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Functional characterization of the soybean cyst nematode effector SCN-27D09 using the model plant pathogenic fungus *Magnaporthe oryzae*-mediated delivery system

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Abstract

Soybean cyst nematode (SCN, *Heterodera glycines*) is widely considered as the model plant-parasitic nematode, which secretes effector proteins to manipulate host responses. In this study, we cloned a dorsal gland-expressed effector protein SCN-27D09 that belongs to the same family as Hg10A07 in SCN. We used the model plant pathogen rice blast fungus (*Magnaporthe oryzae*) to quickly predict and characterize the functions of SCN-27D09. By using *M. oryzae* secretion system in barley, we confirmed that the signal peptide of SCN-27D09 has secretory activity and can guide the protein into the host cells. Heterologous expression of *SCN-27D09* in *M. oryzae* significantly enhanced the susceptibility of barley to *M. oryzae*. SCN-27D09 can inhibit Bax-triggered cell death when expressed in *Nicotiana benthamiana*. Overexpression of *SCN-27D09* in soybean hairy root also increased the susceptibility of soybean plants to SCN. Moreover, yeast two-hybrid and firefly luciferase complementation imaging assays showed that SCN-27D09 interacts with a soybean plant kinase GmIPK-2. Functional characterization of *GmIPK-2* revealed its positive role in soybean resistance, indicating that SCN-27D09 might compromise the function of GmIPK-2 to facilitate nematode infection. Our results not only uncover the biological role of SCN-27D09 in suppressing plant defense responses and therefore promoting nematode parasitism, but also reaffirm the potential application of the model plant pathogenic fungus *M. oryzae* in investigating the pathogenic roles of candidate effectors of phytonematodes.

Keywords: *Heterodera glycines*, SCN-27D09, *Magnaporthe oryzae*, Heterologous expression, Defense suppression

Background

There are more than 4100 nematode species worldwide that cause serious damage to hundreds of crops (Decraemer et al. 2006). Soybean cyst nematode (SCN, *Heterodera glycines*), an obligate and sedentary endoparasitic nematode, is widely considered as the model plant nematode (Niblack et al. 2006). SCN has caused huge economic losses in soybean production in the United States and Canada over two decades (Jones et al. 2013; Allen et al. 2017; Bandara et al. 2020). Effectors mostly produced through esophageal glands play a key role in the plant–nematode interaction (Davis et al. 2008). Plant

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nematodes penetrate plant cell wall and secrete virulent effectors, such as cell wall-degrading enzymes, into plant cells via stylets. The physiological state and defense response of the host plant are thus manipulated by the nematode to facilitate its own development and reproduction (Lilley et al. 2005; Khan and Khan 2021).

With the development and application of various techniques, such as monoclonal antibodies (Smant et al. 1998), mass spectrometry (Filipecki et al. 2021), expressed sequence tags (Alkharouf et al. 2004), genomics, and transcriptomics, a large number of effectors have been identified from plant nematodes. The functions of these effectors are characterized by molecular biology and other methods, and it was found that they have three major functions in the compatible plant–nematode interaction. Firstly, plant nematodes secrete cellulases, such as β-1, 4-endoglucanases, which mostly belong to glycosyl hydrolase family 5 (GHF5), pectate lyases such as PEL-2, and expansins such as HaEXPB2, to degrade or soften plant cell walls and promote nematode parasitism (Smant et al. 1998; Geric Stare et al. 2011; Liu et al. 2016). Secondly, some nematode effectors can mimic host proteins or regulate gene expression in host plants to promote the formation of feeding sites. For example, HgSYV46 has the same function as its homolog CLAVATA3/ESR (CLE) in *Arabidopsis thaliana*, which can positively regulate the development of feeding sites by regulating cell proliferation (Wang et al. 2005; Guo et al. 2017). The effector proteins HsIPT, Hs-Tyr, and HsPDI promote the formation of syncytia by regulating host cell division, hormone homeostasis, and redox balance, respectively (Siddique et al. 2015; Habash et al. 2017; Lilley et al. 2018). Thirdly, plant nematodes utilize effectors such as Ha18764 to hijack the signal transduction processes of PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI) in plants. Hs10A06 was demonstrated to regulate ROS levels and SA signaling pathway to achieve the purpose of inhibiting plant defense responses (Hewezi et al. 2010; Yang et al. 2019). At present, the interaction mechanism between nematode effectors and host plants is being gradually elucidated.

Because transformation systems are still immature in some plants, researchers often investigate nematode effectors using their homologous effectors in sugar beet cyst nematode (BCN, *H. schachtii*) and the corresponding host plant *A. thaliana*. The effector protein 10A07, originally identified from the cDNA library of SCN esophageal gland cells, is such an example. After Hs10A07 is secreted into the *A. thaliana* cytoplasm, it interacts with the interacting plant kinase (IPK) and is phosphorylated at Ser-144 and Ser-231 to promote its trafficking to the nucleus. Then the effector associates with an auxin-responsive protein IAA16 to interfere with the expression of auxin response factors and auxin signaling, thereby promoting the parasitism of nematodes (Hewezi et al. 2015).

To predict and characterize the pathogenic roles of potential nematode effectors on a large scale, we performed studies taking advantage of a model plant pathogen rice blast fungus (*Magnaporthe oryzae*). Herein, we cloned a dorsal gland-expressed nematode effector-coding gene *27D09* (referred to as *SCN-27D09* in the study), which belongs to the same gene family as *10A07*, as an example. We confirmed the interaction between SCN-27D09 and GmIPK-2, a homologous protein of *Arabidopsis* IPK. In addition, we deployed the *M. oryzae*-mediated delivery system to study the functions of this nematode effector. We expressed *SCN-27D09* heterologously in *M. oryzae* and proved that the signal peptide at the N-terminus of SCN-27D09 can guide the secretion of the effector into host plant cells, and found that SCN-27D09 can enhance *M. oryzae* virulence to host plants. We further used the soybean hairy root transformation system to demonstrate the functions of SCN-27D09 in parasitism. Taken together, our findings highlight the pathogenic roles of SCN-27D09 and reaffirm the potential application of the model plant pathogenic fungus *M. oryzae* in functional study of other phytonematode effectors.

