Pattern-triggered immunity against root-knot nematode infection: A minireview

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1 | INTRODUCTION

Root-knot nematodes (RKN) are considered the most economically important plant-parasitic nematodes (PPN) on the planet (Jones et al., 2013). There are more than 100 species and races of Meloidogyne, spanning a wide host-range of more than 2000 plant species. The most prevalent species are Meloidogyne incognita, Meloidogyne javanica, Meloidogyne arenaria, and Meloidogyne hapla. Meloidogyne incognita, M. javanica, and M. arenaria are tropical species, while M. hapla has adapted to temperate climates. In recent years, species such as the guava RKN Meloidogyne enterolobii (Castagnone-Sereno, 2012) have been identified in certain regions of the world for the first time, posing a continued threat to established RKN-resistant crop cultivars.

The RKN life cycle begins as an egg in a gelatinous egg mass laid on the root gall surface (Figure 1A-B). Some currently unknown environmental signal triggers the RKN to undergo a first molt inside the egg, becoming a second-stage juvenile (J2) (Figure 1C). The J2 hatches from the egg and travels through the soil until it finds a host root. The J2 enters the root at the zone of elongation, travels down to the root tip to bypass the Casparian strip, and then back up the vascular cylinder of the root looking for a place to set up a feeding site (Figure 1D). The J2 uses its stylet to secrete effectors into five to nine cells around its head to manipulate cellular function and induce the formation of giant cells for feeding. Once the J2 commits to feeding, it becomes sedentary and undergoes three more molts to the adult female life stage. Most RKN species reproduce via parthenogenesis, so males are rare. The “giant” cells expand up to 400 times their normal size and undergo repeated rounds of acytokinetic mitosis (Caillaud et al., 2008). Giant cell formation requires precise manipulation of the cell wall and the biosynthesis of its components, so that it becomes flexible enough to allow for rapid expansion while retaining enough integrity to remain intact. In addition to giant cells becoming multinucleate, the abundance of other organelles such as mitochondria, Golgi bodies, and endoplasmic reticulum increases (Jones & Payne, 1978). Giant cells are functional transfer cells that serve as the sole nutrition source for the nematode for the rest of its life. The cells neighboring the giant cells are stimulated to divide to give rise to the...
gall. These galls, also called “knots” after which RKN were named, form around and protect the nematode (Figure 1E-F). RKN-induced giant cells and galls disrupt the normal flow of nutrients throughout the plant. As water flow is disrupted and sugars and other photosynthetic products are redirected to the roots, the plant can exhibit above-ground wilting and yield loss (Hofmann & Grundler, 2007).

2 | PHYTOHORMONES IN DEFENSE

Many phytohormones are involved in plant defense against RKN. The three classical phytohormone defense pathways involve salicylic acid (SA), jasmonates (JA), and ethylene (ET). ET interacts with SA and JA to modulate plant defense responses, typically acting synergistically with JA-mediated pathways and antagonistically with SA-mediated pathways (Checker et al., 2018). Other phytohormones involved in defense responses include gibberellins (GA), brassinosteroids (BR), abscisic acid (ABA), and auxin.

ET-, ABA-, JA-, and SA-mediated responses were identified in both compatible and incompatible interactions between tomato and *M. incognita* (Shukla et al., 2018). In the early stages of the incompatible interaction, ABA- and ET-mediated pathways were induced. In the compatible interaction, ABA- and ET-mediated pathways were induced while SA- and JA-mediated pathways were suppressed. Auxin and GA are involved in the incompatible interaction between soybean and *M. javanica* likely influencing ROS production (Beneventi et al., 2013). After RKN infection of tomato, BR-induced MAPK activation functions independently of SA, JA, and ABA pathways (Song et al., 2018). SA and indole-3-butyric acid, an auxin-family phytohormone, induce expression of the WRKY transcription factors (TFs) SIWRKY3 and SIWRKY35 in transgenic tomato roots following RKN infection (Chinnapandi et al., 2019).

