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REVIEW

Integrated signaling networks in plant responses to sedentary endoparasitic nematodes: a perspective

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Abstract Sedentary plant endoparasitic nematodes can cause detrimental yield losses in crop plants making the study of detailed cellular, molecular, and whole plant responses to them a subject of importance. In response to invading nematodes and nematode-secreted effectors, plant susceptibility/resistance is mainly determined by the coordination of different signaling pathways including specific plant resistance genes or proteins, plant hormone synthesis and signaling pathways, as well as reactive oxygen signals that are generated in response to nematode attack. Crosstalk between various nematode resistance-related elements can be seen as an integrated signaling network regulated by transcription factors and small RNAs at the transcriptional, posttranscriptional, and/or translational levels. Ultimately, the outcome of this highly controlled signaling network determines the host plant susceptibility/resistance to nematodes.

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Introduction

Plants are often exposed to biotic stresses derived from viruses, bacteria, fungi, nematodes, and insects. Interactions between host plants and their pathogens determine the degree of pathogenesis observed. Successful pathogens attach to a host plant, penetrate through the physical barriers of the cell wall, and override host plant defenses. Once inside the plant, pathogens can either kill plant cells (necrotrophic pathogens), or live within host tissues without causing plant cell death (biotrophic pathogens). In response, resistant plants have evolved the ability to recognize pathogens and make timely defensive responses.

The interactions between plants and pathogens are summarized as a so called 'zigzag' model where plants are able to recognize pathogen-associated molecular patterns (PAMPs) derived from the pathogen utilizing pattern recognition receptors (PRRs) leading to pattern-triggered immunity (PTI) (Jones and Dangl 2006). PAMPs are invariant epitopes derived from pathogens that are: fundamental to the fitness of pathogens, absent in the host, and recognized by a wide array of potential hosts (He et al. 2007; Schwessinger and Zipfel 2008). PRRs are cell-surface-localized receptors, usually harboring an extracellular leucine-rich repeat (LRR) domain, that recognize conserved pathogen elements (Schwessinger and Zipfel 2008).

Pathogens that successfully suppress PTI responses can release pathogenic effectors into host plants, altering host-cell structures and suppressing defense responses leading to effector-triggered susceptibility (Jones and Dangl 2006).

Specific resistance proteins (R-proteins) have also evolved in response to such effectors, to sense pathogen effectors yielding effector-triggered immunity (ETI) (Jones and Dangl 2006).

Canonical R-proteins are intracellular and often referred to as NBS-LRR proteins because they typically contain nucleotide-binding site (NBS) and leucine-rich repeat (LRR) domains (Hogenhout et al. 2009; Shirasu 2009). While the LRR domain is believed to be responsible for interaction between plant receptors and pathogen effectors, the NBS domain is characterized by NTPase activity and functions as a molecular switch activating subsequent downstream signal transduction during contact with pathogen-derived effectors (Glowacki et al. 2011). R-proteins can directly sense pathogen effectors, or they can detect pathogens through other cofactors, which are direct host targets of pathogens (Glowacki et al. 2011).

Sedentary plant endoparasitic nematodes (SPENs) are biotrophic pathogens that can cause significant yield loss in crop plants. The most well studied and crop-impactful SPENs are root-knot nematodes (RKN, *Meloidogyne* spp.) and cyst nematodes (CN, *Globodera* and *Heterodera* spp.), while reniform nematodes (RN, *Rotylenchulus reniformis* Linford & Oliveira.), a kind of plant semi-endoparasite, are also known to affect important crops such as cotton and soybean (Robinson 2007).

The life cycle of the different SPENs, i.e. RKN, CN, and RN, typically requires around 3 weeks under favorable environmental conditions of optimal soil moisture and temperatures. Eggs generated by female nematodes are deposited into a gelatinous matrix on the host root surface, which protects eggs from dehydration (Williamson and Gleason 2003). There are four juvenile stages, separated by molts, needed for eggs to mature into adults. While the second-stage juvenile of RKN and CN penetrates into the host plant root, for RN it is the female young adult that is the infective stage.

Once inside the host plant, successful nematode parasitism is contingent upon establishment of a nematode feeding site (NFS), which serves as the sole nutrient source on which the nematode lives. The NFS are hypertrophied, multinucleate root cells with enlarged nuclei and dense cytoplasm, which resulted from nuclear division without cytokinesis of infected host cells (giant cells, in the case of RKN) (Williamson 1999), or break down of cell walls between initial feeding cells and neighboring cells (syncytia, in the case of CN and RN) (Williamson and Gleason 2003; Robinson 2007). These sedentary endoparasites ingest nutrient from the NFS through their stylet, a specialized hollow needle-like structure mouthpart. Female nematodes feeding on the host root enlarge and start to produce eggs.

Nematode effectors secreted through the stylet are essential in NFS initiation and maintenance. These effector proteins act as PAMPs or pathogenic effectors aiding nematode parasitism, conditioning host defense responses, and/or modifying host plant physiology (Hewezi and Baum 2013; Mitchum et al. 2013). These nematode effectors are directly secreted into the cytoplasm of host cells to interact with components of the cell cycle, cytoskeleton, and cellular metabolism, or alternatively, they accumulate in host extracellular spaces to degrade plant cell walls and change cell wall architecture (Mitchum et al. 2013).

In this review, signaling and signal transduction involved in plant general biotic defense mechanisms will be reviewed for plant susceptibility/resistance (S/R) to SPENs, and the nature of the integrated signaling network that determines plant responses to nematodes will be defined. Specifically, different *R*-genes mediating nematode resistance and their upstream signaling will be reviewed. The roles of different classes of phytohormones, their synthesis and signaling, and the role(s) of reactive oxygen and nitrogen species (ROS and RNS) generation and signaling in plant responses to SPENs will be reviewed. The emerging and integrative regulatory role of small RNAs in plant S/R to SPENs will be considered then in summary.

R-proteins and their upstream signaling in plant resistance to SPENs

Initial sensing of nematode infestation can occur either extra- or intracellularly and typically involves interaction with an R-protein receptor (typically identified as NBS-LRR proteins) (Fig. 1). Many distinct loci involved in initiating resistance responses to SPENs have been mapped to plant genomes (Table S1). The first cloned locus was $Hs1^{pro-1}$, predicted to encode an extracellular leucine-rich region containing protein. This was followed by the cloning of a series of other *R*-genes including: *Mi-1*, *Gpa2*, *Gro1-4*, "*Hero A*", *CaMi*, and *Ma*, that have all been shown to encode canonical intracellular NBS-LRR type R-protein receptors (Table 1).