Results

SCN-27D09 is secreted into host cells

SCN-27D09 (GenBank accession no. AAM50038) was first identified as a homologous protein of the BCN effector Hs10A07 (GenBank accession no. KP728937) (Gao et al. 2003; Hewezi et al. 2015). The sequence of *SCN-27D09* has an open reading frame of 708 bp, encoding a protein of 235 aa with an N-terminal signal peptide (1–15 aa) and a nuclear localization signal (NLS) peptide (18–93 aa). There is no conserved domain and transmembrane helix in SCN-27D09. Protein sequence alignment revealed that at least four proteins (i.e., 20G04, 10A07, 6E07, and 13A06) are homologous to SCN-27D09 in SCN (Additional file 1: Figure S1). We used the digoxygenin-labeled antisense cDNA probe of *SCN-27D09* to detect the localization of its transcripts. *In situ* hybridization assay revealed strong signals in the dorsal gland of nematode at parasitic stages (Fig. 1). To clarify whether the signal peptide of SCN-27D09 is functional, we used the *M. oryzae*-mediated live cell imaging system to localize this effector in barley (Park et al. 2012; Zhang et al. 2018). The pRP27-SCN-27D09-mCherryNLS vector carrying SCN-27D09 fused in-frame with the mCherry protein and a NLS peptide (mCherryNLS) was transformed into the *M. oryzae* wild-type strain P131. The mCherryNLS could facilitate the visualization of translocated fluorescent protein by concentrating the fluorescence signals in the

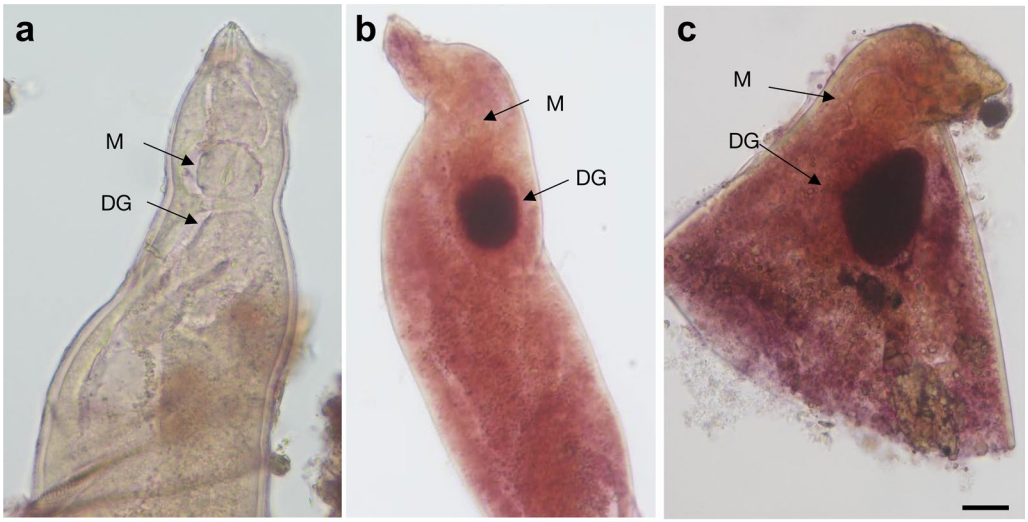

Fig. 1 *SCN-27D09* is expressed in the dorsal gland of plant-parasitic nematodes. *In situ* hybridization was performed to detect tissue localization of *SCN-27D09* transcript using digoxigenin-labeled sense (**a**) and antisense (**b**, **c**) cDNA probes of *SCN-27D09*. Parasitic third- and fourth-stage juveniles of *Heterodera glycines* were used. M, metacorpus; DG, esophageal dorsal gland. Bar = 20 μm

nucleus. The integration of pRP27-SCN-27D09-mCherryNLS into the P131 genome was validated through PCR (Additional file 1: Figure S2). Obvious nuclear localization of red fluorescence signals was detected in conidia and vegetative hyphae of the transformants expressing SCN-27D09-mCherryNLS (Fig. 2a), suggesting that this fusion protein was functional. Interestingly, obvious red fluorescence signals were detected in the nuclei of nearly 40% of the infected barley epidermis cells after infection with *M. oryzae* transformants expressing SCN-27D09-mCherryNLS; while less than 10% of the infected barley epidermis cells were detected with weak red fluorescence signals after infection with the control *M. oryzae* transformants expressing mCherryNLS under the same conditions (Fig. 2b, c). Taken together, these findings suggested that the signal peptide of SCN-27D09 could effectively guide its secretion into the host cells during *M. oryzae* infection.