3 | PLANT IMMUNE PATHWAYS

Plant defenses can protect against RKN. There are two primary signaling pathways involved in a layered host plant defense response against pathogens: pattern-triggered immunity (PTI) and effector-triggered immunity (ETI). In PTI, the first layer of defense, cell surface-localized pattern recognition receptors (PRRs), recognize certain molecules, either damage-associated molecular patterns (DAMPs) or pathogen-associated molecular patterns (PAMPs) (Couto & Zipfel, 2016). PAMPs and the responses they induce in plants are generally conserved among pathogens as well as among plant species, so PTI can protect against a broad range of pathogens. Downstream responses...
to PRR recognition include the production of reactive oxygen species (ROS), callose deposition, expression of defense-related genes, or activation of protein kinase cascades (Boller & He, 2009). Nematode-associated molecular patterns (NAMPs) are a subset of PAMPs that are produced by nematodes. The family of NAMPs that has been found to be evolutionarily conserved among at least 20 nematode species is ascarosides, a type of pheromone. Seven ascarosides have been identified as conserved among M. incognita, M. hapla, and M. javanica, with the most abundant being ascr#18 (Table 1). Ascr#18 has also been identified in cyst and lesion nematodes (Manosalva et al., 2015). Ascr#18 possesses an 11-carbon side chain that is broken down by the plant into ascarosides with shorter side chains using peroxisomal β-oxidation, with ascr#9 being the most abundant. These shorter side-chained ascarosides are excreted through plant roots, where certain blends act as RKN deterrents (Manohar et al., 2020). The immune response triggered by ascr#18 is not nematode-specific. Application of ascr#18 to seven crop plants and Arabidopsis provided modest to strong protection against two viruses, two bacteria, two oomycetes, three fungi, and three nematodes (Klessig et al., 2019). The PRR involved in ascaroside recognition is yet to be identified.

ETI is a second layer of defense mediated by resistance (R) protein recognition of effectors introduced by the pathogen to aid their infection of the host plant (Boller & He, 2009). In plant–nematode interactions, this often manifests as a hypersensitive response once a J2 attempts to initiate a feeding site, leading to localized cell death at the infection site (Dropkin, 1969). A reduction in RKN feeding, development, and reproduction is often achieved. However, host resistance can be achieved at other points in the infection. For example, in soybean, J2s have been documented emigrating from the root of resistant plants after the initial infection (Herman et al., 1991). The molecular basis of this resistance remains to be explored.

Several R genes against RKN have been cloned (Table 1). Canonical resistance to M. incognita was first characterized in tomato. Mi-1.2 encodes a CC-NB-LRR resistance protein with an N-terminal coiled-coil (CC) domain, nucleotide-binding (NB) site, and C-terminal leucine-rich repeat (LRR) domain (Milligan et al., 1998). Resistance conferred by Mi-1.2 is not heat-stable and becomes inactive at soil temperatures above 30°C. Mi-1.2 is cytoplasmically localized, so it likely recognizes something injected into the cell by the nematode. This molecule has not yet been identified. CaMi was cloned from pepper and encodes a protein with predicted NB and LRR domains. When CaMi was transformed into RKN-susceptible tomato cultivars, the plants showed resistance and a hypersensitive response after RKN infection (Chen et al., 2007). The Ma gene was first identified in plum and offered heat-stable and high-level resistance to all RKN species tested. Ma is another classical R-gene, encoding a TOLL/INTERLEUKIN1 receptor (TIR)-NB-LRR resistance protein (Claverie et al., 2011). A fourth R gene, CaRKNR, was cloned from pepper and confers resistance to M. incognita (Mao et al., 2015). CaRKNR encodes another NB-LRR protein sharing 70% identity to Mi-1.2. As genetic technology advances, many more resistance loci are being fine-mapped to find candidate R genes.