These cloned NBS-LRR proteins can also be further classified as TIR-NBS-LRR or CC-NBS-LRR based on their amino-terminus motif (Table 1). Both TIR (toll-interleukin receptor) and CC (coiled-coil) domains are thought to be crucial in the signal transduction of innate immunity (Glowacki et al. 2011). Further investigating how different domains contribute to pathogen sensing and signal transduction will be helpful to understand *R*-gene-mediated signaling in nematode resistance. Specifically, a WRKY-like domain on the carboxyl terminus of the *Ma* gene encoded protein suggests a direct role of the Ma

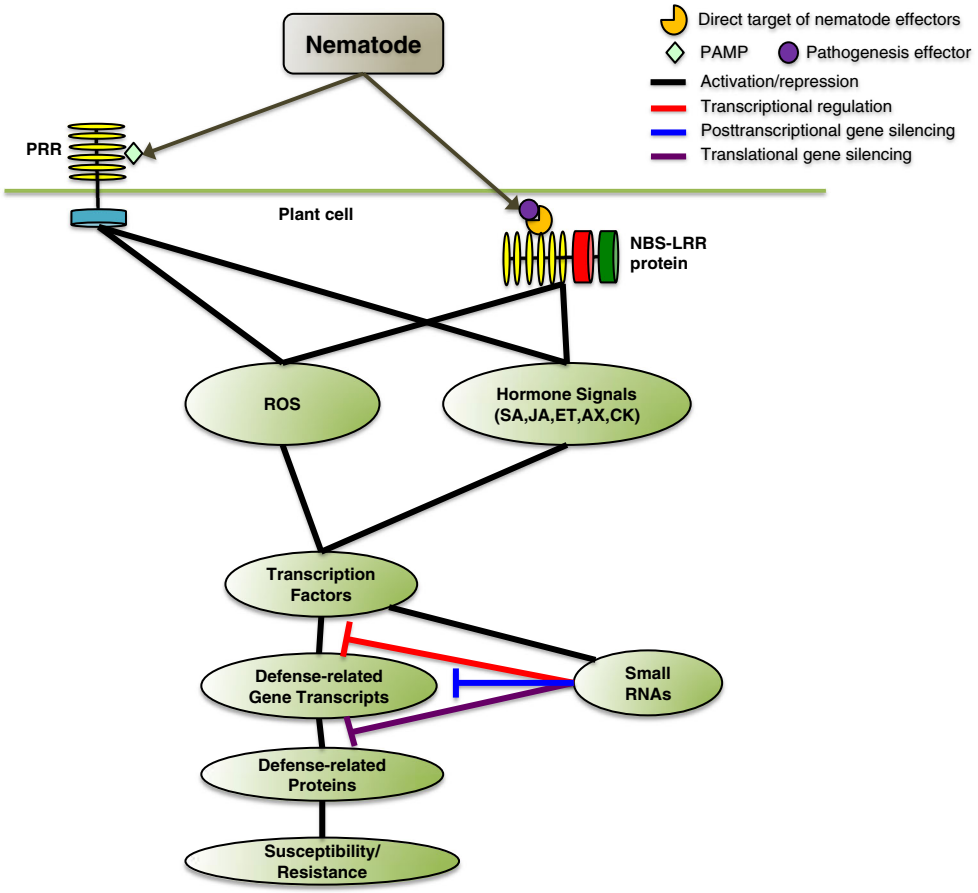

Fig. 1 The integrated signaling network in plant responses to SPENs Sedentary endoparasitic nematodes attack host plants and secrete various effectors functioning as PAMPs or pathogenic effectors. Upon recognition of invading nematodes with plant transmembrane extracellular R proteins or intracellular NBS-LRR proteins, ROS and various hormone-signaling pathways are activated. Different transcription factors and small RNAs regulate plant defense related factors at transcriptional, post-transcriptional, and/or translational levels leading to plant S/R to nematodes

protein in downstream regulation of defense gene expression and plant immunity (Table 1). More discussion and comparison of these R-protein structures can be found in detailed reviews (Fuller et al. 2008; Goverse and Smant 2013; Williamson and Kumar 2006).

Rhg1 and *Rhg4* are two unlinked quantitative trait loci (QTLs) in soybean that appear to condition S/R to SCN and are unique when compared to the canonical *R*-gene loci mentioned above (Hauge et al. 2001). Since the discovery of these QTLs it has been hypothesized that extracellular LRR kinase-type R-proteins found in the coding regions of these QTLs conditioned resistance to SCN (Hauge et al. 2001).

However, transgenic soybean plants with over-expressed or silenced LRR-kinase genes from the *Rhg1* locus showed little change in S/R to SCN (Melito et al. 2010), contradicting the original disclosure. Subsequently, it was shown that *Rhg1*-conditioned SCN resistance was determined by three genes encoding an amino acid transporter, an α-SNAP protein, and a wound-inducible domain protein (Cook et al. 2012). The copy number of a 31 kb repeat sequence containing these 3 genes appears to determine SCN resistance: multiple copies of the repeat produced resistance, while a single copy produced a susceptible phenotype (Cook et al. 2012). While, the precise role of the 31 kb gene repeat remains unclear at this time, it appears that the *Rhg1* locus plays a regulatory role that may involve the expression of some type of as yet undefined *R*-gene. In addition, differentially methylated regions within *Rhg1* correlated with SCN resistance. This fact along with the observation of copy number of the 31 kb repeat-region mentioned above suggest the possibility that some type of epigenetically mediated phenomenon may play a role in *Rhg1*-mediated host plant resistance (Cook et al. 2014) the details of which remain to be elucidated.

Rhg4-mediated SCN resistance or susceptibility is linked to two nucleotide polymorphisms in a single copy gene encoding a serine hydroxymethyltransferase (SHMT) (Liu et al. 2012) in contradiction to the earlier report (Hauge et al. 2001) that the *LRR*-gene near the *Rhg4* QTL locus conditioned SCN resistance. SHMT may affect plant S/R to nematodes through regulation of folate one-carbon metabolism, since folate deficiency may cause parasitizing nematode death and degradation of nematode induced syncytia.

Thus, none of the gene products from *Rhg1* and *Rhg4* resemble either extracellular or intracellular canonical

Table 1 Cloned plant genes for SPENs resistance

Gene/ Loci	Plant	Nematode	Encoded protein(s)	References
CaMi	*C. annuum L.*	RKN: *M. incognita*	CC-NBS-LRR	Chen et al. 2007
Gpa2	*S. tuberosum*	PCN: *G. pallida*	CC-NBS-LRR	Van der Vossen et al. 2000
Gro1-4	*S. tuberosum*	PCN: *G. rostochiensis*, type Ro1	TIR-NBS-LRR	Paal et al. 2004
Hero A	*S. pimpinellifolium*	PCN: *G. rostochiensis* types Ro1, Ro3 and Ro5; *G. pallida* types Pa2 and Pa3, and Luffness	CC-NBS-LRR	Ernst et al. 2002; Sobczak et al. 2005
$Hs1^{pro-1}$	*B. procumbens*	BCN: *Heterodera schachtii*	Amino-terminus leucine-rich region	Cai 1997
Ma	*P. cerasifera*	RKN: all species tested	TIR-NBS-LRR-WRKY	Claverie et al. 2011
Mi-1	*S. peruvianum*	RKN: *M. incognita*, *M. javanica*, *M. arenaria*	CC-NBS-LRR	Milligan et al. 1998; Vos et al. 1998
Rhg1	*G. max*	SCN: *H. glycines* type 0	An amino acid transporter, an α-SNAP protein, and a wound-inducible domain protein	Cook et al. 2012
Rhg4	*G. max*	SCN: *H. glycines* type 0	SHMT	Liu et al. 2012

RKN root knot nematode, *PCN* potato cyst nematode, *BCN* sugar beet cyst nematode, *SCN* soybean cyst nematode, *SHMT* serine hydroxymethyltransferase

R-proteins, and further work is required to elucidate the detailed roles of *Rhg1* and *Rhg4* in SCN resistance.

The best-studied example of an R-protein involvement in nematode resistance is tomato *Mi-1*. RKN resistance mediated by the *Mi-1* gene is dependent on pathogen recognition and resistance signal transmission mediated by an LRR domain internal to the Mi-1 protein (Hwang et al. 2000; Hwang and Williamson 2003) and by an ATPase activity associated with its NBS domain (Tameling et al. 2002). Subsequently, it has been shown that the extended N-terminus of the Mi-1 protein has both negative and positive regulatory roles in the activation of Mi-1 protein (Lukasik-Shreepaathy et al. 2012).

