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Multifaceted Strategies Used by Root-Knot Nematodes to Parasitize Plants-A Review

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Abstract: Root-knot nematodes being omnipresent in agricultural and horticultural soils are tallied among the most important economic pathogens around the world. For successful parasitism, these nematodes use various strategies to control and manipulate the host plant's cell machinery. These strategies include the molecular mimicry of some host genes by some nematode secreted effector proteins, secretion of cell wall digesting enzymes and other effector proteins that are responsible for the suppression of defence by the host plant. All these secretions which are released through the stylet, contribute to the formation of specialized feeding sites or giant cells. The effector proteins interfere with the normal physiology, cytology and biochemistry of the host plant. The present review brings novel insights by summarizing some novel effectors that have been discovered recently like *MgPDI, MiMIF, MiIDL1, MiISE6, Mg16820,* etc. It also discusses some novel mechanisms through which these effector proteins target different pathways of host plants and thus facilitate nematode parasitism.

1 Introduction

Plant parasitic nematodes are ubiquitous, microscopic and hidden enemy of agricultural crops that occur abundantly in the soil. They are biotrophic obligate parasites and may be of sedentary or migratory parasitic behaviour [1]. From phylum Nematoda, about 4,100 species have been recognized as plant-parasitic nematodes [2]. Root-knot nematodes are members of the genus *Meloidogyne*. This genus comprises of more than 100 species with more than 3000 host plants [3]. They are sedentary endoparasites that depend on plants for their reproduction and survival [4]. These nematodes are globally distributed with high diversity in tropics and subtropics. They cause severe damage to cash crops and subsistence crops worldwide [5]. Among the root-knot nematodes (RKNs), the following four species, *M. javanica, M. incognita, M. arenaria* and *M. hapla* pose a major threat to agro-ecosystems, being responsible for at least 90% of all damage caused by phytonematodes [6,7]. In addition to these species, *M. graminicola* also poses a major threat to rice cultivation particularly in Asia, where changes in agricultural practices in response to environmental and socio-economic conditions have led to a dramatic increase in *M. graminicola* populations [8,9].

Root-knot nematodes feed on roots by inducing giant cell (GC) formation to complete their life cycle [10]. These GCs are located close to the vascular system of the root, due to which the availability of water and nutrients to plant decreases [11,12]. These nematodes during feeding cause biochemical and morphological alterations which lead to abnormal growth of plants, gall formation and other deformations [13].

2 Parasitism of Root-knot Nematodes

Root-knot nematodes possess parasitism genes for successful parasitism. The products of these genes play an important role during the plant-nematode interaction [14,15]. They have the ability to alter the expression of plant genes in their favour. Not only they express their genes in the host, but also weaken the defence of plants by suppressing the expression of defence-related genes of host [16]. During parasitism, the second stage juveniles (J2) initiate the parasitic relation with the host by secreting the oesophageal secretions into the root cells through stylet and induce the formation of GCs [17]. These secretions may be deposited either outside the plasma membrane or directly injected into the cytoplasm of the host. Both these cases may result in the mimicry of some proteins that leads to alteration of gene expression of host [18]. The RKNs have evolved themselves with strategies to parasitize the host through secretions. The cell wall digesting enzymes (CWDEs), effectors and the proteins that mimic the proteins of host are key secretions of RKN for their establishment on the host [19,20,21].

3 Secretions of Root Knot Nematodes

The cell walls of host plant cells act as obstacles for the penetration of RKNs. For crossing this barrier, RKNs release several cell wall digesting enzymes that break the structural components of the cell wall (Fig. 1). The other secretions include pectate lyases [22]; expansins [23]; polygalacturonase [24]; endo1,4 β xylanase [25]; 1,4 βendoglucanase [26] and a cellulose binding protein [27]. Among these, expansins and cellulose binding protein does not exhibit enzymatic activity, but they bind to cell wall components and thereby weaken its structure [27]. Majority of the secreted proteins are synthesized and released through three oesophageal glands including two subventral glands that are especially active in infective J2 stage [28]. Apoplasm has been reported to be an important recipient compartment for secretions of RKN [29]. Here they may degrade cell wall or interact with receptors of the plasma membrane to initiate host responses via signalling cascades [30].