Another heat-stable R locus, Mi-3, was identified in Solanum peruvianum, though multiple factors have prevented the gene from being cloned (Yaghoobi et al., 2005). RNAseq of Mi-3-containing tomatoes after M. incognita infection indicated a role for Mi-3 in activating the PTI response (Du et al., 2020). The gene encoding FLS2 (FLAGELLIN-SENSING 2), the PRR that recognizes the bacterial PAMP flg22, was upregulated at 3 days post-inoculation (dpi). In plants grown at 34°C, the soil temperature at which Mi-3 resistance is inactivated, the expression of FLS2 was abolished.

There is also evidence to suggest non-canonical R genes confer resistance against RKN. In soybean, resistance against M. incognita has been mapped multiple times to a region on chromosome 10 in various genetic backgrounds. Xu et al. (2013) fine-mapped this region to a 29.7-kb region containing a block of cell wall-related genes and no canonical R genes. In cotton, a variant of a HAT-like transposase containing a premature stop codon is associated with RKN resistance (Wubben et al., 2019).

4 | IMPROVED METHODS FOR PTI RESEARCH

Ibrahim et al. (2019) provided an excellent review on approaches to studying plant–RKN interactions. One limitation to the existing body of PPN research is the use of Arabidopsis as a model system. Arabidopsis can be infected by some species of RKN; however, it is not a usual target for infection and is a poor host. Thus, parallel studies of economically important nematodes should be done in economically important hosts. Since PTI interest in nematology is relatively new, approaches for the study of PTI in other pathosystems have been applied to study PTI in plant–nematode interactions. Tran et al. (2017) used the bacterial PAMP flg22 to optimize PTI study for root tissue in non-Arabidopsis systems. Several other crop systems have now been used for NAMP work, aided by the development of techniques like NemaWater. NemaWater improves PTI study because it removes the actual nematode from the infection, instead plants are just treated with molecules released by shaking J2s in water overnight (Mendy et al., 2017). When nematodes are absent, effectors will not be released during infection and ETI will not be triggered. The exact components of NemaWater are yet to be identified, though heat-sensitive proteins appear to play a key role in eliciting the defense response. When NemaWater that had been either heated to 37°C for 4 h or treated with proteinase K was applied to roots, the number of adult females developing in the roots decreased while the root fresh weight increased compared to the application of untreated NemaWater (Mendy et al., 2017). Higher-throughput screening approaches for identifying nematode effectors independent of nematode inoculation that suppress PTI have also been adopted. Such screening protocols have led to the identification of Heterodera glycines effectors involved
### Table 1: A list of R genes, plant proteins involved in PTI, ascarosides, and host defense modulating effectors involved in RKN infection

| RKN resistance genes | Gene   | Host species | RKN species                      | Structure       | References                          |
|----------------------|--------|--------------|----------------------------------|-----------------|-------------------------------------|
| Mi-1.2               | tomato | Meloidogyne incognita, Meloidogyne javanica, Meloidogyne arenaria | CC-NB-LRR       | Milligan et al. (1998)              |
| CaMi                 | pepper | M. incognita |                                   |                 | Chen et al. (2007)                  |
| Ma                   | plum   | 30 RKN species | TIR-NB-LRR              |                 | Claverie et al. (2011)              |
| CaRKNR               | pepper | M. incognita |                                   | NB-LRR          | Mao et al. (2015)                   |