Upstream of *Mi-1*, a gene product of the *Rme1* locus is required for RKN resistance, although the *Rme1* sequence has not been cloned as yet, and thus its exact biological function is unknown (Martinez de Ilarduya et al. 2004). In tomato, orthologs of Arabidopsis *HSP90-1* and *SGT1* were also required for *Mi-1*-mediated RKN resistance, as demonstrated by virus-induced gene silencing (Bhattarai et al. 2007). Based on a proposed model of R-protein-mediated signaling (Glowacki et al. 2011), the *Mi-1* encoded NBS-LRR protein, an HSP90-1 protein, and an SGT1 protein form an R-protein signaling complex that can activate downstream signaling pathways by detection of nematode effector-induced conformational changes in the protein coded by the *Rme1* gene that may be an interacting partner of the Mi-1 protein as well as the direct target of nematode effectors (Bhattarai et al. 2007). Similarly, PCN resistance mediated by *Gpa2* also requires the RAN GTP activating protein 2 that functions as a cofactor for *Gpa2* (Sacco et al. 2009).

As pathogen receptors, NBS-LRR proteins detect pathogen effectors and trigger ETI type resistance characterized by hypersensitive responses (HR). In accordance, several nematode effectors that may interact with plant NBS-LRR proteins that are coupled with HR have been identified. These include a PCN SPRY domain-containing protein, RBP-1, that interacts with the Gpa2 protein (Sacco et al. 2009), an RKN protein coded by the *MAP-1* gene that may interact with the tomato Mi-1 protein (Semblat et al. 2001), and another RKN encoded protein product of the *Cg-1* gene that may interact with Mi-1 protein (Gleason et al. 2008). It also could be concluded that there are other unknown nematode effectors interacting with host plant R-proteins, because HR type cell death is frequently identified in plant *NBS-LRR* genes mediating nematode resistance (Chen et al. 2007; Khallouk et al. 2011; Sacco et al. 2009; Sobczak et al. 2005; Williamson 1999).

Moreover, recent findings involving small regulatory RNAs (sRNAs) add a new layer of complexity to the regulation of *NBS-LRR* genes during plant defense

Table 2 Hormone biosynthesis and signaling genes in plant responses to SPENs

Gene/protein	Function	Regulation	Nematode	Tissue	Plant	References
Salicylic acid						
At*NPR1*	Receptor	Required for resistance	SCN; RKN; RN	WR	Arabidopsis; tobacco; cotton	Wubben et al. 2008; Priya et al. 2011; Parkhi et al. 2010
		Contribute resistance	SCN	WR	Soybean	Matthews et al. 2014
At*PAD4*	Signaling	Required for resistance	RKN; SCN	WR	Soybean; Arabidopsis	Youssef et al. 2013; Wubben et al. 2008
EDS1	Signaling	Upregulated	SCN	WR	Soybean	Klink et al. 2007
At*TGA*	Signaling	Contribute resistance	SCN	WR	Soybean	Matthews et al. 2014
ICS	Synthesis	Upregulated	SCN	WR	*SAMT* transgenic soybean	Lin et al. 2013
		Required for resistance	SCN	WR	Arabidopsis	Wubben et al. 2008
NahG	Hydrolysis	Increase susceptibility	SCN	WR	Arabidopsis	Wubben et al. 2008
PR1	Response	Down regulated	RKN	Galls; GC	Tomato; Arabidopsis	Portillo et al. 2013; Barcala et al. 2010
		Induced	RKN	WR	Maize *lox3* mutant	Gao et al. 2008
PR5	Response	Suppressed	SCN; RKN	GC; SN	Arabidopsis; soybean	Barcala et al. 2010
SAMT	Metabolism	Required for resistance	SCN	WR	Soybean	Lin et al. 2013
SNI1	NPR1 suppressor	Contribute susceptibility	SCN	WR	Arabidopsis	Wubben et al. 2008
Jasmonic acid						
AOC	Synthesis	Induced	RKN	WR	Arabidopsis *lox4* mutant; Maize *lox3* mutant	Ozalvo et al. 2013; Gao et al. 2008
AOS	Synthesis	Induced	RKN	WR	Arabidopsis *lox4* mutant; Maize *lox3* mutant	Ozalvo et al. 2013; Gao et al. 2008
γ-thionin	Response	Suppressed	RKN	WR	Tomato transgenic Mj-FAR-1	Iberkleid et al. 2013
COI-1	Receptor	Required for susceptibility	RKN	WR	Tomato	Bhattarai et al. 2008
Multicystatin	Response	Contribute resistance	RKN	WR	Tomato	Fujimoto et al. 2011
OPR	Synthesis	Induced	RKN	WR	Arabidopsis *lox4* mutant; Maize *lox3* mutant	Ozalvo et al. 2013; Gao et al. 2008
PIs	Response	Contribute resistance	RKN	WR	Tomato	Fujimoto et al. 2011
		Suppressed	RKN	WR	Tomato transgenic Mj-FAR-1	Iberkleid et al. 2013
LOX8	Synthesis	Induced	RKN	WR	Maize *lox3* mutant	Gao et al. 2008
Ethylene						
CTR1	Signaling	Contribute susceptibility	RKN	WR	Arabidopsis	Fudali et al. 2013
EIN2	Signaling	Contribute resistance	RKN	WR	Arabidopsis	Fudali et al. 2013
		Contribute susceptibility	CN	WR	Arabidopsis	Wubben et al. 2001
EIN3	Signaling	Contribute resistance	RKN	WR	Arabidopsis	Fudali et al. 2013
		Contribute susceptibility	CN	WR	Arabidopsis	Wubben et al. 2001
EIN4	Receptor	Contribute resistance	RKN	WR	Arabidopsis	Fudali et al. 2013
EIN5	Signaling	Contribute resistance	RKN	WR	Arabidopsis	Fudali et al. 2013
EIN7	Signaling	Contribute resistance	RKN	WR	Arabidopsis	Fudali et al. 2013
ERS2	Receptor	Contribute resistance	RKN	WR	Arabidopsis	Fudali et al. 2013
ETO1	Synthesis	Contribute susceptibility	RKN	WR	Arabidopsis	Fudali et al. 2013
		Contribute resistance	CN	WR	Arabidopsis	Wubben et al. 2001
ETO2	Synthesis	Contribute susceptibility	RKN	WR	Arabidopsis	Fudali et al. 2013
		Contribute resistance	CN	WR	Arabidopsis	Wubben et al. 2001