The RKNs secrete effector proteins that modify or suppress plant cell machinery [31,32] regulate ubiquitination pathways for cell wall deterioration and interfere with plant signalling pathways to promote parastism [33–35]. Effectors can be defined as all pathogen proteins and small substances that can alter the physiology and cell structure of the host. These effectors have different modes of action which mainly include:

3.1. Interfering with the homeostasis of defence related phytohormones.
3.2. Suppression of PTI and/or ETI.
3.3. Manipulation and mimicry of the defence related proteins of the host.
3.4. Manipulation of the host metabolism.

3.1 Interfering with the Homeostasis of Defence Related Phytohormones

Phytohormones such as Indole acetic acid (IAA, also called as auxin), cytokinin, ethylene (ET), salicylic acid (SA), and jasmonic acid (JA) play a necessary role in plant growth and development [36]. The key plant functions like cell elongation and proliferation, differentiation, cell expansion, and response of plants to stress is mediated by auxin and ethylene [37]. These phytohormones play a crucial role in plant-pathogen interactions. As they play an imperative role by assisting coordination of plant response to biotrophs and insects [38], it seems that RKNs manoeuvre the homeostasis of plant hormones for their establishment [39]. Mi-CM is an effector secreted by *M. incognita* that causes manipulation of the auxin pool of the host [40]. Recently research has reported that in *M. incognita* a novel effector protein MiISE6 is involved
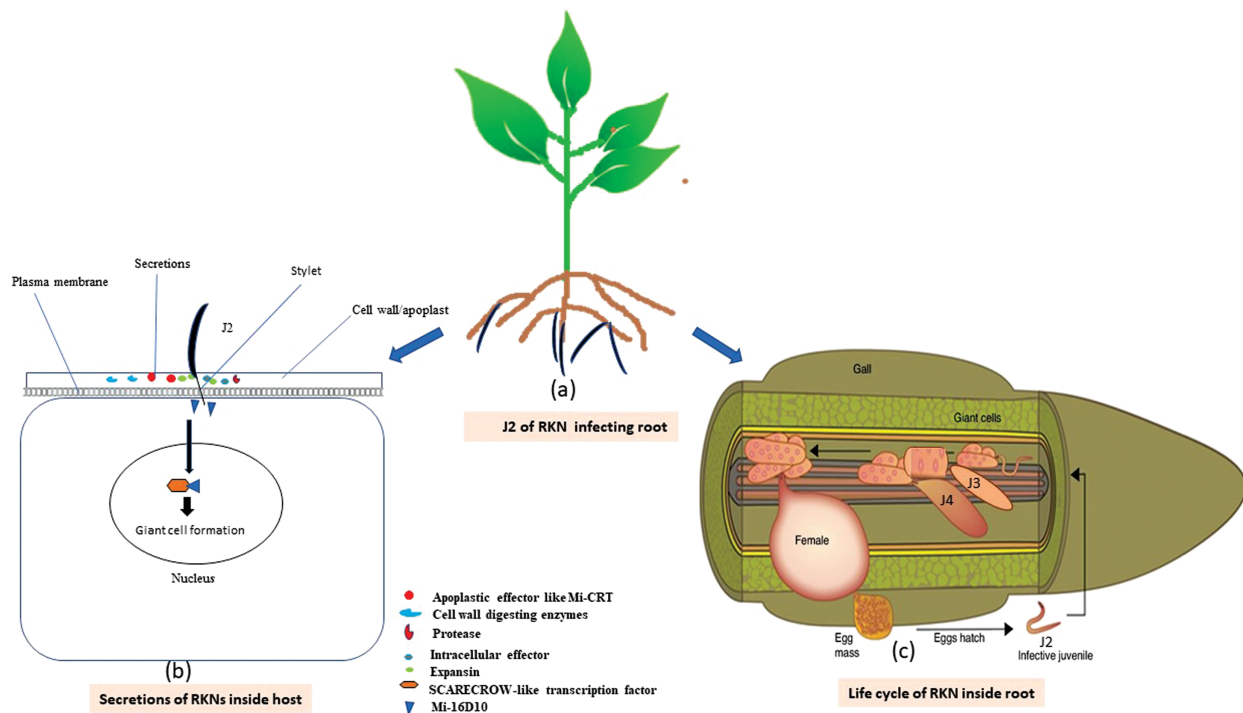
Figure 1: Schematic representation of the potential interactions of RKN with host and its secretions (a) Infection of host root by J2 nematode, (b) Secretion and binding of Mi-16D10 to SCARECROW-like transcription factor and formation of giant cells by RKN, (c) different stages in life cycle of RKN. Illustrations framed with colored shapes at bottom of (b) show secretions of RKNs (Modified from Mitsumasu et al.; Siddiqui et al. [44]