SCN-27D09 suppresses Bax-triggered cell death in *Nicotiana benthamiana*

To explore the potential role of SCN-27D09 in plant defense suppression, we tested the ability of SCN-27D09 to suppress Bax (an apoptosis-promoting protein)-triggered cell death in *N. benthamiana*. We infiltrated the *N. benthamiana* leaves with *Agrobacterium tumefaciens* carrying pYBA1143-SCN-27D09 or the empty vector pYBA1143 for 24 h, and then infiltrated the leaves with *Agrobacterium* harboring Bax or infiltration buffer. Meanwhile, the *A. tumefaciens* strain carrying SCN-27D09, Bax, empty vector, or buffer was injected independently as control. In order to illustrate the suppression effect of SCN-27D09 on Bax-triggered cell death, we also set a gradient (OD_{600} = 0.1, 0.2, 0.3, and 0.4) for the final concentration of Bax. As expected, the infiltration of the empty vector pYBA1143 and then Bax caused significant necrosis after 4 days, whereas the leaves treated with the combination of SCN-27D09 and Bax showed reduced necrosis (Fig. 3a). Cell death suppression effect was more obvious with low infiltration concentration of Bax. As control, only the *A. tumefaciens* strain carrying Bax caused necrosis (Fig. 3b). The results suggested that SCN-27D09 could suppress Bax-triggered cell death in *N. benthamiana*.

Heterologous expression of *SCN-27D09* in *M. oryzae* enhances the fungal virulence

Because SCN-27D09 could be secreted into host cells via *M. oryzae* and suppress Bax-triggered cell death in *N. benthamiana*, we wondered whether overexpression of *SCN-27D09* would enhance the virulence of *M. oryzae*. Thus, we generated and transformed the pRP27-SCN-27D09-GFP vector into the *M. oryzae* strain P131. The integration of pRP27-SCN-27D09-GFP into the P131 genome was validated through PCR (Additional file 1: Figure S3). We then compared the virulence of the resulting transformants on barley leaves with a gradient dilution method. Compared with the wild-type strain P131 and its transformants expressing GFP, three transformants expressing the SCN-27D09-GFP fusion protein

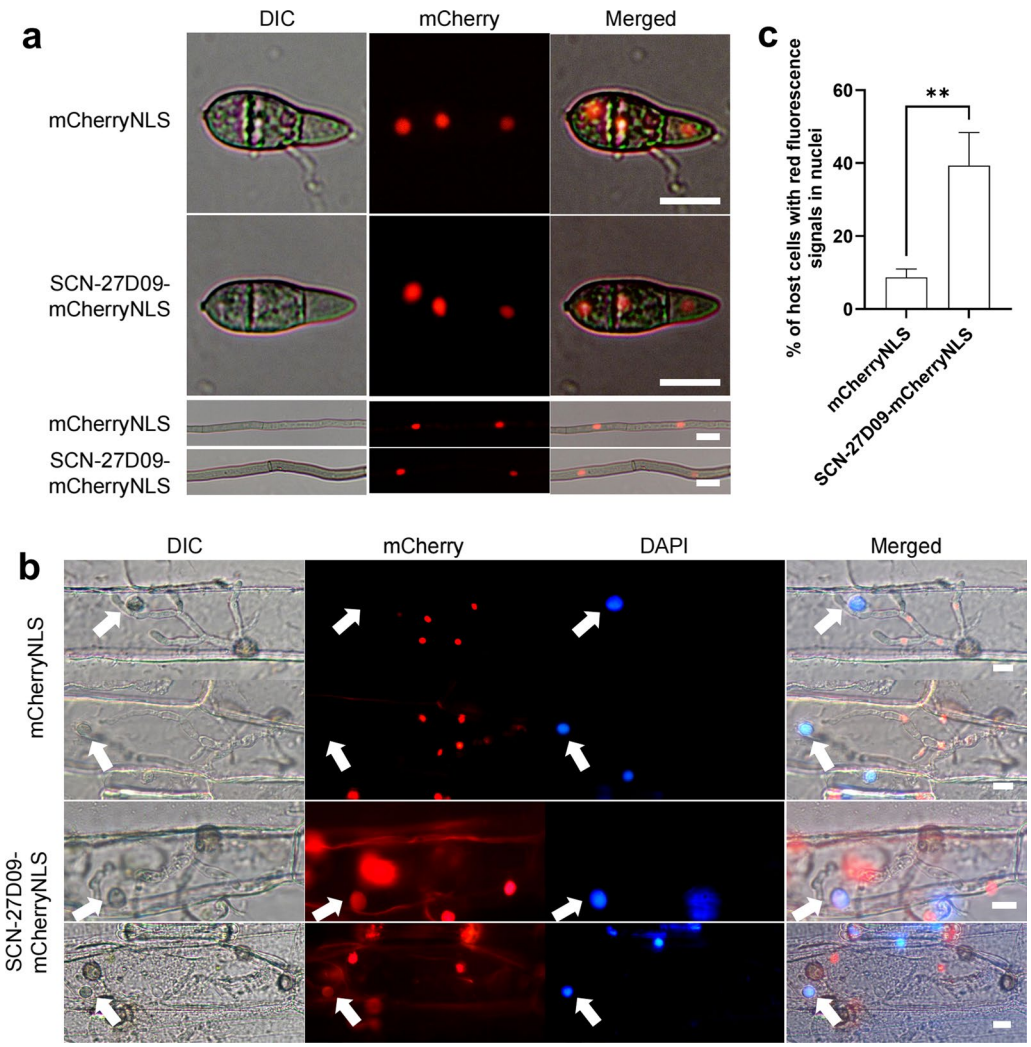

Fig. 2 SCN-27D09 can be secreted into host cells. **a** Fluorescence signals observed in conidia and hyphae of *Magnaporthe oryzae* transformants expressing mCherryNLS and SCN-27D09-mCherryNLS. Bar = 10 μm. Red fluorescence was observed in the nuclei of conidia and hyphae. **b** Nuclear localization of red fluorescent signals in epidermal cells of barley leaves infected with *M. oryzae* transformants expressing SCN-27D09-mCherryNLS but not mCherryNLS at 27 h post-inoculation (hpi). The white arrows point to the barley nuclei. Bar = 10 μm. **c** The percentage of *M. oryzae*-infected barley epidemic cells with red fluorescent signals accumulated in the nuclei. At least 50 *M. oryzae*-infected barley epidemic cells were measured for the strains expressing SCN-27D09-mCherryNLS or mCherryNLS. $^{**}P < 0.01$

formed obvious larger lesions when infecting barley with their conidia (Fig. 4a, b). These findings suggested that heterologous expression of *SCN-27D09* could enhance the virulence of *M. oryzae*.