Table 2 continued

Gene/protein	Function	Regulation	Nematode	Tissue	Plant	References
ETO3	Synthesis	Contribute susceptibility	RKN	WR	Arabidopsis	Fudali et al. 2013
		Contribute resistance	CN	WR	Arabidopsis	Wubben et al. 2001
ETR1	Receptor	Contribute resistance	RKN	WR	Arabidopsis	Fudali et al. 2013
		Contribute susceptibility	CN	WR	Arabidopsis	Wubben et al. 2001
ETR3	Receptor	Contribute resistance	RKN	WR	Arabidopsis; Tomato	Fudali et al. 2013; Mantelin et al. 2013
Auxin						
AUX1	Importer	Induced	RKN & BCN	NFS	Arabidopsis	Mazarei et al. 2003
LAX1	Importer	Induced	BCN	SN	Arabidopsis	Lee et al. 2011
LAX3	Importer	Induced	BCN	SN	Arabidopsis	Lee et al. 2011
LBD16	Response	Support galls and GCs	RKN	WR	Arabidopsis	Cabrera et al. 2014
PIN1	Exporter	Support SN development	BCN	WR	Arabidopsis	Goverse et al. 2000; Grunewald et al. 2009
		Decreased over time	BCN	SN	Arabidopsis	Grunewald et al. 2009
PIN2	Exporter	Support SN development	BCN	WR	Arabidopsis	Goverse et al. 2000
PIN3	Exporter	Support SN development	BCN	WR	Arabidopsis	Grunewald et al. 2009
		Increased over time	BCN	SN	Arabidopsis	Grunewald et al. 2009
PIN4	Exporter	Support SN development	BCN	WR	Arabidopsis	Grunewald et al. 2009x
		Increased over time	BCN	SN	Arabidopsis	Grunewald et al. 2009
PIN7	Exporter	Support SN development	BCN	WR	Arabidopsis	Grunewald et al. 2009
		Decreased over time	BCN	SN	Arabidopsis	Grunewald et al. 2009
Cytokinin						
AHK3	Receptor	Down regulated	SCN	WR	Soybean	Ithal et al. 2007
AHK4	Receptor	Down regulated	SCN	WR	Soybean	Ithal et al. 2007
ARR4	Signaling	Down regulated	RKN	GC	Arabidopsis	Barcala et al. 2010
At-*ARR5*	Signaling	Down regulated	RKN	GC	Arabidopsis	Barcala et al. 2010
		Only expressed in dividing cells around GC	RKN	GC	*Lotus japonicus*	Lohar et al. 2004
ARR9	Signaling	Upregulated	SCN	WR	Soybean	Ithal et al. 2007
At*CKX*	Oxidation	Reduced galls	RKN	GC	*Lotus japonicus*	Lohar et al. 2004
Zm*CKX*	Oxidation	Reduced galls	RKN	GC	*Lotus japonicus*	Lohar et al. 2004

WR Whole root, *GC* Giant cells, *SN* syncytium

responses (Li et al. 2012a, b; Shivaprasad et al. 2012; Zhai et al. 2011), which is discussed in greater detail below in consideration of the role of sRNAs in nematode pathogenesis.

Hormone signaling in plant S/R to SPENs

Downstream of PTI and ETI activation, plant hormone signaling induces or suppresses defense responses to nematodes through regulation of different transcription factors (Fig. 1). Salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) function in plant stress responses (Table 2). Hormones are also involved in plant growth and development related to nematode infection, such as Auxin (AX) and Cytokinin (CK). AX plays an essential role in modulating plant cell morphology regulating NFS development (Table 2). CK also function as modulators in NFS development (Table 2).

Salicylic acid (SA)

SA signaling is required in *R*-gene mediated defense responses to SPENs (Branch et al. 2004; Kandoth et al. 2011; Uehara et al. 2010). Specifically, SA signaling and response genes were strongly induced in tomato plants harboring the *Mi-1 R*-gene (Molinari et al. 2013), the "*Hero A*" gene (Uehara et al. 2010), and the resistance QTL, *Rhg1* (Kandoth et al. 2011). Transgenic tomato plants containing dominant *Mi-1* or "*Hero A*" alleles

expressing *NahG* (encoding SA hydroxylase) exhibited reduced resistance to RKN or CN, respectively (Branch et al. 2004; Uehara et al. 2010). Furthermore, the SA analog benzothiadiazole (BTH), completely restored RKN resistance in *NahG* transformed tomato roots harboring an active *Mi-1* gene, but resistance was not established in susceptible plants lacking a functional *Mi-1* gene (Branch et al. 2004).

Downstream of SA signaling, Arabidopsis *WRKY70* is required for both basal defense and *R*-gene mediated resistance (Eulgem and Somssich 2007). In tomato plants containing *Mi-1* alleles, orthologs of Arabidopsis *WRKY70* were induced after exogenous application of SA. Similarly, attenuated *Mi-1*-mediated resistance against RKN was observed when tomato *WRKY70* was silenced (Bhattarai et al. 2010). However, another WRKY transcription factor, *WRKY72*, was found to control *Mi-1* mediated defense responses and basal resistance to RKN, independent of SA signaling (Atamian et al. 2012). While different *WRKYs* are differentially regulated in roots after nematode infestation (Barcala et al. 2010; Klink et al. 2007; Portillo et al. 2013; Uehara et al. 2010), their specific roles remain elusive. Yet, it is known that WRKY transcription factors regulate the expression of defense related genes in both PTI and ETI (Eulgem and Somssich 2007). Specifically, the down regulation of *WRKY6, WRKY11, WRKY17, and WRKY33* in Arabidopsis roots in response to BCN infestation has been demonstrated to favor nematode and NFS development (Ali et al. 2014).

In addition to *R*-gene-mediated resistance, genes involved in SA biosynthesis, SA signaling, and SA responses also contribute to nematode basal resistance (Table 2). The requirement of endogenous SA accumulation for resistance to SCN was demonstrated in Arabidopsis iso-chorismate synthase gene (*ICS*) mutants and *NahG* transgenic lines where each demonstrates increased SCN susceptibility (Wubben et al. 2008). ICS is a key enzyme in the SA biosynthesis pathway (Vlot et al. 2009). Higher levels of *ICS* expression were detected in *SAMT*-over-expressing (SA methyltransferase) soybean roots, where these plants exhibited resistance to SCN (Lin et al. 2013). SAMT modulates SA levels by converting SA to methyl salicylic acid (MeSA) (Vlot et al. 2009), and MeSA can function as a mobile signal, mediating systemic acquired resistance (SAR) in some plants (Park et al. 2007).

The At*pad4* mutant at the PHYTOALEXIN DEFICIENT locus which is involved in SA signaling showed increased SCN susceptibility; overexpressing wild type At*PAD4* in soybean showed increased resistance to RKN (Wubben et al. 2008; Youssef et al. 2013). PAD4 acts upstream of SA in pathogen responses via interaction with ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1) that has a similar sequence to *PAD4* (Vlot et al. 2009). In soybean roots, *EDS1* transcript levels were induced after infestation by both compatible and incompatible populations of SCN (Klink et al. 2007). These findings together demonstrated that SA-upstream signaling is required in nematode resistance.

Signaling downstream of SA is largely regulated via the NON-EXPRESSOR of PATHOGENESIS RELATED 1 gene (*NPR1*) (Vlot et al. 2009) recently identified as an SA receptor (Wu et al. 2012). In the nucleus, NPR1 interacts with TGA transcription factors which can bind to a *cis*-element required for SA responsiveness (Vlot et al. 2009). Arabidopsis *NPR1*-deficient mutants showed increased susceptibility to SCN, and SUPPRESSOR OF *npr1-1* INDUCIBLE (*SNI1*) deficient mutants exhibited increased resistance to SCN (Wubben et al. 2008). Similarly, transgenic expression of At*NPR1* conferred resistance to RN, RKN, and SCN in cotton, tobacco, and soybean, respectively (Parkhi et al. 2010; Priya et al. 2011). The same effect of SCN parasitism suppression was also observed in soybean root over expressing At*TGA2* (Matthews et al. 2014).