in the interaction between nematode-plant and plays an important role during the early stages of parasitism by interfering with multiple signalling pathways of the plant. It causes suppression of the jasmonate signalling pathway to facilitate nematode parasitism [41]. Another effector protein reported in *M. incognita*, Mi-CM-3 plays an important role in suppressing plant immunity by regulating the SA pathway during the early parasitic stage of *M. incognita* to promote nematode parasitism [34].

3.2 Suppression of PTI and/or ETI

Some of these effectors act by suppressing the pathogen triggered immunity (PTI) and/or effector triggered immunity (ETI). MiMsp40 from *M. incognita* is very important for the suppression of both PTI (including ROS) and ETI mediated plant defences to promote nematode parasitism [42]. One more effector in this category is Mi-CRT, which is a calreticulin, secreted by nematodes into the apoplasm of the host tissue. It causes the suppression of the PTI triggered by the PAMP elf8 in *Arabidopsis thaliana* [43].

3.2.1 Suppression of Host Defence by RKNs

The RKNs use strategies to suppress the defence of the host by disarming the resistance proteins deployed by the plant [44]. At the molecular level, the plants have developed a two-tiered detection system for the recognition of pathogen [45–47].

(i) Plant cells have receptor proteins at external surface called pattern recognition receptors (PRR) that recognize the conserved microbial elicitors, pathogen or microbial associated molecular patterns (PAMPs or MAMPS). On stimulation, these PRR induces the PAMP triggered immunity.

(ii) Virulent pathogens secrete different effectors to overcome the defense mechanisms of the host. These effectors are recognized by intracellular nucleotide-binding (NB)-LRR receptors and can interference with PTI that leads to effector-triggered immunity stimulate (ET1).

The invasion of roots by RKNs cause tissue damage which is likely to generate cell wall fragments that could act as damage associated molecular pattern (DAMPs) and induce a PTI-like basal defence response. DAMPs in plants are mainly cytosolic proteins, peptide, nucleotides, and amino acids, which are released from damaged cells or secreted by intact cells undergoing pathogen invasion. In addition, some oligomeric fragments of plant cell-wall polysaccharides are released when tissues are disrupted by physical injuries or attacks of pathogens and herbivores also function as DAMPs. As the case of PAMPs, DAMPs initiate PRR-mediated immune responses in local sites surrounding of wounding and pathogen invasion and regulate systemic immune signalling [48]. Marhavy et al. [49] reported that when plants are exposed to cellular damage through invading microbes, herbivore feeding or through any mechanical damage, the primary wound responses are communicated to neighbouring cells and distal tissues via mobile signals. In roots a single cell injury caused by nematode attack induces the production of ROS, membrane depolarization and regional up-regulation of ethylene markers. This ethylene signalling acts antagonistically to the nematode establishment and thus constitutes a root immune response against root-knot nematodes.