SCN-27D09 interacts with soybean GmIPK-2

Since Hs10A07 was previously reported to interact with a plant kinase IPK (Hewezi et al. 2015), we hypothesized that SCN-27D09 might also interact with the homologous proteins of IPK from soybean. Blast analysis suggested that there are three homologs of IPK in soybean, and one homolog of IPK named as GmIPK-2 was cloned in this study (Additional file 1: Figure S4). To confirm whether SCN-27D09 interacts with GmIPK-2, yeast two-hybrid assay using vectors GmIPK-2-AD and SCN-27D09-BD was performed. As shown in Fig. 5a, yeast co-transformed with GmIPK-2-AD and SCN-27D09-BD grew well on SD/-Leu/-Trp/-His plate supplemented with X-α-gal, suggesting that SCN-27D09 can physically interact with GmIPK-2 in yeast. The interaction between SCN-27D09 and GmIPK-2 was verified by luciferase complementation imaging (LCI) assay. Strong fluorescence was observed in *N. benthamiana* leaves co-expressing the GmIPK-2-NLuc and SCN-27D09-CLuc

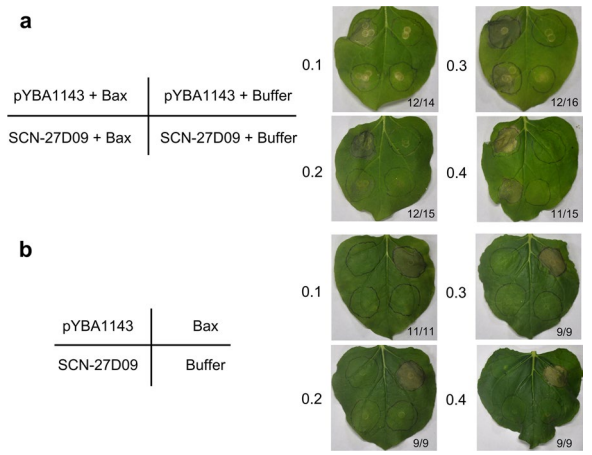

Fig. 3 SCN-27D09 suppresses Bax-triggered cell death in *Nicotiana benthamiana*. **a** Transient expression of *SCN-27D09* suppressed cell death induced by Bax. The corresponding combinations used for infiltration of *N. benthamiana* leaves were indicated on the left panel. **b** Transient expression of Bax induced cell death. Treatments for infiltration of *N. benthamiana* leaves were shown on the left panel. In **a** and **b**, the data (0.1, 0.2, 0.3, and 0.4) indicate the OD_{600} value of *Agrobacterium tumefaciens* carrying Bax. Numbers at the lower-right corner of the pictures represent the number of leaves showing cell death suppression out of the number of leaves agroinfiltrated

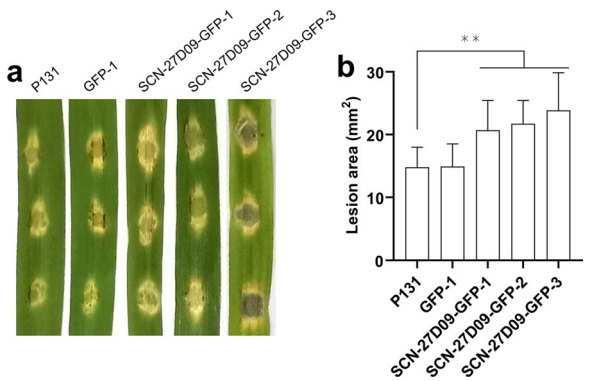

Fig. 4 Heterologous expression of *SCN-27D09* in *Magnaporthe oryzae* enhances the virulence of the fungus. **a** Symptoms on detached barley leaves inoculated with spore suspensions (5×10^4 conidia/mL) of the wild-type *M. oryzae* P131 strain or transformants at 5 dpi. **b** Lesion area on barley leaves shown in **a**. Data are means ± standard deviation. N = 3 (*t* test between SCN-27D09-GFP and control values; $^*P < 0.05$, $^{**}P < 0.01$)

pair, but not in other control pairs including the GFP-NLuc and SCN-27D09-CLuc pair and the GmIPK-2-NLuc and GFP-CLuc pair (Fig. 5b). The luciferase activity of the GmIPK-2-NLuc and SCN-27D09-CLuc pair was also significantly higher than that of the controls (Fig. 5c). Taken together, these results indicated that SCN-27D09 physically interacted with GmIPK-2.

Both SCN-27D09 and GmIPK-2 serve vital roles during SCN parasitism

To characterize the biological role of SCN-27D09 in SCN parasitism, *Agrobacterium rhizogenes* K599-mediated soybean hairy root transformation system was used to overexpress *SCN-27D09* in soybean. Gene expression was confirmed via reverse transcription-quantitative PCR (RT-qPCR). At least 16 positive transgenic roots were inoculated with SCN, and the number of cysts in roots was counted and the fresh weight of roots was weighed at 30 days post-inoculation (dpi). As shown in Fig. 6a, overexpression of *SCN-27D09* increased the numbers of cysts per hairy root line and per gram root in comparison with the control, suggesting the positive role of SCN-27D09 in nematode parasitism.