The best-studied SA responsive defense gene is *PR-1* (pathogenesis related 1) although induction of *PR-2* and *PR-5* are also used as indicators of SA-mediated activation in resistance responses (Vlot et al. 2009). Suppression of *PR-1, PR-2*, and *PR-5* gene expression in *10A06* SCN effector gene transformed Arabidopsis increased susceptibility to SCN (Hewezi et al. 2010). The disruption of SA-responsive defenses may be critical in at least SCN parasitism. Consistent with these findings, the suppression of *PR-1* and *PR-5* in roots where an NFS was successfully established and decrease of SCN parasitism in At*PR-5* over-expressed soybean roots, support the SA-responsive defenses in nematode resistance (Barcala et al. 2010; Hewezi et al. 2010; Matthews et al. 2014; Portillo et al. 2013).

In contrast, *PR-1* was highly induced in the RKN more susceptible *lox3* mutant of maize (Gao et al. 2008). *LOX3* encodes a 9-lipoxygenase that oxidizes fatty acids to oxylipins, including JA (Mosblech et al. 2009) and *lox3 mutant* also shows increased levels of JA and ET responsive and biosynthetic genes (Gao et al. 2008). Exogenous foliar application of the SA analog, BTH in rice only slightly induced RKN resistance compared to the MeJA or the ET-generating compound ethephon (Nahar et al. 2011). Thus, while SA plays a critical role in nematode S/R there are other hormones and interacting signaling pathways involved in plant responses to nematode infestation.

Jasmonic acid (JA)

Blocking JA perception via the *COI* receptor in *Mi-1* resistant tomato plants did not compromise resistance to

RKN (Bhattarai et al. 2008; Mantelin et al. 2013). However, negative crosstalk between the JA- and SA-signaling pathways in *Mi-1*-mediated resistance was consistent with the fact that SA-induced *WRKY70* was suppressed after treatment with the JA derivative MeJA (Atamian et al. 2012).

JA signaling, unlike SA production and signaling appears to be required for susceptibility to SPENs, since it was shown that a mutant JA receptor (*coi-1*) led to significantly lower numbers of RKN egg masses on RKN-susceptible tomato roots (Bhattarai et al. 2008). In addition, JA biosynthesis increased in nematode susceptible tomato genotypes (Bhattarai et al. 2008; Gao et al. 2008; Ozalvo et al. 2013).

Induction of JA biosynthesis genes (Table 2) in the Arabidopsis *lox4* mutant makes plants more susceptible to RKN infestation implicating a link between JA accumulation and nematode susceptibility (Ozalvo et al. 2013). A similar result was found in the RKN susceptible *lox3* maize mutant where JA biosynthesis genes (Table 2) were also induced (Gao et al. 2008). Both *LOX4* and Zm*LOX3* are induced in response to RKN infestation (Gao et al. 2008; Ozalvo et al. 2013). Taken together, it appears that JA biosynthesis may play a positive role in plant susceptibility to nematode (Gao et al. 2008; Ozalvo et al. 2013).

Direct application of JA induces RKN resistance responses in tomato (Cooper et al. 2005) in a dose dependent manner (Fujimoto et al. 2011), and some studies have shown that protease inhibitors may be down-stream regulators of JA-induced nematode resistance. High expression of a multicystatin-type gene and protease inhibitor encoding genes in tomato roots was observed when RKN infection was repressed (Fujimoto et al. 2011). Similarly, JA-responsive protease inhibitor (*Pin2*) and γ–thionin-coding genes were repressed in tomato plants overexpressing the *Mj-FAR-1* gene (Iberkleid et al. 2013). Mj-FAR-1 is a member of RKN-specific fatty acid and retinol binding family protein, and Mj-FAR-1 may play a positive role in plant susceptibility to RKN (Iberkleid et al. 2013).

JA and ET appear to play a greater role in rice resistance to RKN than does SA. Both MeJA and ET treatment induce strong resistance to RKN correlated with strong induction of resistance genes (Nahar et al. 2011). Foliar treatment with JA or ET biosynthesis inhibitors increase rice susceptibility to RKN (Nahar et al. 2011), and genes involved in JA and ET biosynthesis and signaling were mainly suppressed in rice roots and shoots after RKN infestation (Kyndt et al. 2012). Furthermore, JA was found to be an indispensable signal in rice, mediating resistance to RKN, and ET-mediated RKN resistance is dependent on JA biosynthesis (Nahar et al. 2011). ET foliar treatment had no effect on the response to RKN infestation in rice mutants with impaired JA biosynthesis, but JA-induced defense was still functional when ET-signaling was impaired (Nahar et al. 2011).

Ethylene (ET)

ET signaling is not involved in *Mi-1*-mediated RKN resistance (Fujimoto et al. 2011), but ET and ET-signaling affect basal resistance to both RKN and CN (Table 2). ET-treated soybean roots exhibited increased SCN susceptibility (Tucker et al. 2010) while the inhibitors of ET action, 1-methycyclopropene (MCP) and 2,5-norbornadiene (NBD), reduced SCN colonization in soybean roots consistent with ET playing a positive role in nematode susceptibility. Arabidopsis ET-overproducing mutants (*eto1*, *eto2*, and *eto3,* Table 2) demonstrate hyper-susceptibility to CN (Wubben et al. 2001), but the same ET overproducing mutants showed reduced susceptibility (increased resistance) to RKN (Table 2, Fudali et al. 2013). Such opposing results have also been reported with ET signaling mutants in CN and RKN resistance (Table 2). For example, Arabidopsis ET receptor mutant (*etr1*) and ET signaling mutants (*ein2* and *ein3*) showed decreased susceptibility to CN (Wubben et al. 2001); and a gene encoding UDP-glucose-4-epimerase that contributes to CN resistance was negatively regulated by the intermediate ET-signaling genes, *EIN2* and *EIN3* (Wubben et al. 2004). ET-insensitive mutants (*etr1, ers2, ein4*) and tomato (*Nr*) and mutant genes positively regulating ET signaling (*ein2*, *ein3*, *ein5*, and *ein7*) resulted in higher levels of RKN infestation, while the negatively regulating ET signaling mutant (*ctr1*) attracted fewer RKN (Table 2). Thus, it can be concluded that the ET biosynthesis and signaling pathways positively regulate susceptibility to CN, whereas they contribute to RKN resistance (Table 2) although the mechanistic basis of such opposing effects is unclear at this time.

Ethylene Response Factors (ERFs or EREBPs) that specifically bind to a GCC box *cis*-element sequences have been found in many PR-protein coding gene promoters (Wang et al. 2002). *EREBP* transcription factor was induced in soybean resistant reactions but suppressed in susceptible reactions to SCN (Mazarei et al. 2011). One soybean *EREBP* (Gm*EREBP1*) appears to be involved in the induction of different classes of *PR*-genes in roots of both *GmEREBP1*-overexpressing soybean and Arabidopsis plants (Mazarei et al. 2002), although this transgenic overexpression did not confer increased resistance to SCN in Arabidopsis (Mazarei et al. 2007). In addition to ET-induced PR-protein coding genes *GmPR2*, *GmPR3,* and *AtPDF1.2*, SA-responsive PR-protein coding genes (*AtPR1, GmPR1*, and *AtPR2*) and JA-responsive PR-genes (*GmPR3* and *AtPDF1.2*) were also induced in *GmEREBP1* overexpressing plants (Mazarei et al. 2007).