### Plant proteins involved in PTI against RKN

| Class | Arabidopsis thaliana | Solanum lycopersicum | Orthologs | Function                              | References                          |
|-------|----------------------|-----------------------|-----------|---------------------------------------|-------------------------------------|
| SERK  | BAK1                 | SERK1/3               |           | NILR co-receptor                      | Teixeira et al. (2016); Peng and Kaloshian (2014) |
| BIK   | BIK1                 | BIK1/3                |           | Transphosphorylation of RBOH          | Teixeira et al. (2016)              |
| WRKY  | WRKY11/17            |                       |           | Basal defense TFs                     | Teixeira et al. (2016)              |
| MYB   | MYB35/51             |                       |           | Glucosinolate biosynthesis TFs        | Teixeira et al. (2016)              |
| NILR  | NILR1                |                       |           | Plasma-membrane localized PRR        | Mendy et al. (2017)                 |
| RBOH  | RBODH/F              | RBOH1/WF11            |           | Respiratory burst NADPH oxidase       | Song et al. (2018)                  |
| MAPK  | MPK3/6               | MPK1/2/3              |           | Induction of defense gene expression  | Song et al. (2018)                  |

### RKN-associated ascarosides (NAMPs)

| SMID ID | Formula | Carbon side chain length | Association                          | References                          |
|---------|---------|--------------------------|--------------------------------------|-------------------------------------|
| ascr#1  | C_{12}H_{24}O_{6} | 7                        | ascr#18 plant metabolite             | Manohar et al. (2020)              |
| ascr#9  | C_{12}H_{20}O_{6} | 5                        | ascr#18 plant metabolite             | Manohar et al. (2020)              |
| ascr#10 | C_{15}H_{28}O_{6} | 9                        | RKN excreted; ascr#18 plant metabolite | Manosalva et al. (2015); Manohar et al. (2020) |
| ascr#16 | C_{16}H_{30}O_{6} | 10                       | RKN excreted                         | Manosalva et al. (2015)            |
| ascr#18 | C_{17}H_{32}O_{6} | 11                       | RKN excreted                         | Manosalva et al. (2015)            |
| ascr#20 | C_{18}H_{32}O_{6} | 12                       | RKN excreted                         | Manosalva et al. (2015)            |
| ascr#22 | C_{19}H_{32}O_{6} | 13                       | RKN excreted                         | Manosalva et al. (2015)            |
| ascr#24 | C_{20}H_{32}O_{6} | 14                       | RKN excreted                         | Manosalva et al. (2015)            |
| ascr#26 | C_{21}H_{40}O_{6} | 15                       | RKN excreted                         | Manosalva et al. (2015)            |

### RKN effectors involved in modulating plant defenses

| Effector | RKN species | Annotation                                      | Defense pathway target                          | References                          |
|----------|-------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------|
| MjTTL5   | M. javanica |                                  | ROS production/scavenging                        | Lin et al. (2016)                   |
| Mc1194   | Meloidogyne chitwoodi |                                  | extracellular PLCP-related defense                  | Davies et al. (2015)               |
| MeTCTP   | Meloidogyne enterolobii |                                  | Transitionally controlled tumor protein        | Zhuo et al. (2017)                  |
| MiMIF-2  | M. incognita |                                  | Macrophage migration inhibitory factor           | Zhao et al. (2019)                  |
| MiPDI1   | M. incognita |                                  | Protein disulfide isomerase-like                  | Zhao et al., (2020)                 |
| MiEFF1   | M. incognita |                                  | SA- and JA-related defense gene expression         | Jaoanet et al. (2012a); Truong et al. (2021) |
| Mi-CRT   | M. incognita |                                  | Calreticulin                                    | Jaoanet et al. (2012b); Truong et al. (2021) |
| Mg16820  | Meloidogyne graminicola |                                  | Transhretin-like protein                        | Naalden et al. (2018)              |
| MgMO237  | M. graminicola |                                  | JA defense pathway; callose deposition; defense signaling | Chen et al. (2018)                 |
| MgMO289  | M. graminicola |                                  | ROS production/scavenging                        | Song et al. (2021)                  |
in PTI suppression (Pogorelko et al., 2020) and can be easily adapted to other PPN classes, including RKN.