PTI is a multistep response, in which the plant pattern recognition receptors (PPRs) identify the conserved pathogen molecules. This is followed by many events, including activation of the mitogen-activated protein kinase (MAPK) signalling cascade, calcium-dependent protein kinase (CDPK) and the reactive oxygen species (ROS)-generating system [50,51]. During PTI and ETI, the production of ROS in the apoplast is one the earliest plant response during a plant-pathogen interaction [52,53]. The signalling of ROS induces various plant defence responses including activation of defence genes, synthesis of antimicrobial secondary metabolites and strengthening of plant cell walls, and potent toxicity against pathogens. ROS production has variable effects on the response of plants to pathogens in terms of both cell death and resistance. On the one hand, ROS positively correlates with plant resistance by strengthening cell walls via cross linkages, lipid peroxidation, membrane damage, and the activation of defence genes [54,55] while, on the other hand, it is an important susceptibility factor for the successful infection of plants by various pathogens. To combat the oxidative response of host plant, RKNs also employ effector proteins. In this regard the most recent discovery is MGPDI which protects the nematode from H_2O_2 stress and thus help in the reproduction and pathogenicity of *M. graminicola* [56]. The effector, Macrophage migration inhibitory factor (MIF) secreted by from *M. incognita* also causes the suppression of host plant's defence [57]. It promotes parasitism by interfering with the annexin-mediated plant immune responses. Future work will have to provide mechanistic details for the dual role of ROS during plant-nematode interaction. From last few years various PTI-suppressing effectors have been characterized from root-knot nematodes [58]. But the mechanistic details for most of these PTI-suppressing effectors are not fully known.

3.3 Manipulation and Mimicry of the Defence Related Proteins of the Host

Meloidogyne graminicola is one of the most damaging nematodes of rice. A novel effector Mg16820 has been identified from *M. graminicola* that acts in two cellular as an immune suppressor and targets a protein involved in the stress response, therefore indicating an important role for this effector in parasitism [32]. Another novel effector, MiPFN3 (*Meloidogyne incognita* Profilin 3) has been reported from *M. incognita* to be transcriptionally upregulated in the juvenile stage of the nematode and can be injected into the host plant through stylet where it promotes nematode parasitism [59]. Kim et al. [21], reported a novel effector, MiIDL1 that produces a functional IDA mimic that appears to play a role in successful gall development on *Arabidopsis* roots. IDA (INFLORESCENCE DEFICIENT IN ABSCISSION) is a signalling peptide that regulates cell separation in Arabidopsis including floral organ abscission and lateral root emergence.

It has been reported after the genome wide overview of gene expression that majority of the nematode regulated genes involved in plant defence pathways showed suppression [60–62]. The effector MgGPP plays a role in *M. graminicola* parasitism on rice. It is secreted into host plants and targeted to the ER, where it undergoes some modifications like *N*-glycosylation and C-terminal proteolysis to subjugate plant immunity and promote parasitism [63]. The effector MiSGCR1 has been reported with a potential role to facilitate parasitism during the early stages of plant-nematode interaction [64]. Some of the novel mechanisms related to RKN parasitism have also been recently revealed. A good example can be cited in Lin et al. [33]. It reported an effector MjTTL5 from *M. javanica* that facilitates parasitism by exploiting the ferredoxin: thioredoxin system of the host. DnaJ proteins have been discovered in *M. arenaria* as potential effectors [65]. These proteins have a predicted nuclear localization signal (NLS). In sedentary plant parasitic nematodes, it has been proposed that these kinds of molecules have important roles in host cell remodeling, establishing the feeding sites and counteracting plant defences [66]. The genome-wide overview of gene expression of the nematode regulated genes involved in plant defence pathways show suppression [61,62]. Tab. 1 summarizes some important effector proteins of RKNs.