These studies demonstrate widely varying roles of ET biosynthesis and signaling in S/R to SPENs that vary according to the specific nematode investigated. ET may have pleiotropic effects in plant resistance to nematodes because: (1) unique mechanisms are required for different nematode species in host attraction, as see where CN and RKN responded differently in ET biosynthesis and signaling mutants; (2) complex unknown crosstalk between ET and JA or SA signaling pathways occur during nematode infestation, as suggested by induction of different classes of *PR* proteins in *GmEREBP1* transgenic plants. Thus, it is currently difficult to determine a precise role for ET in nematode infestation, but it is clear that ET does play important roles in specific nematode pathogenesis and possibly indirectly in resistance.

Auxin (AX)

AX insensitive mutants appear to be resistant to CN compared to their wild type counterparts (Goverse et al. 2000), and AX levels increase transiently in the expanding NFS and cells surrounding the NFS (Goverse et al. 2000; Karczmarek et al. 2004; Absmanner et al. 2013). In particular, AX responsive elements were found in the *cis*-element of *NtCel7* gene (Wang et al. 2007). *NtCel7*, a tobacco endo-β-1,4-glucanase gene, functions in cell-wall degradation and is strongly induced in both RKN and CN feeding cells (Wang et al. 2007). Taken together, these studies suggest that a local and transient accumulation of AX in feeding cells upon nematode infection support NFS establishment and nematode parasitism.

Polar AX transport manipulates AX distribution in feeding cells during nematode infestation (Table 2). At the beginning stages of CN infection, AX accumulates in the infection site by induced *LAX3*/*AUX1*-mediated AX import and reduced *PIN1*-mediated AX export, whereas when a syncytium is expanding, *PIN3* and *PIN4* facilitate the lateral transport of AX to the cells surrounding the initial syncytium (Grunewald et al. 2009; Lee et al. 2011). Specifically, *LAX3* was demonstrated to be a direct target of the nematode secreted protein Hs19C07 (Lee et al. 2011). Binding to Hs19C07 can activate *LAX3*, leading to subsequent syncytia development (Lee et al. 2011).

AX effects on nematode S/R are also mediated through AX response factors (ARF) by activating or repressing AX-responsive genes (Matthews et al. 2013). Members of the ARF gene family are distinctly and dynamically regulated in host Arabidopsis plants in response to BCN infestation compared to uninfected plants (Hewezi et al. 2014). AX accumulation and *ARF*s appear to play a transient role in NFS initiation and early development (Goverse et al. 2000; Karczmarek et al. 2004; Absmanner et al. 2013). However, continued high expression of *ARF*s in fully developed syncytia (Hewezi et al. 2014) and AX-responsive mature root galls supports a functional role of AX and *ARF*s in mature NFS as well (Cabrera et al. 2014). It should be noted that *ARF*s are targets of several microRNAs and small interfering RNAs (see sRNA regulation section below, and Table 3). The detailed role of such sRNA regulation of *ARF*s in plant responses to SPENs has not been extensively investigated to date, but given the role of *ARF*s in NFS growth and development this area is likely to yield significant information on plant/nematode interactions in the future.

Downstream of *ARF*s, the AX responsive gene *LATERAL ORGAN BOUNDARIES-DOMAIN 16* (*LBD16*), which showed activation upon RKN infestation, was implicated in the induction of both root galls and lateral roots (Table 2, Cabrera et al. 2014). This study established the first molecular link between root gall induction and lateral root formation (Cabrera et al. 2014).

In contrast to the above studies that suggested a dependence of NFS initiation and morphogenesis on AX, *AtWRKY23,* which acts downstream of *ARF*s was induced in early syncytium development during BCN infection independent of AX (Grunewald et al. 2008). This result suggests the involvement of other pathways in the regulation of early plant responses to nematode infestation. AX is known to interact synergistically with ET in general, but ET-mediated nematode susceptibility was independent of AX (Wubben et al. 2004; Fudali et al. 2013). This inconsistency of AX dependence in uninfected and infected plant signaling pathways suggests the possibility of nematode secreted effectors bypassing plant AX signaling to modulate plant responses (Lee et al. 2011). It is also possible that AX-like compounds found in nematode secretions can manipulate plant S/R to nematodes (Hewezi and Baum 2013; Mitchum et al. 2013).

Cytokinin (CK)

CK signaling components are also differentially regulated during SCN infestation (Table 2). It was found that in RKN infested *L. japonicus* roots, the CK-inducible gene *ARR5* was strongly induced in rapidly dividing small cells around the giant cell but absent in mature galls (Lohar et al. 2004). Similarly, significantly fewer and smaller galls were formed on transgenic *L. japonicus* roots, when CK was degraded by overexpressed CK oxidase (Lohar et al. 2004).

In addition to functioning downstream of *R*-gene mediated nematode resistance, different hormones are also important players in plant basal defense responses. Stress hormones SA, JA, and ET regulate plant S/R to SPENs mainly through *PR* genes or other resistance related factors; growth hormones AX and CK primarily affect nematode parasitism though manipulation of NFS initiation and

Table 3 Examples of soybean cyst nematode responsive small RNAs

Small RNA	Regulation	Target function
miRNA		
gma-miR1510ab-3p[a]	Down in S & R	HEX TF
gma-miR1515[a]	Down in S & R	Autophagy protein
gma-miR156[a]	Down in S & R	SBP domain protein
ath-miR156[b]	Down 4dpi; up 7dpi	
gma-miR159bcf[c]	Down in S & R	MYB TF
gma-miR160[c]	Down in S & R	ARF
ath-miR160[b]	Down 4dpi	
gma-miR162[a]	Down in S & R	Embryo-related protein
ath-miR164[b]	Down 4dpi & 7dpi	NAC
gma-miR164[a]	Down in S & R	
gma-miR166a-5p[a]	Down in S & R	HD-ZIP TF
gma-miR167[a]	Down in S & R	ARF
ath-miR167[b]	Down 4dpi & 7dpi	
ath-miR168[b]	Down 4dpi; up 7dpi	AGO protein
gma-miR169[b]	Down in S; up in R	Nuclear factory
ath-miR169[b]	Up 7dpi	
gma-miR171b[a]	Down in S & R	Polyubiquitin protein; TCP family TF
ath-miR171b[b]	Down 4dpi; up 7dpi	
gma-miR172[a]	Down in S & R	Heat shock cognate protein; AP2 TF
ath-miR172a[b]	Up 7dpi	HSP, AP2 TF; TCP family TF
gma-miR319[c]	Up in S & R	TCP family TF, plasma membrane intrinsic protein
gma-miR390b[a]	Up in S; down in R	Unknown protein
gma-miR394a[a]	Down in S; up in R	NADP+
ath-miR396a[b]	Down 4dpi; up 7dpi	GRF
gma-miR397ab[c]	Down in S & R	60 s ribosomal protein; multicopper oxidase
ath-miR398a[b]	Down 4dpi & 7dpi	CSD
gma-miR408[c]	Down in S & R	Oxidoreductase
gma-miR482a-5p[a]	Down in S & R	NA
gma-miR5374[a]	Down in S; up in R	Disease resistance protein-like protein MsR1
gma-miR5674[a]	Down in S & R	PPR-containing protein
siRNA		
ath-siRNA41[b]	Up 4dpi & 7dpi	Similar to TOR1
ath-siRNA46[b]	Up 4dpi & 7dpi	Disease resistance protein
ath-siRNA52[b]	Down 4dpi; up 7dpi	MAPKKK13
ath-siRNA29[b]	Up 4dpi; down 7dpi	LTR/Gypsy
ath-siRNA32[b]	Down 4dpi & 7dpi	RC/Helitron
ath-siRNA50[b]	Up 4dpi; down 7dpi	Glycosyl hydrolase family 17 protein
ath-siRNA9[b]	Up 4dpi & 7dpi	Oxidoreductase