## 5 | DOWNSTREAM MANIFESTATIONS OF PTI

### 5.1 | ROS production

Figure 2 illustrates some PTI responses upon RKN infection. For PTI to be triggered, PRRs must recognize the PAMPs or DAMPs. Few PRRs have been identified in RKN infections. A leucine-rich repeat receptor-like kinase (LRR-RLK) NILR1 (NEMATODE-INDUCED LRR-RLK 1) is needed for NAMP perception in Arabidopsis (Mendy et al., 2017). The SERK (SOMATIC EMBRYOGENESIS RECEPTOR KINASE) family of LRR-RLKs contains several proteins involved in RKN-induced PTI. Tomato roots with either SlSERK3A or SlSERK3B silenced showed increased susceptibility to RKN (Peng & Kaloshian, 2014). Another member of the SERK family is BAK1 (BRASSINOSTEROID INSENSITIVE-ASSOCIATED KINASE 1). BAK1 phosphorylates cytoplasmic BIK1 (BOTRYTIS-INDUCED KINASE 1), which then phosphorylates RBOHD (RESPIRATORY BURST OXIDASE D) to catalyze superoxide radical formation (Teixeira et al., 2016). At the beginning of this pathway, nilr1 mutants lacked the ROS burst response to NemaWater application (Mendy et al., 2017). In a separate assay, Arabidopsis bak1-5 mutants showed significantly more galls than wild-type (WT) plants after RKN infection (Teixeira et al., 2016). Arabidopsis bik1 mutants and rbohD/rbohF double mutants also showed significantly more galls than WT (Teixeira et al., 2016). Other genes involved in H2O2 production include RBOH1 (RESPIRATORY BURST OXIDASE HOMOLOG 1) and WFI1 (WHITEFLY INDUCED 1), both encoding NADPH oxidase. Silencing of either of these genes in tomato increased the number of galls compared to WT plants (Song et al., 2018).

There are several ROS types—superoxide radicals (O2•−), hydrogen peroxide (H2O2), singlet oxygen (1O2), and hydroxyl radicals (OH•). O2•− are typically the first ROS to be produced, created by the loss of a single electron from O2 (Gill & Tuteja, 2010). These radicals can become H2O2 through several pathways, ultimately causing a univalent reduction. One pathway is demonstrated by the rice protein copper/zinc-oxide dismutase 2 (Cu/Zn-SOD2, OsHPP04). Cu/Zn-SOD2 delivers Cu(I) to Cu/Zn-SOD2. Cu(I) is required for the activation of SOD enzymes. Once SOD is active, it can catalyze the dismutation of O2•− via:

\[
O_2\cdot^- + Cu(II)ZnSOD \rightarrow O_2 + Cu(II)ZnSODO_2^- + Cu(II)ZnSOD + 2H^+ \rightarrow H_2O_2 + Cu(II)ZnSOD.
\]

This process both creates non-radical H2O2 and decreases the formation of radical OH• (Gill & Tuteja, 2010). This pathway is targeted by RKN through effectors like MgMO289 from *M. graminicola*. MgMO289 increases the activity of Cu/Zn-SOD2, in turn increasing the amount of H2O2 (Song et al., 2021). Accumulation of H2O2 can lead to cell death, a desired outcome of PTI.

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**Figure 2** Model of a PTI response pathway and nematode effector target points. NAMP recognition by PRRs (A), ROS production (B), calcium signaling (C), defense gene expression (D), phytohormone signaling (E).
For the increased SOD activity to benefit the nematode, a catalase would presumably be required to further dismutate H$_2$O$_2$ to H$_2$O and O$_2$ unless the nematode has another use for the H$_2$O$_2$.