With the same system, overexpression of *GmIPK-2* significantly reduced the numbers of cysts per hairy root line and per gram root in comparison with the control (Fig. 6b). Meanwhile, the RNAi approach was further used to assay the function of GmIPK-2, and a 303 bp conserved fragment near the N-terminal of *GmIPK-2* was selected to construct a hairpin structure. In comparison with the control, obvious more cysts per hairy root line and per gram root were observed in the *GmIPK-2*-silencing lines (Fig. 6c). Taken together, the overexpression and RNAi assays suggested that GmIPK-2 played positive roles in host resistance against nematodes.

Discussion

In the arms race between nematodes and host plants, successful parasitism of plants by nematodes is attributed to the molecular dialogue between nematode effectors and their corresponding targets in hosts. At present, the most studied effectors of plant-parasitic nematodes are proteins with N-terminal signal peptide, which are believed to be secreted through the classic endoplasmic reticulum-Golgi protein secretion system (Lee et al. 2004) and enter the host cell cytoplasm via the nematode stylet (Wang et al. 2010; Hewezi and Baum 2013; Hewezi et al. 2015). Plant-parasitic nematodes cannot be genetically modified by traditional transformation methods, and current researches mainly rely on *in vitro* RNA interference and host-induced gene silencing techniques to functionally characterize nematode effectors. However, due to the low silencing efficiency and poor stability combined with the presence of off-target risk, these techniques have greatly reduced the reliability of functional verification (Jackson et al. 2003). Moreover, it is difficult to perform genetic transformation in some crops. To predict and characterize the pathogenic roles of potential

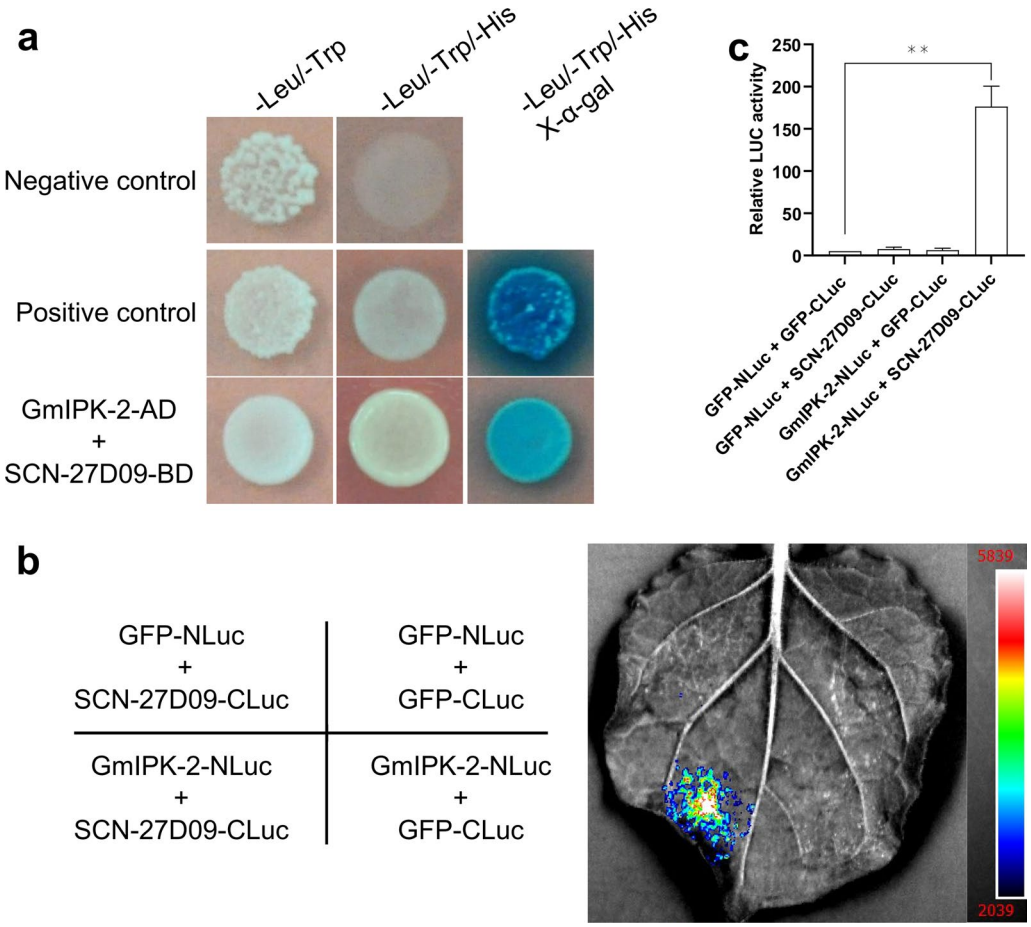

Fig. 5 SCN-27D09 interacts with GmIPK-2. **a** SCN-27D09 interacted with GmIPK-2 in yeast two-hybrid assay. The interaction between GmIPK-2-AD and SCN-27D09-BD activated the expression of *HIS3* and *LacZ* reporter genes. The colony of positive control turned blue. **b** SCN-27D09 interacted with GmIPK-2 in luciferase complementation imaging (LCI) assay. GmIPK-2-NLuc and SCN-27D09-CLuc were transiently expressed in *N. benthamiana* by co-infiltration. The combination of GFP-NLuc and GFP-CLuc was used as negative control. The luminescence was detected with a low-light cooled CCD imaging apparatus at 36 h after infiltration. **c** Quantification of the LUC activity in the leaves shown in **b**. N = 3 (*t* test between GmIPK-2-NLuc/SCN-27D09-CLuc and control group values; $^{**}P < 0.01$)

nematode effectors on a large scale, we deployed an effector delivery platform assisted with a model plant pathogenic fungus *M. oryzae*.