Down downregulated, *Up* upregulated, *S* susceptible genotype, *R* resistant genotype

[a] was tested using Solexa sequencing

[b] was tested using RT-PCR

[c] was tested using Solexa sequencing and RT-PCR

(Hewezi et al. 2008; Li et al. 2012b; Hewezi et al. 2012)

development. The specific role of each hormone in plant responses to nematodes is a bit more complex showing both plant and nematode species specificity as well as differences that depend on infestation timing. In addition, using different gene/proteins for studying hormone effects on plant-nematode interactions could lead to different conclusions because there are complex interactions between various hormone-signaling pathways. Moreover, nematode secretions should also be considered when interpreting these plant responses, since nematode secreted effectors are known to manipulate plant development and defense responses by interacting with or mimicking plant genes/proteins (Hewezi and Baum 2013; Mitchum et al. 2013).

ROS generation and signaling in plant S/R to SPENs

ROS are chemically reactive molecules containing oxygen, including singlet oxygen, superoxide, hydrogen peroxide

(H_2O_2), and hydroxyl radical (Ray et al. 2012). Plants constantly produce ROS as a byproduct of metabolic processes such as photosynthesis and respiration (Tripathy and Oelmuller 2012). Steady-state levels of ROS are tightly regulated by competing ROS generation and scavenging mechanisms. ROS over-accumulation usually causes oxidation of lipids, proteins, and DNA as well as other components often leading to cell death (Tripathy and Oelmuller 2012). When challenged by pathogens, ROS production increases rapidly in what is often called the oxidative burst, which can lead to local cell death (Tripathy and Oelmuller 2012). The local burst of ROS in response to pathogen infection could also be transferred systemically in a cell-to-cell auto-propagating manner, integrating with other signaling pathways generating a SAR response (Baxter et al. 2014; Tripathy and Oelmuller 2012).

In response to RKN infection, both RKN susceptible and resistant tomato plants showed nematode penetration into their roots (Melillo et al. 2006, 2011). However, 48 h post infection (hpi), significantly fewer RKN were observed in *Mi-1*-mediated incompatible infested roots than in compatible infested roots (Melillo et al. 2006). In accordance, an oxidative burst was observed at the root infection site as soon as 12 hpi for both compatible and incompatible responses, but this was only prolonged in incompatible responses (*Mi-1* tomato responses to avirulent RKN) until 48 hpi when cell death became evident (Melillo et al. 2006, 2011).

NADPH oxidases were the main source of ROS production in plant incompatible responses and subcellular localization of H_2O_2 production for incompatible responses followed a pattern consistently observed in HR (Melillo et al. 2006). Collectively, these observations show that, as one of the most sensitive signals monitoring cellular metabolic changes, ROS accumulation occurs rapidly in response to RKN infection, and temporal and spatial differences in ROS (particularly H_2O_2) accumulation are crucial in determining the extent of RKN pathogenesis in host plants.

Rapid apoplastic generation of ROS has been mainly associated with pathogen resistance (Baxter et al. 2014; Tripathy and Oelmuller 2012). Consistent with this, RKN genes encoding ROS scavenging enzymes (clade B peroxiredoxins) were more actively transcribed in parasitic stages to protect RKN from the oxidative responses of the host, and knockdown of these genes resulted in reduced RKN parasitism (Dubreuil et al. 2011).

Conversely, a recent study demonstrated a negative role for ROS in cell death and BCN resistance in Arabidopsis (Siddique et al. 2014). Loss of two specific NADPH oxidase genes, *RbohD* and/or *RbohF*, required for ROS production at 24 hpi after BCN infestation, resulted in reduced BCN parasitism, smaller syncytium size, and enhanced cell death (Siddique et al. 2014). In addition, the suppression of cell death in *RbohD* and/or *RbohF* mutants was demonstrated to be independent of SA accumulation, and an antagonistic relationship between SA and ROS was suggested because SA responsive genes were induced in *RbohD/F* double mutants but were suppressed in *RbohD* overexpressing Arabidopsis (Siddique et al. 2014).

ROS also work in concert with nitric oxide (NO), the main reactive nitrogen species (RNS) found in biological systems, to control plant responses to SPENs (Melillo et al. 2011; Yu et al. 2012). NO is a gaseous nitrogen-containing free radical, endogenously produced by plants serving as an important mediator of defense responses (Bellin et al. 2013). In tomato plants demonstrating an incompatible reaction to RKN, the peak generation of ROS was preceded by production of NO (Melillo et al. 2011). Similarly, in *P. thunbergii* responding to pine wood nematode, endogenous NO levels increased coincident with a rapid increase in H_2O_2 levels, whereas H_2O_2 levels decreased when pretreated with an NO scavenger (Yu et al. 2012).

ROS generation is always associated with plant defense responses, and rapid apoplastic generation of ROS often leads to an HR-type cell death, thus restricting the spread of the infection leading to pathogen resistance (Baxter et al. 2014). However, newly identified ROS suppression of cell death and support of BCN parasitism suggests an activation of HR-type cell death can occur through other unknown signaling mechanisms (Feng and Shan 2014). This new finding is also consistent with ROS having distinct roles in plant S/R to SPENs.

Small RNAs may be important regulators in plant S/R to SPENs

Small regulatory RNAs (20–24 nucleotides in length) are emerging as important aspects of plant defense responses resulting from epigenetic, transcriptional, posttranscriptional, and/or translational gene regulation (Shukla et al. 2008; Ruiz-Ferrer and Voinnet 2009; Katiyar-Agarwal and Jin 2010). The primary classes of plant sRNAs are the short interfering RNAs (siRNAs) and the micro RNAs (miRNAs) although there are other emerging classes of sRNA that have not yet been extensively investigated in the context of pathogenesis (Axtell 2013).

miRNAs are the best studied class of sRNAs. miRNAs are derived from single stranded RNA transcripts, which form hairpin loop structures (Axtell 2013). Both miRNAs and siRNAs are processed from their double-stranded RNA precursors by DICER-like proteins (DCLs) and the resulting miRNAs and siRNAs are loaded into Argonaute (AGO) proteins to form an RNA-induced silencing complex (RISC) that can bind to target RNAs or DNAs (Axtell 2013).

Plant miRNAs and siRNAs play important roles in plant biotic stress responses by regulating genes involved in plant PTI and ETI (Katiyar-Agarwal and Jin 2010), hormone signaling (Liu and Chen 2009), ROS generation and signaling (Shukla et al. 2008), and various other types of signaling (Ruiz-Ferrer and Voinnet 2009). Thus, sRNAs are positioned to integrate various aspects of the pathogenesis responses into regulatory networks. Current studies further suggest that miRNAs and siRNAs are playing such regulatory roles during nematode pathogenesis in host plants (Fig. 1; Tables 3, S2).

Genes encoding proteins associated with miRNA or siRNA biogenesis and/or function including *DCL*s, *AGO*s, *RDR*s, and genes encoding DNA methylase proteins, as well as histone methylation and deacetylation-related genes, are regulated in RKN-induced tomato root galls and RKN-infected rice roots (Ji et al. 2013; Portillo et al. 2013). DNA and histone methylation and histone acetylation are important mechanisms mediating epigenetic gene regulation in plants (Sahu et al. 2013). Taken together, these results are consistent with miRNA and siRNA biogenesis and function playing an important role in plant responses to SPENs.