5.2 | Mitogen-activated protein kinase activation

ROS production leads to the activation of additional defense pathways, including mitogen-activated protein kinase (MAPK) signaling cascades. The loss of either of these steps can prevent PTI and increase susceptibility to RKN. RKN infection increases the expression of MPK1 in tomato within 24 h of infection (Song et al., 2018) (Figure 2). Application of 0.2 μM 24-epibrassinolide (EBR) to leaves, an active BR of tomato, watermelon, muskmelon, and cucumber, significantly decreased the number of galls formed after RKN infection. EBR application activates MPK1, MPK2, and MPK3. Silencing of MPK1, MPK2, or MPK3 decreases the resistance conferred by EBR application.

5.3 | Alteration of gene expression

PTI signaling pathways can result in many different manifestations to accomplish the greater goal of plant defense. A key goal of PTI is to alter gene expression to aid in defense against the invading pathogen. One mechanism for regulating gene expression employs transcription factors (TFs). In Arabidopsis, the TFs WRKY11 and MYB51 are induced upon RKN infection, with this induction dependent on BAK1 (Teixeira et al., 2016) (Figure 2). In resistant coffee roots, CoWRKY11 was upregulated at 6 dpi with M. incognita (Albuquerque et al., 2017). Various types of non-coding RNAs are also involved in regulation of gene expression. Small interfering RNAs (siRNAs) are 21–24 nucleotide long RNAs resulting from double-stranded RNA (dsRNA) that mediate gene expression through different pathways—transcriptional gene silencing (TGS) or post-transcriptional gene silencing (PTGS) (Brodersen & Voinnet, 2006). When siRNAs were sequenced in Arabidopsis roots infected with M. incognita, more clusters of siRNAs were found in galls than in uninfected roots (Medina et al., 2018). One pathway for TGS, the RNA-dependent DNA methylation pathway (RdDM), was implicated in PTI through transcriptome data. Genes involved in RdDM were upregulated in rice roots infected with RKN (Kynadt et al., 2012a). Micro-RNAs (miRNAs) have been shown to affect the expression of genes involved in JA synthesis after RKN infection in tomato (Zhao et al., 2015). Nematode-responsive long non-coding RNAs (lncRNAs), another tool for regulation of gene expression, have been identified in tobacco. These included those shared between resistant and susceptible genotypes, or specific to genotypes (Li et al., 2018).

Another method for increasing gene expression is DNA hypomethylation (Atighi et al., 2020). When rice was treated with NemaWater prepared from M. graminicola, hypomethylation of gene promoter regions was noticeable after 72 h. There was a positive correlation between hypomethylation of promoter regions at 72 h post-NemaWater application and genes upregulated 7 dpi with M. graminicola J2s. This demonstrates the delayed effect of hypomethylation on gene expression. As the gene promoter region is demethylated and more accessible to the transcriptional machinery, gene expression is increased. The hypomethylation response was also demonstrated with M. incognita NemaWater treatment of RKN-susceptible tomato plants.

6 | TRANSCRIPTOMICS OF PTI RESPONSES

Since one goal of PTI is altered gene expression, a common way to examine PTI is through transcriptome analysis after RKN infection. Several studies have used this approach to correlate gene expression with host defense responses; however, not every differentially expressed gene (DEG) identified in such a study is definitively involved in PTI. Changes in gene expression can result from multiple aspects of host defense responses, as well as the RKN infection process itself. Upon NAMP recognition, PTI will alter the expression of genes in multiple defense pathways. RKN secrete effector proteins through the stylet to facilitate the infection process, ultimately leading to altered gene expression (Table 1). Upon recognition of these effectors, ETI can also alter gene expression. Thus, each DEG must be carefully considered to attribute the most likely source of the expression change. In coffee, M. incognita infection was shown to affect immune signaling genes in resistant and susceptible genotypes in the early stages of infection (Albuquerque et al., 2017). A set of 88 defense-related genes were isolated as differentially expressed at 5 and 6 dpi, including WRKY TFs involved in plant immune responses. In the compatible interaction, most DEGs were downregulated at 5 dpi when feeding sites are being initiated. In the incompatible interaction, most DEGs were upregulated at 6 dpi as the hypersensitive response occurs. After M. incognita infection in tomato, the majority of the DEGs genes in the incompatible interaction have defense-related functions. In the compatible interaction, DEGs are involved in many cellular processes, including cell wall modification, plant development, primary metabolism, and solute transport (Shukla et al., 2018).