Here, we demonstrated that SCN-27D09 could be transferred to host cells using *M. oryzae*-mediated live cell imaging method (Park et al. 2012; Zhang et al. 2018). In the past few years, there have been some reports on the investigation of signal peptide secretion activity of nematode effectors using the rice blast system. Ha18764 from *Heterodera avenae* and MiISE5 from *Meloidogyne incognita* can be secreted into the host cells via this system (Shi et al. 2018; Yang et al. 2019). We also used this system to investigate the function of SCN-27D09. Heterologous expression of *SCN-27D09* significantly enhanced the virulence of *M. oryzae* on barley, in consistent with the increased susceptibility to SCN by overexpression of *SCN-27D09* in soybean. Because *M. oryzae* could be genetically manipulated very easily, this effector delivery system provides a useful method for large-scale functional characterization of phytonematode effectors.

Bax is a Bcl-2 family protein that can induce cell death in tobacco leaves, and the cell death phenotype it induced is physiologically similar to the hypersensitive response in plants (Lacomme and Santa Cruz 1999). The ability of effectors to inhibit Bax-triggered cell death has been regarded as one of the indicators of suppression of basal immunity in host plants (Jamir et al. 2004; Dou et al. 2008; Wang et al. 2011). A number of cyst nematode effectors have been shown to inhibit Bax-triggered cell death, such as Ha-annexin (Chen et al. 2015), HaVAP1 and HaVAP2 (Luo et al. 2019), and HaCRT1 (Liu et al. 2020). Ha18764 was reported to not only suppress cell death triggered by various elicitors, but also suppress cell death induced by putative effectors of *H. avenae* (Yang

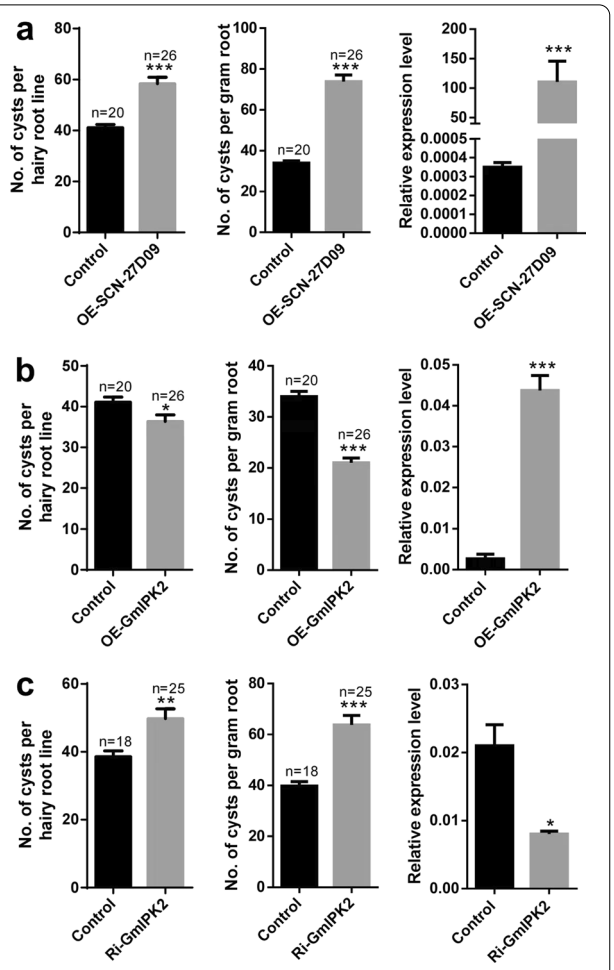

Fig. 6 GmIPK-2 plays a positive role against soybean cyst nematode. **a** The numbers of cysts per hairy root line (left panel) and per gram root (middle panel) in Williams 82 (control) and *SCN-27D09* overexpression line (OE-SCN-27D09). Relative expression level of *SCN-27D09* was shown on the right panel. **b** The numbers of cysts per hairy root line and per gram root in Williams 82 (control) and *GmIPK-2* overexpression line (OE-GmIPK-2). Relative expression level of *GmIPK-2* was shown on the right panel. **c** The numbers of cysts per hairy root line and per gram root in Williams 82 (control) and *GmIPK-2* RNAi line (Ri-GmIPK-2). Relative expression level of *GmIPK-2* was shown on the right panel. Data are means ± standard error. Significant differences were calculated by Student's *t* test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$)

et al. 2019). In this study, the expression of *SCN-27D09* partially inhibited Bax-triggered cell death, indicating that SCN-27D09 may be involved in suppressing plant innate immune responses. Plants have also evolved a multi-layered immune system to subvert nematode evasion, whether SCN-27D09 is involved in this multiple-layer defense suppression remains to be examined.

Previous studies showed that BCN effector 10A07 interacted with the plant kinase IPK in *A. thaliana* cytoplasm, and phosphorylation of 10A07 at Ser-144 and Ser-231 mediated its nuclear translocation (Hewezi et al. 2015). The two phosphorylation sites are conserved in 10A07 and 27D09. Here, we confirmed that SCN-27D09 physically interacted with GmIPK-2. The results suggested the sequence conservation during the evolution of nematodes and hosts. Furthermore, we found that silencing of *GmIPK-2* resulted in enhanced susceptibility, while overexpression of *GmIPK-2* increased soybean resistance against SCN. This indicates that GmIPK-2 plays an important role in the defense response of soybean to SCN infection, which seems to be contradictory to a previous report on the role of IPK in *Arabidopsis*, in which the phosphorylation of 10A07 by IPK promoted nematode parasitism by interfering with auxin signaling (Hewezi et al. 2015). This might indicate functional differentiation and diversification of IPKs in different plants. Although IPK can be targeted by both 10A07 and 27D09, the underlying molecular mechanism for the interactions might be distinct and needs further elucidation.