The biogenesis and functioning of miRNAs and siRNAs were also demonstrated to be required in plant S/R to SCN (Hewezi et al. 2008). Responses to SCN were examined in several single, double, and triple mutants of Arabidopsis. The genes examined included genes coding for the *DCL*s and *RDR*s, various isoforms of which are involved in the production of specific miRNAs and siRNAs. Mutation in these sRNA-producing genes all displayed decreased SCN susceptibility compared to wild type (Hewezi et al. 2008).

Predicted targets of differentially expressed miRNAs and siRNAs indicated specific roles of sRNAs in nematode pathogenesis in host plants (Tables 3, S2). Genes encoding R-proteins, ARFs, Heat Shock Proteins, ROS scavenger Cu/Zn superoxide dismutases, and various transcription factors are all predicted to be the targets of one or more differentially expressed miRNAs or siRNAs (Tables 3, S2). Among the differentially expressed sRNAs in response to nematode infestation, Arabidopsis miR396 was down-regulated 4 days post SCN infestation and up-regulated 7 days post infestation (Hewezi et al. 2008). Targets of miR396, including Arabidopsis *GRF* (Growth Regulating Factors) exhibited the opposite expression trends to miR396 post SCN infestation (Hewezi et al. 2008).

To investigate the role of miR396/*GRF* in plant responses to SCN, Arabidopsis mutants deficient in *GRF* genes or overexpressing miR396 were examined, and overexpression of miR396 and/or reduced *GRF* gene expression resulted in reduced SCN susceptibility (Hewezi et al. 2012). Furthermore, miR396-overexpressing Arabidopsis roots produced smaller syncytia with fewer SCN infections. Similar characteristics were also observed in miR396 binding site-deficient mutants (Hewezi et al. 2012).

Since the *GRF* gene family positively controls cell proliferation and size, the coordinated expression of miR396 and its target *GRF* genes are critical in syncytia development during SCN infection. Moreover, almost half of the genes differentially expressed in syncytia overlapped with genes differentially regulated in *GRF* deficient and miR396 resistant Arabidopsis mutants, indicating that miR396/*GRF* is an essential regulatory system in reprogramming of gene expression in SCN-induced syncytia (Hewezi et al. 2012).

sRNA expression changes in response to RN infestation have been investigated in cotton (Li and Locy, unpublished result). It was found that specific miRNAs and siRNA sequences (including cotton miR396 and miR482 among others) exhibit distinct expression patterns in response to RN infestation in cotton genotypes differing in RN resistance and susceptibility. The spectrum of sRNA target genes derived from differentially expressed sRNAs include genes previously implicated in plant innate immunity, hormone signaling, ROS generation and signaling, as well as sRNA biogenesis and function, and in epigenetic regulation. This analysis supports the idea that sRNAs serve to integrate a signaling network that regulates most, if not all, of the various signaling pathways discussed above.

Viral (Shivaprasad et al. 2012), bacterial (Shivaprasad et al. 2012), or fungal (Zhu et al. 2013) infection of tomato or diploid cotton (*G. raimondii*) all resulted in suppression of specific miRNAs and induction of their target *R*-genes (*NBS-LRR* genes). Co-expression of miRNAs and their *NBS-LRR* targets in tobacco caused decreased resistance to TMV (Li et al. 2012a). Based on bioinformatics and experimental data generated from different plant species, some *NBS-LRR* genes can produce clustered secondary siRNAs from their mRNA transcripts in a phased manner, and miRNA targeting is required for the production of secondary siRNAs (Shivaprasad et al. 2012; Zhu et al. 2013; Zhai et al. 2011; Li et al. 2012a). Furthermore, some secondary siRNAs (i.e. trans-acting siRNAs) can also target other defense related genes (Shivaprasad et al. 2012) while others have shown involvement in AX signaling regulation of root development (Fahlgren et al. 2006; Marin et al. 2010; Yoon et al. 2010). Since NBS-LRR proteins and AX signaling clearly have a role in endoparasitic nematode S/R, it is reasonable to presume that miRNA/siRNA signaling plays important roles in integrating nematode signaling systems.

Support for such an hypothesis comes from a bioinformatic analysis of sRNA regulatory networks involved in RN signaling in cotton. A RN-responsive miRNA (miR482

family) was predicted to target an NBS-LRR protein-coding mRNA that could be cleaved into a cluster of secondary phased siRNAs (Li and Locy, unpublished results). These siRNAs also target a series of transcription factors and other proteins many of which are known pathogenesis-related genes involved in signaling pathways. This provides preliminary support for the involvement of sRNA regulatory network in cotton-RN interactions through miRNAs and NBS-LRR protein coding genes producing secondary siRNAs that have been implicated in plant innate immunity as described for other pathogens (Zhai et al. 2011; Li et al. 2012a; Shivaprasad et al. 2012; Fei et al. 2013).

Overall, these studies suggested that the miRNA regulation of *NBS-LRR* gene expression via the production of secondary siRNAs is playing an important role in nematode pathogenesis in host plants, although the exact nature of such regulatory network remains to be defined. It is possible that host plants can defend themselves by down regulating specific miRNAs leading to the expression critical *R*-genes involved in nematode resistance. The suppression of *NBS-LRR* gene expression via the sRNA pathway serves as a protective mechanism for plants since large increases in NBS-LRR transcripts and protein levels could trigger cell death and/or plant HR (Qiao et al. 2013). It is also possible that infecting nematodes secrete specific effectors that induce sRNA regulatory network suppressing the expression of *NBS-LRR* genes and thus promoting nematode parasitism. Since the delivery of sRNAs between host plants and infecting nematodes can suppress the expression of genes essential for nematode pathogenesis and development (Fairbairn et al. 2007; Klink et al. 2009; Charlton et al. 2010; Dalzell et al. 2010; Li et al. 2010), it is possible that nematode effector RNAs can act as the initiators of the plant sRNA regulatory networks, although this remains to be established.

Conclusions

In conclusion, SPENs resistance in host plants appears to be initiated when plant R-proteins sense nematode secreted effectors. Detection of nematode effectors leads to massive downstream reprogramming of gene expression through various hormones, ROS, and NO signaling pathways. However, each hormone's role in particular plant-nematode interaction is unique. Detection of nematode by R-proteins also leads to a localized cell necrosis at the nematode infection site, and a local burst of ROS and NO are causally correlated with HR observed in *R*-gene-mediated nematode resistance, although ROS play a supportive role in parasitism when incompatible *R*-gene interactions are involved.

It is clear that, instead of functioning independently, different signaling factors work in concert with each other in a highly controlled regulatory network. The specific nature of nematode produced factors interacting with plant factors also plays an important role in mediating the behavior of the regulatory network developing plant responses. sRNAs are emerging as important regulators of pathogen resistance that are implicated in the regulation of crucial regulatory nodes in plant defense responses, such as *NBS-LRR* genes. Based on these findings, it could be concluded that sRNA might be the hub of plant S/R to nematodes, but additional, continuing studies are required to reveal and demonstrate the specific roles of sRNAs in various plant-nematode interactions.

The application of emergent next generation sequencing technologies and network analysis strategies will not only support the implication of canonical signaling pathways in plant S/R to nematodes, but will also play a key role in implicating pathway cross talk and integration as we move forward.

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