These studies also provide an opportunity to evaluate the RKN transcriptome in both compatible and incompatible interactions throughout the infection process. RKN effectors identified through RNAseq of infected tomato plants indicate several target suppression of the host defense response, in addition to those involved in feeding site development (Shukla et al., 2018). As RNAseq has become more commonplace in recent years, additional work is currently being done to profile similar transcriptome data from both host plants and nematodes during compatible and incompatible interactions. One use for this data is to compare differential expression of genes involved in PTI pathways to expression data of effectors produced by the nematode in the different host compatibilities.

7 | IMPLICATIONS OF INFECTION BY MULTIPLE PATHOGENS

It has been shown that the wounding created by PPN entering plant roots can increase susceptibility to other pathogens (Rocha et al., 2009).
et al., 2020). While the PTI response is primarily localized in the infected region, RKN infection also alters gene expression throughout the plant (Hofmann et al., 2010). As defense-related genes are upregulated in infected roots, the same genes are down-regulated in shoot tissues (Kynadt et al., 2012b), increasing susceptibility to other pathogens whose infection mechanism is based in shoot organs. In rice, infection by *M. graminicola* increases susceptibility to *Magnaporthe oryzae* (rice blast), a pathogen that can cause 30–100% yield loss (Kynadt et al., 2017). This interaction of defense responses throughout the plant has possible ramifications in breeding resistant varieties if the PTI response against RKN in the roots hinders a plant’s adapted ETI response against a different pathogen concurrently infecting the shoots.

8  |  SIMILARITIES TO OTHER PLANT-PARASITIC NEMATODE INFECTIONS

Ascarosides have been found to be conserved across PPN species. However, the differences in feeding lifestyles of different species affect the approaches used by the host plant for PTI. Even differences between migration patterns of sedentary endoparasites would alter PRR action and PTI. RKN travel intercellularly through roots, causing relatively little damage on their journey. Arabidopsis mutants of DAMP-associated PRRs PEPR1, PEPR2, and DORN1 do not show increased susceptibility to RKN infection (Teixeira et al., 2016), indicating that DAMP perception is likely not a crucial aspect of PTI against RKN. Cyst nematodes travel intracellularly through the cortex, causing wounding damage along the way. Damage-related basal defense responses are associated with the early stages of cyst nematode infection. Expression of *PGIP1* and *PGIP2* (polygalacturonase-inhibiting proteins that inhibit cell wall degradation by nematodes) is induced in Arabidopsis after infection with *Heterodera schachtii* (sugar beet cyst nematode), an expression pattern not seen during the migratory stage of *M. incognita* infection (Shah et al., 2017). Cell wall modification enzymes are almost always associated with RKN infections in both the host and nematode and the intricacies of their expression patterns will yield key insights into the molecular process of RKN infection and host resistance.

9  |  EFFECTORS MODULATING PLANT DEFENSE

RKN secrete a variety of effector proteins through the stylet, some of which serve to modulate plant defenses (Table 1). These effector proteins are expressed in specialized esophageal gland cells, the dorsal gland, and two subventral glands (Mitchum et al., 2013). RKN effectors have an N-terminal signal peptide to mark them for secretion.

Mi-EFF1 is secreted through the stylet into giant cells, where it targets nuclei (Jaouannet et al., 2012b). Truong et al. (2021) confirmed via yeast two-hybrid screens (Y2H) and bimolecular fluorescence complementation (BiFC) that Mi-EFF1 interacts with a universal stress protein (ATUSP, AT3G53990) and two cytosolic glyceraldehyde 3-phosphate dehydrogenases (AtGAPC1, AT3G04120; AtGAPC2, AT1G13440). Certain Arabidopsis mutants in AtGAPC1 or AtGAPC2 showed decreased susceptibility to *M. incognita*. Quantitative RT-PCR analysis of these mutants indicated that AtGAPC1 and AtGAPC2 regulate the expression of SA-, JA-, and ET-mediated defense pathways.