Conclusions

Identification and functional characterization of nematode effectors are essential to better understand the mechanisms underlying nematode virulence and host defense, which could potentially provide strategies for designing future crops with improved nematode resistance. We demonstrated the important roles of SCN effector SCN-27D09 in suppressing host defense and promoting nematode parasitism. GmIPK-2 is directly targeted by SCN-27D09. Heterologous expression of nematode effector-coding genes in the model plant pathogenic fungus *M. oryzae* can be used as a strategy to study these effectors, providing more perspectives for the study of pathogenic roles of candidate effectors from phytonematodes.

Methods

In situ hybridization

In situ hybridization was performed with third-stage (Par-J3) and fourth-stage (Par-J4) juveniles of *H. glycines* as described previously (Jaouannet et al. 2018). Nematodes were isolated according to de Boer et al. (1998). Digoxigenin-labeled sense and antisense cDNA probes of *SCN-27D09* were synthesized using a DIG DNA labeling kit (Roche, USA). Hybridization was carried out at 42 °C overnight and the signals were detected using anti-DIG antibody conjugated with alkaline phosphatase (1:500). Nematode samples were photographed with an Olympus BX63 light microscope. The primers used for probe preparation are listed in Additional file 2: Table S1.

Functional analysis of the signal peptide of SCN-27D09 in barley leaves

In order to prove whether the signal peptide of SCN-27D09 has a secretory function, we generated the pRP27-SCN-27D09-mCherryNLS vector, and the empty vector pRP27-mCherryNLS was used as a negative control. The two vectors were individually transformed into the protoplasts of the *M. oryzae* wild-type strain P131 and the positive transformants were cultured on oatmeal tomato agar (OTA) after fluorescence screening. Spore suspensions were prepared with 0.025% Tween 20 and adjusted to a concentration of 1×10^5 conidia/mL. The conidia were spotted on the back of 5-day-old barley leaves, which were subsequently incubated for 27 h in a humid and dark environment at 28 °C (Liu et al. 2021). The nuclei were stained with 1 μg/mL 4',6-diamidino-2-phenylindole (DAPI), and the amount of SCN-27D09 entering the barley cell nuclei was counted and photographed with a fluorescence microscope (Nikon ECLIPSE 90i). Filter sets used were as follows: mCherry (excitation 510–560 nm; emission 575–590 nm) and DAPI (excitation 330–380 nm; emission 400–420 nm) (Zhang et al. 2018).

Molecular identification of *M. oryzae* transformants harboring SCN-27D09

In order to verify the positive transformants of *M. oryzae*, we extracted the genomic DNA of transformants for PCR verification. Mycelia were harvested into a tube containing 500 μL DNA extraction solution (1 M KCl, 100 mM Tris–HCl, and 10 mM EDTA, pH 8.0) and ground with a homogenizer (MP FastPrep®-24). After centrifugation at 2,400 *g* for 5 min at room temperature, the supernatant was collected and 300 μL isopropanol was added. After centrifugation, the pellet was dissolved in 30 μL sterile water. The extracted DNA can be directly used as a template for PCR assay. *Actin* was used as a reference gene for PCR detection (Kong et al. 2012). All primers used for PCR are listed in Additional file 2: Table S1.

Cell death analysis

Empty vector pYBA1143 and vectors carrying SCN-27D09, Bax, and silencing suppressor p19 were individually transformed into *A. tumefaciens* EHA105. To determine the suppression of Bax-triggered cell death by SCN-27D09, equal volume of suspensions carrying SCN-27D09 or empty vector ($OD_{600} = 1.0$), and p19 ($OD_{600} = 0.5$) were mixed and infiltrated into the *N. benthamiana* leaves. After 24 h, *A. tumefaciens* suspension carrying Bax ($OD_{600} = 0.1$, 0.2, 0.3, and 0.4) or infiltration buffer was injected into the same site. At the same time, the *A. tumefaciens* strain carrying SCN-27D09, Bax, pYBA1143, or buffer was injected alone as control. The experiments were repeated twice with similar results. The pictures were taken at 4 days after Bax infiltration (Lakhssassi et al. 2020; Liu et al. 2020).

Virulence assay

In order to test the virulence of *M. oryzae* strains in which the SCN effector is heterologously expressed, we generated the pRP27-SCN-27D09-GFP vector and subsequently transformed the resulting vector or the empty vector pRP27-GFP into the wild-type strain P131. Three positive transformants of SCN-27D09 were used for subsequent inoculation. Conidia grown on OTA medium for 7 days were washed with 0.025% Tween 20 and filtered through two layers of lens wiping paper, and 10 μL spore suspension (5×10^4 conidia/mL) was drop-inoculated onto a detached leaf of barley plants (one-week-old). Three leaves were used for each transformant. Samples were incubated in a moist cassette at 28 °C for 24 h, and then maintained in a 12 h light/12 h dark cycle for 4–6 days. The pictures were taken at 6 dpi and the leaf areas of 9 lesions on 3 leaves were measured using Image J software. GraphPad Prism 9.0.0 was used to analyze the data (Yang et al. 2010; Gao et al. 2019) by Student's *t* test. The experiment was performed three times with similar results.

Yeast two-hybrid assay

GmIPK-2 from soybean and *SCN-27D09* from soybean cyst nematode were cloned and fused into pGADT7 and pGBKT7 vectors, respectively. Competent cells of yeast strain AH109 were prepared by the LiAc method, and then the recombinant bait vectors and AD vectors were transformed into AH109 using the PEG/LiAc method. After incubation on SD-Leu/-Trp and SD-Leu/-Trp/-His plates at 30 °C for 3 days, single colonies that grew well were cultured on SD-Leu/-Trp/-His plate containing X-α-gal. The colonies containing interacting proteins turned blue.