Table 1: List of some novel and important effectors secreted by RKNs

Effector	Nematode Species	Putative Function	References
DnaJ	*M. arenaria*	Host cell remodeling	[65]
MiMIF	*M. incognita*	Suppression of annexin- mediated immune response	[57]
MgPDI	*M. graminicola*	Counteract Host ROS	[67]
MiISE5	*M. incognita*	Interferes with JA signaling pathway	[41]
MiISE6	*M. incognita*	Targets host nucleus	[68]
Mi IDL1	*M. incognita*	Gall development	[21]
Mi-CM-3	*M. incognita*	Manipulates salicylic acid pathway	[34]
Mg16820	*M. graminicola*	Suppression of host defense	[32]
MiPFN3	*M. incognita*	Disrupts actin filaments of host	[59]
MgMO237	*M. graminicola*	Manipulates Host defense proteis	[69]
MiSGCR1	*M. incognita*	Facilitate parasitism	[64]
Mg-GPP	*M. graminicola*	Suppression of host resistance	[61]
MhTTL2 and Mh265	*M. hapla*	Facilitate parasitism	[70]
Mi-sp12	*M. incognita*	Defense suppression	[71]
Mi-Msp40	*M. incognita*	Defense Suppression	[42]
Mj TTL 5	*M. javnica*	Suppress host immune response	[33]
M-jNULG1a	*M. javanica*	Unknown	[33]
Mi-EFF1	*M. incognita*	Manipulates nuclear functions of host	[43]
Mi-asp2	*M. incognita*	Predigestion of peptidic nutrients	[29]
Mi-16D10	*M. incognita*	Transcriptional reprogramming	[40]
Mi-CM	*M. incognita*	Affects auxin pool of host	[40]

3.4 Manipulation of the Host Metabolism

The induction of GCs by RKNs directly affects the metabolic pathways of the host plant because these cells act as excellent sink of various metabolites and nutrients. A highly dense cytoplasm without vacuoles, a high density of organelles, and endopolyploidy, all indicate the highly active metabolism of GCs [72]. These cells also develop extensive ingrowths into the vascular system of neighbouring cells that represent a strategy for the most efficient and direct access to nutrient requirements. Redirecting the root xylem to the GCs would allow direct acquisition of essential amino acids and other solutes that are normally assimilated in the roots and transported to transpiring tissues for redistribution in the phloem [73]. Eloh et al. [74] reported that the infection of RKNs alters the primary metabolic pathways of the host plant. They observed a reduction in glycine and fumaric acid. The former is catalyzed by glycine decarboxylase complex during the process of photorespiration. The reduction in fumaric acid which is an intermediate in kerb's cycle reflects higher demand for the reducing power equivalents required in defence response of the host plant. RKN infection alters the process of translation in host roots [75]. Baldacci et al. [76] demonstrated that glutathione (GSH) metabolism differs in a neoformed gall and an uninfected root of the *Medicago truncatula*. The change in GSH metabolism also caused the modification in starch and γ-aminobutyrate metabolism and of malate and glucose content in GSH-depleted galls. By analysing these changes in host metabolic pathways, new and more efficient management strategies against these pests can be developed.

4 Conclusion and Perspectives

Because of the enormous yield losses caused by RKNs to agricultural crops, the understanding of plant-RKN interaction has become of outmost importance. These RKNs use various strategies for their establishment on host. From the molecular and genetic approaches, it has now become evident that these RKNs, like other plant pathogens produce effector proteins and suppressors. For understanding the molecular aspects of plant-RKN interactions, the identification of effector proteins is a major challenge. Further, the targets of some novel effectors like MILSE6 need to be studied in order to elucidate its role during plant-nematode interaction. Another effector Mg16820, secreted by the root-knot nematode *M. graminicola* has been proven to be secreted in both the apoplast and the cytoplasm of GCs during the early stages of parasitism, and has immune-suppressing capacities in both cell compartments. The mechanism behind the suppression of both the PTI and ETI responses at two different subcellular localizations is not understood and requires further study. Nowadays omics approaches are being followed for characterizing the nematode secretions at genomic level [77], effector immunocytochemistry and the cellular imaging of feeding sites [78,79], transcriptome level using RNAseq [80], and the proteome level [81]. For computational identification of effector proteins various bioinformatical approaches such as Ortho-MCL [82] could be very fruitful [83]. Currently research is being done to explore the plant targets of these effector proteins whose identification will be a landmark breakthrough in this field.

Future analysis of the strategies that RKNs use to reprogram the host plant and the effector proteins mainly responsible for this reprogramming, are expected to highlight the molecular mechanisms involved in this process. The understanding of these mechanisms may provide a good sense to manage these RKNs that otherwise have become a threat to world agriculture.

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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