Mi-PDI1 is a protein disulfide isomerase-like protein expressed in the subventral glands and secreted during migration and feeding (Zhao et al., 2020). Y2H, BiFC, and coimmunoprecipitation (Co-IP) showed that Mi-PDI1 interacts with a tomato stress-associated protein (SISAP2). Overexpression of Mi-PDI1 is followed by decreased expression of the SA-marker AtPR1a and increased expression of JA and ET markers PDF1.2a and PR4 (Zhao et al., 2020).

Mi-MIF2 (macrophage migratory inhibitory factor-like) interacts with two annexins (AnnAt1, AnnAt4) in Arabidopsis (Zhao et al., 2019). Annexins regulate various biotic and abiotic stress responses. Mi-MIF2 manipulates the plant’s annexins to prevent an immune response.

The *M. incognita* effector MiMsp40 is expressed in the esophageal glands, with expression peaking in the J2 stage. Overexpression of *MiMsp40* in Arabidopsis increases susceptibility to RKN (Niu et al., 2016). This indicates a role for MiMsp40 in the suppression of PTI and/or ETI in the early stages of infection. This role was further supported by lack of callose deposition and expression of marker genes associated with elf18-triggered immunity after application of the bacterial elicitor elf18 to MiMsp40-overexpressing Arabidopsis.

Another *M. incognita* effector gene, Misp12, peaks in expression in the mature female stage and is localized to the cytoplasm (Xie et al., 2016). In planta, RNAi of Misp12 significantly decreased the number of galls, eggs, and remaining nematodes in infected roots. Overexpression of Misp12 in *N. benthamiana* led to down-regulation of defense genes associated with the SA defense pathway. These results suggest a role for Misp12 in the suppression of plant immune responses like programmed cell death against giant cells.

In *M. graminicola*, the rice RKN, several effectors have been identified as interacting with rice proteins during infection. MgMO237 reaches maximum production by the nematode around the J3/J4 stages. In giant cells, it interacts with a 1,3-β-glucan synthase (OsGSC), a cysteine-rich repeat secretory protein (OsCRRS55), and a pathogenesis-related Betvl family protein (OsBetvl) (Chen et al., 2018). MgMO289 is also upregulated in the nematode J3/J4 stages. This effector targets copper metallochaperone heavy metal-associated plant protein 04 (OshPP04), a chaperone protein for cytosolic Cu/Zn-SOD2. This likely interrupts the cell’s superoxide radical scavenging system to suppress immunity (Song et al., 2021).

10  |  CONCLUSION

Significant progress has been made in understanding the PTI response in plant–RKN interactions. Much of this research has been carried out in compatible interactions, but understanding the PTI response in incompatible interactions can give insight into the mechanisms used by nematodes to overcome this resistance. Identifying effectors is crucial to understanding incompatible host–nematode interactions.
and should remain a priority. However, understanding complex interactions requires viewing them from several different angles. New innovations in research practices and technology have given us many new angles to use and PTI research is one of them. Understanding the plant responses that nematodes need to overcome to successfully infect host plants will aid in understanding the functions of effectors. PTI also could give valuable insight into non-host interactions, possibly explaining molecular aspects of host specificity in RKN and other nematode species. This is just conjecture and stands to be shown, but there are many possible benefits to continued work on PTI in plant-nematode interactions.

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Kelly Goode conceived the manuscript and had a primary role in writing the manuscript with input from Melissa G. Mitchum. Both authors contributed to images, reviewed, edited, and approved the final manuscript.

DATA AVAILABILITY STATEMENT
Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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