, and in transgenic *Arabidopsis* overexpressing *AbPFN3*, *OsAAC1* expression was positively regulated. AAC1 proteins are typically localized to the inner mitochondrial membrane. Intriguingly, our study reveals that *AbPFN3* expression alters the subcellular localization of OsAAC1. Plant AACs catalyze the exchange of cytosolic ADP for mitochondrial ATP, thereby linking ATP synthesis in the matrix to ATP-consuming cellular processes. Profilins possess nucleotide-exchange activity that accelerates ADP-to-ATP exchange on G-actin by approximately 1,000-fold, replenishing the ATP-actin pool and promoting actin filament elongation at free barbed ends (Witke 2004). We speculate that AbPFN3, through its interaction with OsAAC1, increases AAC1 abundance or relocalization, thereby enhancing intracellular ATP transport efficiency. Consequently, we hypothesize that AbPFN3 modulates the actin cytoskeleton through dual mechanisms: directly by binding actin (similar to MiPFN3) and indirectly by interacting with AAC1 to elevate cytoplasmic ATP levels.

BAP31 is a ubiquitously expressed transmembrane protein that interacts with partners in the endoplasmic reticulum, outer mitochondrial membrane, cytoplasm, and mitochondria. In this study, the AbPFN3-OsBAP31 interaction signal was detected in the cytoplasm, and BAP31 was positively regulated in transgenic *Arabidopsis*. Overexpression of the tobacco BAP31-like gene *NtPBL*, which interacts with Nt-4/1 in the cytoplasm, impairs the plant's ability to maintain homeostasis during pathogen attack and under abiotic stress (Pankratenko *et al.* 2017). Thus, *AbPFN3*-induced upregulation of *BAP31* during RWTN infection may compromise the host's homeostatic responses to nematode challenge. Additionally, transient expression of AbPFN3 in rice protoplasts resulted in chloroplast-localized fluorescence. Although several putative chloroplast-localized interactors were identified *via* Co-IP/MS (Table 1), subsequent validation assays did not detect interaction signals within chloroplasts.

To assess the role of *AbPFN3* in RWTN parasitism, we generated transgenic *A. thaliana* lines overexpressing *AbPFN3*. Overexpression of *AbPFN3* altered plant growth, resulting in taller plants compared to wild-type (WT) *A. thaliana*. Previous studies have reported that overexpression of certain nematode effectors induces phenotypic changes in plants; for example, rice overexpressing *M. graminicola MgMO237* exhibits elongated primary roots (Chen *et al.* 2018), while *A. thaliana* overexpressing *M. enterolobii MeTCTP* shows reduced lateral root formation. In our study, 10 *SAUR* family genes displayed aberrant expression levels in transgenic *Arabidopsis* relative to WT, though only OsSAUR50 was confirmed to interact with AbPFN3. Van Mourik *et al.* (2017) demonstrated that phenotypic effects of individual *SAUR* knockouts can be compensated by feedback upregulation of other *SAUR* genes, often resulting in no net phenotypic change. The dysregulation of other *SAUR* genes in our transgenic lines may therefore stem from such feedback mechanisms. Previous studies have shown that the root-knot nematode effector MiPFN3 suppresses AtACT1-induced dwarfism. In this study, a putative Actin-7 protein was identified as a potential interactor of AbPFN3 (Appendix C), suggesting functional conservation between AbPFN3 and MiPFN3. Given that plant phenotypes are governed by multiple genes, the observed phenotype in our transgenic *Arabidopsis* likely results from the combined influence of *Actin-7* modulation and feedback effects involving *SAUR50*.

To date, few effectors that alter plant physiology and development have been characterized. The best-studied examples are plant peptide-hormone mimics, including CLAVATA3/EMBRYO SURROUNDING REGION-RELATED (CLE)-like peptides, C-terminally encoded peptides (CEPs), rapid alkalization factors (RALFs), INFLORESCENCE DEFICIENT IN ABSCISSION (IDA) peptides, and, most recently, nematode-encoded mimics of plant peptides containing sulfated tyrosine (PSYs), which promote cell expansion and proliferation during root development (Bobay

et al. 2013; Tucker and Yang 2013; Rutter *et al*. 2014; Kim *et al*. 2018; Zhang *et al*. 2020). The first profilin characterized in parasitic nematodes, MiPFN3 from root-knot nematodes, modulates the plant actin cytoskeleton (Leelarasamee *et al*. 2018).

5. Conclusion

Here, we identify AbPFN3 from RWTN as an effector that alters host physiology and development through interactions with AAC1 and SAUR50. Furthermore, AbPFN3 interacts with BAP31, potentially dampening the host immune response and thereby promoting nematode infection. This work significantly expands the known molecular interaction network of profilins in parasitic nematodes and their host plants.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation and writing of this manuscript.

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