Luciferase complementation imaging (LCI) assay

The LCI assay was performed as described previously (Chen et al. 2008). Using homologous recombination, SCN-27D09^{-SP} and GmIPK-2 were constructed into pCAMBIA1300-CLuc and pCAMBIA1300-HA-NLuc, respectively. At the same time, *GFP* was sub-cloned into the two vectors as negative control. All vectors were transformed into *A. tumefaciens* strain GV3101 and cultured overnight. After centrifugation at 1,900 *g* for 10 min, the pellet was re-suspended in infiltration buffer containing 100 μM acetosyringone, 10 mM 2-(N-morpholino) ethanesulfonic acid, and 10 mM $MgCl_2$ to reach $OD_{600} = 1.0$. The two strains were mixed at a volume ratio of 1:1 and then allowed to stand in the

dark at room temperature for 3–5 h. Then the suspensions were injected into *N. benthamiana* leaves that have grown for about 4 weeks. After 36 h, 1 mM D-luciferin was applied to the back of the leaves. The luminescence was detected with CCD imaging system (Tanon 5200). Punch the leaves with a puncher (0.6 cm in diameter) and place them in a 96-well white ELISA plate containing 50 μL sterile water for fluorescence scanning with a luminometer (SpectraMax i3x). Draw a bar graph based on the scanned value to show the intensity of fluorescence generated by luciferase after decomposing the substrate and to judge the interaction degree between the target proteins.

Soybean hairy root transformation

The overexpression vector was constructed by directly cloning the *GmIPK-2* and *SCN-27D09* full-length cDNA into the binary expression vector pCAMBIA1300-OE using *Xba*I and *Sal*I restriction sites. To generate silencing vectors, a 303-bp fragment of *GmIPK-2* was cloned into pMD18-T and then into the RNAi binary vector using *Asc*I/*Swa*I and *Bam*HI/*Avr*II restriction sites, respectively. The soybean cultivar used in this study is 'Williams 82' (W82), which is susceptible to SCN. SCN (HG type 0) was maintained in sand: soil mixture (v/v = 3:1) in a 28 °C greenhouse. Cysts were extracted from infested soil at 35 dpi using sieves and centrifugation. Second-stage juveniles (J2) were hatched from the eggs by incubation with water at 28 °C for 2–3 days. Seeds of W82 cultivar were planted in vermiculite for 4 days. *A. rhizogenes* strain K599 was used to inoculate the soybean hypocotyls as described previously (Guo et al. 2015; Toth et al. 2016). After 3 days of co-cultivation in the dark, the explants were grown under the light for about 10 days until hairy roots developed from the hypocotyls. The GFP-positive roots were selected under an Olympus SZX16 fluorescence stereomicroscope. The plants were transplanted to sand: soil mixture (v/v = 3:1) to recover for 5 days. Then 300–400 pre-parasitic J2s were inoculated to each plant. The number of cysts and root fresh weight were evaluated at 30 dpi. The experiments were performed three times with at least 16 independent hairy roots for each treatment. The data were analyzed by Student's *t* test using GraphPad Prism 9.0.0.

RNA extraction and RT-qPCR

Total RNA was extracted from GFP-positive soybean transgenic roots using TRIpure reagent (Aidlab, Beijing, China) and first-strand cDNA was synthesized using HiScriptII QRT SuperMix (Vazyme, Nanjing, China) following the manufacturer's instructions. Gene-specific primers were designed and quantitative PCR was performed using iCycler iQ 5 thermal cycler (Bio-Rad, California, USA) real-time PCR system and AceQ qPCR SYBR Green Master Mix (Vazyme, Nanjing, China). Soybean *SKP16* was used as the internal control (Wang et al. 2021). A dissociation curve was generated to verify that a single product was amplified. The experiments were performed twice. Relative gene expression was determined using $2^{-\Delta\Delta Ct}$ method compared with the internal control. The data were analyzed by Student's *t* test using GraphPad Prism 9.0.0. All primers used for RT-qPCR are listed in Additional file 2: Table S1.

Abbreviations

A. rhizogenes: *Agrobacterium rhizogenes*; *A. thaliana*: *Arabidopsis thaliana*; BCN: Beet cyst nematode; CLE: CLAVATA3/ESR; DAPI: 4′,6-Diamidino-2-phenylindole; dpi: Days post-inoculation; ETI: Effector-triggered immunity; GHF5: Glycosyl hydrolase family 5; hpi: Hours post-inoculation; IPK: Interacting protein kinase; J2: Second-stage juvenile; LCI: Luciferase complementation imaging; *M. oryzae*: *Magnaporthe oryzae*; *N. benthamiana*: *Nicotiana benthamiana*; NLS: Nuclear localization signal; OTA: Oatmeal tomato agar; Par-J3: Parasitic third-stage juvenile; Par-J4: Parasitic fourth-stage juvenile; PTI: PAMP-triggered immunity; RT-qPCR: Reverse transcription-quantitative PCR; SCN: Soybean cyst nematode.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42483-022-00138-w.

Additional file 1: Figure S1. Amino acid sequence alignment of 27D09 and the other four putative effectors in SCN. **Figure S2**. PCR verification of pRP27-mCherryNLS and pRP27-SCN-27D09-mCherryNLS transformants. **Figure S3**. PCR verification of pRP27-GFP and pRP27-SCN-27D09-GFP transformants. **Figure S4**. Multiple sequence alignment and phylogenetic analysis of IPK and its homologous proteins in soybean and *Arabidopsis thaliana*.

Additional file 2: Table S1. PCR primers used in this study.

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Authors' contributions

NY, QY, WL, DC, JJ, HZ, GW, SL, WH, and HP performed the experiments. All authors analyzed the data. DP, JY, XG, and LK designed the study. NY and QY wrote the draft manuscript. LK, XG, and JY revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Not applicable.

Declarations

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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